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**DEVELOPING A PORTABLE, HIGH-SPEED, LOW-
COST DESKTOP PHOTOACOUSTIC TOMOGRAPHY
IMAGING SYSTEM**

**KALVA SANDEEP KUMAR
SCHOOL OF CHEMICAL AND BIOMEDICAL ENGINEERING
2019**

**DEVELOPING A PORTABLE, HIGH-SPEED,
LOW-COST DESKTOP PHOTOACOUSTIC
TOMOGRAPHY IMAGING SYSTEM**

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A thesis submitted to the Nanyang Technological University in
partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Authorship Attribution Statement

This thesis contains material from 4 papers published in the following peer-reviewed journals in which I am listed as an author.

Chapter 2 is published as Sandeep Kumar Kalva, and Manojit Pramanik, “Experimental validation of tangential resolution improvement in photoacoustic tomography using a modified delay-and-sum reconstruction algorithm,” *Journal of Biomedical Optics* 21(8), 086011 (2016).

The contributions of the co-authors are as follows:

- I have designed all the necessary phantoms, conducted all the simulation and experimental studies required for validating the idea of this project, analyzed the data and prepared manuscript drafts and figures.
- Asst. Prof. Manojit Pramanik developed the idea of the project and the reconstruction code, supervised the work, assisted in analyzing the data and revised the manuscript drafts and figures.

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The contributions of the co-authors are as follows:

- I was involved in initial discussions in developing the idea of this project, developed the set-up, conducted all the experimental studies required for implementing this idea, analyzed the data and prepared manuscript drafts and figures.
- Asst. Prof. Manojit Pramanik proposed the idea of the project, suggested in improvising the set-up, supervised the work, assisted in analyzing the data and revised the manuscript drafts and figures.

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The contributions of the co-authors are as follows:

- I was involved in initial discussion of the idea of this work. I have developed the calibration code, validated using the simulation and experimental studies, analyzed the data and prepared manuscript drafts and figures.
- Mr. Zhe Zhi Hui was involved in discussions and assisted in writing the calibration code in MATLAB.
- Asst. Prof. Manojit Pramanik proposed the idea of calibrating the reconstruction radius, assisted in implementation and analysis of the code, and revised the manuscript drafts and figures.

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- I was involved in initial discussions of the idea of this project, developed the experimental set-up, characterised its performance by conducting all experimental studies, analysed the data and prepared manuscript drafts and figures.
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Dedicated to my parents.

Abstract

Photoacoustic tomography (PAT) is a non-invasive, hybrid biomedical imaging modality. Conventional PAT system uses Nd:YAG-OPO laser as excitation source and a single-element ultrasound transducer (SUT) for detecting the generated photoacoustic (PA) waves from the imaging object. Usually Nd:YAG-OPO laser is bulky, heavy and needs an optical table to deliver non-fluctuating laser beam and it needs external optics (reflectors/prisms) to direct the laser beam onto the sample (there are portable OPO lasers from OpoTek, EKSPLA, and various other companies that do not require optical table but they are more expensive than conventional Nd:YAG-OPO lasers). Also, the detecting element of the SUT is placed at orthogonal direction to the laser illumination for deep tissue imaging. Due to this configuration, the length of the transducer body and the connecting cable requires a lot of space inside the water (coupling medium) tank, thereby, increasing the size of the PAT scanner area. All these (laser, additional optics, SUT alignment) make the whole PAT imaging system very large in size and non-portable. Therefore, it is limited to bench-side laboratory imaging purposes. Moreover, this Nd:YAG laser is very expensive and has low pulse repetition rate of (10-100) Hz. So, one SUT takes several minutes to acquire a single cross-sectional PA data. This in turn, inhibits real-time imaging. The acquired PA data using this SUT is reconstructed conventionally using a simple delay-and-sum algorithm to obtain cross-sectional PA images. When this technique is employed for reconstruction, there will be blurring in tangential direction due to the effect of SUT aperture size, thereby, resulting in the degradation in quality (poor tangential resolution) of the PA images, especially when the target is far from the scanning center.

These are some of the limitations in using a conventional PAT system in terms of

size, cost, speed and image quality that inhibit the translation of the PAT system into clinics for real-time high-speed imaging applications. Thus, there is a need for a low-cost, portable and high-speed PAT imaging system that can render better quality images.

To minimize the size of the overall PAT system, initially we have reduced the space occupied by the SUT in the PAT scanner. To achieve this, we have placed the transducer vertically instead of conventional horizontal direction and augmented it with an external acoustic reflector to the transducer body. This will reflect/direct the incoming PA waves from the imaging object to the detector element of the vertically placed SUT. We have validated the effectiveness of using the acoustic reflector augmented SUT (SUTR) for PA imaging using phantom and *in vivo* studies. Further to overcome the space occupied by the bulky Nd:YAG-OPO laser, we have used pulsed laser diode (PLD) as an alternate excitation source.

With advancement in laser technology, pulsed laser diodes have come into existence and been used effectively for PA imaging over the past few years. These PLD lasers are compact in size, light in weight and less expensive compared to the conventional Nd:YAG lasers. Taking these advantages into consideration, we have used a PLD laser directly inside the PAT scanner by mounting it on top of the imaging area (PLD laser is placed directly above the imaging object at the scanning centre). Due to this we saved a humongous amount of space occupied by the previously used Nd:YAG-OPO laser (placed outside the scanning area). In addition, there was no requirement of using any additional optics in this configuration as the laser source directly illuminates the sample. Hence, by employing the PLD excitation source, we have made the PAT system more compact, portable and easy to use for bed-side imaging. Moreover, the pulse repetition rate of this PLD laser is very high (2 kHz) compared to Nd:YAG laser (10 Hz). This is more advantageous for high speed imaging. Therefore, to improve the imaging speed, we have used PLD along with 8 SUTRs rotating in 45° (instead of 1 SUTR rotating in 360°) and obtained a high scan speed of 0.5 s *in vivo*.

Experimentally, all these transducers cannot be placed exactly at the same distance from the scanning center manually. Hence the acquired PA data from each transducer needs to be reconstructed with the corresponding radii while using delay-and-sum algorithm. This requires the exact location of each transducer from the scanning center. Hence, we proposed a calibration method to find the scanning radius of each transducer. We validated the efficacy of this method using numerical and experimental data.

To improve the image quality in terms of tangential resolution, we have used a modified delay-and-sum algorithm, an improved version of conventional simple delay-and-sum algorithm for reconstructing the PA images and observed three-fold improvement in tangential resolution. In addition to it, this algorithm also preserved the shape of the target object which is very useful for diagnosis and treatment purposes.

Overall, by combining all the innovations described earlier, we have developed an affordable, portable, high-speed desktop PLD based PAT imaging system. Using this system we achieved a high spatial resolution of $165\ \mu\text{m}$ and an *in vitro* imaging depth of 3 cm. We also demonstrated its dynamic imaging ability at a high scan speed of 2 frames/s by monitoring the fast uptake and clearance process of indocyanine green (ICG) dye in rat cortical vasculature.

