Co-registration of Ultrasound and Photoacoustic Radar Imaging and Image Improvement for Early Cancer Diagnosis

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Mechanical and Industrial Engineering
University of Toronto

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Abstract

Unlike mainstream biomedical photoacoustics, mostly based on pulsed laser excitation, this work explores the alternative photoacoustic radar (PAR) modality, using intensity-modulated continuous-wave laser sources driven by frequency-swept (chirp) waveforms. A one-dimensional, axisymmetric model, and quantitative blood oxygenation analysis were developed for analyzing experimental results. Signal processing tools are investigated to enhance system parameters such as signal-to-noise ratio (SNR), dynamic range and spatial resolution. Besides applying pulse compression through matched-filtering to generate high peak power cross-correlation response, SNR can be improved by leveraging the distinct phase channel to filter the PAR amplitude channel acquired by the cross-correlation of the PA response and the reference waveform. Phase-filtering is also investigated as a spatial-resolution improvement technique. An in-vivo study of a cancer cell-injected mouse recorded a 14-15 dB SNR gain for the phase-filtered image compared to the amplitude and phase independently, while the phase PAR image produced ~340 μm spatial resolution compared to ~840 μm for the amplitude image.

Additionally, to accelerate clinical acceptance and use, the combination of the PAR system with clinically-accepted ultrasound is explored. PAR tomography is investigated toward in-vivo
functional analysis and the effects of the number of scan lines on image quality, resolution and contrast, examined.

Furthermore, addressing assumptions of linear fluence dependence in conventional multispectral photoacoustic imaging is crucial for obtaining accurate spatially-resolved quantitative functional information by exploiting known chromophore-specific spectral characteristics. A study conducted introduces the non-invasive phase-filtered wavelength-modulated differential photoacoustic radar technique to address this issue by eliminating the effect of the unknown wavelength-dependent fluence. It employs two laser wavelengths modulated out-of-phase to significantly suppress background absorption while amplifying the difference between the two photoacoustic signals. This facilitates (pre)malignant tumor identification and hypoxia monitoring with significant improvement in dynamic range, SNR and spatial resolution, as minute changes in total hemoglobin concentration and oxygenation are detectable.
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Chapter 1
Introduction to Photoacoustics for medical imaging

In this chapter, the concept of the photoacoustic (PA) effect is introduced with a brief history of its use and progress in biomedical research. Key features and merits of this method are highlighted culminating in the rationale for its adoption in medical imaging, contrasted against current preclinical/clinical imaging modalities. Areas of application and related research are discussed before rounding off the chapter with the objectives and outline.

1 Introduction to Photoacoustics for medical imaging

1.1 Background and History of Photoacoustic imaging

Photoacoustic (PA) imaging is an emerging non-ionizing, non-invasive imaging modality being investigated for early cancer diagnosis[1], [2]. Optical imaging of tissue offers potential benefits in distinguishing different structures. Electromagnetic (EM) waves in the optical spectral range have energies that can interact with various biomolecules and wavelengths that are analogous with the size of cell and tissue micro-structures. Tissue is a highly scattering media in the optical spectral range; resulting in spreading of light beams and loss of directionality. Optical imaging therefore, either has limited imaging depth with ballistic photons (approximately one photon mean free path (MFP) i.e. ~1 mm) or limited resolution with diffuse light (~1 cm)[3], [4].

PA imaging overcomes these limitations by applying the Photoacoustic effect – the generation of acoustic waves by the absorption of EM energy, which was first discovered by Alexander Graham Bell in 1880[5]. Upon illumination, biomolecules absorb photons which thermoelectrically induces pressure waves, resulting in subsequent emission of less-scattering acoustic waves that propagate to the tissue surface for detection as illustrated in Figure 1.1.
Figure 1.1: Schematic illustrating the Photoacoustic effect

Two types of EM sources are employed in PA imaging – pulsed and continuous-wave (CW). Pulsed PA imaging is frequently used in biomedicine as it provides high signal-to-noise ratio (SNR) and allows direct detection of the PA source distance through time-resolved signals[6]. Optical wavelengths in the visible and near-infrared (NIR) region (~550–1100 nm) are mostly used in biomedicine with the latter providing greater penetration depth.

1.1.1 History

PA imaging has a somewhat sporadic history, particularly in its early years. After Alexander Bell discovered the PA effect, little progress was made in research and technological development until the 1960s with the development of the laser (the high peak power, spectral purity, and directional light source needed in PA techniques). Applications in biomedicine began in the 1970s and slowly progressed until the 1990s where many pioneering works emerged with the first images appearing[7], demonstrating the PA effect in optically scattering media and biological tissue.

The first truly compelling in vivo images were obtained in the early to mid-2000s. There has been major growth since then in the development of instrumentation, image reconstruction algorithms, functional and molecular imaging capabilities as well as in vivo application in biological research and clinical medicine[6], [8], [9].
1.1.2 Fundamentals/Principles

Non-destructive and non-ionizing
Absorbed photons cause a slight temperature rise (~< 0.1 K[10]) during the PA process which is well below that needed to cause physical damage or physiological changes to tissue. Furthermore, unlike other imaging modalities like Positron-Emission tomography (PET), only non-ionizing radiation within safety limits stipulated by regulatory bodies like the American National Standards Institute (ANSI) (Laser Institute of America), is often used. These features make PA imaging suitable for in vivo applications.

Imaging contrast
PA image contrast depends on optical properties (specifically absorption) and not on the mechanical and elastic properties of tissue, like in ultrasound. Optical absorption is a function of molecular composition. PA imaging can, therefore, provide greater tissue differentiation and specificity than US because differences in optical absorption between different tissue types can be much larger than those in acoustic impedance. For example, strong optical absorption of hemoglobin makes PA imaging particularly well suited for imaging microvasculature, which due to weak microvessels echogenicity, can be a challenge to observe with pulse-echo US.

Figure 1.2: Absorption coefficient spectra of endogenous tissue chromophores[11].
Oxyhaemoglobin (HbO₂) – red (150 gl⁻¹), deoxyhaemoglobin (Hb) – blue (150 gl⁻¹), water – black (80% by vol. in tissue), lipid(a) – brown (20% by volume in tissue), lipid(b) – pink, melanin – black dashed, Collagen – green, and elastin – yellow.
Photoacoustics is intrinsically suitable for spectroscopic (multi-wavelength) detection in living tissue since absorption values of endogenous tissue chromophores can be highly sensitive to wavelength as seen in Figure 1.2. PA imaging can, thus, be used to deduce certain physiological parameters, such as oxygen saturation and concentration of hemoglobin. Spectral dependence of optical absorption enables selective enhancement of chromophore-specific contrast by tuning laser excitation wavelength to its peak absorption.

Furthermore, exogenous contrast agents can be used to improve optical absorption. Provided absorption contrast agents are properly conjugated to bio-peptides, proteins, antibodies, hormones, drugs or other bioactive agents, PA imaging at a molecular level is possible by implementing optical absorbent biomarkers or targeted molecular probes such as nanoparticles, to enhance the absorption contrast of diseased tissue areas[3], [12].

Spatial Resolution and Penetration depth

Ultrasound (US) scattering is 2–3 orders of magnitude weaker in biological tissue than light scattering and can therefore provide better resolution than optical imaging at depths greater than ~1 mm[3]. Spatial resolution in most PA imaging ultimately depends on the frequency content of acoustic waves arriving at the detector. Maximum frequency content of the PA wave is limited by band-limiting of propagating PA wave due to frequency-dependent acoustic attenuation in soft tissue. Spatial resolution therefore scales with depth, which both scale with ultrasonic frequency.

Penetration depth is ultimately limited by optical and acoustic attenuations, both significant in soft tissue but optical attenuation dominates. Optical attenuation depends on wavelength-dependent absorption and scattering coefficients and is therefore best characterized by the effective attenuation coefficient ($\mu_{\text{eff}}$). At depths beyond several MFPs in homogeneous scattering media where light diffuses, irradiance also decays exponentially ($\mu_{\text{eff}}$ being the exponential constant) with depth[3], [8]. This presents a major challenge for PA imaging, i.e. the need to detect extremely weak US signals due to PA signal attenuation of several orders of magnitude to penetrate deep in tissue.

However, PA imaging, unlike its pure optical counterparts, actually detects the absorbed photons and can therefore attain significantly greater penetration depth (several centimeters) where absorption of diffuse photons generate ultrasound in the 1–50 MHz range, still allowing for high spatial resolution (as illustrated in Figure 1.3) since US scattering is much less in tissue.
1.2 Cancer diagnosis

Cancer remains a major health problem worldwide[14] and the second leading cause of death (over 595,000 cancer-related deaths – nearly one in 4 deaths) estimated in 2016 in the United States alone[15]. The early detection and treatment of cancer greatly increase the chances of survival[3]. This has been a great motivator for increased research in the field of PA imaging. Cancer is known to be caused by genetic mutations and alterations (via tumorigenesis) largely in oncogenes and tumor suppressor genes that results in an uncontrolled growth of cells[16]–[18]. Hyper-proliferating cancerous cells drastically alter nutrient utilization[19] such that characteristic metabolic changes are redirected to support the large biosynthetic demands of cell growth and proliferation. Thus resulting in the rapid development of a dense microvascular network via angiogenesis to perpetuate tumor growth (Figure 1.4) during the multistage development of invasive cancers (premalignant and malignant phases alike)[20], and a drop in intratumoral oxygenation levels making it hypoxic. This leads to upregulation of the hypoxia inducible factor (HIF1), a heterodimeric transcription factor. Vascular endothelial growth factor (VEGF) is an important transcriptional target of HIF1, which combined with other cytokines, induces tumor neovascularization allowing growth beyond the oxygen diffusion-imposed size limitation[17]. VEGF is present in almost every type of human tumor, with particularly high concentration around tumor blood vessels and in hypoxic regions of the tumor, and causes hyperpermeability in normal blood vessels[21], [22].
PA imaging is sensitive to oxygenated hemoglobin concentration, which is a critical diagnostic parameter for the metabolic state of lesions. Through enhanced laser light absorption in the 650-1100 nm (NIR) tissue optical spectral range, early cancer detection can be facilitated. Molecular and spectroscopic imaging is possible by applying multi-wavelength optical sources to the conventional PA modality [23]–[26].

Figure 1.4: Tumor-progression pathway depicting the angiogenic switch for tumor growth and metastasis. Tumors often start growing as (a) avascular nodules (dormant) before reaching a steady-state level of cell proliferation and apoptosis. (b) Perivascular detachment and vessel dilation precede (c) angiogenic sprouting, in response to VEGF, that leads to (d) new blood-vessel formation and maturation, which (e) continues as long as the tumor grows, and the blood vessels specifically feed hypoxic and necrotic areas of the tumor providing it with essential nutrients and oxygen.[27]

Consequently, assessing tumor hypoxia (a hallmark of cancer diagnostics) is a critical issue in cancer therapy management[28]–[30] and, therefore, of value to radiation oncologists, surgeons, and biotechnology and pharmaceutical companies involved in developing hypoxia-based treatment strategies to improve patient outcomes. Though several tumor hypoxia assessment modalities exist, none has been clinically approved for routine practice, impeding the clinical
development of hypoxia-based therapies. Hence, there is a need for a suitable tumor hypoxia assessment and monitoring modality for clinical applications. Polarographic electrodes, the current benchmark for tumor hypoxia detection and characterization[28], are highly invasive and require significant technical skill making measurement repeatability extremely challenging. It also overestimates hypoxia in necrotic sample regions due to the inability of the probe to discriminate between viable and necrotic tissue[31]. An alternative direct \( \text{pO}_2 \) measurement method is phosphorescence quenching that relies on the interaction of oxygen molecules with phosphorescent dyes[32]–[35]. Since analysis is based on phosphorescence lifetime (not signal intensity), oxygen response is independent of tracer concentration. Real-time tissue oxygenation profile is easily attained due to the high temporal resolution of this modality. Other direct assessment modalities include electron paramagnetic resonance (EPR)[36]–[38], \(^{19}\text{F}-\)magnetic resonance spectroscopy (MRS)[39], and Overhauser enhanced magnetic resonance imaging (OMRI)[40]. Some modalities that report on the physiological processes (directly) involving oxygen molecules require the use of contrast agents such as in contrast-enhanced color duplex sonography (CDS)[41]–[44] and MRI-based measurement[45]–[47] and photoacoustic lifetime imaging (PALI), or radiolabeled reporters like in hypoxia positron emission tomography (PET)[48].

Among the several measurement methods, existing non-invasive approaches that rely on endogenous markers and are thereby appropriate for \textit{in vivo} clinical applications include near-infrared spectroscopy (NIRS)[49], [50], blood oxygen level-dependent MRI (BOLD-MRI)[51] and photoacoustic tomography (PAT)[26], [52]–[57]. NIR spectroscopy quantifies an \( \text{Hb}/\text{HbO}_2 \) ratio using the different absorption spectra of deoxyhemoglobin (Hb) and oxyhemoglobin (HbO\(_2\)). A 3D oxygen distribution in breast cancer patients was reconstructed using Diffuse Optical Tomography (DOT)[58], a variation of NIR spectroscopy. BOLD-MRI, on the other hand, estimates temporal changes of blood oxygenation[59], [60] using endogenous paramagnetic deoxyhemoglobin. Limited tissue penetration and low spatial resolution[61] are however significant drawbacks for NIRS whereas BOLD-MRI, though capable of providing useful complementary qualitative information, is prone to the influence of other independent variables not related to tissue oxygenation due to its dependence on deoxyhemoglobin concentration[59] instead of \( \text{pO}_2 \) directly. Biomedical PAT detects less-scattering acoustic waves generated from absorbed EM energy with comparable axial resolution (~mm) and signal-to-noise
ratio (SNR) making it auspicious for hypoxia assessment[2], [52], [54] and early cancer diagnosis[1], [2].

1.3 Motivation for preclinical/clinical use of PA imaging

PA imaging is a hybrid modality that combines high optical contrast and spectroscopic-based specificity with high ultrasonic spatial resolution and depth penetration. Due to much higher optical scattering in tissue than ultrasound scattering, optical methods are only applicable at very small depths (~1 mm) and suffer from poor resolution, even though they produce good contrast. US provides greater penetration depth and better resolution but lacks good contrast when anatomically unapparent since it is based on mechanical inhomogeneity. Varying acoustic frequency allows for scalable resolution and imaging depth in soft tissue.

A major advantage photoacoustics offers in preclinical/clinical applications is its intrinsically spectroscopic imaging capability[23]–[26] devoid of the depth limitations associated with optical methods. This enables the interrogation of endogenous tissue chromophores for functional imaging in vivo, such as deducing oxygen saturation and total hemoglobin concentration, which is obviously very attractive for clinicians and patients. The large difference in optical absorption between blood and other tissues enables PA imaging to detect tissue angiogenesis associated with rapid tumor growth, particularly in the early stages of cancer development. Furthermore, the relative molecular concentration of different blood and tissue components, particularly deoxyhemoglobin (Hb) and oxyhemoglobin (HbO₂), can be used to differentiate tumors from healthy tissue and to determine tumor malignancy[62], using their uniquely distinct optical absorption spectra before and after the isosbestic point over different laser wavelengths. Properly chosen laser excitation wavelengths, therefore, allow for selective enhancement of chromophore-specific contrast and spatial resolution for cancer diagnostics [1], [63].

Upscaling applications like in vivo metabolic imaging, based solely on endogenous contrast from small animals to humans can be revolutionary in screening, diagnosis and treatment of metabolic diseases like cancers and cerebral disorders.

PA imaging possesses several other valuable features such as non-ionizing radiation use (unlike x-ray mammography, for instance), relatively fast image acquisition speeds and in vivo imaging, making it conducive for imaging biological tissue.
To-date, no clinical PA imaging technology is available for cancer patient screening. Facilitating effective clinical translation will require passing rigorous regulatory approval as well as technical improvement. PA imaging is conducive for combined imaging with other imaging modalities, particularly ultrasound and pure optical modalities to simultaneously acquire multi-modal images to augment diagnostic value through complementary information provided. Achieving PA imaging functionality on a commercial US instrument, inherently made possible by the use of ultrasonic transducers to detect laser-induced acoustic signals in PA imaging, could accelerate clinical acceptance and use. Commercial US imagers also possess sophisticated software for rapid image acquisition that could dramatically speed-up PA imaging. Real-time hybrid (US-pulsed laser PA) imaging systems have been reported for human hand vasculature, sentinel lymph node detection in the breast and cardiovascular dynamics in small animals[6], [8]. However, efficient PA–US integration and image co-registration is difficult as US imagers operate on frequency-domain (FD) principles.

1.4 Contributions in Biomedical PA imaging

Biomedical PA imaging relies on the optical absorption properties of tissue to provide concentration maps of chromophores with remarkable contrast and specificity as well as spatial resolution due to its tissue-specific absorption of monochromatic light, thereby increasing the chances of early cancer detection. Some significant contributions in the Literature are discussed in this section as a prelude to the objectives of the thesis.

1.4.1 Breast cancer (deep) PA imaging

Breast cancer remains a leading cause of death among women worldwide[15]. Photoacoustic Tomography (PAT), a computed reconstruction-based PA imaging modality, is not limited by focused transducers or lenses with fixed imaging regions and as such, is the most versatile PA imaging modality; conducive for breast imaging. Various system designs have been developed and real-time imaging can be obtained with US transducer arrays. Detection geometry and reconstruction algorithms significantly affect the image quality of PAT. Three detection geometries commonly used for image reconstruction include: spherical, cylindrical and planar. Applications that allow the region of interest (ROI) to be enclosed by the detection surface offer the highest practically achievable image fidelity due to the large solid angular aperture realizable. Kruger’s group [9] employs a (hemi)spherical scanner design for in vivo whole breast imaging as
shown in Figure 1.5, illustrating a ROI enclosed by the detection surface to obtain the most exact image in practice. An opening at the bottom of a liquid-filled hemispherical detector bowl allows 800 nm pulsed excitation laser light (10 Hz pulse repetition frequency (PRF)) to enter along its vertical axis. 128 unfocused 5 MHz 3 mm-diameter piezoelectric (PZT) elements are distributed spirally over the detector bowl surface. A smaller optically and acoustically transparent bowl holding the breast is inserted into the detector bowl, allowing independent rotation of the detector bowl without disturbing the breast. The study reported a near-isotropic spatial resolution of \( \sim 250 \mu m \) over a \( 6.4 \times 6.4 \times 5 \text{ cm}^3 \) field of view (FOV) with a fast image acquisition time of 24-s using 240 angular steps per revolution.

Also shown in Figure 1.5b is a maximum intensity projection (MIP) image of vasculature in the left breast of a 57 year old patient volunteer with sub-millimeter vessels visible to a depth of \( \sim 4 \) cm (marked by the hollow arrow in Figure 1.5b), demonstrating the deep tissue imaging capability of PAT.

![Figure 1.5: PAT breast scanner with hemispherical detection geometry[9]. (a) System schematic. (b) Lateral projection (FOV = 64 x 50 mm\(^2\)) MIP of left breast of a patient volunteer.](image)

PAT, however, often has reconstruction artefacts and reduced spatial resolution since detection aperture is almost always truncated in practice and thus, only part of the wavefront is recorded. An example of such scanning methodologies involves the use of an arc transducer array for breast imaging[64].

Several reconstruction methodologies like back-projection methods, filtered back projection-type algorithms, etc. have been developed[8]. Other factors, besides image reconstruction that use sensitive US detectors and improved algorithms, are also to be considered. The dynamic range
and SNR of PA signals are affected by non-uniformity of EM radiation due to high optical scattering and heterogeneity of overlying skin and tissue (in *in vivo* functional imaging as well as other deep PA imaging and spectroscopic attempts). Studies have been done to tackle this problem by applying broad illumination[4], [65] to generate relatively uniform EM radiation within a section of the breast or reducing breast thickness by mildly compressing it, as done in the Twente PA Mammoscope[6].

Among other studies being investigated for PA imaging of breast cancer include the identification of sentinel lymph nodes (vital for determining the stage of breast cancer) to guide needle biopsy (that traditionally uses a radioactive tracer and has poor spatial resolution[6]) via accumulating injected optically absorbing contrast agents such as methylene blue, and the use of pulsed microwaves that could complement optical excitation and possibly improve penetration depth[6], [8]. Optical molecular markers for VEGF (targeting) have also been explored for sensitivity and contrast optimization *in vitro* in cell cultures and explanted human tumors, and *in vivo* in animal models.[66]–[68]

### 1.4.2 Other applications

**Skin imaging**

PA imaging has shown its capacity for imaging skin with applications in visualizing the vasculature (dermal and sub-dermal) and other features like melanin content in skin. This suggests potential for clinical assessment of skin pathologies such as accurate diagnosis and staging of tumors like malignant melanomas, and assisting in their surgical removal. An example is a Fabry-Pérot interferometer (FPI)-based PAT system (optical-detection method) that obtains a 3D image of the human palm (20 mm² FOV; sub–100 μm spatial resolution; ~4 mm penetration depth; ~10 min acquisition time)[69]. Upon contact with the tissue surface, the optical thickness of the planar FPI film is modulated by arriving acoustic pressure. Once a CW laser beam illuminates it, FPI encodes the variation in optical thickness in the generated back-reflected interference signal and ultrasonic pressure mapping on the tissue surface is obtained by 2D raster scanning the focused laser beam. Volumetric distribution of tissue absorption is acquired using a reconstruction algorithm. This system was also applied in tumor vasculature, mouse brain and embryo imaging. Other preclinical applications include burn depth assessment, wound healing, plastic surgery and superficial soft tissue damage (e.g. onset of pressure sore and ulceration)[8].
Small animal imaging

The many advantageous features of PA imaging including non-ionizing radiation use, high contrast, good (sub-millimeter) resolution in centimeters of depth, relatively fast image acquisition speeds and most importantly, in vivo imaging, make it very attractive for small animal studies (preclinical applications).

A PAT cylindrical scanner was used to illustrate the ability of PAT to visualize dynamic and functional properties of the nervous system[70]. 532 nm laser light transversely irradiates a rat head surface along the axis of rotation whilst a single 3.5 MHz PZT transducer, driven by a computer-controlled step motor around the rat head, detects PA signals in the imaging plane at each scanning position. The estimated spatial resolution of the system was ~200 µm. When applied to whisker stimulations in the cerebral cortex of rats, PAT images of the rat superficial cortex were taken without whisker stimulation and subtracted from two PAT images taken while stimulating the left or right whiskers, respectively to produce two function maps. The images (see Figure 1.6) clearly show cerebral changes in response to left or right whisker stimulation. The open-skull photograph of the rat cortical surface is provided.

Figure 1.6: Non-invasive functional imaging of cerebral hemodynamic changes in response to whisker stimulation[70]. (a) Non-invasive PAT image of vasculature in superficial layer of rat cortex. (2.0 x 2.0 cm FOV) (b, c) Non-invasive PAT images corresponding to the left- and right-side whisker stimulations, respectively. {a, b, c} acquired with the skin and skull intact and {b, c} acquired by superimposing functional whiskers image on a. (d) Open-skull photograph of rat cortical surface. B: bregma, L: lambda, M: midline, A: activated regions corresponding to whisker stimulation (4 mm x 4 mm).
PA imaging has also been employed for *in vivo* whole-body images of a mouse using an arc array transducer that clearly shows internal organs like the spleen, liver and kidney; achieving a resolution of 0.5 mm and image acquisition time of 8 min[8].

### 1.4.2.1 Multimodal imaging

As previously mentioned, PA imaging can be combined with current biomedical imaging and detection modalities such as ultrasound and optical methods to provide not only convenient multi-function detection but also accurate diagnosis through complementary information and quantitative capabilities. Advancements made in this area include a hand-held dual-mode PA–US imaging system[71] (a pair of optical fiber bundles attached to a commercially available diagnostic linear array probe), and a PA–US combined system for imaging stent placement during surgical insertion and in follow-up thereafter[72]. The former provides combined 2D images in real-time (image frame rate of 10 frames per second), intended for sentinel lymph node detection in the breast (vital for determining the stage of breast cancer) while the latter shows a cardiovascular application [6], [8], [73]. Real-time hybrid/dual-mode (US*-pulsed* laser PA-based) imaging systems have also been reported for human hand (finger) vasculature[74], [75] to help facilitate the diagnosis and characterization of inflammatory joint diseases such as rheumatoid arthritis (RA). Also under investigation are PA modes such as tomography for imaging human finger joints to detect the inflamed synovium (indicator for RA)[65] and detect osteoarthritis (OA)[76]. Imaging deeper into tissue remains an on-going quest with exogenous contrast agents being employed (~5.2 cm in chicken breast tissue[77]).

### 1.4.2.2 Quantitative (functional) PA imaging

Recent work presented by Li et al. [78] employ functional photoacoustic tomography(fPAT) to obtain functional images of breast tissue demonstrating high resolution detection and characterization of breast cancer, as well as quantitative total hemoglobin concentration (tHb) and oxygen saturation (SO$_2$). The fPAT system employs a tunable pulsed laser (10 Hz PRF) positioned underneath the examination table and scanned in 2D (indicated by the red dashed arrows in Figure 1.7b) to attain a large illumination area, and a radially adjustable ring-shaped transducer array (indicated by the red solid arrows in Figure 1.7b). The system generates tHb and SO$_2$ maps in the coronal plane for an invasive mammary carcinoma in the left breast as shown in
Figure 1.7c compared to the healthy right breast corroborated by MRI. The study reports a penetration depth of 5.6 cm and a spatial resolution of 0.5 mm.

Functional PA imaging has also been investigated for the visualization of tHb and SO$_2$ in the rat brain in vivo[79].

![Figure 1.7: (a) fPAT prototype, and (b) top view of fPAT breast interface. Red solid arrows show radially adjustable breast interface diameter; red dashed arrows indicate light scanning path. An invasive mammary carcinoma with ductal-carcinoma-in situ (DCIS) in the left breast[78]. (c) Coronal tHb and SO$_2$% maps for the left and right breasts. (d) Reconstructed image from MRI for both breasts (performed 7 days before fPAT exam). Arrows indicate suspicious lesion area.](image)

1.4.3 Frequency Domain Photoacoustic Radar (FD-PAR) imaging

Unlike pulsed PA, CW or frequency-domain PA (FD-PA) offer compact, inexpensive CW laser diodes with a wide wavelength selection making them attractive for portable, sensitive PA imagers. The FD modality also possesses depth-selective imaging capabilities[80] and can
generate high peak power cross-correlation response through matched filtering. Energy compression, typically, ms-long frequency chirps compacted into a narrow correlation peak, significantly increases SNR[81], [82]. SNR of FD-PA can also be greatly enhanced by coherently averaging multiple (typically 50–500) chirps and increasing laser power while decreasing chirp duration (exposure) congruently, to remain within the maximum permissible exposure (MPE) guidelines stipulated by regulatory bodies like the American National Standards Institute (ANSI)[83]. In principle, axial resolution in the pulsed mode is better due to larger bandwidth but the signal’s bipolar shape is a downside. On the contrary, due to the lack of baseline oscillation in cross-correlation FD-PA and the possibility of combining the FD phase signal with the amplitude signal, the FD mode has been shown to yield better or similar axial resolution to pulsed laser PA without the ultrasonic wake distortions which follow the laser pulse[84]. Additionally, FD-PA possesses superior contrast even after (high-pass) filtering to enhance the contrast of the pulsed response[84]. Moreover, low peak-power CW laser irradiation of deeply embedded tumors is an added laser safety advantage (less of a health risk). Table 1.1 provides a summary contrasting FD-PA with pulsed (TA)-PA.

Table 1.1: Comparison between the proposed CW FD-PA modality and pulsed (TD)-PA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FD-PA</th>
<th>TD-PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal-to-noise ratio (SNR)</td>
<td>High peak power CC response through matched filtering via energy compression; Coherently averaging multiple (typically 50–500) chirps and increasing laser power while also decreasing chirp duration, to remain within MPE</td>
<td>Proper high-pass filtering of the signal (minimizes but does not eliminate baseline oscillations)</td>
</tr>
<tr>
<td>Absorber (µₐ=2 cm⁻¹) in 3 cm of tissue; SNR=14 dB [25]</td>
<td>Estimated SNR=25 dB (ignoring acoustic attenuation and band-pass effects)</td>
<td>At low frequency (500 kHz), pulsed laser energy of 100 ml/cm⁻¹; 30 averages (does not enhance SNR): - SNR=11.8 dB (poor contrast and SNR due to deterministic baseline oscillations induced by radiation impinging on transducer). - SNR (HPF)=22.1 dB - FWHM of the TD-PA peak=0.86 µs (1.27 mm); 0.82 µs (1.21 mm) - HPF</td>
</tr>
<tr>
<td>Sample µₑffective similar to breast tissue at 1064 nm with black absorber, 16 mm deep [84]; At low frequency (500 kHz), 6.5 W/cm⁻¹ CW laser intensity (200–800 kHz); total laser exposure time of 800 ms; - higher contrast and SNR=23.1 dB - axial resolution (FWHM of signal peak)=2.43 µs (3.60 mm).</td>
<td>At high frequency (3.5 MHz): - experimental SNR=26.1 dB (small abs.: 24.8 dB); SNR (HPF) improved by 4 dB - FWHM=0.164 µs (0.243 mm; HPF);</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SNR=23.2 dB (small abs.: 20.5 dB) - axial resolution=0.46 µs (0.68 mm)</td>
<td>compromised due to the rarefaction zone (N-shape) that follows the compression peak.</td>
</tr>
<tr>
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<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Image resolution</strong></td>
<td>Lack of baseline oscillation in CC FD-PA; Combine FD phase with amplitude signal - yields sub-mm axial resolution (better than or similar to TD-PA) without the ultrasonic wake distortions which follow the laser pulse [84]. Two plastisol pieces (µa=9 cm⁻¹) ~0.9 mm apart; 1 cm deep in low-conc. Intralipid solution (0.05%)[84]; - FWHM=0.46 µs (0.68 mm; ampl.), 0.34 µs (0.50 mm; phase) and 0.30 µs (0.44 mm; combined).</td>
<td>In principle, better due to larger bandwidth but the signal’s bipolar shape is a downside.</td>
</tr>
<tr>
<td><strong>Maximum Detectivity (Depth limit for detection)</strong></td>
<td>Depth-selective imaging capabilities [80]</td>
<td>Cannot be chirped - cannot be used for depth-selective PA imaging. Estimated PA capability of 1 mm tumor at 7 cm depth in human tissue; only attained ~2 cm deep in clinical trials[64].</td>
</tr>
<tr>
<td></td>
<td>For a 2–6 cm⁻¹ absorption coefficient range, chirp-modulated FD-PA with much lower optical power: - comparable (or better; &gt;25 mm at low frequency (500 kHz)) with mm-scale axial resolution due to superior SNR. [85]</td>
<td>- Maximum imaging depth=18–23 mm Est. imaging feasibility=35–45 mm (provided efficient signal conditioning is implemented to suppress the background interference signal) - deeper detectivity at higher frequency (3.5 MHz)</td>
</tr>
<tr>
<td><strong>Accuracy of oxygenation measurement</strong></td>
<td>PAR amplitude and phase-based images instead of one in pulsed PA imaging; Higher diagnostic reliability at each probed subsurface depth with more localized, higher dynamic range, optical fluence-independent phase[82]</td>
<td>Mixed PA and surface optical property image influenced by optical fluence; - Multi-wavelength TD-PA may inaccurately (over)estimate the hypoxic level with depth (as the two single-wavelength PAR amplitude in section 6.3.2.5 does), due to the effect of variations in surface absorption between the different wavelengths, and consequently, the significant variations in fluence with depth.</td>
</tr>
<tr>
<td><strong>Rate of acquisition and processing</strong></td>
<td>Frame rate improvement for real-time imaging (i.e. ~25 Hz); additional NI cards for data collection</td>
<td>High repetition rate pulsed lasers enable high frame rate imaging</td>
</tr>
<tr>
<td><strong>Cost and size</strong></td>
<td>Compact, inexpensive CW laser diodes with wide wavelength selection (particularly, in the NIR range)</td>
<td>Larger (footprint) pulsed lasers</td>
</tr>
<tr>
<td><strong>Practicability of systems</strong></td>
<td>Attractive for portable, sensitive PA imagers suitable for clinical applications</td>
<td></td>
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</tbody>
</table>
The reconstructed image is the spatial cross-correlation function between the PA response (detected acoustic signal) and the reference signal used for laser source modulation (the radar principle), hence the name *FD-PA radar (FD-PAR)* as first proposed and implemented by our group[85]–[87]. The optical absorption of a chromophore corresponds to the total energy of the signal within the chirp duration, as indicated by the magnitude of the cross-correlation signal. The subsurface position (i.e. depth information) of the chromophore is obtained from the delay time of the resulting peak by analyzing the response signal to identify the presence of signals with characteristics of the known reference signal (incident laser waveform).

### 1.5 Objectives and Outline of thesis

This work aims to provide a preclinical/clinical co-registered PAR–US imaging system with comparable axial resolution (~mm) and SNR for improving early cancer diagnosis and care. The combination of the FD-PAR imaging system with US using a portable commercial US system is investigated. Leveraging both amplitude and phase information acquired from PAR imaging can provide higher diagnostic power. Additionally, making use of the different laser diodes in our lab (the Center for Advanced Diffusion-Wave and Photoacoustic Technologies (CADIPT)) for spectroscopic PA imaging could also allow for functional PA imaging to measure chromophore distributions and physiological changes, such as oxygen saturation and vascular blood volume to facilitate early cancer detection. Moreover, image improvement techniques including filtering, amplification and tomography are explored, with phase filtering investigated as a spatial resolution improvement tool.

The effects of the number of scan lines on image quality, resolution and contrast is studied in PAR tomography, paving the way for its use in our non-invasive differential functional PAR technique, i.e. wavelength-modulated differential photoacoustic radar (WM-DPAR). The WM-DPAR modality is investigated as a fluence-independent technique for obtaining accurate spatially-resolved quantitative functional information.

At each stage of system development, blood and tissue-mimicking phantom imaging as well as *in vivo* animal (i.e. cancer-injected mice and rats) testing is conducted under an on-going CADIPT collaboration agreement with the Princess Margaret hospital.
Chapter 1 of this thesis provides an introduction of the PA concept and its adoption in biomedical imaging in spite of its challenges.

Detailed in Chapter 2 are the experimental set-up and instrumentation of the PA radar and commercial US imaging systems used in this work. Some preliminary work done for system quality and synchronization as well as signal processing tool for system and image improvement are presented. Safety standard requirements for laser irradiation on skin is also provided.

In Chapter 3, theoretical analysis of acoustic wave generation by laser irradiation for one-dimensional (1D), and axisymmetric conditions is presented using FD analysis. Also described is the analysis for quantifying SO₂. Results interpretation and data analysis will hinge on these theoretical models.

The implementation of the FD-PAR imaging system and integration with US is presented in Chapter 4. Preliminary results are provided to validate the application of previously discussed image improvement techniques in vivo.

Chapter 5 investigates PAR tomography and how image quality, resolution and contrast are affected by altering the number of scan lines used. Different instrument configurations are considered for system optimization. Three-dimensional PAR imaging is also introduced.

Chapter 6 details our non-invasive differential PAR modality, WM-DPAR, for improved sensitivity to minute changes in SO₂ and tHb. WM-DPAR imaging is validated by demonstrating in vivo functional PAR imaging of a cancer cell-injected rat.

Chapter 7 concludes the thesis with a summary pulling together and highlighting key findings of the work.
Chapter 2
Materials and Instrumentation

Chapter 2 discusses the experimental set-up and instrumentation of the PA radar system in detail while briefly describing the set-up of the commercial US imager. Preliminary experiments/study conducted validate the quality of both modalities and their capacity for synchronization as a vital step for effective system handshaking and maintaining data integrity. Signal processing techniques, particularly matched filtering in the FD mode, as well as image improvement techniques including apodization (gleaned from advancements in US), and amplification are presented. Furthermore, phase filtering is introduced as a spatial resolution improvement tool. Finally, some dominant properties of tissue and safety parameters for human exposure to laser irradiation are expounded.

2 Materials and Instrumentation

2.1 Commercial US set-up

Ultrasound (US) imaging, a widely used modality in clinical applications for tumor detection in patients[1], [88], can be leveraged to help fast-track the clinical acceptance and use of PA imaging by equipping it with PA functionality. In PA imaging, the pressure waves generated in the photoacoustic effect can be detected by conventional US array transducers that convert the mechanical acoustic waves to electrical signals, making PA highly compatible with US imaging. Obtaining both US and PA images, and by extension their co-registration, is simplified since the same transducer is employed for both modalities.

Figure 2.1 shows the portable commercial US imager (SonixTOUCH, Analogic Corporation, Peabody, Massachusetts, USA) used throughout this work in obtaining pure US images. The SonixTOUCH was acquired with a data acquisition add-on box called the SonixDAQ, which allows for the parallel collection of raw pre-beamformed channel data; the raw signal from each element of the ultrasonic transducer prior to any processing. Ideally, the modular design of the SonixDAQ, in particular, we hoped could facilitate easy modification and process optimization for co-registering PA and US.
2.1.1 Ultrasonic transducers

Using existing commercial US array transducers in PA imaging is convenient, relatively inexpensive, and exploits advances made in diagnostic US imaging. Various acoustic detectors are used including single-element, and virtual point (ring-based or high-NA-based) transducers. Scanning single-element transducers is cheap and thus widely used in PA imaging but can be time consuming (acquisition times of minutes to hours). Commercial US array transducers, as well as specially-designed array detectors for PA imaging, have therefore been developed. These US detectors, often piezoelectric-based having low thermal noise and high sensitivity, are capable of providing a wideband detection (up to 100 MHz[3]). However, the detection sensitivity of a piezoelectric US array drops with decreasing element size and a higher frequency array offers higher spatial resolution at the expense of SNR. Optical detectors have been developed to overcome this limitation. They are capable of noncontact measurement and rapid large area-monitoring as they are commonly based on PA-pressured-induced surface displacement or refraction index changes[3] rather than element size. Large planar and line detectors much larger than the region of interest (ROI) are also being explored to achieve exact image reconstruction.
Commercial US imagers also possess sophisticated software for rapid image acquisition that could potentially speed-up PA imaging dramatically, if developed to be compatible with that modality. However, efficient PA and US integration and image co-registration is difficult as digital signal processing (DSP) in US imagers operates on frequency-domain (FD) principles. This renders the use of FD-PAR imaging ideal for integrated image co-registration.