Contents

Acknowledgements	vi
Abstract	ix
Contents	xii
List of Figures	xiv
List of Tables	xv
Abbreviations	xvi
 Chapter 1 Introduction	 1
1.1 Introduction to various biomedical imaging modalities	1
1.2 Photoacoustic imaging	7
1.3 Challenges associated with circular-view PAT systems	12
1.4 Outline of thesis	17
 Chapter 2 Improving tangential resolution	 20
2.1 Introduction	20
2.2 Materials and Methods	22
2.2.1 Modified delay-and-sum reconstruction algorithm	22
2.2.2 Numerical simulations	24
2.2.3 Experimental method	26
2.3 Results and Discussions	28
2.3.1 Simulation results	28
2.3.2 Experimental results	31
2.4 Conclusion	36
 Chapter 3 Compact PAT scanner using acoustic reflector	 37
3.1 Introduction	37
3.2 Materials and Methods	39
3.2.1 Photoacoustic tomography imaging system	39
3.2.2 Phantom experiments	41
3.2.3 <i>In vivo</i> experiments	41
3.2.4 Comparison of reconstructed images	42
3.3 Results and Discussions	42
3.3.1 Phantom experimental results	42
3.3.2 <i>In vivo</i> experimental results	46
3.4 Conclusion	49
 Chapter 4 Calibrating scanning radius in a multi-SUT based PAT system	 50
4.1 Introduction	50
4.2 Methods	53
4.2.1 Numerical Simulations	53

4.2.2	Calibration Method	54
4.2.3	Experimental Method	56
4.2.4	Comparison of Reconstructed Images	57
4.3	Results and Discussion	58
4.4	Conclusion	68
Chapter 5	Desktop PLD-PAT-G2 system	69
5.1	Introduction	69
5.2	PLD-PAT-G2 system	70
5.3	Results and Discussions	72
5.3.1	Phantom experiments	72
5.3.2	<i>In vivo</i> experiments	75
5.4	Conclusion	78
Chapter 6	Conclusion and future directions	80
6.1	Conclusion	80
6.2	Future directions	83
	List of publications	86
	References	88

List of Figures

Figure 1.1	Schematic diagrams of various transducers with different geometry	13
Figure 2.1	Schematic diagram of simulation and experimental set ups	25
Figure 2.2	Simulations results for all three numerical phantoms	29
Figure 2.3	Experimental results for point source phantom for flat and focused USTs	31
Figure 2.4	Experimental results for circular shaped phantom for flat and focused USTs	33
Figure 2.5	Experimental results for ‘N’ shaped blood vessel phantom for flat and focused USTs	34
Figure 3.1	USTR, UST, three phantoms and schematic of experimental set up	39
Figure 3.2	Cross-sectional reconstructed PAT images of the point source phantom	43
Figure 3.3	Cross-sectional reconstructed PAT images of triangular shaped hair phantom	44
Figure 3.4	Cross-sectional reconstructed PAT images of ‘N’ shaped blood vessel phantom	45
Figure 3.5	Reconstructed cross-sectional PAT images of rat brain	46
Figure 4.1	Schematic of radius calibration and numerical phantoms	53
Figure 4.2	Schematic diagram of multi-SUT PACT system	56
Figure 4.3	Multi-SUT point target images	59
Figure 4.4	MultiSUT Derenzo	61
Figure 4.5	MultiSUT Blood Vessel	64
Figure 4.6	MultiSUT experimental point source	66
Figure 5.1	Schematic of PLD-PAT-G2 system	71
Figure 5.2	Reconstructed images for triangular shaped horse hair phantom	73
Figure 5.3	Deep-tissue imaging	74
Figure 5.4	Reconstructed images of <i>in vivo</i> rat brain vasculature	76
Figure 5.5	Dynamic imaging of ICG uptake and clearance	77

List of Tables

Table 1.1	Comparison of existing medical imaging modalities	6
Table 1.2	Comparison of various imaging modalities	11
Table 4.1	Transducer Specifications	55
Table 4.2	PCC values of reconstructed initial pressure rise distribution ^a	62

Abbreviations

AM	Anesthesia machine
AMP	Amplifier
ANSI	American National Standards Institute
AR	Acoustic resolution
CT	Computed tomography
CV	Cerebral veins
DAS	Delay-and-sum
DAQ	Data acquisition
DOT	Diffuse optical tomography
FDA	Food and drug administration
FDG	Fludeoxyglucose
fMRI	Functional magnetic resonance imaging
FWHM	Full width at half maximum
GPU	Graphics processor unit
ICG	Indocyanine green

LDPE	Low-density polyethylene
LDU	Laser driving unit
LED	Light emitting diode
MRI	Magnetic resonance imaging
NA	Numerical aperture
NIR	Near-infrared
OAI	Optoacoustic imaging
OCT	Optical coherence tomography
OPO	Optical parametric oscillator
OR	Optical resolution
PA	Photoacoustic
PACT	Photoacoustic computed tomography
PAE	Photoacoustic endoscopy
PAI	Photoacoustic imaging
PAM	Photoacoustic microscopy
PAT	Photoacoustic tomography
PC	Pearson correlation
PCC	Pearson correlation coefficient

PET	Positron emission tomography
PLD	Pulsed laser diode
SM	Stepper motor
SNR	Signal-to-noise ratio
SPECT	Single photon emission tomography
SS	Sagittal sinus
SUT	Single-element ultrasound transducer
SUTR/USTR	Acoustic reflector augmented single-element ultrasound transducer
TAT	Thermoacoustic tomography
TS	Transverse sinus
TTL	Transistor-transistor logic
UI	Ultrasound imaging
US	Ultrasound
UST	Ultrasound transducer
UV	Ultraviolet

Chapter 1

Introduction

1.1 Introduction to various biomedical imaging modalities

Simple way to look inside human body is by cutting it open through surgery or by using an endoscope, to image internal organs in the body. These are invasive procedures where the body needs to be cut and put something in it or both for direct optical viewing. This results in potential damage or trauma to the body. Medical imaging is a platform that helps us to see inside the human body in ways that are less invasive than surgery or endoscopy. Some of the well-established medical imaging modalities in clinical use today are projection radiography, X-ray computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), and ultrasound imaging. All these imaging modalities do not involve cutting the body or putting a physical device into it to see inside. Moreover, these medical imaging techniques allow us to see things that are not visible to the naked eye. Each imaging modality reveals different information as the signal received from them is intrinsically different. However, all these biomedical imaging modalities have their advantages and disadvantages. Therefore, scientists are always in search of new

imaging modality. Optical imaging is a strong contender. For the past few decades, research in optical imaging modalities has been in good progress and many attempts were made to translate them into clinics. In the recent years, a novel hybrid biomedical imaging modality known as photoacoustic imaging, combining both optical and ultrasound imaging, has been gaining significant attention with a great promise of being translated into clinics.

Radiography was the first medical imaging modality that came into clinics after the discovery of X-rays by Wilhelm Roentgen in 1895. In radiography imaging, when X-rays are incident on the patient, different tissues present inside the body absorb this energy in various amounts. Bone absorbs the maximum radiation compared to soft tissue and air. Therefore, the bone appears white in color whereas soft tissue appears gray and air as black in the X-ray image. X-ray imaging finds applications in diagnosing fractures, bone diseases, pneumonia, pulmonary edema, intestinal obstructions, renal or gall bladder stones etc. [1]. This imaging modality is widely used in clinics till date. X-ray mammography is the most commonly used technique for screening breast cancer in women. X-ray radiography provides only structural information and does not provide any 3D information. In 1972, Godfrey N. Hounsfield developed the first **computed tomography** that generates 3D volumetric images using X-ray projections in multiple angles and tomographic reconstruction [2]. CT finds applications in assessment of lesions, evaluation of trauma of gastrointestinal, vascular, bone, neurology systems etc. The major disadvantage of radiography and CT is that X-rays are ionizing radiation that cause cancer. Moreover the radiation dosage is high in CT compared to X-ray radiography which inhibits repetitive long-term studies. Also, it is expensive in

terms of acquisition and maintenance compared to X-ray.

MRI works on the phenomenon of nuclear magnetic resonance (NMR) where the magnetic moments of nuclei in the presence of external strong magnetic field and their response to certain radio frequency waves are detected. When a patient is exposed to strong and uniform magnetic field (1.5 T) inside MRI scanner, the magnetic moments of abundant hydrogen nuclei (1H) present inside the body get aligned. When radio frequency wave is incident then these protons absorb these energy and come back to equilibrium state through T1 (spin-lattice) and T2 (spin-spin) relaxation by emitting radio frequency signal when it is off. These signals are then encoded to produce anatomical information. Different tissue contrast is achieved by the rate of relaxation time taken by various tissues to reach equilibrium state. MRI is widely used for diagnosing brain tumours, breast tumours, staging of prostate cancer, assessment of congenital heart diseases, in angiography etc. [3–6]. Functional MRI (fMRI) provides physiological/functional information in order to measure the brain activity by detecting variations in the blood flow [7–9]. Unlike X-ray CT imaging, MRI doesn't use ionizing radiation making it safer to use and also it generates high resolution images comparatively. However due to strong magnetic fields employed in MRI, this imaging modality is not suitable for patients with pacemaker implants or any other non-removable metallic implants. During MRI scan, the subject needs to be still for several minutes to hours which creates discomfort to the patient mainly to those who are claustrophobic.

Nuclear medicine is a branch of radiology where a compound (glucose, ammonia, water etc) containing radioactive isotope (Carbon-11, Fluorine-18, or Zirconium-89) is

administered into patient's body. This isotope gets distributed throughout the body and undergoes radioactive decay to emit X-rays and/or gamma rays. The radiation detectors are employed to detect these rays and nuclear medicine images are reconstructed to provide information about the physiological condition of the patient rather structural information [10]. In **PET** imaging, when a positron-emitting radionuclide is introduced into the body, a pair of gamma rays are emitted indirectly. Most frequently used molecular compound is fludeoxyglucose (FDG) labelled with fluorine-18. These emitted gamma rays are detected using a ring of detectors around the patient. This information is reconstructed to obtain a three-dimensional image (a series of tomographic emission images). Unique advantage of PET over other morphological imaging modalities such as CT or MRI is that it reveals functional information describing the changes in biological processes happening inside the human body. Therefore, PET imaging modality is majorly used along with CT or MRI modalities to obtain metabolic information from PET scan and structural information from CT or MRI scan. PET imaging can be used for detecting myocardial ischemia in coronary heart disease, labelling amyloid plaques in Alzheimer's, capturing increased metabolism in cancer and tumour [11, 12]. However, spatial resolution is limited in PET compared to CT and MRI. Also, major drawback of PET is that the production cost of compound radioisotopes with short half-life is very high, difficult to prepare and the maintenance cost is also high due to limited equipment usage in hospitals. Moreover, administering these radioactive materials inside human body is not safe. In **SPECT** imaging modality, gamma rays emitted from the patient are recorded using a gamma camera over various projection angles around the patient. Tomographic images are then reconstructed from this projection data. SPECT scan is less costlier compared to PET or CT scan. SPECT imaging modality is widely utilized

for detecting blood flow alterations to the brain, bone, or heart, leading to brain injury, cardiovascular disease, and epilepsy [13, 14].

For more than half a century, **ultrasound (US)** imaging has been practised in clinics. It is a non-invasive, non-ionizing technique which is relatively inexpensive and portable as well. In 1942, ultrasound was first used to diagnose brain tumours [2]. In ultrasound imaging, a hand held probe is used to send pulses of sound waves above the audible frequency range (20-20 KHz) into the tissue. Due to the differences in acoustic impedances inside the body, part of the transmitted sound wave is reflected back to the transducer and detected as an echo. The echo is larger when the difference in acoustic impedance is greater. This mode of imaging is also known as pulse echo imaging [15]. The recorded echo data is computed to generate an ultrasound image. US imaging finds applications in cholecystitis, appendicitis, pancreatitis, ectopic pregnancy, pelvic masses, aortic aneurysms, deep vein thrombosis [1]. Unlike CT, US imaging is a non-ionizing technique, and therefore it is much more safer to use. Also, it is portable and less expensive compared to CT and MRI. This US imaging modality can be used to depict rhythmic motion of the heart and the ultrasound probe is very flexible to image in all possible directions such as oblique, transaxial, or sagittal views. Major disadvantage of using this imaging modality is its operator dependent and interpretation of ultrasound images requires expertise. Also, the imaging depth is still limited to $\sim 10\text{-}20$ cm which is not suitable for imaging full body.

Although all these clinically available biomedical imaging modalities are well established techniques for diagnostic and therapeutic purposes, each modality has some

Table 1.1: Comparison of existing medical imaging modalities

Modality	X-ray	CT	MRI	PET	SPECT	US
Source	X-rays	X-rays	Electric, magnetic fields	High energy γ -rays	Low energy γ -rays	High frequency sound
Spatial resolution	(50-200) μm	(50-200) μm	(25-100) μm	(1-2) mm	(1-2) mm	(50-500) μm
Imaging depth	Full body	Full body	Full body	Full body	Full body	(10-20) cm
Imaging speed	Minutes	Half minute to minutes	Minutes to hour	Seconds to minute	Minutes	Real time
Information	A	A, F	A, F	F, M	F	A, F
Cost	Low	High	Very high	Very high	High	Low
Safety	Ionizing	Ionizing	Safe	Ionizing	Ionizing	Safe

A - anatomical; F - functional; M - metabolism.

disadvantages (Table 1.1). Currently there has been a shift in the interest of the researchers towards **optical imaging** modalities where the light in ultraviolet (UV) - visible - near infra red (NIR) regions is used to obtain structural and functional information of the human body. The major advantage of using light in medical applications is it's a non-ionizing and safe radiation unlike conventional established clinical modalities like X-ray radiography, CT, PET, SPECT that use harmful ionizing radiation. The broad spectrum of optical wavelengths provide anatomical, functional, molecular, metabolic and genetic information. When a light is irradiated on any tissue, some photons get absorbed by the tissue and some photons get scattered to different directions. We can study angiogenesis which is a hallmark of cancer through optical absorption properties of the tissue chromophores for e.g., blood. Also, optical scattering reveals information about nuclei etc. In biological tissues scattering is a dominant characteristic. This makes deep optical imaging with high resolution quite impossible. Many microscopic and tomographic optical imaging modalities have been developed for rich optical contrast and high spatial resolution imaging of biological tissues. Some of the optical imaging

modalities include fluorescence microscopy, confocal microscopy, multi-photon microscopy, and optical coherence tomography (OCT). However, the imaging depth of these techniques is limited to ~ 1 mm inside the biological tissue as they are dependent on ballistic photons. In contrast, by employing diffuse optical tomography (DOT) imaging modality that uses multiple scattered photons for imaging, we can image up to several centimetres deep inside the biological tissue. But, the strongly scattered light in tissues limits the spatial resolution provided by DOT technique at deeper penetration depths. Thus, using optical imaging techniques deep tissue imaging with higher spatial resolution is still a challenge.

Hence, there is a void to be filled by an imaging modality that can provide high spatial resolution images at much deeper imaging depths and also which is safer to use, portable as well as affordable. Photoacoustic imaging, a new hybrid biomedical imaging modality combining light excitation and acoustic detection, connects this gap of deeper imaging with high spatial resolution and better optical contrast .

1.2 Photoacoustic imaging

Photoacoustic or optoacoustic imaging (PAI/OAI) is a non-invasive hybrid biomedical imaging modality that has been rapidly advancing from laboratory to preclinical and clinical stages over the past few decades [16–21]. It combines optical imaging (rich contrast) and ultrasound imaging (high spatial resolution) in a single modality. This PAI modality works on the phenomenon of photoacoustic effect. Alexander Graham Bell reported first the observation of sound generated by modulated light (photophonic

phenomenon/photoacoustic effect) in 1880 [22]. When pulsed laser of nano-second duration is incident on any biological tissue, the photons are absorbed by the intrinsic tissue chromophores like oxy-hemoglobin (HbO_2), deoxy-hemoglobin (HbR), melanin, water (H_2O), lipids etc. Due to this, there is a local rise in temperature (in the order of few milli-degrees/milli-kelvin) in the tissue resulting in thermoelastic expansion of the tissue. This in turn generates pressure waves around the tissue known as photoacoustic (PA) waves over a wide-band ranging from 1-100 MHz. These waves are detected using wide-band ultrasound transducer(s) (UST). PA images generated from these data provides information about internal structures and their functional, molecular, metabolic and genetic information of vasculature, hemodynamics, oxygen saturation levels, gene expression etc., [18, 23–26].