2.2 PAR experimental set-up

![Diagram of experimental set-up of FD-PAR system](image)

Figure 2.2: Schematic of experimental set-up of FD-PAR system

A schematic of the experimental set-up of our FD-PAR imaging system is shown in Figure 2.2. The system employs frequency-swept (chirped) waveforms to drive the intensity-modulated (coded) compact CW laser source. A Fourier transform (FT) pair relates the frequency- and time-domain descriptions. The sample is illuminated by an 805-nm CW diode laser (Laser Light Solutions (LLS), NJ, USA), while the generated PA signals are detected using a standard commercial 64-element phased array transducer (Analogic Corporation, Peabody, MA, USA with 2 to 4 MHz frequency range and 0.254 mm pitch). Laser modulation is achieved by generating linear frequency modulated (LFM) chirp signals (0.5 to 4 MHz, 1-ms long) using a NI PXI-5442 (National Instruments, Austin, Texas) signal-generation card. The sample and transducer surfaces are fully-submerged in water for acoustic coupling, and any mirrors are also under water to minimize heat accumulation on the optics. A pre-amplifier (5662; Olympus Panametrics, CA, USA) was used for PA signal amplification. Sequential data acquisition and signal processing are performed using modular analog-to-digital converters, Lab View (National Instruments, Austin, Texas)[73], and Matlab software. Using four programmable switch boards, an economical and flexible architecture was achieved through a synthetic receive aperture and
multiplexer system. It allows for parallel readout of a subarray of eight elements sequentially multiplexed over the entire array in reasonable time. Increasing the subarray size and total number of channels is also possible due to the ease of hardware expansion permitted by its modularity.

For spectroscopic imaging, several portable laser diodes in our lab (Center for Advanced Diffusion-wave and Photoacoustic Technologies (CADIPT)) with different emission wavelengths in the 650-1100 nm range are added to the basic PAR set-up. A RPMC 680-nm laser (LDX-3230-680; MO, USA) is employed and so is an 808-nm CW laser diode (Jenoptik JOLD-120-QPXF-2P, Goeschwitzer, Jena, Germany), primarily as a replacement when repairs were needed after the LLS 805-nm CW diode laser malfunctioned. The 680-nm laser is integrated with a customized laser driver (VFM5-25; MESSTEC, BY, DE) on an aluminum heat sink. A 3.5 MHz single-element ultrasonic transducer (C383; Olympus Panametrics, CA, USA) that revolves around the sample is used to detect the PA signals generated when conducting PAR tomography.

2.2.1 US and PAR system quality and parameter optimization

A useful first step to integrating the FD-PAR imaging system with a portable commercial US imager is to ensure the proper functioning and optimization of each system separately. The SonixTOUCH imager, requiring some repairs at the onset, afforded the opportunity to better understand its internal mechanisms by performing an in-house refit (due to an expired warranty).

To ascertain the quality of the FD-PAR imager, two experiments were performed – the first to image a plastisol sample (with low absorption coefficient, < 0.5 cm$^{-1}$ and ~1500 m/s speed of sound) with three 2-mm graphite rods embedded at ~7mm below the surface, and the second to image a sample with three 3-mm plastic tubes containing black ink staggered at ~1-cm depth intervals. The LLS 805-nm diode laser is used to illuminate both samples from above and the Analogic 3-MHz phased array transducer is used to detect the PA signals generated. The sample and transducer surface are fully-submerged in water. Signal processing was performed using Lab View and Matlab software, and data acquisition system (NI PXle-1065).

The set-up of the first experiment is shown in Figure 2.3b with the photograph of the sample provided in Figure 2.3a. Graphite inclusions were clearly identifiable (indicated by white dashed
rectangles) in the pure US image obtained from the commercial SonixTOUCH imager at 4 MHz presented in Figure 2.3c, while at 4-MHz (and 3-MHz) laser end frequency, only two graphite rods were seen in the PA images (indicated by white dashed arrows in Figure 2.3d) due to the laser beam’s limited diameter. The left-most bright spot observed is an artefact as deduced from the PA signals collected by the transducer elements. PA images were also rigged with distortion and significant artefacts along the center of images and on either side of inclusions, resulting in poor resolution.

![Image of Plastisol sample with three 2-mm graphite rods embedded within.](image)

Figure 2.3: (a) Plastisol sample with three 2-mm graphite rods embedded within. (b) Experimental set-up, (c) Pure US image, and (d) PA image at 4 MHz laser end frequency. FO: Optical fiber; T: Transducer; S: Sample–plastisol sample embedded with three graphite rods.

Furthermore, the second experiment investigated the concept of the point spread function (PSF) – degree of spreading of absorbers at various imaging depths. Here, a measure of the quality of the imaging system is provided via the degree of spreading of the imaged inclusions. Figure 2.4a shows the experimental set-up. Each tube was illuminated separately to tackle the laser beam diameter limitation. Both top and bottom edges of the topmost and middle plastic tubes were seen (Figure 2.4b and c) while only one bright spot was observed for the deepest tube potentially due to significantly reduced laser beam intensity at that depth (Figure 2.4d).
As expected, the image spread of the absorbers were observed to increase with depth; the topmost tube having a width of ~1.5 cm, the middle ~2 cm, and the deepest ~3 cm. Improving the image reconstruction algorithm to better distinguish inclusions and reduce artefacts, thereby reducing the degree of spreading, requires a better understanding of its fundamental details as well as improvement techniques like de-convolving the PSF data with the original image data. Various apodization functions are also investigated for potential image improvement. These improvement techniques are further discussed in Section 2.3.2. An exponential apodization function was applied in the images provided in Figure 2.4b–d.
2.2.2 US and PAR Synchronization

Synchronization of processes and data is another crucial step to ensure effective handshaking as well as maintain data integrity between the US and PAR system modalities. The SonixDAQ add-on to the SonixTOUCH imager for parallel raw pre-beamformed channel data collection permits the possibility of applying a processing method optimized for the co-registration of PAR and US. An internal and external trigger were employed to confirm the adequate synchronization of the DAQ with the SonixTOUCH and particularly, the PAR system, respectively. The external trigger was provided by a function generator simulating chirp matching the LLS laser signal used in the PAR system. The instant the trigger is applied in both cases, a spike is recorded in all elements.

Figure 2.5: (a) Photograph of sample – plastisol with graphite rods embedded within, (b) Schematic of Synchronization set-up, (c) US image obtained for sample, (d) Excerpt of results of synchronization of the SonixDAQ with internal trigger from the SonixTOUCH, and (e) Excerpt of results of synchronization of SonixDAQ with external trigger
The schematic of the experimental set-up for synchronization is provided in Figure 2.5b. A simple plastisol sample with two graphite rods ~1.3 cm apart embedded within (see Figure 2.5a), compatible with both US and PA was employed and the US image taken to corroborate is shown in Figure 2.5c. Figure 2.5d and e show the first sixteen out of 64 elements of the results obtained from synchronizing the DAQ with the internal (SonixTOUCH) and external (PAR) trigger, respectively.

Working with two imaging technologies allows for potentially leveraging their capabilities to improve each other, e.g. learning from the reconstruction techniques of more well-known and clinically applied commercial US systems to improve the PA system. Theoretically, the modular design of the data acquisition, processing and reconstruction software of the SonixTOUCH US imager is meant to facilitate easy modification/improvement. The software includes core procedures like beamforming, scan-conversion and coordinate transformation for display.

2.3 Signal-processing

Pulsed PA imaging applies straightforward time-of-flight acoustic transient measurements for depth determination[3], [8]. However, among the different signal processing tools that can be applied to determine chromophore depth in the FD mode, matched-filtering compression has been the principal method employed in this work.

Also presented are image improvement techniques investigated for artefact reduction as well as image reconstruction algorithm improvements to better distinguish absorbers.

2.3.1 Matched-filtering Compression

In an effort to increase operating range while compensating for the weak acoustic pressure wave response due to the limited transmission power, FD-PA can employ frequency modulated (chirped) waveforms and pulse compression by matched filtering[89]. Matched filtering enables pre-known radar signal immersed in white Gaussian noise (WGN) to be detected in order to perform optimum detection decisions (i.e. target or no target), estimate target parameters (i.e. range, velocity, etc.) within minimum root-mean-square errors, or obtain maximum resolution among a group of targets[89]. Waveform design is an integral part of radar system development. After detectability requirements have been satisfied, range resolution conditions could be met by coding the transmitted signals with wideband modulation information. Thus, depth-resolved
imaging is facilitated by employing frequency-swept (chirped) waveforms to increase the bandwidth. The received chirped acoustic wave is compressed via cross-correlation processing through FD analysis into a narrow correlation peak (with a temporal width of the inverse of the bandwidth), determining the axial resolution of the system at a certain delay time. SNR is thus, amplified with comparable resolution, contrast and imaging depth to pulsed PA as demonstrated in our group’s work[84], [85], [90].

The signal processing flowchart for matched filtering provided in Figure 2.6 shows the evaluation of the cross-correlation in FD involving the multiplication of the transducer output, \( s(t) \), by the complex conjugate of the reference chirp input signal, \( r(t) \) (i.e. matched filter[91]), using the fast Fourier transform (FFT), and then obtaining the inverse fast Fourier transform (IFFT) of the result. In addition to the in-phase cross-correlation (amplitude), the cross-correlation of the detected signal with the quadrature of the input waveform (phase) is evaluated by eliminating the negative frequencies to make the signal analytic.

Figure 2.6: Schematic of signal processing flowchart for matched-filtering[84].

2.3.2 Image improvement techniques

Image quality improvement has received a great deal of attention in PA imaging with different techniques being explored, including short-lag spatial coherence[92], [93], adaptive beamforming[94], [95] and pixel based focusing[96]. The concept of phase filtering[97], through which PA signal spatial resolution can be improved by filtering the amplitude with the more localized phase, is introduced to PAR imaging.

As previously discussed, the commercial SonixTOUCH US imager possesses sophisticated software that can be adopted to improve the reconstruction algorithm of the PAR system. The data acquisition, processing and reconstruction software contained in the SonixTOUCH US system are primarily built in Matlab. Examples of these include code for creating a beamformed image from pre-beamformed data (in which select sections of each scanline were chosen,
providing an inherent apodization over the data similar to applying a Hanning window),
converting post-beamformed radio frequency (RF) data to scan-converted B-mode (in which an
envelope of the signal is calculated via Hilbert transformation, log compressed to achieve the
desired dynamic range for display and decimated to decrease the sampling rate), and coordinate
transformation for display (in which an image is resized appropriately using different
interpolation techniques depending on whether the array probe is linear or curved).
Applying apodization and coordinate transformation are therefore, considered for PAR imaging.

2.3.2.1 Apodization functions

Figure 2.7: PA images of the deepest ink-tube with and without apodization; (inset) shows
photograph of imaged sample. Apodization functions applied: (a) exponential apodization, (b) no
apodization, (c) Hamming window, and (d) Hanning window.

Different apodization functions are studied and applied in the PAR reconstruction algorithm for
comparison and potential image improvement. Among those apodization functions analyzed, the
expansional, Hamming, and Hanning windows are contrasted in Figure 2.7a, c and d, respectively, using results of the deepest ink-tube from the PSF experiment (three 3-mm plastic tubes containing black ink staggered at ~1-cm depth intervals) discussed in Section 2.2.1. The exponential apodization function showed the most improvement in artefacts and image spreading. The image obtained without apodization (Figure 2.7b) is provided for comparison.

2.3.2.2 Normalization and Amplification

Normalization often stems from a need to directly compare images with varying color bars. It thereby, involves the scaling of image intensity values within a common range.

Moreover, image (pixel intensity) amplification is explored for further image improvement. The image is first scaled to values within \([0, 1]\) and then squared (apply exponentiation). The challenge however, is knowing how drastic the amplification (i.e. exponentiation) should be. It should be noted that over-amplification could lead to loss of relevant information or difficulty in identification afterwards.

These techniques are applied to phantom and \textit{in vivo} imaging results hereafter to validate their capability for absorber spread and artefact reduction.

2.3.3 Phase-filtering as a spatial resolution improvement technique

Furthermore, another image improvement technique investigated employs phase contribution to provide more localized images in order to improve resolution.

Phase contrast is more highly localized because phase lag is based on the energy centroid location of converted ultrasound through optical absorption across subsurface regions determined by the optical absorption depth. The PA ultrasonic centroid location does not depend on actual photon flux nor does it involve surface optical contrast due to reflection or absorption, unlike the PAR amplitude. As such the phase lag is a truly photoacoustic imaging channel as opposed to a mixed PA and surface optical property image generated from the PAR amplitude [80]. Moreover, it does not depend on optical fluence, a major advantage over time-domain modalities.

Amplitude (in-phase cross-correlation) and phase (the cross-correlation of the detected signal with the quadrature of the input waveform) images are combined by filtering the amplitude with
the inverse of the standard deviation (SD) of the phase. The two samples imaged to validate this methodology include a small 2-mm graphite piece attached at the end of a glass rod, and a thin black plastic thread with significant absorption coefficient (Figure 2.8).

Figure 2.8: Experimental set-up for (a) the graphite piece, and (b) the plastic thread. FO: Optical fiber; T: Phased array Transducer; G: Graphite; PT: Thin black Plastic Thread

The pure US images of the graphite piece and the plastic thread obtained from the SonixTOUCH US imager at a frequency of 4 MHz are provided in Figure 2.9. Both absorbers (indicated by the white arrows) can be identified (albeit with significant artefacts) from the images at depths of ~2 cm and ~2.25 cm from the transducer surface, respectively.

Figure 2.9: Pure US image of (a) the graphite piece, and (b) the plastic thread
The amplitude and phase images as well as a phase-filtered combination thereof obtained by the PAR system (averaging 300 signals) are provided below. Imaging and data acquisition take much longer and require significantly more memory space since the inverse of the SD of the phase is also recorded. Images obtained without apodization but with amplification (as previously discussed) to further reduce absorber spread and artefacts in the image are presented. The first set in Figure 2.10 corresponds to the graphite piece whilst the last set in Figure 2.11 corresponds to the plastic thread. The graphite piece and plastic thread are clearly seen at ~2 cm and ~2.25 cm (indicated by white short-dashed arrows) from the transducer surface, respectively, in the reconstructed PAR images matching those in the pure US images.

Figure 2.10: Reconstructed (a) Amplitude, (b) Phase, and (c) Phase-filtered PAR images of a 2-mm graphite piece attached at the end of a glass rod.

Figure 2.11: Reconstructed (a) Amplitude, (b) Phase, and (c) Phase-filtered PAR images of the high-absorbing plastic thread.

The phase images provide better resolution and contrast of the absorbers owing to their localization. The combined images show considerable improvement in resolution and artefacts.
2.3.4 Tissue (and phantoms) optical and acoustic properties

The PA system sensitivity, dynamic range (contrast) and detection range are significantly affected by the optical (and acoustic) properties of tissue (and tumor). The most important optical properties include the absorption coefficient, $\mu_a$, the scattering coefficient, $\mu_s$, and the anisotropy factor, $g$. These parameters have been documented in the literature for some tissue and body parts over some wavelengths[98], [99].

The anisotropy factor demonstrates the directional probability of light scattering defined by

$$g = \frac{\int_{4\pi} q(\theta) \cos \theta d\Omega}{\int_{4\pi} q(\theta) d\Omega} \quad (2-1)$$

where $-1 \leq g \leq 1$. It is the mean cosine of the scattering function, $q(\theta)$, of the photon over all spatial directions described by the solid angle, $\Omega[100]$. Negative values of $g$ imply net backward scattering while positive values imply net forward scattering. Typical values of $g$ in human tissue range between 0.7–0.9, implying that majority of the photons (specifically at scattering depths effectively without ballistic light) will only deviate slightly from their original direction upon scattering[101], thereby allowing the PA modality to image deep tissue inaccessible to pure optical methods. The reduced scattering coefficient applies this parameter:

$$\mu'_s = \mu_s (1 - g) \quad (2-2)$$

As depicted in Figure 1.2, wavelength-dependent absorption coefficients of water, oxyhemoglobin (HbO$_2$) and deoxyhemoglobin (Hb) are most dominant (besides melanin) in the commonly employed near-infrared (NIR) wavelength window in PA imaging due to low scattering. For diagnostic purposes, HbO$_2$ and Hb are vital and thus, validate the selection of the 805-nm (808-nm) and 680-nm wavelengths for this work. Generally, $\mu'_s$ for soft tissue ranges from 2–20 cm$^{-1}$, and $\mu_a$ ranges between 0.5–11 cm$^{-1}$ (even lower in the breast; 0.03–0.05 cm$^{-1}$), in the 650–1000 nm wavelength range[98], [99].

As previously discussed, optical attenuation is best characterized by the effective attenuation coefficient, $\mu_{eff}$, due to its dependence on wavelength-dependent absorption and scattering coefficients. The effective attenuation coefficient is defined as
Acoustic attenuation and the speed of sound are the main critical acoustic parameters of tissue. Frequency-dependent acoustic attenuation in soft tissue influences spatial resolution (as acoustic intensity changes) with penetration depth. A mean speed of sound of ~1540 m/s is commonly used in ultrasound imaging with <10% deviation in (soft) tissue[102], [103].

\[
\mu_{\text{eff}} = \sqrt{3\mu_a (\mu_a + \mu_s^0)} \tag{2-3}
\]

2.3.5 Maximum Permissible Exposure to CW laser irradiation

Human exposure to EM radiation must be limited for safety reasons. Maximum permissible exposure (MPE) is the level of EM radiation a person may be exposed to without hazardous effects or biological changes. Regulatory bodies, such as the American National Standards Institute (ANSI; Laser Institute of America) have stipulated guidelines for laser exposure to the eye and skin[83]. Their laser safety standards depend on the optical wavelength and exposure duration (and exposure aperture).

Table 2.1: Excerpt of MPE for skin exposure to a laser beam[83]

<table>
<thead>
<tr>
<th>Wavelength, $\lambda$ ((\mu m))</th>
<th>Exposure Duration, $t$ (s)</th>
<th>MPE $\text{(J-cm}^{-2}\text{)}$</th>
<th>MPE $\text{(W-cm}^{-2}\text{)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible and Near Infrared</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.400 to 1.400</td>
<td>10^{-4} to 10^{-7}</td>
<td>2 $C_A \times 10^{-2}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10^{-7} to 10</td>
<td>1.1 $C_A t^{0.25}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 to $3 \times 10^4$</td>
<td>0.2 $C_A$</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 provides part of the standard that evaluates the MPE for skin exposure to visible and near-infrared wavelengths. $C_A$ is a laser wavelength-dependent correction factor given by[83]:

\[
C_A = \begin{cases} 
1.0, & 0.400 < \lambda < 0.700 \\
10^{2(\lambda - 0.700)}, & 0.700 < \lambda < 1.050 \\
5.0, & 1.050 < \lambda < 1.400
\end{cases}
\]

For a wavelength of $\lambda = 805$ nm, the parameter $C_A$ has a value of 1.622 and thus, for a CW case with an exposure duration of 0.1 s (i.e. chirp duration being 1 ms and 100 chirps applied), the MPE according to Table 2.1 is calculated as:
\[ MPE_{\text{Train of chirps}} = 1.1 \, C_A \times 0.1^{0.25} = 1.003 \, J/cm^2 \]

compared to the lower \( MPE_{\text{Single pulse}} = 0.02 \, C_A = 0.0324 \, J/cm^2 \) for a short pulse (with duration 5.2 ns) at the same wavelength.