PAI has various advantages over pure optical and pure ultrasound imaging techniques such as: (i) it is label-free imaging as it provides intrinsic optical contrast, (ii) it can image much deeper inside the tissue up to 12 cm [27], compared to other optical imaging modalities like fluorescence microscopy [28], optical microscopy, confocal microscopy [29], multi-photon microscopy, Raman microscopy, optical coherence tomography and diffuse optical tomography, (iii) it is free from speckle artefacts unlike US and OCT imaging, (iv) it is faster, compact and less expensive compared to X-ray, CT, MRI, PET, (v) it provides structural and functional information using multiple wavelengths, (vi) it is a multi-scale, multi-contrast imaging modality used for visualizing organelles to organs, (vii) ultrasound scattering is 2-3 orders of magnitude less compared to optical scattering in biological tissue, hence, ultrasound signal can be detected from much deeper compared to optical signal, and (viii) the sensitivity of optical

absorption for PA imaging is two orders of magnitude greater compared to that optical imaging techniques like OCT and confocal microscopy [18]. With all these advantages, PAI stands as a potential tool for clinicians in oncology, neurology, cardiology, vascular biology, ophthalmology, gastroenterology and dermatology [18, 28, 30–34]

For effective generation of PA waves, two conditions must be met: thermal and stress confinements [35]. According to the thermal confinement, the pulse width τ_p needs to be lesser than the heat dissipation time τ_{th} after the absorption of electromagnetic energy and stress confinement states that the pulse width τ_p should be lesser than the time for the stress to transit the heated region τ_s . When both these constraints are met, thermal expansion results in initial pressure rise p_0 inside the tissue which is given as:

$$p_0 = \left(\frac{\beta c^2}{C_p}\right) \mu_a F = \Gamma A \quad (1.1)$$

where β is isobaric volume expansion coefficient in K^{-1} , c is the acoustic speed, C_p is the specific heat at constant pressure in $J/(K Kg)$, μ_a is the absorption coefficient in cm^{-1} , F is the fluence in J/cm^2 , A is the local energy deposition density in J/cm^3 : $A = \mu_a F$ and Γ is Gruneisen coefficient: $\Gamma = (\beta c^2 / C_p)$. Mathematically, the photoacoustic wave propagation equation is given as [19]:

$$(\nabla^2 - c^{-2} \frac{\partial^2}{\partial t^2}) p(\vec{r}, t) = -(\beta / C_p) \frac{\partial H(\vec{r}, t)}{\partial t} \quad (1.2)$$

Here, $p(\vec{r}, t)$ is the acoustic pressure at position $\vec{r}$ and time t and H is the heating function which is defined as thermal energy converted per unit volume per unit

time. The left hand side of the equation represents the wave propagation, whereas right hand side describes the source term. The source term is related to the first order time derivative of H . Hence, time-invariant heating doesn't generate pressure waves but only time-variant heating does generate the pressure waves. Therefore, rapid laser heating is must for effective PA wave generation.

Photoacoustic imaging is a multi-scale imaging modality which makes it possible to image from various microscopic organelles to macroscopic organs with better contrast. Broadly there are three embodiments of PAI, namely: (i) photoacoustic microscopy (PAM), (ii) photoacoustic endoscopy (PAE), (iii) photoacoustic computed tomography (PACT) or photoacoustic tomography (PAT). PAM is further classified into optical-resolution PAM (OR-PAM) and acoustic-resolution PAM (AR-PAM) depending on whether optical (laser excitation) or acoustic (ultrasound detection) focus is tighter. In OR-PAM, the optical focus is much finer/tighter than the acoustic focus whereas in AR-PAM acoustic focus is finer. To increase measurement sensitivity, these dual foci are confocally configured in both OR-PAM and AR-PAM techniques [36, 37]. PAE is utilized to image internal organs like esophagus and colon. Using this technique we can image upto 7 mm deep (dorsal region of rat colon *ex vivo*) which is much greater than conventional optical endoscopy [38]. However, imaging with higher resolution is limited to shallower depths using these PAM and PAE imaging systems. This limitation is circumvented by using PAT/PACT systems that can image several centimetres deep inside the tissue and hence stands a better chance in clinical imaging applications [33, 39–43].

Major motivation to develop PAT imaging system is summarized in Table 1.2

Table 1.2: Comparison of various imaging modalities

Modalities	OCT [44]	DOT [44]	Ultrasound imaging (UI, 3 MHz) [45]	PAT [27,46,47]
Resolution	Excellent ($\sim 10 \mu\text{m}$)	Poor ($\sim 5 \text{ mm}$)	Excellent & scalable ($\sim 0.55 \text{ mm}$)	Excellent (=UI)
Contrast	Good	Excellent	Poor for early cancers	Excellent
Imaging depth	Poor ($\sim 1 \text{ mm}$)	Good ($\sim 5 - 10 \text{ cm}$)	Good & scalable ($\sim 6 \text{ cm}$)	Excellent ($\sim 11.6 \text{ cm}$)
Speckles	Strong	None	Strong	None
Scattering	Strong ($\sim 100/\text{cm}$)	Strong ($\sim 100/\text{cm}$)	Weak ($\sim 0.1/\text{cm}$)	Very weak

PAI is sensitive to the optical absorption contrast given by the various intrinsic chromophores present inside the biological tissue such as haemoglobin, lipids, melanin, water etc. However, in some scenarios the signal from the intrinsic chromophores situated at higher depths may not strong enough. To enhance the contrast, other exogenous or extrinsic contrast agents can be used. These contrast agents can also be used for targeted molecular imaging [48]. There are several types of contrast agents that have been explored for PA imaging like organic dyes, inorganic dyes, nanometre-sized contrast agents, such as gold nanoparticles, nanospheres, nanocages, carbon nanotubes etc [49]. Methylene blue (MB) and indocyanine green (ICG) are the food and drug administration (FDA) approved organic dyes that have been explored for sentinel lymph node (SLN) imaging, imaging of brain vasculature, urinary bladder etc., [50–52]. Nanoparticle based contrast agents are used for tumour imaging [53]. These nanoparticles can be designed for targeted molecular imaging by coating their surface with specific antibodies which is very helpful for therapeutic monitoring during treatment of cancer [54,55]. Several contrast agents have been explored for PA imaging applications but only few are FDA approved. This is due to the limitations posed by the contrast agents that needs

to overcome for effective clinical usage. The contrast agents needs to be designed so that they are biocompatible, not toxic, have sufficient systemic circulation times and efficient renal clearance.

1.3 Challenges associated with circular-view PAT systems

Based on the type of transducer and the detection geometry, photoacoustic computed tomography (PACT/PAT) systems can be classified into the following categories: spherical-view PAT [56], cylindrical-view PAT [57], planar-view PAT [58], circular-view PAT [42, 59], and linear-view PAT [60] systems [61, 62]. The schematic of different types of transducers based on their detection geometry is shown in Fig. 1.1. Using the spherical, cylindrical, and planar geometry based PAT systems, 3-D images can be formed. These 3-D PACT systems can be commonly used for volumetric imaging studies. Whereas linear and circular geometry based PAT systems can be used for obtaining 2-D images. These 2-D PAT systems can also be used to obtain 3-D volumetric images by scanning vertically along the image plane. However, the spatial resolution will be much poorer in the third dimension than their in-plane resolution [63].

Conventional circular scanning geometry/circular-view based PAT systems use Q-switched Nd:YAG-OPO/Dye lasers as excitation source and a single-element UST (SUT) to acquire the PA waves from the target object in full 360 degree [64]. Although these Nd:YAG based lasers deliver high per pulse energy (~ 100 mJ), they are very expensive, bulky, heavy and require an optical table to house in order to deliver light effec-

Figure 1.1: Schematic diagram of (a) spherical-view PAT, (b) cylindrical-view PAT, (c) Planar-view PAT, (d) circular-view PAT, and (e) linear-view PAT systems.

tively without any fluctuations. Also, they require external optics to guide the beam onto the imaging sample, making the PAT imaging system non-portable and limited to bench-side laboratory imaging applications. Currently there are portable OPO lasers (e.g., Opotek) but they are even more expensive than conventional OPO lasers [65,66]. Moreover, the pulse repetition rates of these lasers are very low in the order of 10-100 Hz which limits the imaging speed of one SUT to several minutes $\sim(2-5)$ for PA data acquisition. This inhibits dynamic imaging of physiological processes occurring inside the body. Moreover, the SUT is usually placed in horizontal direction (detector element facing the scanning center) orthogonal to the laser illumination [67,68]. Due this placement, it requires more space inside the water tank (water acts as a coupling medium) because of the length of the transducer body and part of the connecting cable. Therefore, the SUT rotates in bigger scanning radius which in turn increases the size of the PAT scanner area, thereby, together with bulky Nd:YAG-OPO laser, additional guiding optics, the overall volume of the PAT imaging system increased. These are some of the challenges faced using conventional PAT imaging system in terms of cost, portability

(size) and imaging speed.

With the advancement in UST technology, various types of array USTs are developed such as linear [69–72], semi-circular [73], circular [74–80], and spherical [81–83] array USTs that can be used in different scanning geometries (planar/spherical/circular/cylindrical) [84]. Our focus is on circular scanning geometry based PAT systems. State-of-the-art circular scanning geometry based PAT system employs full-ring (circular) array UST which provides an acquisition frame rate of 50 Hz (1 frame in 0.02 seconds) which is very helpful for visualizing rapidly changing phenomenon *in vivo* [85]. But these circular-ring array transducers are custom-made and are not readily available in the market. Also they require complex back-end receiving electronics and parallel signal amplifiers, thereby, increasing the overall cost of the PAT system. Hence, SUTs are still preferred as they are cheap and easily available in the market.

In tomographic PA imaging, the PA data obtained using the SUT is reconstructed to map the initial pressure rise which correlates to the optical absorption density distribution from the measured PA data i.e., to map the optical absorption heterogeneity of the tissue. Various reconstruction algorithms can be used to map the initial pressure rise distribution inside the tissue [86–103]. Broadly the reconstruction algorithms for PAT are classified into analytical algorithms and model-based iterative image reconstruction algorithms. Analytical algorithms include filtered back-projection, and Fourier transform based reconstructions [19]. These analytical algorithms are based on the spherical Radon transform (useful for solving 3-D PA reconstruction problem) and are used for obtaining just structural information without any quantitative information. These ana-

lytical algorithms require a large set of data points around the imaging object. Either using transducer arrays with more number of detector elements or using SUT for longer acquisition time, we can collect large number of data points. For obtaining more accurate quantitative PA images, model-based techniques are preferred [104, 105]. Model-based reconstruction involves inversion of a model matrix that is generated either using impulse response or discretizing the solution of wave equation. These model-based iterative reconstruction techniques doesn't require large number of data points which in turn reduces the long acquisition times or usage of expensive array transducers. However, analytical based reconstruction techniques are faster than model-based reconstruction techniques [106]. Traditionally, a simple delay-and-sum (DAS) algorithm implementing back-projection technique is used to reconstruct the cross-sectional PA images. While using this reconstruction, due to the large aperture (detecting element) size of the SUT, there will be blurring in the tangential direction. This blurring effect (poor tangential resolution) is more prominent for the reconstructed objects nearer to the detecting surface of the SUT. As a result, the objects closer to the transducer looks distorted in shape. Therefore the quality of the reconstructed PA image degrades and it needs to be improved.

These are some of the challenges for a conventional PAT imaging system in terms of portability, affordability, imaging speed and image quality, to be translated into clinics for dynamic imaging applications.

Over the past few years, attempts were made to overcome some of these challenges. A fast and compact diode-pumped laser was used for deep tissue PAT imaging [107].

This diode-pumped Nd:YAG laser (Montfort Laser GmbH) has a miniature footprint of $13 \times 14 \times 7 \text{ cm}^3$ weighing only 1.6 Kg. It occupies only 4.5% volume and 6.7% weight of conventional Nd:YAG-OPO laser and delivers pulse energies up to 80 mJ at 1064 nm wavelength. This compact Montfort laser can easily overcome space-constrained problems faced by the conventional lasers. In addition, this diode-pumped Nd:YAG laser doesn't require any cooling system as they have higher energy conversion efficiency (> 20%) unlike flash-lamp based Nd:YAG lasers with 1-2% efficiency. However, a major limitation of this laser is fixed output wavelength (1064 nm) which inhibits certain functional and molecular imaging applications. Also, the beam quality is relatively poor compared to conventional Nd:YAG laser. Moreover, the imaging speed is still limited by the pulse repetition rate (50 Hz) of the laser when used with SUT. Recently, high speed imaging using SUTs was demonstrated by using slip-ring based PAT system [108]. This system uses Nd:YAG pumped Ti:Sapphire laser for optical excitation and an electric slip ring for transmitting electric power or signal through rotators attached to two focused SUTs. The schematic of the slip-ring based PAT system can be seen in Fig.1 of Ref [108]. A scanning speed of 1.5 min per frame was achieved using this system. However, the imaging speed is still limited by the low repetition rate of conventional Nd:YAG pumped Ti:Sapphire laser. Also, electromagnetic shielding is a challenge for transmitting weak PA signals with high SNR due to the structure and mechanism of the electric slip-ring. Special care needs to be taken to reduce noise. Moreover the portability of the slip-ring based PAT system is still limited due to the conventional Nd:YAG based laser.

Hence, there are still certain limitations in using the above discussed diode-pumped

laser based and slip-ring based PAT imaging systems. Not all the limitations in terms of portability, affordability, imaging speed and image quality are overcome in a single PAT imaging system. Therefore, there is a huge requirement for a low-cost, high-speed, portable photoacoustic tomography imaging system that can provide better spatial resolution.

1.4 Outline of thesis

In **Chapter 2**, we demonstrated the improvement of tangential resolution in PAT using a modified delay-and-sum reconstruction algorithm for various shaped phantom and *in vitro* data. This algorithmic technique was proposed previously by our group in which the conventional DAS reconstruction algorithm was modified to improve the tangential resolution and was validated only for simulation data. We experimentally validated this modified DAS algorithm and showed a threefold improvement in tangential resolution for images obtained using non-focused (flat) as well as cylindrically focused SUTs. We also preserved the shape of the target objects using this modified DAS algorithm, with no compromise in signal-to-noise ratio (SNR) levels.

In **Chapter 3**, we demonstrated the design of compact PAT circular scanner using a commercially available acoustic reflector augmented to the traditional SUT (SUTR), and placed the SUTR in vertical position instead of conventional horizontal plane. This reduced the water tank size and also load on the motor. As a result, we have minimized the PAT scanner area which reduced the overall volume of the conventional PAT system. We compared the quality (SNR levels and resolution) of reconstructed cross-sectional PA images of phantom and *in vivo* data obtained using SUTR with that of images ob-

tained using conventional SUT in case of both flat and focused transducers and found to be similar.

In **Chapter 4**, we proposed a calibration method for obtaining scanning radii of all SUTs in a multiple-transducer based PAT system. This method avoided the usage of conventional, user-dependent trial-and-error method in finding the scanning/ reconstruction radius for all transducers. These individual scanning radii obtained using the proposed calibration method were used in the DAS reconstruction algorithm for reconstructing each SUT datum. This improved the distortion in shape of the objects that appeared when only single scanning radius is used for all transducers. We showed the efficacy of this method by comparing the quality of 2,4,8-SUTs based reconstructed images with that of 1-SUT based image using numerical phantoms and experimental point source phantom (only 2,4-SUTs scenarios) and found to be similar. Usually one SUT takes several minutes to acquire cross-sectional PA data in a circular scanning PAT system. By using 'N' number of SUTs of similar type, we improved the imaging speed by 'N-fold' which is very useful for dynamic imaging applications.

In **Chapter 5**, we presented the design and engineering of a portable, high-speed, low-cost, desktop pulsed laser diode based photoacoustic tomography imaging system and characterized its performance in terms of speed, spatial resolution and imaging depth. We have used PLD laser with 2 KHz repetition rate at ~ 816 nm wavelength along with 8 SUTRs and developed a second generation desktop PLD-PAT system for high-speed imaging. We compared the quality of the images obtained using 8-SUTRs with that of images obtained using 1-SUTR in terms of SNR for various scanning speeds

of 30 degree/sec, 45 degree/sec and 90 degree/sec both *in vitro* and *in vivo* and found to be similar. We demonstrated a single frame acquisition in as short as 0.5 second scan speed with a spatial resolution of 165 μm . Also, *in vitro* deep tissue imaging up to 3 cm inside the tissue was demonstrated. Dynamic imaging ability of this desktop PLD-PAT system was demonstrated as we monitored the fast wash-in and wash-out process of Food and Drug Administration (FDA) approved indocyanine green (ICG) dye in rat cortical vasculature.

Finally in **Chapter 6**, we summarized this work and recommended future directions.