Additionally, “the average irradiance of the pulse train shall not exceed the MPE applicable for the total pulse train”[83] in the case of repetitive pulses, a vital condition both for pulsed lasers as well as CW lasers where a duty cycle pattern for chirps could exist, for instance, due to data acquisition and processing limitations.
Chapter 3
Theory of PA Wave Generation, Propagation and Detection

This chapter presents the theoretical platform for adequate analysis and interpretation of results and data acquired from experiments conducted in this work. Using FD analysis, the acoustic wave generation via laser irradiation is investigated along with other model considerations such as acoustic attenuation, ultrasonic transducer function, and cross-correlation. Furthermore, two approaches for oxygenation quantification, the two single-wavelength FD-PAR and two-wavelength differential PAR methods, applied in this work are discussed.

3 Theory of PA Wave Generation, Propagation and Detection

In order to better understand in vivo PA imaging, the diffusive propagation of laser light in turbid media that results in ultrasonic wave generation and propagation should be determined. Fourier analysis is employed for this purpose as well as to evaluate the velocity potential when absorber and surrounding media are considered. A one-dimensional (1D) model and its extension to a two-dimensional axisymmetric case is therefore, provided.

3.1 One-Dimensional PA wave generation

Subsurface absorbers (e.g. chromophores) absorb laser chirps (photons) when optically stimulated, resulting in the transmission/propagation of generated less-scattering acoustic waves through the medium (and overlying scattering medium) following thermoelastic energy conversion. To investigate PA wave generation and propagation, a two-layer structure made up of a chromophore overlaid by a layer of scattering medium as shown in Figure 3.1 is used.
Following Mandelis et al.’s approach[104], a one dimensional (1D) PA wave generation and propagation analysis is provided using the Fourier method. The effect of heat conduction and viscosity dissipation in PA is negligible[105], as is transverse wave generation due to its large acoustic attenuation in tissue[106]. Under thermal confinement conditions, the velocity potentials in the absorbing (a) and scattering (s) media, respectively, is governed by the (inhomogeneous) Helmholtz equation:

\[ \nabla^2 \tilde{\phi}_a(\vec{r}, f) + k_a^2 \tilde{\phi}_a(\vec{r}, f) = \frac{\beta_a \mu_a}{\rho_a C_p} I(\vec{r}, f)e^{-\mu_s z} \]  

\[ \nabla^2 \tilde{\phi}_s(\vec{r}, f) + k_s^2 \tilde{\phi}_s(\vec{r}, f) = 0 \]  

where the tilde indicates the Fourier transform (FT); \( k_a = \omega/c_a \) and \( k_s = \omega/c_s \) are the absorbing and scattering media wavenumbers, respectively; \( \omega = 2\pi f \) is the angular frequency; \( c_a, c_s \) are the speed of sound in the absorbing and scattering media, respectively; \( \vec{r} \) is the position vector; \( \tilde{\phi}_a, \tilde{\phi}_s \) are the FT of the velocity potentials in the absorbing and scattering media, respectively; \( \mu_a \) is the absorption coefficient in the absorbing medium (e.g. blood vessel, tumor); \( \beta_a \) is the thermal expansion coefficient; \( \rho_a \) is the density of the absorbing medium; \( C_p \) is the specific heat capacity; \( I(f) = \tilde{I}_o(f)e^{-\mu_{eff}L} \) is the optical intensity reaching the absorbing medium at a frequency, \( f \), estimated using the exponential attenuation; \( \tilde{I}_o(f) \) is the incident laser intensity; and \( \mu_{eff} \) is the effective optical attenuation coefficient of the turbid medium (e.g. tissue).
In the 1D (z-axis) case, the radiation boundary condition for outward moving waves only, as well as the pressure and velocity continuity at the interface \( \bar{p}_a(z = 0, f) = \bar{p}_s(z = 0, f) \);
\[
\frac{d\phi_a(z,f)}{dz}\bigg|_{z=0} = \frac{d\phi_s(z,f)}{dz}\bigg|_{z=0}
\]
are applied to give the PA pressure detected at the transducer location \( z = -L \) using the laser-induced pressure transient generated at the absorber surface inside a turbid medium as

\[
\bar{p}_s(-L, f) = -j\omega \rho_s \bar{p}_s(-L, f) = \frac{\Gamma_a e^{-\mu_s Z}}{1 + \rho_a c_a} \frac{\mu_a}{\mu_c \bar{c}_a + j\omega} e^{-j k_s L} \tilde{I}_0(f)
\]

where \( \Gamma_a = \beta_a c_a^2 / \rho_p \) is the Grüneisen coefficient (efficiency of thermoacoustic excitation).

### 3.2 Linear chirp PAR detection

The validity and sensitivity of the FD-PAR (and thereby, WM-DPAR) modality is tested by imaging long cylindrical structures such as graphite rods, and plastic tubes carrying heparinized sheep blood at various \( \text{SO}_2 \) levels, some embedded in tissue-mimicking phantoms as well as performing \textit{in vivo} imaging of tumors (containing blood vessels) in live animals (mice and rats).

The theory of the PA response of a long cylinder (axisymmetric model) is therefore of relevance in this work.

Laser radiation diffusion in turbid media for an axisymmetric model using the diffuse-photon-density wave (DPDW) was shown to (asymptotically) converge to the previously discussed 1D solution (Figure 3.1) assuming uniform irradiation using a Gaussian laser beam intensity profile and the laser beam size is large \( (W \rightarrow \infty) \) due to scattering within the turbid medium or deliberate expansion (and low frequencies become insignificant)[97].

#### 3.2.1 FD-PAR modality

Other theoretical considerations necessary in the analysis including the effect of acoustic attenuation on the model, modeling the ultrasonic transducer, and cross-correlation are herein presented.

The model can be incorporated with the acoustic attenuation effect by applying the complex wavenumber in the scattering medium instead[97], thereby modifying equation (3-3) as
\[ \tilde{p}_s(-L, f) = \frac{\Gamma_a e^{-\mu_a i L}}{1 + \frac{\rho_a c_a}{\rho_s c_s}} \mu_a c_a + j \omega e^{-j k_s L} e^{-\alpha_s' i L} \tilde{I}_o(f) \] 

(3-4)

where \( \alpha_s' \) is the acoustic attenuation coefficient (Np/cm) in the scattering medium. For human breast, for instance, \( \alpha_s' = \alpha_s f = 0.75 \) dB/MHz·cm \( \times f = 0.0863 \) Np/cm[106]; with \( \alpha_s \) reported in the 1–10 MHz range (at 37°C).

The Krimholtz-Leedom-Matthaei (KLM) model[107] has been applied in modeling the effect of the transducer[97]. It can be applied for a receiver instead of the broadly applied transmitter mode. The transducer function, \( \tilde{H}_{tr} \) is normalized with respect to the sensitivity, \( \eta \) of the transducer at peak frequency. A sensitivity of 31.8 \( \mu V/Pa \) was measured at the center frequency for the 3.5 MHz transducer using a calibrated hydrophone. The detected PA response voltage, \( V_{tr} \) can be determined using the transducer transfer function model and PA formulation in equation (3-4):

\[ V_{tr}(t) = \mathcal{F}^{-1}\{\tilde{p}_s(f) \cdot \eta \tilde{H}_{tr}(f)\} \] 

(3-5)

where \( \mathcal{F}^{-1} \) is the inverse Fourier transform.

In the FD method, the cross-correlation of the PA response and the reference waveform used for laser source modulation is used to determine the delay time needed for image reconstruction. Hence, the detected signal is multiplied by the complex conjugate of the input signal resulting in the elimination of the DC part of the input signal and normalization of the waveform amplitude to produce the reference signal, \( \tilde{I}_{ref}^*(f) \).

\[ R(t) = \mathcal{F}^{-1}\{\tilde{V}_{tr}(f) \cdot \tilde{I}_{o}^*(f)\} = \mathcal{F}^{-1}\{\tilde{p}_s(f) \cdot \eta \tilde{H}_{tr}(f) \cdot \tilde{I}_{o}^*(f)\} \]

\[ = \mathcal{F}^{-1}\left\{ \frac{\Gamma_a e^{-\mu_a i L}}{1 + \frac{\rho_a c_a}{\rho_s c_s}} \mu_a c_a + j \omega e^{-j k_s L} e^{-\alpha_s' i L} \tilde{I}_o(f) \cdot \eta \tilde{H}_{tr}(f) \cdot \tilde{I}_{ref}^*(f) \right\} \] 

(3-6)
The rectangular bandwidth approximation (that gives the average amplitude of matched-filtered spectrum), $I_0(f) \cdot I_{ref}^*(f) \approx A_I(T_{ch}/4B_{ch})$ can be applied for the chirp bandwidth in combining the in-phase and quadrature signals (total spectral energy):

$$R_{\max} \left( t = \frac{L}{c_s} \right) \approx \Gamma_c \mu_a \eta e^{-\mu_{eq} L} A_I T_{ch} \left[ \frac{f_c + B_{ch}}{2} \int_{f_c - B_{ch}}^{f_c + B_{ch}} \left( e^{-\alpha_{eq} L} \left( \frac{\lambda}{\mu_a c_a + j \omega} \right) \tilde{r}_{tr}(f) \right) df \right]_{\max} \quad (3-7)$$

where $T_{ch}$ is the chirp duration; $B_{ch}$ is the frequency bandwidth; $f_c$ is the center frequency of the chirp; ‘max’ denotes the maximum of the real and imaginary parts, and the time delay corresponding to the peak value at distance $L$ is determined by setting $t = L/c_s$.

For simplicity, particularly in soft tissue, we assume negligible acoustic impedance mismatch between water and tissue, i.e. $\rho_a c_a \approx \rho_s c_s$ which only minimally affects the peak value of the signal.

### 3.2.2 WM-DPAR modality

WM-DPAR imaging employs chirp-modulated laser beams at two different wavelengths to generate PA signals from a chromophore. As shown in Figure 3.2, the first wavelength is selected so that the absorption difference between oxy- ($\text{HbO}_2$) and deoxy- (Hb) hemoglobin coincide (i.e. at the isosbestic point of 798 nm; though the same system principles apply at 805 nm (readily available laser), as it is still very close to the isosbestic point) while the other such that the absorption difference is large (for instance, at 680 nm). The two chirps with identical sweep frequency, approximately have a 180° phase difference, which ideally causes the signal responses to cancel each other out at a specific (arbitrary) blood oxygenation level and thus, helps suppress background absorption and other noise, while amplifying the difference between the two simultaneously generated signals. Minute changes in total hemoglobin concentration (tHb) and hemoglobin oxygenation (SO$_2$), which are useful in the determination of benchmarks of tumor formation such as angiogenesis and hypoxia, can be detected as a result.
As previously mentioned, the delay time is determined by the cross-correlation of the output and linear frequency modulation (LFM) chirp input signals in the FD modality. The differential PA signal (and the cross-correlation function) can be obtained by summing the two respective detected PA signals from lasers 1 and 2.

The two LFM (sinusoidal) input chirps can be described as

$$I_{o1}(t) = A_1 \left[1 + \cos \left(\omega_c t + \frac{\pi B_{ch}}{T_{ch}} t^2\right)\right], \quad -\frac{T_{ch}}{2} < t < \frac{T_{ch}}{2} \quad (3-8)$$

$$I_{o2}(t) = A_2 \left[1 + \cos \left(\omega_c t + \frac{\pi B_{ch}}{T_{ch}} t^2 + \varphi\right)\right], \quad -\frac{T_{ch}}{2} < t < \frac{T_{ch}}{2} \quad (3-9)$$

where $I_{o1}, I_{o2}$ are the laser intensities of the two lasers (1 and 2); $A_1, A_2$ are the corresponding average laser intensities calculated by measuring the laser power and beam spot size; $\omega_c = 2\pi f_c$ is the center angular frequency of the chirp; $T_{ch}, B_{ch}$ are the duration and frequency bandwidth of the chirp, respectively; and $\varphi$ is the phase difference.

The differential PA signal can be obtained by adding the two detected PA signals from the two lasers – when laser 1 and laser 2 are superimposed. Consequently, the differential cross-correlation function stimulated by the above-mentioned LFM chirps is the sum of the respective...
cross-correlation functions of the two lasers and can be written using equations (3-4), (3-5), (3-8), and (3-9) as

\[
R_{\text{diff}}(t) = \mathcal{F}^{-1}\{\bar{\mathcal{V}}_{tr}(f) \cdot \bar{I}_o^*(f)\} = \mathcal{F}^{-1}\{\bar{\mathcal{P}}_s(f) \cdot \eta \bar{H}_{tr}(f) \cdot \bar{I}_o^*(f)\} = \mathcal{F}^{-1}\left\{ \frac{\Gamma_a}{1 + \frac{\rho_a c_a}{\rho_s c_s}} \left[ \frac{e^{-\mu_{\text{eff},1} L} \mu_{a1}}{\mu_{a1} c_a + j \omega} \bar{I}_{o1}(f) \right] - \frac{e^{-\mu_{\text{eff},2} L} \mu_{a2}}{\mu_{a2} c_a + j \omega} \bar{I}_{o2}(f) \right\}
\]

(3-10)

The resulting differential PA signal is multiplied by the complex conjugate of the input signal resulting in the elimination of the DC part of the input signal and normalization of the waveform amplitude to produce the reference signal, \( \bar{I}_{\text{ref}}(f) \). Again, applying the rectangular bandwidth approximation, \( \bar{I}_{o1}(f) \cdot \bar{I}_{\text{ref}}^*(f) \approx A_t(T_{ch}/4B_{ch}) \) for the chirp bandwidth and evaluating at a delay time of \( t = \frac{L}{c_s} \), results in:

\[
R_{\text{diff}}\left( t = \frac{L}{c_s} \right) \approx \frac{\Gamma_a \eta}{1 + \frac{\rho_a c_a}{\rho_s c_s}} \frac{T_{ch}}{4B_{ch}} \left[ e^{-\mu_{\text{eff},1} L} \mu_{a1} \right] \left[ e^{\frac{B_{ch}}{2}} - e^{-\frac{B_{ch}}{2}} \right] \left[ e^{-\mu_{\text{eff},2} L} \mu_{a2} \right] e^{-\alpha s f_{\text{ch}}} \bar{H}_{tr}(f) \right] \int df
\]

(3-11)

Assuming the effect of the transducer over the chirp range is negligible, i.e. \( \bar{H}_{tr}(f) = 1 \), the transducer spectrum behaves as a top hat bandpass filter for an ideal case, i.e. acoustic attenuation is negligible, and chromophore absorptions are small, i.e. \( (\mu_{\text{at}} c_a/\omega)^2 \ll 1 \), which is often the case in the NIR window, the differential PA expression becomes

\[
R_{\text{diff}}\left( t = \frac{L}{c_s} \right) \approx A_1 \mu_{a1} R_1 e^{j \theta_1} - A_2 \mu_{a2} R_2 e^{j \theta_2} e^{-j \varphi}
\]

(3-12)

where the amplitude component,
The real term of the amplitude component in equation (3-13) is much smaller than the imaginary term, i.e. 
\[
\left( \mu_{a1} c_a B_{ch} / \left[ 4 \pi^2 \left( f_c^2 - B_{ch}^2 / 4 \right) \right] \right)^2 \ll \left( \frac{1}{2\pi} \ln \left( \frac{f_c - B_{ch}/2}{f_c + B_{ch}/2} \right) \right)^2,
\]
reducing the amplitude component to
\[
R_i = \frac{\Gamma \eta e^{-\mu_{eff,iL}}}{N} \int \frac{f_c + B_{ch} / 2}{f_c - B_{ch} / 2} \left( \mu_{a1} c_a - j \omega / \omega^2 \right) df
\]

To obtain a zero baseline at a specific SO\textsubscript{2} level (for specific \( \mu_{a1}, \mu_{a2} \)), two conditions need to be satisfied. Firstly, both signals should have equal amplitudes, i.e. \( A_1 \mu_{a1} R_1 = A_2 \mu_{a2} R_2 = A \mu_a R \), which can be shown using equation (3-14), and secondly, an adequate phase difference is required between the two signals. For \( R_{\text{diff}} \left( t = \frac{L}{c_z} \right) = 0 \), the phase relationship is given using equation (3-12) and applying condition 1 by
\[
e^{j \theta_1} = e^{j(\theta_2 - \varphi)} \Rightarrow \varphi = \theta_2 - \theta_1
\]

The phase of the cross-correlated signal for each wavelength can then be evaluated similarly to equation (3-13) as
\[ \theta_i \left( t = \frac{L}{c_s} \right) = \tan^{-1} \left( \frac{R_{\lambda_i, \text{imag}}(t)}{R_{\lambda_i, \text{real}}(t)} \right) \approx \tan^{-1} \left\{ \frac{1}{2\pi} \int_{f_c - B_{ch}/2}^{f_c + B_{ch}/2} \frac{B_{ch}}{f_c} - \frac{1}{f_c} \, df \right\} \]

\[ \approx \tan^{-1} \left\{ \frac{2\pi \left( f_c^2 - B_{ch}^2/4 \right)}{\mu_{ai} c_a B_{ch}} \ln \left( \frac{f_c - B_{ch}/2}{f_c + B_{ch}/2} \right) \right\} \]

(3-16)

The phase is seen to be independent of the Grüneisen coefficient and fluence \( \left( A_i e^{-\mu_{eff,i} L} \right) \).

Applying the reciprocal argument identity, i.e. \( \tan^{-1}(1/x) = \pm \frac{\pi}{2} - \tan^{-1}(x) \), and the first two terms of the Taylor series expansion for \( \tan^{-1} \), the phase change can be approximated:

\[ \phi \left( t = \frac{L}{c_s} \right) = \theta_2 - \theta_1 \approx \frac{c_a B_{ch} (\mu_{a2} - \mu_{a1})}{2\pi \left( f_c^2 - B_{ch}^2/4 \right)} \ln \left( \frac{f_c - B_{ch}/2}{f_c + B_{ch}/2} \right) \]

(3-17)

Using the expression in equation (3-17), the phase difference can be tuned such that the differential signal for (human) blood is zeroed at a SO\(_2\) level of \(~98\%\) making the generated signal increase monotonically with decreasing SO\(_2\). Therefore, to image a blood sample with 45\% hematocrit (Hct), for instance, the phase difference can be calculated to be \(~176^\circ\) using absorption coefficients of blood[109] at this SO\(_2\) level when two LFM chirps with a 300 kHz–3MHz frequency range are applied to modulate the 680 nm and 808 nm lasers.