Chapter 2

Improving tangential resolution¹

2.1 Introduction

In a circular scanning PAT, axial resolution is along the radial direction and is spatially invariant, whereas tangential resolution is along the tangential direction and is spatially variant [109, 110]. The axial resolution is mainly dependent on the transducer bandwidth, whereas the tangential resolution is dependent on both transducer bandwidth as well as transducer aperture size (active area). When the target object is far from the scanning center (i.e., when it is closer to the detector surface), the tangential resolution is poorer for a detector with larger aperture. One of the easiest ways to get rid of this problem is to move the UST very far from the scanning center so that the imaging region is far from the detector surface. However, this increases the scanning radius unnecessarily and practically may not be feasible due to the space constraints. Also, the further detector is moved, the more its sensitivity decreases. To improve the tangential resolution without increasing the scanning radius, a negative acoustic lens concept was proposed, which increases the acceptance angle of the large detector and improves the tangential

¹Part of the work presented in this chapter has been published in *Journal of Biomedical Optics*. **S. K. Kalva**, and M. Pramanik, "Experimental validation of tangential resolution improvement in photoacoustic tomography using a modified delay-and-sum reconstruction algorithm," *Journal of Biomedical Optics* 21(8), 086011 (2016).

resolution [111–113]. There are some practical challenges with this method. Attaching the in-house made acoustic lens to the detector surface without the formation of any air bubbles is difficult. Also, absorption of ultrasound signal and the impedance mismatch between the negative lens and the acoustic coupling medium (water/mineral oil) results in loss of signal. Another problem is that it is very difficult to make an in-house acoustic lens for cylindrically focused detectors, which are also used in circular scanning PAT for better elevation resolution (slice thickness) [114]. A custom-made detector with curved piezo surface or a negative lens attached to the piezo surface inside the transducer can overcome these problems associated with an in-house acoustic lens, but typically such custom-made detectors are very expensive. Another method to improve tangential resolution is to employ high numerical aperture (NA)-based virtual point detectors that provide a wide acceptance angle, high sensitivity, and negligible aperture effect [115, 116]. However, these types of transducers are also home-made, and are not readily available for purchase from the market. So to overcome these difficulties, a modified delay-and-sum reconstruction method was proposed to improve the tangential resolution without using any negative acoustic lens or any custom-made detectors [117]. Simulations showed promising results for this modified delay-and-sum reconstruction algorithm together with conventional ultrasound detectors with large apertures. In this chapter, the modified delay-and-sum reconstruction algorithm was experimentally validated for various shaped phantoms. Additionally, it was demonstrated that the modified reconstruction algorithm can also be used with a cylindrically focused ultrasound detector. Both numerical simulations and experimental data were shown to validate the effectiveness of the modified delay-and-sum reconstruction method.

Three different types of phantoms were used for demonstration purposes: (a) point sources (pencil leads of 0.5-mm diameter) placed at different distances from the scanning center, (b) large circular objects [low-density polyethylene (LDPE) tubes of 5-mm inner diameter filled with black Indian ink], and (c) “N”-shaped LDPE tubes (0.38-mm inner diameter) filled with mice blood to mimic blood vessels, embedded inside chicken tissue. Both flat and cylindrically focused transducers were used and showed that with modified delay-and-sum reconstruction method tangential resolution can be improved more than three times for both these transducers. Moreover, with modified reconstruction, the object shapes are also preserved.

2.2 Materials and Methods

2.2.1 Modified delay-and-sum reconstruction algorithm

The modified delay-and-sum reconstruction algorithm is given in detail elsewhere [117]. The algorithm is briefly summarized here. For a delta light illumination $\delta(t)$, the initial pressure rise at a position $\vec{r}$ on a tissue is given by $p_0(\vec{r}) = \Gamma(\vec{r})A(\vec{r})$, where $A(\vec{r})$ is a spatial light absorption function and $\Gamma(\vec{r})$ is the Gruneisen parameter of the tissue. The main objective of the PAT image reconstruction is to estimate the initial pressure rise $p_0(\vec{r})$ inside the tissue from a set of measured acoustic signals $p(\vec{r}_0, t)$. The acoustic pressure $p(\vec{r}_0, t)$ at position $\vec{r}_0$ and time t , due to initial pressure source $p_0(\vec{r})$, obeys the following photoacoustic wave equation in an acoustically homogenous medium:

$$\nabla^2 p(\vec{r}_0, t) - \frac{1}{c^2} \frac{\partial^2}{\partial t^2} p(\vec{r}_0, t) = -p_0(\vec{r}) \frac{\partial \delta(t)}{\partial t} \quad (2.1)$$

where c is the speed of sound. The initial pressure rise can be obtained by means of backprojection as [86]:

$$p_0(\vec{r}) = \int b(\vec{r}_0, t = \frac{|\vec{r} - \vec{r}_0|}{c}) \frac{d\Omega_0}{\Omega_0} \quad (2.2)$$

where, Ω_0 is the solid angle subtended by entire surface S_0 with respect to $\vec{r}$ (reconstruction point inside S_0). $\Omega_0 = 2\pi$ for a planar geometry and $\Omega_0 = 4\pi$ for spherical and cylindrical geometries. $d\Omega_0$ is the solid angle subtended by detection element dS_0 with respect to reconstruction point at $\vec{r}$. The term $\frac{d\Omega_0}{\Omega_0}$ is weighting factor that contributes to the reconstruction from the detection element dS_0 [Figs. 1 and 2 of Ref. [86]]. $b(\vec{r}_0, t)$ is the backprojection term given by:

$$b(\vec{r}_0, t) = 2p(\vec{r}_0, t) - 2ct \frac{\partial p(\vec{r}_0, t)}{\partial t} \quad (2.3)$$

In the conventional delay-and-sum algorithm, the backprojection equation is implemented by recording the pressure wave as the backprojection term: $b(\vec{r}_0, t) = p(\vec{r}_0, t)$. For large aperture detectors, the recorded pressure signal at $\vec{r}_0$ can be represented as a surface integral over the detector aperture [109].

$$p'(\vec{r}_0, t) = \int \int p(\vec{r}_0', t) W(\vec{r}_0') d^2 \vec{r}_0' \quad (2.4)$$

where, $W(\vec{r}_0')$ is weighting factor contribution from different elements of the detector surface. During conventional delay-and-sum reconstruction, large-aperture detectors are considered as a point detector, typically at the center of the transducer surface. This introduces artifacts in the reconstructed image. In the modified delay-and-sum

reconstruction algorithm, the entire surface area of the detector was considered during backprojection. The signal recorded in the transducer $[p'(\vec{r}_0, t)]$ was backprojected from the entire surface of the detector instead of backprojecting from $\vec{r}_0$. So that the entire integrating surface used to receive the PA signal was used in the modified reconstruction method [117]. Since all the simulations were done in two dimensions (2-D), the recording surface of the transducer is a line segment instead of a circular surface (Fig. 1 of Ref. [117]). As such, many small segments on the line have been considered from which the recorded PA signal $p'(\vec{r}_0, t)$ is backprojected instead of a single center point of the line. The same process is repeated for all transducer positions. For backprojecting, the first term of Eq. (4) was used for both conventional and modified delay-and-sum reconstruction techniques. All elements of the line contribute equally for detecting a signal (as the size of the small segments of the transducer is small). Therefore, the weighing factor $W(\vec{r}_0')$ was considered to be unity. For the experimental data, even though the recorded PA signals are in 3-D, all the reconstructions were done in 2-D. Since the projection of the cylindrically focused transducer is also a line on a 2-D plane, the same algorithm can be used for the data acquired with cylindrically focused UST as well. For experimental validation, both flat USTs and cylindrically focused USTs have been used.

2.2.2 Numerical simulations

Numerical simulations were done using k-wave tool box in MATLAB [118]. The simulation geometry used is shown in Fig. 2.1(a). The computational grid was of 820 x 820 pixels (0.1 mm/pixel) with a perfectly matched boundary layer. Circular scanning geometry with 40 mm scanning radius was used. The imaging region was the circular

Figure 2.1: (a) Schematic diagram of the k-wave simulation geometry in MATLAB. 820x820 pixels (0.1 mm/pixel) were used for all the simulations. Scanning radius 40 mm. UST: ultrasound transducer. (b) Point source numerical phantom. Five point targets were placed at a distance of 0 mm, 8 mm, 16 mm, 24 mm, and 32 mm from the scanning center. (c) Circular shaped numerical phantom (5 mm diameter): one at center and the other at 15 mm from the center. (d) 'N' shaped blood vessel numerical phantom (vessel width 0.38 mm). (e) Schematic diagram of the experimental set up. DAQ: Data acquisition card, R/A/F: Receiver, amplifier and filter for photoacoustic signal. P1, P2, P3, and P4 are uncoated prisms. L1: Plano concave lens. Rotating disc: connected to UST and controlled by motor. The UST and the sample are immersed in water for ultrasound coupling. (f) Point source phantom made using pencil leads of 0.5 mm diameter. (g) Circle shaped phantom made using LDPE tubes (5 mm diameter) filled with black Indian ink. (h) Blood vessel phantom of 'N' shape made with LDPE tubes of 0.38 mm inner diameter filled with mice blood embedded inside chicken breast tissue. On top of this another layer of chicken tissue of 6 mm thickness was placed as shown in inset.

shaded region (in blue) of radius 40 mm as shown in Fig. 2.1(a). PA data were acquired at 400 sensor locations by placing an UST on the scanning circle. We have used a 2.25 MHz center frequency with 70% nominal bandwidth detectors. In all the simulations, sound speed was chosen as 1500 m/s. For simplicity, an ideal medium (acoustically homogeneous) was considered that exhibited no properties of absorption or dispersion of sound energy. SNR was maintained at 40 dB by adding 1% noise to the simulated data. The three numerical phantoms used were point targets, circular shape, and N-shaped blood vessel phantom [Figs. 2.1(b) – 2.1(d)]. Figure 2.1(b) shows the five point targets used, located at 0, 8, 16, 24, and 32 mm from the scanning center. The second numerical phantom [Fig. 2.1(c)] consists of two circles of 5 mm diameter located at 0 and 15 mm from the scanning center. The third numerical phantom [Fig. 2.1(d)] consists of an N-shaped blood vessel numerical phantom of 0.38 mm diameter. The simulated PA data were generated with a time step size of 40 ns and a total of 1250 time steps for point source numerical phantoms (1010 time steps for circular and blood vessel numerical phantoms). Once the forward PA data were generated using the k-wave, both traditional delay-and-sum and the modified delay-and-sum reconstruction methods were used for reconstructing the cross-sectional PAT images and further analysis were made. All reconstructions were done using a desktop with i7 Intel 64 bit processor (3.4 GHz) and 8 GB RAM running the Windows 10 operating system.

2.2.3 Experimental method

The schematic diagram of the experimental setup is shown in Fig. 2.1(e). A Q-switched Nd:YAG laser was used to deliver laser pulses of 10 Hz at 532 nm wavelength with a 5 ns laser pulse width. The laser energy density on the object was $\sim 3.18 \text{ mJ/cm}^2$, which

is much lower than the American National Standards Institute (ANSI) safety limit [119] of 20 mJ/cm^2 at 532 nm wavelength. A non-focused UST (Olympus NDT, V306-SU) and a cylindrically focused UST (Olympus NDT, V306-SU-NK, CF = 1.90 in.) with 13 mm diameter active area and 2.25 MHz central frequency with $\sim 70\%$ nominal bandwidth were used to acquire the PA signal. The UST acquires the data around the sample, a full 360 degree in circular configuration for a continuous data acquisition time of 480 s with a rotational speed of 0.75 deg/s. The acquired PA signals were regrouped into 800 A-lines [120]. The acquired PA signal was first amplified and filtered by a pulse amplifier (Olympus-NDT, 5072PR) and then recorded using a data acquisition (DAQ) card (GaGe, compuscope 4227) inside a desktop. All PA data were acquired with 25 MHz sampling rate. The data acquisition was synchronized with the laser illumination with a transistor-transistor-logic (TTL) sync signal from the laser.

The phantoms used for the experiments were as shown in Figs. 2.1(f) – 2.1(h). The first phantom was the point source phantom consisting of five pencil leads (0.5 mm) held using pipette adhered on an acrylic slab [Fig. 2.1(f)]. The leads were placed at a distance of 0, 8, 16, 24, and 32 mm from the scanning center. The second phantom was a circular-shaped phantom made using LDPE tubes of 5 mm inner diameter filled with black Indian ink [Fig. 2.1(g)]. The tubes were placed at 0 and 15 mm from the scanning center and attached to an acrylic slab at the bottom. The third phantom was a blood vessel phantom made using LDPE tubes of 0.38 mm inner diameter filled with mice blood embedded inside a layer of chicken breast tissue in the form of an N shape [Fig. 2.1(h)]. On top of this layer, another chicken breast tissue layer of 6 mm thickness was placed as shown in Fig. 2.1(h) inset. All samples were placed inside

the water bath for better ultrasound coupling. Once again, after the PA data acquisition, both traditional delay-and-sum and the modified delay-and-sum reconstruction methods were used for reconstructing the cross-sectional PAT images and further analysis was made. The tangential resolution improvement was shown quantitatively. The tangential resolution was calculated as the full width at half maximum (FWHM) of the point spread function along the tangential direction. The SNR was also calculated for all the targets from the reconstructed PAT images. The SNR is defined as the amplitude of the PA signal from the target divided by the standard deviation of the background noise $SNR(indB) = 20\log_{10}V/n$, where V is the average PA signal amplitude from the target, and n is the standard deviation of the background noise.

2.3 Results and Discussions

2.3.1 Simulation results

Figure 2.2 shows the reconstructed images of three numerical phantoms using conventional reconstruction and modified reconstruction algorithms. Figure 2.2(a) shows the conventionally reconstructed PAT images of the five point targets. Figures 2.2(c) – 2.2(g) show the zoomed in images of point targets 1 to 5, respectively. As can be clearly seen from these reconstructed PAT cross-sectional images, there is an elongation of the point target in the tangential direction. The further the point is from the scanning center (or the closer the point to the UST), the larger the elongation (or poorer the tangential resolution). Figure 2.2(b) shows the reconstructed PAT images using the modified delay-and-sum algorithm. It is evident that with modified reconstruction, the elongation along the tangential direction has been reduced. Figures 2.2(h) – 2.2(l) show

Figure 2.2: (a - l) Simulations results for point source phantoms. (a) Conventionally reconstructed PAT images of 5 point targets. (b) Reconstructed using modified delay-and-sum reconstruction algorithm. (c-g): Zoomed in point targets 1-5 in (a). (h-l): Zoomed in point targets 1-5 in (b). (m) Comparison of the tangential resolution and SNR between conventional and modified reconstruction algorithm as a function of distances from the scanning center. (n-s): Simulation results for circular shaped numerical phantom. (n) Conventionally reconstructed PAT images of two circles at different distances from scanning center. (o) Reconstructed modified reconstruction algorithm. (p,q) Zoomed in individual circles in (n). (r,s) Zoomed in individual circles in (o). (t) Conventionally reconstructed PAT image of 'N' shaped blood vessel numerical phantom. (u) Reconstructed using modified reconstruction. Red arrow points to the places where improvements can be visible clearly.

the zoomed in images of the point targets 1 to 5, respectively. From the zoomed in images, it is even clearer that with modified reconstruction, the tangential elongation is drastically improved. Figure 2.2(m) shows the tangential resolution and SNR at each point target position for Figs. 2.2(a) and 2.2(b). From this comparison plot, more than three times improvement in terms of tangential resolution and a relatively higher SNR is observed depending on the location of the point target in the scanning region. So, in addition to the improvement in tangential resolution, SNR was also improved with modified reconstruction algorithm.