It should be mentioned that values applied in the analysis should be adjusted for the appropriate blood sample. For example, similar absorption is seen at SO\(_2=78\%\) for human blood but at 84\% for sheep blood. Adequate adjustments should also be made to account for the effect of the transfer function of the transducer (in reducing the effective chirp bandwidth).

### 3.3 Hypoxia monitoring

A PA image is an estimate of the distribution of initial acoustic pressure arising from the optical absorption when tissue is irradiated with a light pulse. Using the 1D model solution, the generated PA pressure can be simplified as

\[ \bar{p}(z, f) \propto \Gamma_a h(z, f) \propto \Gamma_a \mu_a \Phi(z, f ; \mu_a, \mu_s, g) \]

(3-18)
where \( z \) is the spatial/position variable, \( \Gamma \) is the Grüneisen parameter of the tissue, \( \mu_a, \mu_s \) are the absorption and scattering coefficients, respectively, and \( \Phi \) is the wavelength-dependent fluence distribution that depends on \( \mu_a \) (and \( \mu_s \)), resulting in the nonlinear dependence of the absorbed energy density, \( h \), on chromophore concentrations.

The absorption coefficient of blood (a mixture of oxy- (HbO\(_2\)) and deoxy- (Hb) hemoglobin) is dominated by the optical absorption of hemoglobin. Applying the Beer-Lambert law[63], [110], it is thus, represented as

\[
\mu_a(\lambda, C_{thb}, SO_2) = \ln(10) \left[ \epsilon_{HbO_2,\lambda} C_{HbO_2} + \epsilon_{Hb,\lambda} C_{Hb} \right]
\]
\[
= \ln(10) \left[ \epsilon_{HbO_2,\lambda} C_{HbO_2} + \epsilon_{Hb,\lambda} \left( C_{thb} - C_{HbO_2} \right) \right]
\]
\[
= \ln(10) C_{thb} \left[ SO_2 \epsilon_{HbO_2,\lambda} + (1 - SO_2) \epsilon_{Hb,\lambda} \right]
\]

where \( C_{thb} = C_{HbO_2} + C_{Hb} \) [111] is the total hemoglobin concentration (and therefore \( SO_2 \) can be defined as \( SO_2 = C_{HbO_2}/C_{thb} \)), and \( \epsilon_{HbO_2}, \epsilon_{Hb} \) are the molar extinction coefficients of oxy- and deoxy- hemoglobin at the specific wavelength, \( \lambda \).

Therefore, the absorption coefficients of hemoglobin for the two laser wavelengths (\( \lambda_1 = 680 \text{ nm} \) and \( \lambda_2 = 808 \text{ nm} \)) used in the WM-DPAR modality are given by

\[
\mu_{a,\lambda_1=680\text{ nm}} = \ln(10) C_{thb} \left[ SO_2 \epsilon_{HbO_2,680} + (1 - SO_2) \epsilon_{Hb,680} \right]
\]

\[
\mu_{a,\lambda_2=808\text{ nm}} = \ln(10) C_{thb} \epsilon_{Hb,808}
\]

Applying an overlap of the extinction coefficient of HbO\(_2\) and Hb at \( \lambda_2 = 808 \text{ nm} \), causes the \( \epsilon_{HbO_2,808} - \epsilon_{Hb,808} \) term to vanish. The total hemoglobin concentration can be determined using equation (3-21) (knowing \( \mu_{a,\lambda_2=808\text{ nm}} \)[112]) and thereby, \( SO_2 \) can be obtained by rearranging equation (3-20):

\[
SO_2 = \frac{\mu_{a,680\text{ nm}} - \ln(10) C_{thb} \epsilon_{Hb,680}}{\ln(10) \left[ C_{thb} \left( \epsilon_{HbO_2,680} - \epsilon_{Hb,680} \right) \right]}
\]

However, for a reference blood sample (in \textit{in vivo} analysis), the \( \mu_{a,680} \) and \( \mu_{a,808} \) are often unknown or involve some invasive procedure. Therefore, alternative methods are needed.
Using the quotient of two single-wavelength PAR amplitude measurements in the quantification of \( \text{SO}_2 \), allows for the elimination of the effect of the variation in \( \Gamma_a \) between different tissue types, without assuming uniform \( \Gamma_a \) as commonly used throughout the tissue [113]. The quotient approach, herein referred to as the two single-wavelength PAR method, also eliminates the effect of the \( C_{tHb} \) (or hematocrit level) and system transfer function, by careful, painstaking pressure normalization using laser fluence from differing laser-dependent Gaussian-shaped beam profiles due to varying spot sizes required for adequate reliability. It is unlikely that the fluence distribution remains unchanged through a change in wavelength to an absorption peak as used in our methodology. The \( \text{SO}_2 \) expression is, therefore, obtained by

\[
\frac{p_{680}/\Phi_{680}}{p_{808}/\Phi_{808}} = \frac{\mu_{a,680}}{\mu_{a,808}} = \frac{SO_2 \varepsilon_{HbO_2,680} + (1 - SO_2)\varepsilon_{Hb,680}}{SO_2 \varepsilon_{HbO_2,808} + (1 - SO_2)\varepsilon_{Hb,808}} \tag{3-23}
\]

leading to

\[
SO_2 = \frac{\varepsilon_{Hb,680} - \varepsilon_{Hb,808}}{\alpha R - \gamma} R \tag{3-24}
\]

where

\[
R = \frac{p_{680}/\Phi_{680}}{p_{808}/\Phi_{808}} \quad \alpha = \varepsilon_{HbO_2,808} - \varepsilon_{Hb,808} \quad \gamma = \varepsilon_{HbO_2,680} - \varepsilon_{Hb,680}
\]

Conversely, applying our two-wavelength differential PAR amplitude method using the WM-DPAR imager, the effect of the unknown wavelength-dependent fluence, which is a significant concern in conventional linear spectroscopy, can be eliminated using a ratio of two out-of-phase differential PAR measurements. This modality, however, depends on the local hematocrit level, unlike the two single-wavelength PAR amplitude mode. Slight measurement errors are introduced when the typical range of \( C_{tHb} \) from the literature (34%–57% [114], [115]) for 13-week old female rats is considered, as used in our experiment. The differential PAR amplitude signal can be described using (3-18) as
\[ p_{\text{diff}} \propto \mu_{a,680} - k \mu_{a,808} \]
\[ = \ln(10) C_{\text{thb}} \left[ SO_2 \left( \varepsilon_{\text{HbO}_2,680} - k \varepsilon_{\text{HbO}_2,808} \right) + (1 - SO_2) \left( \varepsilon_{\text{Hb},680} - k \varepsilon_{\text{Hb},808} \right) \right] \]

where \( k \) is a system constant experimentally determined from the amplitude ratio \( (A_{680}/A_{808}) \) and phase difference \( (\theta_{680} - \theta_{808}) \) applied between the two laser wavelengths. This experimental determination is no easy feat without prior knowledge of the \( \mu_{a,680} \) and \( \mu_{a,808} \) for a reference blood sample, which is often unknown or involves some invasive procedure. It is important to note here that \( k \) must also be carefully selected with respect to the specific chromophore characteristics (for example, HbO\( _2 \)/Hb absorption spectra, in our case) for accurate results and data integrity. Processing the differential PAR signals relative to a reference differential PAR measurement, enables the determination of \( SO_2 \) devoid of the effects of the \( k \) determination and without another calibration (proportionality between differential PAR signal and \( k \)) in order to make use of a relative \( k \) instead. \( SO_2 \) is, thus, determined as

\[ SO_2 = \frac{SO_2^{\text{ref}} (\alpha p_{\text{diff}} + \beta \ln(10) C_{\text{thb}}) + \varepsilon_{\text{Hb},808} (p_{\text{diff}} - p_{\text{diff}}^{\text{ref}})}{\alpha p_{\text{diff}}^{\text{ref}} + \beta \ln(10) C_{\text{thb}}} \]

where \( \beta = \varepsilon_{\text{HbO}_2,680} \varepsilon_{\text{Hb},808} - \varepsilon_{\text{Hb},680} \varepsilon_{\text{HbO}_2,808} \).

In order to estimate the total hemoglobin concentration in addition to oxygenation, three wavelengths would be required. This requires an extra CW laser, thereby increasing the system footprint. Supplementary wavelengths are required for other added parameter characterizations, such as melanin, lipids, etc. The three-wavelength DPAR system produces two independent two-wavelength differential PAR measurements. Relative \( k \) is needed to estimate absolute \( C_{\text{thb}} \)-independent \( SO_2 \) in the three-wavelength DPAR modality, since the effects of \( k \) cannot be eliminated by processing the signals as done in the two-wavelength DPAR mode.[116] System calibration for \textit{in-vivo} tumor detection, thus involves an oxygenated sample calibration (i.e. relatively easy using higher \( SO_2 \) surrounding tissue), and a more difficult deoxygenated sample calibration.
Chapter 4
Co-registered PAR and US imaging toward early cancer detection

This section focuses on the frequency-domain photoacoustic radar (FD-PAR) imaging of absorbers in turbid media and their comparison and/or validation as well as co-registration with their corresponding ultrasound (US) images. The separate PAR, US and co-registered images the system acquired can reveal (complementary) morphological information with comparable imaging axial resolution and signal-to-noise ratio (SNR) to potentially facilitate early cancer detection. Live animal testing is performed twice; the first to further validate the ability of the various image improvement techniques including normalization, filtering and amplification introduced earlier (Section 2.3.2) to reduce absorber spread and artefacts in vivo, and the second to illustrate the application of phase-mediated image improvement. In addition, co-registered images generated from the US and PAR images provide more information than the US and PAR images independently. The significance of this work lies in the fact that achieving PA imaging functionality on a commercial ultrasound instrument could accelerate clinical acceptance and use. This work is aimed at functional PA imaging of small animals in vivo.

4 Co-registered PAR and US imaging toward early cancer detection

Due to strong induced signals and straightforward signal processing algorithms, most conventional PA imaging modalities have almost entirely employed pulsed laser PA with time-resolved measurements of acoustic transients for subsurface tumor detection [3], [8]. Alternatively, our FD-PAR imaging, using compact, inexpensive continuous wave (CW) laser diodes with a wide wavelength selection, has been shown to possess superior contrast and depth-selective imaging capabilities, as well as enhanced SNR via pulse compression through matched filtering[82], [117]. The FD-PAR modality uses intensity-modulated (coded) CW laser sources driven by frequency-swept (chirped) waveforms. The spatial cross-correlation function between the PA response and the reference signal used for laser source modulation is used to produce the reconstructed image.

Moreover, we investigate the combination of the FD-PAR imaging system with the portable commercial SonixTOUCH US system (widely used in clinical applications to detect tumors in
patients), which is especially simplified since US imagers operate on frequency-domain principles and the same conventional US transducer array is employed for detection in both modalities, thereby accelerating the integration of the PAR accessory into the commercial US imager.

4.1 Applying image improvement techniques

The co-registered US and FD-PAR image reported explores capitalizing on the strengths of both modalities for accurate tumor localization and detection. Filtering methods as well as image normalization and amplification techniques were applied to improve FD-PAR imaging parameters such as SNR, contrast and spatial resolution. A cancer cell-injected mouse was imaged to demonstrate \textit{in vivo} imaging as well as illustrate co-registration and image improvement.

4.1.1 \textit{In vivo} animal imaging I

With the image improvement tools (i.e. apodization, normalization, and amplification) discussed in Section 2.3.2 in hand, live animal testing to demonstrate \textit{in vivo} imaging of a tumor injected into the right leg of a nude mouse was conducted. The experimental set-up is shown in Figure 4.1. The 805-nm diode laser (Laser Light Solutions (LLS), NJ, USA) is employed to illuminate the sample at an output power of 6 W over a 2-cm beam diameter, producing a \(~1.91\) W/cm\(^2\) power density (less than half the calculated MPE for our CW system). The standard commercial 64-element, Analogic 3.3-MHz phased array transducer (Analogic Corporation, Peabody, MA, USA) compatible with the SonixTOUCH US imager is used to detect the generated PA signals. Linear frequency modulated (LFM) chirp signals (0.5 to 4 MHz, 1-ms long) were generated for laser modulation using a signal-generation card. The sample and transducer surfaces were fully-submerged in water to achieve acoustic coupling. Data acquisition and signal processing were performed using a data acquisition system and Lab View (National Instruments (NI), Austin, TX, USA), as well as our in-house developed Matlab software. Acquiring amplitude data takes \(~320\) ms. System (hardware) modularity permits the subarray size and total number of channels to be increased for consecutive/ real-time imaging. A FD beamforming algorithm was employed for the PAR image reconstruction.
In order to perform *in vivo* animal testing, animal protocols were approved by the Division of Comparative Medicine (DCM) of the Faculty of Medicine, University of Toronto, and animal handling was performed according to the guide for animal care and laboratory use. A mouse was injected in its right thigh with cultured human hypopharyngeal head and neck squamous cell carcinoma FaDu cells. When a tumor became apparent, the experiment was performed with the animal fully anesthetized by applying 1.4 L/min of oxygen and 1 L/min of isofluorane gas, while its body temperature was kept constant using an IR lamp and a temperature controller.

The laser beam and transducer positioned in front of the thigh were moved together along the thigh to locate the tumor within. The generated PA image was optimized by adjusting the transducer once the tumor was located. Spatial registration of the two modalities was achieved by the use of the zero insertion force (ZIF) connector that fixed the transducer to the FD-PAR and SonixTOUCH systems, allowing the PAR and US images to be produced at the same location with the US image providing structural guidance to the PA image.
Figure 4.2: Pure US image showing the bottom edge of the plastic seat (white arrow) and the region of interest (white dashed oval).

The SonixTOUCH US imager was used to obtain the pure US image of the cancer cell-injected mouse at a frequency of 3.3 MHz as shown in Figure 4.2. The tumor is difficult to distinguish among all the other body parts reflected such as bone, muscle and fat (indicated by dashed oval).

The reconstructed PAR images in Figure 4.3, on the other hand, clearly indicate the tumor located at ~2.5 cm from the transducer surface, i.e. less than 3 mm below the skin. The PA image is highly sensitive to the presence of increased blood flow in the tumor, and is essentially much less sensitive to the presence of the surrounding body parts unlike the US image, thereby providing much clearer information regarding the tumor (i.e. better contrast and sensitivity). This leads us to hypothesize that the cancer may have increased the overall vascularity of the region of interest (ROI), which is later corroborated by CD31 immunohistochemical analysis (section 6.3.2.5). The arrow in the US image matches the white dashed arrow in the PAR image, suspected to be the raised right foot of the mouse.
Figure 4.3: (a) Reconstructed PAR image (exponential apodization); (b) Improved PAR image (applying Nuttall & Poisson (exponential) apodization functions); (c) Post-amplification improved PAR image of tumor in right thigh of nude mouse.

An exponential filter is applied in the PAR image in Figure 4.3a, with significant improvements observed in contrast and spatial resolution when the PA data is adequately normalized.

To further improve the image, various apodization functions and function combinations were tested. An improved image was obtained by jointly applying Nuttall and Poisson (exponential) apodization functions with a targeted decay of 60 dB over half of the window length of the latter. The spread of the absorbing tumor as well as artefacts are reduced as shown in Figure 4.3b. Absorber spread and artefacts were further reduced by amplifying pixel intensities (Figure 4.3c) where the image was first scaled to values within [0, 1] and then squared. It is important to note, however, that some relevant information could be lost or become difficult to identify afterwards.
as seen from the loss of information (white dashed arrows) in the previous images, depending on how drastic the amplification is. Therefore, care must be taken in applying amplification.

A cross-correlation signal (A-scan) corresponding to the amplitude PAR images is shown in Figure 4.4, where the strong signal amplitudes (at ~16-µs delay time) indicate the presence of the tumor. A scan of the mouse thigh further confirmed this by revealing similar increased signal strength at that same location when placed within the range of the transducer. The spatial extent of the tumor is represented in the images provided.

![Figure 4.4: Cross-correlation signal from one element of the transducer over the tumor location.](image)

The co-registered image of the US and PAR images is provided in Figure 4.5. Tumor information obtained in the improved PAR image is overlaid on the US image to provide enhanced tumor diagnostics, indicating the position of the tumor relative to the other body parts outlined in the US image as well as the spatial extent of the tumor.
4.2 Co-registered PAR and US in vivo animal testing II

The merits of ultrasound and cross-correlation (radar) frequency-domain photoacoustic imaging are leveraged for accurate tumor detection. The PAR image generated from the amplitude of the cross-correlation between detected and input signals was filtered with the standard deviation (SD) of the phase of the correlation signal, resulting in significant improvement in image spatial resolution, SNR and contrast. The application of phase-mediated image improvement is demonstrated by imaging a cancer cell-injected mouse. A 14–15 dB SNR gain was recorded for the phase-filtered image compared to the amplitude and phase independently, while ~340 μm spatial resolution was seen for the phase PAR image compared to ~840 μm for the amplitude image.

Image improvement techniques such as filtering using apodization functions as often done in conventional US imaging, and normalization, are applied. Furthermore, PAR produces two images (amplitude and phase-based) instead of one in pulsed PA imaging. This feature results in higher diagnostic reliability at each probed subsurface depth and phase can be used for further...
image improvement, as it tends to be more localized and of higher dynamic range (contrast) than amplitude. The optical absorption depth determines the energy centroid location of converted ultrasound through optical absorption across subsurface regions, on which phase lag is based. Unlike the PAR amplitude that produces a mixed PA and surface optical property image, the PA ultrasonic centroid location neither depends on actual photon flux nor involves surface optical contrast due to reflection or absorption, and as such provides a truly PA imaging channel. It possesses an important advantage over time-domain modalities as it does not depend on optical fluence. Phase-filtered imaging is, therefore, investigated as a spatial resolution improvement technique in this work. The PAR amplitude image is filtered with the inverse of the SD of the phase, with the resultant envelope cross-correlation signal possessing higher contrast and spatial resolution than its separate parts. A smoother trace is also obtained, thereby diminishing the need for added FD windowing. However, combination amplitude–phase data acquisition and imaging takes longer than amplitude imaging alone, since the SD of the phase must also be recorded.

4.2.1 Animal preparation

*In vivo* imaging of cancer cells injected into the left thigh of a nude mouse was performed. Human hypopharyngeal head and neck squamous cell carcinoma FaDu cell lines were obtained from the American Type Culture Collection (Manassas, VA), and cultured in MEM F-15 supplemented with 10% fetal bovine serum. $1 \times 10^7$ cultured cells were injected into the left thigh of the mouse, three weeks prior to imaging. The animal was fully anesthetized throughout the experiment by administering 1.4 L/min of oxygen and 1 L/min of isofluorane gas. An IR lamp and a heating and temperature controller were used to regulate the animal body temperature (at a constant level). The experiment was performed under the guidelines of animal protocol 20010465 approved by the Division of Comparative Medicine (DCM) of the Faculty of Medicine, University of Toronto. Animal handling was also performed according to guidelines for laboratory animal care.

4.2.2 Experimental procedure

Our system employs the LLS 805-nm CW diode laser to illuminate the sample at an output power of 5 W, and the standard commercial 64-element, Analogic 3.3-MHz phased array transducer (with 2 to 4 MHz frequency range and 0.254 mm pitch) to detect the PA signals generated. A power density of $\sim 1.59 \text{W/cm}^2$ is obtained from the 5-W laser power illuminating
over a 2-cm beam diameter, which is less than half of the calculated MPE of 4.10 W/cm² for our CW system. Laser modulation was achieved by generating LFM chirp signals (0.5 to 4 MHz, 1-ms long) using a NI PXI-5442 (National Instruments, Austin, Texas) signal-generation card.

A schematic of the experimental set-up is shown in Figure 4.6. The sample and transducer surfaces were fully-submerged in water for acoustic coupling. Sequential data acquisition and signal processing were performed using modular analog-to-digital converters, NI Lab View, and Matlab software. Parallel readout of a subarray of eight elements sequentially multiplexed over the entire array is achieved in reasonable time due to the economical and flexible architecture (that uses four programmable switch boards) featuring a synthetic receive aperture and multiplexer system. The subarray size and total number of channels can be increased as a result of the ease of hardware expansion permitted by its modularity.

Amplitude data acquisition took ~320 ms. To perform consecutive imaging, the laser should be off 55% of the time, meaning a 392 ms relaxation time for each 320 ms of exposure. This results in a frame rate of ~1.40 Hz. The frame rate can be improved for real-time imaging (i.e. ~25 Hz) by using additional NI cards for data collection. PA image reconstruction was done by a FD beamforming algorithm similar to that employed in conventional US images making it adequate for integration with clinical US systems for co-registration.

![Figure 4.6: Schematic of experimental set-up](image)

To find the tumor inside the thigh, the transducer placed in front of the thigh and the laser beam were moved in tandem along the body part and PA images were produced. Upon finding the tumor location, the transducer was adjusted to optimize the image. The tumor was located less than 3 mm below the skin, based on visual inspection of the tumor after euthanasia.
Measurements were performed at three laser energy levels (3 W, 4 W, 5 W) including scans of the ROI at 5 W. The images obtained are consistent. As the transducer was fixed by connecting the zero insertion force (ZIF) connector to the SonixTOUCH system, the US image was also produced at the same location, ideal for spatial co-registration of the two modalities, with the US image providing structural guidance to the PA image and tumor location information with respect to its surrounding tissues. The effectiveness of the phase-filtered imaging technique was also demonstrated via this experiment.

4.2.3 Results and Discussion

4.2.3.1 Histopathological validation

To confirm the presence of the tumor in the left thigh of the mouse, histological validation was performed. Hematoxylin and eosin (H&E) staining was performed on the removed tissue and the stained sections were reviewed under a microscope with x200 magnification as shown in Figure 4.7. H&E staining of the tissue reveals a high density of cancer cells that possess dark nuclei (Figure 4.7(a)) while normal tissues (Figure 4.7(b)) are seen as the (clear) pink sections, illustrating the presence of the tumor interlaced with the surrounding normal muscle tissue. This leads us to hypothesize that the cancer may have increased overall vascularity of the area of interest.

Figure 4.7: Xenografts of (a) cancer cells with dark nuclei, and (b) normal tissue.
4.2.3.2 US and PAR images

A photograph of the position of the mouse placed in its seat, relative to the transducer, is provided in Figure 4.8(a). The tumor region is identified with the red circle. The pure US image of the cancer cell-injected mouse was obtained from the SonixTOUCH US imager at a frequency of 4 MHz as shown in Figure 4.8(b). The tumor is difficult to distinguish among all other body parts reflecting the US such as bone, muscle and fat (indicated by the dashed oval). The bright spot shown by the white arrow is suspected to be the bottom of the plastic seat for the mouse.

![Figure 4.8: (a) Experimental set-up showing mouse secured to the seat and the transducer positioned in front of it. (b) Pure US image obtained from commercial SonixTOUCH imager.](image)

On the contrary, the reconstructed PAR images in Figure 4.9(a) clearly indicate the location of the absorber (tumor) at ~1.7–2.2 cm from the transducer surface. Unlike the US image, the PA image is less sensitive to the presence of the surrounding body parts, but is highly sensitive to the presence of increased blood flow in the tumor, thereby providing much clearer information regarding the tumor, manifested as better contrast and sensitivity than its pure US counterpart. Figure 4.9(a), (b), (c) show the amplitude only, phase only and phase-filtered images, respectively, obtained by the PAR system.
Figure 4.9: (a) Amplitude, (b) Phase, and (c) Phase-filtered PAR images of the left mouse thigh

4.2.3.3 Phase-filtering

The phase image (Figure 4.9(b)) shows a more localized tumor with better contrast than the amplitude image while an even more improved image is obtained in the phase-filtered image (Figure 4.9 (c)), evaluated by filtering the amplitude with the inverse of the SD of the phase. The tumor in the phase-filtered image seems just as localized and particularly more accentuated (higher contrast) due to the suppressed background noise resulting from phase-filtering. The white dashed arrows in the PAR images of Figure 4.9 are placed at locations suspected to be blood vessels on the back of the mouse.

To understand the effectiveness of phase-filtered PAR imaging, cross-correlation signals (A-scans) corresponding to amplitude and phase PAR images in Figure 4.9 are shown in Figure 4.10
where the strong signal amplitude (at ~13-µs delay time) indicates the presence of the tumor. The peak at ~10-ms delay is the element’s view of microvasculature near the back of the mouse (toward the tail). The A-scan for the phase (Figure 4.10b) illustrates its superior localization relative to the amplitude with narrower peaks seen at corresponding delay times. This was further confirmed by performing a scan of the mouse thigh which revealed similar increased signal strength at that same location when it was put within the range of the transducer. The spatial extent of the tumor is further represented in the images provided.

Figure 4.10: (a) Amplitude and (b) Phase cross-correlation signal from one element of the transducer over tumor location

From the cross-correlation (PA response) data obtained from the experiment, the SNR for the phase-filtered image shows a 14 dB and 15 dB increase over the amplitude and phase alone,
respectively. Spatial (axial) resolution is estimated from the full-width at half maximum (FWHM) of the signal to be \(~840\) µm for amplitude PAR and improved to \(~340\) µm for the corresponding phase PAR. Due to the low spatial (transverse/ lateral) resolution of the transducer (and its depth dependence), the images generally suffer from poor lateral resolution.

4.2.3.4 Coregistration of PAR and US

Figure 4.11 provides the co-registered US and PA phase images. Tumor information obtained in the phase-filtered PAR image is overlaid on the US image to provide enhanced tumor diagnostics (higher diagnostic power). The zoomed image (Figure 4.11(b)) of the ROI in Figure 4.11(a), indicates the position of the tumor relative to the other body parts delineated in the US image and illustrates the spatial extent of the tumor.

Figure 4.11: (a) Phase-filtered PAR image superimposed on the pure US image of the left thigh of the mouse. (b) Zoomed image of the region of interest.
Chapter 5
PAR Tomography

In this chapter, photoacoustic radar (PAR) tomography is explored as an image enhancement methodology. How image quality, resolution and contrast are affected by altering the number of scan lines (or the step sizes) of the transducer is investigated. The ability of PA system to produce adequate results where physical constraints exist is therefore, ascertained. For example, in live animal testing when the transducer motion is restricted by the seat/bed structure or the configuration of the set-up to accommodate the necessary conditions to be compliant with the required animal protocol. Also introduced is three-dimensional (3D) PAR imaging of a tissue and blood-mimicking phantom.

5 PAR Tomography

5.1 Tissue and Blood-mimicking phantoms

The FD-PAR tomography system employs a similar set-up to the one previously described for FD-PAR imaging (in Section 2.2) with the 3.5-MHz single-element transducer (C383; Olympus Panametrics, CA, USA) used for detection instead of the transducer array. The transducer can be revolved around the sample for data collection from multiple locations at multiple angles. The intensity-modulated LLS 805-nm diode laser (driven by frequency-swept waveforms over a 300 kHz–3 MHz range) irradiates the sample. The sample and transducer surfaces were fully-submerged in water to achieve acoustic coupling. Data acquisition and signal processing were performed using a data acquisition system and Lab View, as well as our custom Matlab software.

Two graphite rods of different sizes (2-mm and 0.7-mm) arranged side-by-side were imaged using FD-PAR tomography. Various sampling step sizes were applied to explore the effect of reducing the number of scan lines on image quality, resolution and contrast.

Other tissue and blood-mimicking phantoms including a cylindrical absorber (black ink mixed into plastisol) embedded in a plastisol scatterer, a cylindrical plastisol sample with three holes along its circumference filled with blood, a cylindrical scattering-plastisol sample with three graphite rods arranged in a triangle embedded within, and an irregularly shaped plastisol scatterer holding two tubes filled with sheep blood at different oxygenation levels, as well as ex...
\textit{vivo} samples as seen in Section 5.2.1 are analyzed. Results are presented for the last two phantoms; the first to investigate the discrimination of closely-situated inclusions in turbid media and the second to explore PAR tomography using a transducer array. Creating the plastisol samples involved the mixture of polyvinyl chloride plastisol (PVCP) with various amounts of titanium dioxide ($\text{TiO}_2$) powder and black plastic color (BPC) to simulate scattering and absorption properties of tissue, respectively.

5.1.1 Image improvement

5.1.1.1 Effect of step size on image quality

In this experiment, the ability of the PAR tomography system to distinguish between two closely located absorbers, as often is the case of blood vessels, is investigated. Figure 5.1 provides a photograph of the sample used.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{graphite_rods.jpg}
\caption{Two graphite rods of different sizes (I – 0.7mm and II – 2mm) arranged side-by-side}
\end{figure}

Figure 5.2 shows the experimental results of the two graphite rods of different sizes (I – 0.7-mm rod and II – 2-mm rod) arranged side-by-side acquired with the FD-PAR tomography system. As observed in Figure 5.2c, the larger diameter rod, II, is not illuminated internally as the PA signal originates in regions close to the surface of the rod. Optical intensity is significantly reduced beyond the surface due to strong absorption at the surface.
Figure 5.2: Reconstructed tomographic images of two closely-positioned graphite rods of different sizes obtained using (a) 91 (quarter) equally-spaced scan lines, (b) 182 (half) equally-spaced scan lines, and (c) 364 equally-spaced scan lines, in one revolution.

The images (Figure 5.2a, b, c), although obtained without filtering, normalization or amplification as done in the previously discussed single-location FD-PAR imaging, show good quality and spatial resolution. Interference patterns are observed to increase as the number of scan lines decreases. However, the system still produces adequate results even when as few as one-quarter of the number of the original scan lines in a complete revolution (~1° step-size) are used.

System optimization, particularly with respect to alignment, as well as the orientation and position of the laser fiber optic and transducer is crucial in producing images of good quality.
5.1.1.2 PAR tomography in turbid media

In imaging the cylindrical scattering-plastisol sample (with a high reduced scattering coefficient, \(\sim 4 \text{ cm}^{-1}\) and a low absorption coefficient, < 0.05 cm\(^{-1}\)) with three graphite rods arranged in a triangle embedded within, a frequency-sweep range of 300 kHz–5 MHz is applied to the laser. Two illumination configurations (top and side) are tested for system optimization. Figure 5.3a provides the experimental set-up showing illumination from above. A schematic of the sample depicting the triangular arrangement of the graphite rods is illustrated in Figure 5.3b.

Figure 5.3: (a) Experimental set-up showing sample illuminated from above. (b) Schematic of the phantom. Reconstructed PAR images of Plastisol-Graphite triangle phantom (reduced scattering coefficient: \(\sim 4 \text{ cm}^{-1}\), absorption coefficient: < 0.05 cm\(^{-1}\)) (c) illuminated from above, and (d) illuminated from the side. FO: Optical fiber; P: Phantom (Plastisol-Graphite triangle); T: single-element Transducer

The experimental result acquired using top-illumination is shown in Figure 5.3c while irradiation from the side using a protected silver mirror produced Figure 5.3d. The PAR images provide some useful information, albeit with significant artefacts from scattering in the turbid medium (more complex sample). Applying the previously discussed image improvements methods such
as apodization and amplification could provide great enhancement. The triangular configuration
of the graphite inclusions is observed when illuminated from above whereas the sample
boundary is better outlined when illuminated from the side. The two configurations therefore,
provide complementary information about the phantom, illustrating the significance of
multidirectional illumination.

5.1.1.3 PAR tomography using transducer array

The experimental set-up for imaging the irregularly shaped plastisol scatterer holding two tubes
filled with sheep blood at different oxygenation levels is shown in Figure 5.4b. A frequency-
sweep range of 300 kHz–5 MHz is applied to the laser, irradiating the sample from the side. The
3.3-MHz transducer array is used for detection and is revolved in tandem with the laser around
the sample. A schematic showing the cross-section of the phantom is provided in Figure 5.4a.

The oxygenated blood is injected directly from the supplier’s bottle which from prior repeated
measurements using a gas analyzer (CCA-TS; OPTImedical, GA, USA) produced an
oxygenation range of 93–99%. Sodium dithionite (Na₂S₂O₄) was mixed in with a small amount
of the sheep blood to deoxygenate it (beyond the lower SO₂ limit (i.e. 70%) quantifiable by the
gas analyzer) and injected into the tube. The deoxygenated blood tube was then sealed. Both
tubes are surrounded by a common high-scattering plastisol with reduced scattering coefficient,
\(\sim 4 \text{ cm}^{-1}\).

Figure 5.4: (a) Schematic of the phantom. (b) Experimental set-up. (c) Reconstructed PAR image
of Plastisol-Blood tubes phantom illuminated from the side. FO: Optical fiber; P: Phantom
(Plastisol-Blood tubes); T: array Transducer

Figure 5.4c shows the PAR image of the phantom using thirty-six scan lines of the array
transducer in a complete revolution. Spatial registration was achieved by combining the
tomographically constructed data over the thirty-six scan lines for each of the 64 elements separately. Again, significant artefacts exist without apodization or amplification. More scan lines are necessary to eliminate the interference patterns observed. The experiment was repeated using the single-element transducer but memory limitations prevented appropriate data acquisition for adequate comparison. The reflectivity of the tube also influenced the results and as such less-reflective tubes were employed in subsequent phantoms.

5.2 Future outlook

Extending the FD-PAR modality to three-dimensional (3D) imaging can advance imaging deep structures (by several centimeters) with improved localization, unattainable with 2D imaging. 3D PA imaging can provide detailed and problem-oriented information about soft tissue, providing more extensive and accurate assessments of the captured structures. Upgrading the system for 3D functionality entails enhancements with regards to light delivery optimization, transducer array design parameters and signal processing.

5.2.1 Three-dimensional (3D) PAR imaging

3D imaging combines multiple scans computationally and allows for image manipulation thereafter to evaluate anatomical structures, aid diagnosis and surgical planning, for instance.

The experimental set-up is shown in Figure 5.5b. The LLS 805-nm diode laser is employed to illuminate the sample at an output power of 2.5 W over a 2-cm beam diameter, producing a ~795.7 mW/cm$^2$ power density (within safe MPE limits for our CW system). LFM chirp signals (0.3 to 4 MHz, 1-ms long) were generated for laser modulation using a signal-generation card. The Olympus 3.5-MHz single-element transducer is revolved around the sample for tomographic data collection at multiple angles. This process is repeated at different vertical scan positions to obtain the multiple scans required for 3D image processing. Acoustic coupling is achieved by fully submerging the sample and transducer surfaces in water. A data acquisition system comprising of our custom Lab View and Matlab software is used for data acquisition and signal processing.

A thin layer of chicken breast injected with heparinized sheep blood was fastened to a seat as shown in Figure 5.5a and successively imaged at ten equally-spaced heights using FD-PAR
Though, the transducer revolved over 180° around the sample (~1° step-size) due to physical constraints, the adequate image quality, resolution and contrast were still generated as established in the preceding section.

Figure 5.5: (a) Photograph of chicken breast injected with blood. (b) Experimental set-up. (c) 3D Matlab stack image of blood-injected chicken sample. (d) 3D Fiji image of blood-injected chicken sample (centered at ROI). 2D slices in (c) and (d), obtained tomographically (183 steps in 1/2 revolution) are equally-spaced 0.025 in (0.635 mm) apart. Image size [1 cm x 1 cm].

FO: Optical fiber; S: Sample on inclined seat; T: Single-element transducer

Figure 5.5c provides a stack of the scans (X-Y plane) acquired for the blood-injected chicken breast sample using the FD-PAR tomography system. The scan slices are equally spaced at 0.025 in (0.635 mm) apart. The images are captured at a -60° azimuth, 14° elevation and 50% transparency. The high intensity region showing the absorber (i.e. blood) location varies for each scan. The largest width observed is ~5 mm, which is dimensionally consistent with the sample (Figure 5.5a). Using an open source image processing package called Fiji (ImageJ), the 3D image of the blood-injected chicken breast sample is constructed from its scans as shown in Figure 5.5d.
These results show promise as a great first step towards 3D PAR imaging. However, system enhancements including light delivery optimization via multidirectional illumination, multi-element transducer design parameters and signal acquisition and processing for adequate data analysis can significantly improve the frame rate and advance imaging depth.
Chapter 6
Quantitative WM-DPAR imaging

In this chapter, we introduce wavelength-modulated differential photoacoustic radar (WM-DPAR) imaging for non-invasive early cancer detection via sensitive characterization of functional information such as hemoglobin oxygenation (SO$_2$) levels. Well-known benchmarks of tumor formation such as angiogenesis and hypoxia can be addressed this way. WM-DPAR imaging employs two laser wavelengths simultaneously to eliminate background noise by canceling out variations caused by the local fluence (capable of undermining spectral interpretation of PA images), thus yielding high sensitivity and specificity for accurate tissue hypoxia assessment. The WM-DPAR imaging system provides three images concurrently: separate images for the two applied wavelengths as well as the differential image. Further developments in image improvement techniques such as filtering and normalization are also investigated.

Experimental results presented demonstrate WM-DPAR imaging of sheep blood phantoms in comparison to single-wavelength FD-PAR imaging. Furthermore, the WM-DPAR imaging of a head and neck carcinoma tumor grown in the thigh of a nude rat demonstrates the functional PA imaging of small animals in vivo. The PA appearance of the tumor in relation to tumor vascularity is investigated by immunohistochemistry. Phase-filtered WM-DPAR imaging is also illustrated, maximizing quantitative SO$_2$ imaging fidelity of tissues.

6  Quantitative WM-DPAR imaging

6.1  Introduction

Tumor hypoxia has become a hallmark of cancer diagnostics and a critical issue in cancer therapy management[28]–[30]. Cancer is known to be caused by genetic mutations, resulting in uncontrolled cell growth[18] raising nutrient and oxygen consumption compared to normal tissue[19], and therefore rapidly develops a dense microvasculature via angiogenesis to perpetuate tumor growth, and a drop in oxygenation levels within the tumor making it hypoxic. Dilated and tortuous abnormal vessels form in the locally increased microvascular density region. Chances of survival increase considerably when detected and treated early[3].
The spectroscopic imaging capability of PA imaging is leveraged to allow for the potential measurement of critical diagnostic parameters for the metabolic state of lesions and therefore, early cancer diagnosis. Biomedical PAT detects less-scattering acoustic waves generated from absorbed EM energy with comparable axial resolution and SNR facilitating accurate tumor hypoxia assessment[63]. While most conventional biomedical PA imaging have almost entirely employed high-power pulsed lasers, FD-PAR imaging has seen significant development as an alternative technique. It employs a CW laser source, intensity-modulated and driven by frequency-swept waveforms.

The WM-DPAR imaging modality herein described operates on the same FD principles using compact, inexpensive CW lasers, instead of the bulkier and expensive pulsed lasers used in conventional biomedical PA. WM-DPAR imaging utilizes chirp modulated CW laser sources at two distinct wavelengths for which absorption differences between oxygenated (HbO₂) and deoxygenated (Hb) hemoglobin is minimum for one wavelength (isosbestic point, 805 nm) and maximum for the other (680 nm). The laser sources are modulated out-of-phase with each other to suppress background absorption and amplify the difference between the two simultaneously generated signals thereby enabling the system to be sensitive to minute changes in SO₂ levels as well as total hemoglobin concentration (tHb). By regulating the signal amplitude ratio and phase shift, the system can be tuned to applications such as cancer screening, identifying pre-malignant tumors, and hypoxia monitoring (quantifying SO₂).

The WM-DPAR imager possesses higher diagnostic power and reliability at each probed subsurface depth as it provides three pairs of images (each pair being amplitude and phase-based) congruently, instead of one in pulsed PA imaging. It provides separate images for the two applied wavelengths as well as the differential image. Since the phase tends to be more localized and of higher dynamic range (contrast) than amplitude as well as being optical fluence independent, it can be used to further improve the SNR and spatial resolution of images[82].

6.2 Optical output test

In order to ascertain the compatibility of the two laser diodes needed for the differential PA, a comparison between the driver and photodiode output is made for both wavelengths. The LLS 805 nm diode laser and the 680 nm laser illuminate the mirror from above, and a 200–1100 nm
Si photodetector (DET10A, ThorLabs) is used for detection in the experimental set-up shown in Figure 6.1a. A frequency-sweep range of 300 kHz–5 MHz is applied to both lasers.

Figure 6.1b, c show the optical output plot over the frequency range of interest. The output amplitudes of both lasers are seen to be adequate and relatively uniform over the entire desired frequency range.

6.3 Quantitative phase-filtered WM-DPAR imaging

Before extending WM-DPAR imaging experiments to the in vivo stage, tissue phantoms including circulating sheep blood and sub-surface controlled absorbers (e.g. wrapping blood tube circulating sheep blood with chicken breast to simulate overlying tissue) are used.
6.3.1 Tissue and Blood-mimicking phantom

The validity and sensitivity of the WM-DPAR modality is tested in comparison to single-wavelength FD-PAR tomography by imaging plastic tubes (~2.67-mm inner diameter; 3.86-mm outer diameter) carrying heparinized sheep blood at various SO$_2$ levels[118].

6.3.1.1 Experimental set-up and imaging procedures

The photograph and block diagram of the experimental set-up are shown in Figure 6.2. Though the detailed descriptions of the foundations of the WM-DPAR (and FD-PAR) technique have been previously discussed (see Sections 1.4.3, 2.2, and 2.3.1), a brief summary is provided for convenience.

Figure 6.2: (a) Photograph, and (b) Block diagram of the experimental set-up of the WM-DPAR system. Las 1: Laser 1 (680 nm) fiber optic; Las 2: Laser 2 (805 nm) fiber optic; B: circulating blood (in tube); T: Single-element Transducer

The RPMC 680-nm (LDX-3230-680; MO, USA) laser and LLS 805-nm (LLS8800; Somerset, NJ, US) diode laser are employed for illumination in the experiment. The 680-nm laser, with a maximum optical power (out-of-fiber) of ~1.4 W, is integrated with a customized laser driver (VFM5-25; MESSTEC, BY, DE) on an aluminum heat sink, while the 805-nm laser, with a maximum power of 15 W, is modulated using a NI PXI-5442 (National Instruments, Austin, TX, USA) signal-generation card. A frequency-sweep range of 300 kHz–3 MHz is applied to the lasers. Both laser beams were collimated into 0.8-mm diameters via two collimators (F230SMA-B; ThorLabs, NJ, USA) and then directed to coincide at the same spot on the sample (with an acute incidence angle) by two protected silver mirrors with 25.4 mm diameter (PF10-03-P01; ThorLabs, NJ, USA). A convex lens placed in front of the 805-nm diode beam ensures an
identical beam size for both lasers. A 3.5 MHz single-element ultrasonic transducer (C383; Olympus Panametrics, CA, USA) that revolves around the sample is used to detect the PA signals generated. Most components of the set-up had freedom of motion in all directions (X, Y and Z). The sample and transducer were fully submerged in water for acoustic coupling, and the mirrors were also under water to minimize heat accumulation on the optics. A pre-amplifier (5662; Olympus Panametrics, CA, USA) was used for PA signal amplification. The NI PXIe-1065 (National Instruments, Austin, TX, USA) data acquisition system collects the data needed for image reconstruction via signal processing using custom Lab View (National Instruments, Austin, TX, USA) and Matlab software code.

The sample was placed at a distance of ~32–33 mm from the surface of the transducer, and 200 chirps were coherently averaged to enhance the SNR of the PA signals. Moreover, to optimize the WM-DPAR method, the power of the two lasers was tuned to 1 W (unity amplitude ratio; ~0.318 W/cm$^2$ power density over a 2-cm beam diameter) and the phase difference to 180° so that the differential PA signal of HbO$_2$ was minimized.

The need arose to manually correct a synchronization lapse between the two laser outputs due to the difference in drivers. A measurement is therefore, initially run to approximately evaluate the delay between the two laser outputs, the chirps adjusted accordingly (i.e. the 805 nm shifted by the delay lapse, in our case) and fed back into the function generator, and then the measurement rerun. Unfortunately, this process must be repeated each time before imaging with the aforementioned setup.

Sodium dithionite (Na$_2$S$_2$O$_4$) was mixed in with a small amount of the sheep blood and a small volume was repeatedly injected into the circulation tubing to deoxygenate the blood and obtain the various oxygenation levels. The blood was allowed to circulate for ~10–15 min after each injection for uniform deoxygenation. A section of the blood-containing tubing was then imaged at every deoxygenation step. A gas analyzer (CCA-TS; OPTImedical, GA, USA) was used to measure blood parameters including SO$_2$ and tHb of the specific blood sample.
6.3.1.2 Results and discussion

Three sets of images were obtained for different blood oxygenation levels—95.2% (I), 84.7% (II) and 73.0% (III). The images were first obtained without normalization or amplification. Upon normalization, the dynamic range for the differential PAR image was observed to improve over the 680-nm (and significantly over the 805 nm) FD-PAR mode as shown in Figure 6.3 (and confirmed in Figure 6.4b). Each image is scaled to values within [0, 1] using the maximum amplitude in its respective column (680 nm, 805 nm and Differential). A common maximum was applied to the 680 nm and 805 nm images to facilitate adequate comparison (and portrayal of the
sample). The absorber (blood at various SO\textsubscript{2} levels) is clearly observed at the same location consistently with a diameter of \(~2.7\text{ mm}\) showing dimensional integrity with the tube dimensions. The maximum intensity of the image corresponds to the maximum signal amplitude obtained for a given mode, an indication of the presence of an absorber. The maximum image intensity at the ROI increases with decreasing SO\textsubscript{2} levels for the 680-nm wavelength, which validates the spectral trend of increasing absorption difference between HbO\textsubscript{2} and Hb at this wavelength. At the isosbestic point (805-nm), the maximum intensity at the ROI is similar for decreasing SO\textsubscript{2} levels, as expected. The differential PAR mode was tuned to zero local fluence-induced variations at SO\textsubscript{2} level I, thereby resulting in enhanced sensitivity and specificity for accurate hypoxia monitoring (Figure 6.3). An increasing trend is thus observed for the differential PAR mode for the decreasing SO\textsubscript{2} levels with improved dynamic range.

![Figure 6.4: (a) Sensitivity and (b) Dynamic range comparison of the tuned differential PAR Amplitude signals with the corresponding single-wavelength FD-PAR signals](image)

Figure 6.4 clearly shows the significant improvement in the sensitivity and dynamic range of the WM-DPAR system to changes in SO\textsubscript{2} levels and tHb. Background absorption and noise are highly suppressed (due to the out-of-phase modulation of the lasers) and the difference between the two PA signals is amplified. From the reconstructed images in Figure 6.3 (c, f, i), it is seen that the WM-DPAR method provides more resolved, better contrast and dimension-consistent images of the blood in the tube as the SO\textsubscript{2} level decreases compared to the 680-nm and 805-nm FD-PAR modes separately. The differential PAR amplitude exhibited a 24.42\% signal change per unit SO\textsubscript{2}, demonstrating superior sensitivity compared to the single-wavelength FD-PAR
measurements of 5.98% and 0.70% signal change per unit SO\textsubscript{2}, for the 680 nm and 805 nm modes, respectively. Optical fluence is significantly reduced as light travels towards the center of the tubing since it is strongly absorbed at the boundary of the blood and the tube. This is depicted, in particular, by the images of the deoxygenated blood (SO\textsubscript{2} level III; Figure 6.3(g, h, i)) showing the high signal intensity ring at the boundary of the blood region and the tube diminishing with depth. The increasingly weaker signal surrounding the highly absorbing blood ring is likely a result of the spectral attenuation from the plastic tubing and the PA-generated US reflection off its surfaces. This is why it appears brighter/stronger with increased intensity of the generated PA signal (which is also reduced in the differential PA image compared to the 680 nm FD-PAR mode).

### 6.3.1.2.1 System Optimization

System optimization, particularly with respect to coupling the laser beams to be incident on the same spot on the sample and correcting for any delay time discrepancies between the two lasers are crucial in producing high quality images. A coupler will help better align the laser beams to the same spot. In addition to the single amplitude channel (also used in existing modalities), the WM-DPAR mode can provide two complementary amplitude and phase channels which can further enhance the spatial resolution and SNR of images[82], and thus the reliability of hypoxia monitoring.

### 6.3.2 In vivo tumor hypoxia imaging

The clinical development of hypoxia modification therapies necessitates accurate noninvasive techniques for tumor hypoxia assessment (thereby, also facilitating clinical approval for routine practice). As previously discussed, noninvasive modalities that rely on endogenous markers such as near-infrared (NIR) spectroscopy/tomography, and blood oxygen level-dependent MRI (BOLD-MRI), though suited for in vivo clinical assessment of tumor hypoxia are limited; NIR in tissue penetration and low spatial resolution, and BOLD-MRI in susceptibility to the influence of other independent variables not related to tissue oxygenation.

In the preceding section, the feasibility of the WM-DPAR imaging modality was demonstrated using heparinized sheep blood at decreasing oxygenation levels circulating in plastic tubing. The method was shown to be more sensitive to minute changes in SO\textsubscript{2} and tHb than single-
wavelength FD-PAR imaging, with significant improvement in sensitivity, dynamic range (contrast), SNR and spatial resolution.

In this section, the WM-DPAR modality is further applied for accurate quantitative absolute SO₂ imaging \textit{in vivo} and monitoring of a rat in contrast with a two single-wavelength FD-PAR method. This is particularly valuable for clinical applications, as benchmarks of tumor formation such as angiogenesis and hypoxia can be detected.

Additionally, PA imaging is investigated for microvessel quantitation as a prognostic parameter for head and neck squamous cell carcinoma as done in pathology by analyzing immunohistochemically (IHC) stained tissue specimens under a microscope\cite{119}–\cite{121}. Ultrasound (US), though useful in the diagnostic assessment largely for palpable mass analysis, distinguishing between solid masses and cysts, and guiding biopsies, is usually unable to evaluate early cancer, as it often shows no anatomical and morphological differences between the tumor and healthy tissue as yet and is operator-dependent. A comparison is made between US and PA images with histopathological corroboration.

\subsection*{6.3.2.1 Experimental set-up and procedure}

In this setup, the WM-DPAR system employs the RPMC 680-nm laser and the CW 808-nm laser diode (Jenoptik JOLD-120-QPXF-2P, Goeschwitzer, Jena, Germany) instead, for illumination. The 680-nm laser, with a maximum optical power (out-of-fiber) of \(~1.4\) W, is integrated with the customized MESSTEC laser driver on an aluminum heat sink, while the 808-nm laser, with a maximum power of 5 W, is modulated using a software function generator. A frequency-sweep range of 300 kHz–3 MHz is applied to the lasers. In optimizing the system, a coupler is employed to better align both laser beams to the same spot and then a collimator (F230SMA-B; ThorLabs, NJ, USA) is used for collimating into a 0.8-mm diameter. The Olympus 3.5 MHz single-element ultrasonic transducer revolves around the sample to detect the generated PA signals. Most components of the set-up have freedom of motion in all directions (X, Y and Z). The sample and transducer were fully submerged in water for acoustic coupling. A pre-amplifier (5662; Olympus Panametrics, CA, USA) was used for PA signal amplification. The NI PXIe-1065 (National Instruments, Austin, TX, USA) data acquisition system collects the data needed for image reconstruction via signal processing using custom Lab View and Matlab software code. The block diagram of the experimental set-up is shown in Figure 6.5.
6.3.2.2 Cancer cell preparation

Human hypopharyngeal head and neck squamous cell carcinoma FaDu cell lines were obtained from the American Type Culture Collection (Manassas, VA), and cultured in MEM F-15 supplemented with 10% fetal bovine serum. The right thigh of the nude rat was injected subcutaneously with $4.8 \times 10^6$ cultured cells/100 µl and imaged consecutively over a three week period (results presented for one session). The animal was fully anesthetized throughout the experiment by administering 1.4 L/min of oxygen and 1 L/min of isofluorane gas. An IR lamp and a heater with a thermostat were used to regulate the animal body temperature. The experiment was performed under the guidelines of animal protocol 20011459 approved by the Division of Comparative Medicine (DCM) of the Faculty of Medicine, University of Toronto. Animal handling was also performed according to guidelines for laboratory animal care.

FaDu cells were prepared by Dr. Wei (Willa) Shi at the Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Canada.

6.3.2.3 Animal tumor model

Two ~10–12 week-old (200–250 g) adult female NIH-rnu immunosuppressed nude rats were purchased from Charles River, Wilmington, MA. One of the rats was subcutaneously injected (~1 mm beneath the skin) while the other was intramuscularly injected ~7 mm below the skin for the purpose of deep imaging investigation.
A digital caliper was employed in the tumor and thigh dimension measurements. Anterior-posterior (A–P) and medial-lateral (M–L) measurements of both thighs began right before cancer cell injection (day 0) and continued prior to imaging on subsequent scheduled days. The subcutaneously injected rat developed an anatomically identifiable bump four days after injection, whose length, width and height (raised above the skin) dimensions were also measured thereafter. For the intramuscularly injected rat, tumor growth and size were estimated using the anterior-posterior and medial-lateral measurements obtained. Imaging commenced on day 6 and day 7 for the subcutaneously and intramuscularly injected rats, respectively and were conducted approximately every other day (excluding weekends). Table 6.1 show the imaging schedule followed. Each rat was returned to quarantine as stipulated in our animal protocol at the end of each imaging day until euthanized after ~three weeks.

Table 6.1: Experimental schedule and details of the rats and tumor models.

US: ultrasound; FD-PAR: frequency-domain photoacoustic radar; WM-DPAR: wavelength-modulated differential photoacoustic radar

<table>
<thead>
<tr>
<th>Animal</th>
<th>Injection site (beneath skin)</th>
<th>Day after tumor injection (Day 0)</th>
<th>Imaging modalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 1</td>
<td>Right thigh: Subcutaneous (~1 mm)</td>
<td>Day 0</td>
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<td></td>
<td>Day 4</td>
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<td></td>
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<td>Day 6</td>
<td>US; WM-DPAR</td>
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<td></td>
<td></td>
<td>Day 10</td>
<td>US; FD-PAR; WM-DPAR</td>
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<td>Day 13</td>
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<td>Day 19</td>
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<td>Day 21</td>
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<tr>
<td>Rat 2</td>
<td>Right thigh: Intramuscular (~7 mm)</td>
<td>Day 0</td>
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<td></td>
<td></td>
<td>Day 4</td>
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<td></td>
<td>Day 7</td>
<td>US; WM-DPAR</td>
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<td></td>
<td></td>
<td>Day 11</td>
<td>US; FD-PAR; WM-DPAR</td>
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<td>Day 14</td>
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<td>Day 20</td>
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<tr>
<td></td>
<td></td>
<td>Day 24</td>
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</tbody>
</table>

The length, width and height of the bulge above the skin surface that developed as a result of subcutaneous injection of the first rat is illustrated in Figure 6.6c, showing significant tumor
growth in all three measured dimensions between day 0 and day 4. The tumor continues to grow at a slightly slower rate (in length and width) until it plateaus after day 13. In Figure 6.6a, the A–P measurements of the tumor-bearing right thigh and the healthy left thigh track each other whereas the corresponding M–L measurements diverge depicting the height of the tumor bulge. However, the trend is inverted in the intramuscularly injected rat case (Figure 6.6b), with the M–L measurements of the tumor-bearing right thigh and the healthy left thigh tracking each other while the A–P measurements diverge as the tumor-bearing thigh has higher measurements. The difference in thigh growth (A–P) between the tumor-bearing thigh and the healthy thigh was however, not significant until day 7 in the intramuscular case, indicating that rat growth was likely the reason for the size change over that period. The tumors were therefore, seen to grow differently depending on the injection site in spite of similar initial thigh dimensions.

![Figure 6.6](image_url)

**Figure 6.6:** Caliper measurements of growth of tumor-bearing right thigh and healthy left thigh of (a) subcutaneously injected rat, and (b) intramuscularly injected rat, as well as (c) tumor growth in right thigh of subcutaneously injected rat. A–P: anterior-posterior; M–L: medial-lateral
6.3.2.4 Imaging procedures

WM-DPAR imaging utilizes chirp modulated laser beams at two distinct wavelengths for which absorption differences between HbO$_2$ and Hb coincide for one wavelength (isosbestic point, 805 nm) and is maximum for the other (680 nm) to simultaneously generate two signals detected using a standard commercial array transducer as well as a single-element transducer that scans the sample. The superior accuracy of the WM-DPAR method in SO$_2$ quantification of an *in-vitro* blood-containing plastisol phantom over other PA modalities has also been shown in [122] and verified using a gas analyzer (gold standard).

The sample (rat thigh) is positioned near the center of rotation, 25 mm from the surface of the transducer, and 100 chirps are coherently averaged to enhance the SNR of the PA signals.

Moreover, to optimize the WM-DPAR method, the power of the two lasers is tuned to achieve a unity amplitude ratio (~240 mW/cm$^2$ power density over a 2-cm beam diameter, which is within safe maximum permissible exposure (MPE) limits) and the phase difference to 180$^\circ$ so that the differential PA signal of HbO$_2$ is minimized (zeroed). The validity and sensitivity of the WM-DPAR modality is tested in comparison to single-wavelength FD-PAR tomography by imaging the subcutaneously cancer cell-injected right thigh of a nude rat. The morphological size of the tumor region of interest (ROI) is measured at the time of imaging to be ~12 mm (anterior-posterior) x 12 mm (medial-lateral) x 3 mm (height above skin) using a vernier caliper.

A horizontal field of view (FOV) of the ROI of 50 mm is achieved within a ~20 min measurement time. A relaxation time (in which the laser is off) of 55% of the imaging time is required for consecutive imaging. Parallel acquisition of detection elements was not possible in this version. Providing additional National Instruments (NI) cards and detection elements, simultaneous acquisition for data collection can improve the frame rate for real-time imaging (i.e. ~25 Hz). The system is also adequate for integration with clinical US systems for co-registration since a FD beamforming algorithm similar to that employed in conventional US imaging can be used for PA image reconstruction. The reconstructed image is the spatial cross-correlation function between the PA response and the reference signal used for laser source modulation (the radar principle).
6.3.2.5 Results and discussion

Figure 6.7: (a) Removed tumor showing its length, width and height. (b) Photograph of experimental set-up showing rat secured to the seat and the relative position of the irradiation source and transducer to it. (c) Pure US image obtained from commercial SonixTOUCH imager.

FO: Optical fiber; T: Transducer

A photograph of the position of the rat placed in its seat, relative to the transducer, is provided in Figure 6.7(b). The post-surgery tissue specimen obtained for histopathological analysis measuring at 10 mm x 10 mm x 4 mm is also shown in Figure 6.7(a). The red circle marks the tumor ROI. The presence of a palpable mass is evident in a raised lump at the ROI thirteen days after injecting the cancer cells (at the time of imaging). The pure US image of the cancer cell-injected rat was obtained using the SonixTOUCH US imager at a frequency of 4 MHz as shown in Figure 6.7(c). The tumor, though not immediately obvious and distinguishable from other tissue such as muscle and tendon, can be seen as an unsharply delineated, irregular mass with inhomogeneous echo internal structure (indicated by the white dashed oval).

Histopathological studies and validation were conducted to confirm the presence of the cancerous tumor and its vascularity in the right thigh of the rat. Hematoxylin and eosin (H&E) staining and IHC staining were performed on the removed tissue and the stained sections were reviewed under a microscope with x20 magnification as shown in Figure 6.8. A biomarker for
blood vessel endothelium (CD31) is used on tissue specimens. H&E staining shows a cellular neoplasm arranged in nests and sheets (Figure 6.8(a)). The surrounding healthy tissue shows eosinophilic hyaline staining. Matching CD31 IHC staining reveals an increased overall spread of tumor blood microvessel density over the entire lesion as a result of the cancer (Figure 6.8(b)), appearing more pronounced at the lesion border than in certain areas. Areas with high vascularity are observed in the lesion periphery (Figure 6.8(c)–black border; (d)–green border) and those with mostly lower vascularity are in the center (Figure 6.8(e)–blue border; (f)–red border) of the lesion.

Figure 6.8: Post-surgical histopathological analysis via xenografts in which (a) the H&E stained tumor slide shows the cancer region, and (b) the CD31 stained slide shows an inhomogeneous vascular distribution as highlighted in the selected areas (c)–(f) at the tumor borders and center.

Although the location of the PA tomography FOV (perpendicular to the skin/tumor) does not provide composite 3D images for direct comparison with Figure 6.8 (tumor slices parallel to the skin used for H&E and IHC staining), the histopathological analysis corroborates the PAR
imaging results, confirming (pre)malignancy and providing spatial features of the tumor (slice) useful as an image location reference.

The reconstructed PAR images in Figure 6.9 clearly indicate the location of part of the tumor and other relevant absorbing markers (like the skin, not included for clarity) from the transducer surface. The PA vertical FOV is through the center of the dashed oval shown in the US image (Figure 6.7(c)). The image maximum intensity corresponds to the maximum signal amplitude obtained for a given mode, indicating the presence of an absorber. The PAR images show a confined region of high intensity measuring ~3 mm (only part of the tumor due to illumination limitations). The PAR images, unlike their US counterparts, are less sensitive to the presence of the surrounding tissue, but are highly sensitive to the presence of increased blood flow in the tumor. Thus, the PAR images exhibit better contrast and sensitivity, providing much clearer information regarding the tumor. This is particularly significant for much earlier stage tumor imaging: PAR images (not shown) reveal the tumor only six days after cancer-cell injection while the tumor is indistinguishable in the corresponding US image. Normalized amplitude-only and phase-only images are obtained for the single-wavelength 680-nm (Figure 6.9(a), (d)) and 808-nm (Figure 6.9(b), (e)) methods as well as the differential PAR modality (Figure 6.9(c), (f)) using the WM-DPAR system. Normalization is achieved by scaling each image to values within [0, 1] using the maximum intensity. A common maximum is applied to the 680-nm and 808-nm images to facilitate adequate comparison and portrayal of the tumor. The 680-nm PAR amplitude image (Figure 6.9(a)) shows much higher intensity for Hb compared to HbO\textsubscript{2}, while the 808-nm PAR amplitude image (Figure 6.9(b)) exhibits higher intensity for tHb in general. The differential PAR mode is tuned to zero local fluence-induced variations at the highest SO\textsubscript{2} level, in order to enhance sensitivity and specificity for accurate hypoxia monitoring. Differential PAR amplitude, therefore, increases with decreasing SO\textsubscript{2} with improved dynamic range. A relative intensity map of Hb and HbO\textsubscript{2} is obtained in Figure 6.9(c), though embedded in significant background intensities. This demonstrates the inability of the differential PAR amplitude to clearly differentiate the reduced relative intensity map of Hb and HbO\textsubscript{2} from the background noise in a complex system, such as imaging in vivo. The phase images (Figure 6.9(d), (e), (f)) show a more localized tumor with better contrast and spatial location consistency than the amplitude images (Figure 6.9(a), (b), (c)).
Figure 6.9: Normalized reconstructed PAR images using the 680-nm laser only (a, d), PAR images using the 808-nm laser only (b, e), and differential PAR images (c, f) using both lasers for in vivo imaging of a nude rat subcutaneously injected with cancer cells. Laser irradiation is from above at ~45° to the transducer (revolving counterclockwise over 180° from the bottom of the images).

Filtering the amplitude with the inverse of the SD of the phase produces images with improved contrast and resolution. The tumor in the phase-filtered images shown in Figure 6.10 for the single-wavelength 680-nm, single-wavelength 808-nm and differential PAR modalities, respectively, seems just as localized and particularly more accentuated (higher contrast) due to the suppressed background absorption and noise resulting from phase-filtering. Coupled with the amplified signal difference between the out-of-phase modulated lasers used in the differential PAR modality, further enhancement in the sensitivity and dynamic range of the phase-filtered differential WM-DPAR image (Figure 6.10(c)) is observed. This results in higher fidelity in size and shape reconstruction, which enables more accurate PA visualizations to be generated. Hence, the phase-filtered WM-DPAR image provides more highly resolved, better contrast and dimension-consistent images of the tumor compared to the single-wavelength 680-nm and 808-nm FD-PAR modes separately.
Figure 6.10: Normalized reconstructed phase-filtered PAR images using (a) the 680-nm laser only, (b) the 808-nm laser only, and (c) the differential PAR modality using both lasers for in vivo imaging of a nude rat subcutaneously injected with cancer cells.

Figure 6.11 shows the quantitative SO$_2$ images obtained using the two single-wavelength FD-PAR method (Figure 6.11(a), (c)) compared to the WM-DPAR modality (Figure 6.11(b), (d)). A baseline SO$_2$ level of 92% is applied for HbO$_2$ with the typical SO$_2$ range in healthy rats being 89%–92% [123]–[125]. From the quantitative imaging results of the imaged FOV calculated using equation (3-26) with $C_{tHb} = 44\%$, the tumor shows lower SO$_2$ levels than the surrounding tissue in the differential PAR amplitude case (Figure 6.11(b)), though not falling below the nominally normal SO$_2$ range. Applying equation (3-24), the two single-wavelength FD-PAR amplitude mode (Figure 6.11(a)) overestimates the hypoxic level with depth, as can be deduced by inspection of the PAR amplitude images in Figure 6.9(a), (b), (c). This is due to the effect of variations in surface absorption between the different wavelengths, and consequently, the significant variations in fluence with depth. Simultaneous detection and acquisition would be a useful improvement to better and more adequately interrogate and observe the entire ROI instead of a single scanline.

Upon filtering the quantitative SO$_2$ two single-wavelength and two-wavelength differential PAR amplitude images with the inverse of the SD of the phase from the combined 808-nm and 680-nm, and differential PAR measurements, respectively, the results are significantly improved with better correlation to the qualitative phase-filtered PAR images. A combination of the inverse of the SD of the phase from the 808-nm and 680-nm modes is employed, instead of that of the 808-nm measurement only, to allow for dimensional integrity of the tumor. The two single-wavelength phase-filtered SO$_2$ PAR image (Figure 6.11(c)) is, therefore, greatly improved with
better spatial consistency of the tumor compared to the single-wavelength phase-filtered 680-nm and 808-nm FD-PAR modes separately. Furthermore, the two-wavelength phase-filtered differential SO$_2$ PAR image (Figure 6.11(d)) shows significantly improved sensitivity with adequate size and shape fidelity for accurate quantitative SO$_2$ visualization.

The experimental results obtained show that single-wavelength PAR images may be qualitatively incomplete and quantitatively unreliable, whereas differential PAR modality can provide the most reliable and quantitatively accurate images.

![Figure 6.11: Oxygenation levels within the tumor using (a) two single-wavelength PAR amplitude measurements, (b) two-wavelength differential PAR amplitude method, (c) two single-wavelength phase-filtered PAR measurements, and (d) two-wavelength phase-filtered differential PAR method.](image)

6.3.2.6 Deep Tissue Imaging

Current literature has employed exogenous contrast agents to achieve imaging depths of ~5 cm in chicken breast tissue[77], and used three dimensional imaging for sub-millimeter vessels in the breast to be visible to a depth of ~4 cm[9]. A plastic tube containing black ink in water (low-
scattering medium) was identifiable at ~4 cm with our system while investigating the PSF (degree of spread with depth in Section 2.2.1). At depths beyond ~1 mm (optical limit; MFP), where light diffuses (losing directionality) and irradiance decays exponentially with depth[3], [8], PA imaging is capable of detecting absorbed photons achieving significantly greater penetration depth (several centimeters) while still allowing high spatial resolution as US scattering is much less (two to three orders of magnitude) in tissue.

The pair of images (amplitude- and phase-based) produced concurrently in PAR imaging to provide higher diagnostic power and reliability at each probed subsurface depth, allows for leveraging the more localized phase to improve the spatial resolution and contrast of images[82]. Phase contrast is more highly localized because phase lag is based on the energy centroid location of converted ultrasound through optical absorption across subsurface regions determined by the optical absorption depth. The PA ultrasonic centroid location does not depend on actual photon flux nor does it involve surface optical contrast due to reflection or absorption, unlike the PAR amplitude. As such the phase lag is a truly photoacoustic imaging channel as opposed to a mixed PA and surface optical property image generated from the PAR amplitude [80]. Moreover, it does not depend on optical fluence, a major advantage over time-domain modalities.

Figure 6.12: (a) Removed tumor showing its length, width and height, (b) Photograph of the rat showing the bulge 14 days (red circle) after IM injection, (c) Pure US image obtained from commercial SonixTOUCH imager, and Post-surgical histopathological analysis via xenografts in which (d) the H&E stained tumor slide shows the cancer region with a necrotic center, and (e) the CD31 stained slide shows an inhomogeneous vascular distribution.
Under the aforementioned animal protocol 20011459, the intramuscularly injected (~7 mm below the skin surface) rat thigh was imaged consecutively over a three-week period using our PAR imager. The PAR results presented are those of the ROI 7 days after injecting the FaDu (cancer) cells, though anatomically unapparent at this stage as depicted by the pure US image of the ROI (indicated by the white dashed oval) acquired using the SonixTOUCH US imager at a frequency of 4 MHz (Figure 6.12(c)). Tumor growth resulted in an anatomically identifiable bulge at the injection site 14 days after injection (Figure 6.12(b)), helping guide tumor location thereafter. Histopathological analysis via H&E (Figure 6.12(d)) and IHC (CD31; Figure 6.12(e)) staining of the extracted tumor shown in Figure 6.12(a) also confirm the presence of the tumor.

Figure 6.13: Normalized reconstructed phase-filtered PAR images using (a) the 680-nm laser only; (b) the 808-nm laser only; and (c) the differential PAR image using both lasers for in vivo imaging of a nude rat IM injected with cancer cells.

The tumor in the phase-filtered images shown in Figure 6.13(a), (b), (c) for the 680-nm, 808-nm and differential PAR modalities, respectively, is localized, portrays the spatial extent of the tumor, and possesses adequate spatial resolution and contrast due to the suppressed background absorption and noise as a result of phase-filtering. Additionally, the phase-filtered WM-DPAR image (Figure 6.13(c)) exhibits further improvements in sensitivity and dynamic range due to the amplified signal difference between the out-of-phase modulated lasers used in the differential PAR modality, resulting in higher size and shape fidelity. The phase-filtered differential PAR image, therefore, generates highly resolved, better contrast and dimension-consistent images of the tumor compared to the 680-nm and 808-nm PAR modes separately.
Extending the WM-DPAR modality to three-dimensional imaging, in addition to appropriate wavelength selection, light delivery optimization via multidirectional illumination, multi-element transducer design parameters and signal processing can advance imaging depth penetrations of several centimeters.
Chapter 7
Conclusions

A summary of the highlights and key findings of this work is provided in this concluding chapter. Several experiments were conducted in this work to illustrate the effectiveness of the photoacoustic radar (PAR) modality in providing adequate results with comparable relevant system features including comparable signal-to-noise ratio, dynamic range (contrast) and spatial resolution. In live animal testing, results are often contrasted with their ultrasound counterparts and corroborated via histopathology.

7 Conclusions

7.1 Coregistration of PAR and US toward early cancer detection

This work demonstrates frequency-domain photoacoustic radar (FD-PAR) imaging of absorbers in turbid media and their comparison and/or validation as well as co-registration with their corresponding ultrasound (US) images. The merits of US and cross-correlation (radar) frequency-domain photoacoustic imaging are leveraged for accurate early tumor localization and detection. The FD-PAR modality uses intensity-modulated (coded) continuous wave (CW) laser sources driven by frequency-swept (chirp) waveforms. The spatial cross-correlation function between the PA response and the reference signal used for laser source modulation produce the reconstructed image. The feasibility of this combined modality is demonstrated by imaging cancer cell-injected mice, producing images of comparable signal-to-noise ratio (SNR), contrast and spatial resolution. Filtering methods as well as image normalization and amplification techniques were investigated to improve FD-PAR imaging parameters such as signal-to-noise ratio (SNR), contrast and spatial resolution, and hence, further reduce absorber spread and artefacts.

The co-registered image produced from the US and PAR images provides more information than both images independently; working cooperatively toward the optimization of spatial resolution and image acquisition speed than either modality independently.

This work is aimed at functional PA imaging of small animals \textit{in vivo} and is significant in the potential acceleration of clinical acceptance and use of PA imaging if adequately integrated on a
commercial US instrument. Further studies are underway to fully amalgamate both US and PAR modalities in a single clinical imager.

7.2 Phase-filtering as a spatial resolution improvement technique

Phase-mediated image improvement is further explored in this work. Experimental results presented show enhancements in SNR, contrast and spatial resolution (providing clearer information regarding a tumor) via phase-filtered PAR imaging owing to the high localization of the phase. The PAR amplitude image generated from the amplitude of the cross-correlation between detected and input signals is filtered with the standard deviation (SD) of the phase of the correlation signal generated in the FD, resulting in strong improvement of image spatial resolution, SNR and contrast. Application of phase-mediated image improvement is illustrated repeatedly throughout this work by performing in vivo live animal testing. In one such experiment involving the imaging a cancer cell-injected mouse, a 14–15 dB SNR gain was recorded for the phase-filtered image compared to the amplitude and phase independently, while ~340 μm spatial resolution was seen for the phase PAR image compared to ~840 μm for the amplitude image.

Applying sophisticated US-inspired software could speed-up PA imaging and offset the slower phase-filtered data acquisition.

FD-PAR tomography was also presented as an image improvement technique, providing images of high quality and improved spatial resolution. The effects of reducing the number of scan lines (or angles) on image quality, resolution and contrast were investigated. Adequate image quality was still achievable with a quarter of the original number of scan lines used. The validity of this modality was demonstrated with experiments on PVC-plastisol samples as well as ex vivo chicken breast phantoms before extending it to the evaluation of the oxygenation level of in-vitro sheep blood and in vivo animal (qualitative and quantitative) imaging.

The concurrent use of phase-filtering and PAR tomography have provided significant improvements in crucial system parameters.
7.3 Quantitative WM-DPAR hypoxia imaging

It was shown that the wavelength-modulated differential photoacoustic radar (WM-DPAR) modality can be more sensitive to minute changes in total hemoglobin concentration (tHb) and oxygenation (SO₂), than single-wavelength FD-PAR imaging. This is valuable for clinical applications as benchmarks of tumor formation such as angiogenesis and hypoxia, can be detected. The feasibility of the method was demonstrated with experiments on heparinized sheep blood at decreasing oxygenation levels circulating in plastic tubing. Image normalization techniques were applied to improve WM-DPAR imaging parameters such as dynamic range, SNR, contrast and spatial resolution.

The imaging of cancer cells injected into the right thigh of a nude rat demonstrated the validity of phase-filtered WM-DPAR modality for the functional imaging of small animals in vivo. Experimental results presented demonstrate live animal testing and show enhancements in SNR, contrast and spatial resolution, providing clearer information regarding the tumor via phase-filtered differential PA imaging owing to the high localization of the phase, minimization of background noise and amplification of signal differences.

The WM-DPAR modality was also used in the quantification of SO₂, showing greater sensitivity to minute changes in tHb and SO₂, than single-wavelength FD-PAR imaging. This is valuable for clinical applications, as benchmarks of tumor formation such as angiogenesis and hypoxia, can be detected. The WM-DPAR images were further improved in terms of dimensional integrity, dynamic range (contrast), SNR, and spatial resolution by applying image normalization techniques and phase-filtering. Potential problems (less quantitative reliability) with consecutive single-wavelength imaging contrast widely used in multispectral PA imaging were revealed and a method for quantitative differential PA imaging that yields precise contrast based on the level of tumor hypoxia was presented. Further studies are underway to extend the WM-DPAR modality to three-dimensional quantitative tumor hypoxia imaging and fully amalgamate both US and WM-DPAR modalities into a single imager.
References


X. Wang, X. Xie, G. Ku, L. V Wang, and G. Stoica, “Noninvasive imaging of hemoglobin concentration and oxygenation in the rat brain using high-resolution photoacoustic


Appendices

Appendix A: List of Publications


5. E. Dovlo, B. Lashkari, S. Soo S. Choi, and A. Mandelis, “Co-registration of ultrasound and frequency-domain photoacoustic radar images and image improvement for tumor detection,” in *SPIE BiOS*, 2015, p. 93160J.