Next, it was demonstrated that the modified delay-and-sum reconstruction algorithm preserves the shape of the target object. For this, a numerical phantom with two large

circles was used. Figure 2.2(n) shows the conventionally reconstructed PAT images of the two circles, one at the scanning center and the other at 1.5 cm from the scanning center. Figure 2.2(o) shows the PAT images reconstructed using modified delay-and-sum. As can be observed from the zoomed in images of each circle [Figs. 2.2(p) – 2.2(s)], the shape of the circular target object has been distorted in the tangential direction (the further the object from the scanning center or the nearer the object to the UST, the greater is the degradation). After using the modified reconstruction algorithm, it was noticed that the shape of the object was preserved as shown in Figs. 2.2(q) and 2.2(s). The distortion in the tangential direction for the circular object has been reduced. SNR improvement can also be observed here. For the circle at the scanning center using the conventional reconstruction algorithm, SNR was calculated to be 41.36 dB and using the modified reconstruction algorithm it was 45.58 dB. For the circle 1.5 cm away from the scanning center, SNR was calculated to be 34.68 and 35.32 dB using traditional and modified reconstruction techniques, respectively. Comparatively, the SNR was better apart from preserving the target shape with the modified reconstruction algorithm.

Finally, a numerical phantom in the shape of an N was simulated and reconstructed using the conventional and modified reconstruction algorithms as shown in Figs. 2.2(t) and 2.2(u). This phantom was used to mimic the blood vessel. It can be observed that the shape has been retained in the modified reconstruction technique (red arrows). In this numerical phantom, also, there was no SNR degradation (28.49 dB using traditional and 28.68 dB using modified reconstruction algorithms). Therefore, all the simulation results were demonstrated that the modified delay-and-sum reconstruction algorithm can help to improve the tangential resolution without using any external lens or other

techniques proposed earlier, without any compromise in the SNR values. In section 2.3.2, it was demonstrated that this algorithm can be applied in real-experimental data as well.

2.3.2 Experimental results

Figure 2.3: Experimental results using unfocused UST of 2.25 MHz for point source phantoms. (a) Conventionally reconstructed PAT images of 5 point targets (b) Reconstructed using modified delay-and-sum reconstruction algorithm. (c-g): Zoomed in point targets 1-5 in (a). (h-l): Zoomed in point targets 1-5 in (b). (m) Comparison of tangential resolution and SNR between conventional and modified reconstruction algorithm as a function of distances from the scanning center for unfocused UST. (n-y): Experimental results using cylindrically focused UST of 2.25 MHz for point source phantom. (n) Conventionally reconstructed PAT images. (o) Reconstructed using modified reconstruction algorithm. (p-t) Zoomed in point targets 1-5 in (n). (u-y) Zoomed in point targets 1-5 in (o). (z) Comparison of tangential resolution and SNR between conventional and modified reconstruction algorithm as a function of distances from the scanning center for cylindrically focused UST. Red arrows point to the places where improvements can be visible clearly.

As described earlier, three different types of phantoms were used for the experimental work as well. PAT data were collected for all the objects with two different USTs, both flat as well as cylindrically focused USTs. The PAT images of the sample were reconstructed using both conventional reconstruction algorithm and modified delay-and-sum reconstruction algorithm. Figure 2.3(a) shows the conventionally reconstructed

PAT images of the point targets (pencil leads located at 0, 8, 16, 24, and 32 mm from the scanning center) obtained using an unfocused UST. As can be seen in the zoomed images [Figs. 2.3(c) - 2.3(g)], the target object elongation increases in the tangential direction when it is nearer to the detector surface or away from the scanning center. But with the modified reconstruction algorithm, improvement was evident from the zoomed images [Figs. 2.3(h) – 2.3(l)]. There was a more than threefold improvement in the tangential resolution. The quantified tangential resolution and SNR values versus distances from the scanning center are shown in Fig. 2.3(m). It can be clearly seen that the modified reconstruction technique improves the tangential resolution without any compromise in the SNR values compared to the conventional reconstruction technique. Experiments were repeated for the same pencil leads configuration using a cylindrically focused UST. A similar trend in tangential resolution can be observed as shown in Figs. 2.3(n) – 2.3(z). For the cylindrically focused UST, we observed more than threefold improvement in terms of tangential resolution and higher SNR. It is clearly shown that using this modified reconstruction algorithm we obtained a better SNR in addition to the improvement in tangential resolution.

Next, it was shown that with this modified reconstruction algorithm, the shape of the target object can also be retained. LDPE tubes (5 mm inner diameter) were filled with black Indian ink, one placed near the scanning center and the other at ~ 1.5 cm from the scanning center. Figures 2.4(a) and 2.4(b) show the reconstructed PAT images using conventional and modified reconstruction algorithms for the data collected using unfocused UST. In Fig. 2.4(d), there is a shape distortion for the circular object which is reconstructed conventionally. From Fig. 2.4(f), the shape preservation can be observed

Figure 2.4: Experimental results using unfocused UST of 2.25 MHz for large circular phantom (LDPE tube of 5 mm inner diameter filled with India black ink). (a) Conventionally reconstructed PAT images. (b) Using modified reconstruction algorithm. (c,d): Zoomed in individual circles in (a). (e,f): Zoomed in individual circles in (b). (g-l): Experimental results using cylindrically focused UST of 2.25 MHz for the same phantom. (g) Conventionally reconstructed PAT images. (h) Reconstructed using modified reconstruction algorithm. (i,j) Zoomed in individual circles in (g). (k,l) Zoomed in individual circles in (h). Red arrows point to the places where improvements can be visible clearly.

when the modified reconstruction algorithm was used. Similarly, for the data collected using cylindrically focused UST, shape of the target object was preserved [Figs. 2.4(g) – 2.4(l)]. In addition to preserving the shape of the target, we were able to maintain the SNR levels. In the case of unfocused UST, SNR was 33.42 and 34 dB using conventional and modified reconstruction algorithms, respectively, for the tube at the center. For the tube at 15 mm away from the center, the SNR was 37.41 and 32.76 dB using conventional and modified reconstruction techniques, respectively. Similarly, in the

case of cylindrically focused UST, for the tube at the scanning center, SNR was 29.58 and 30.8 dB using traditional and modified delay-and-sum reconstruction, respectively. For the tube at 1.5 cm away from the center, SNR was 41.54 and 35.17 dB, respectively, using conventional and modified reconstruction algorithms.

Figure 2.5: Experimental results for ‘N’ shaped blood vessel network (LDPE tube of 0.38 mm inner diameter filled with mice blood placed within chicken tissue) using unfocused UST. (a) Reconstructed PAT images with conventional reconstruction algorithm. (b) Using modified reconstruction algorithm. (c,d) Experimental results for the same phantom using cylindrically focused UST. (c) Using conventional reconstruction algorithm. (d) Using modified reconstruction algorithm. Red arrows point to the places where improvements can be visible clearly.

Next, a study was conducted to demonstrate that this modified reconstruction algorithm can be used for more realistic phantom imaging with tissue samples. For this study, LDPE tubes of 0.38 mm inner diameter filled with mice blood were embedded inside a chicken breast tissue that was cut into a circular shape [Fig. 2.1(h)]. The tubes were embedded inside the tissue to form a blood vessel network in an N shape of 2 cm

x 2 cm dimensions. On top of this layer of tissue, a layer of 6 mm thick chicken breast was placed. Figures 2.5(a) and 2.5(b) show the PAT images reconstructed using conventional and modified reconstruction techniques, respectively, for the data collected using unfocused UST. Figures 2.5(c) and 2.5(d) show the corresponding PAT images for the cylindrically focused UST. From Figs. 2.5(a) and 2.5(c), we can notice that there is a distortion (curved and blurred lines) in the N shape as indicated by the red arrows. Improvement of shape can be seen in the reconstructed images in Figs. 2.5(b) and 2.5(d). There is no curving of the lines in the N shape and also we can see the entire shape of the tubes (corresponding red arrows). The distortion in the shape of the blood vessel phantom was reduced in the modified reconstructed PAT images and the SNR was also maintained. From the reconstructed cross-sectional PA images of the blood vessel network in tissue phantom, SNR was calculated to be 22.96, 22.54 dB in the case of unfocused UST and 21.62, 21.79 dB in the case of cylindrically focused UST, using standard and modified delay-and-sum reconstruction algorithms, respectively.

Hence, this modified delay-and-sum algorithm helped in improving the tangential resolution of the object nearer to the detector surface (further from the scanning center). There was no requirement for attaching any negative acoustic lens to the detector surface or use of any virtual point detectors. Moreover, there was no loss of signal due to the presence of the lens. For the 800 A-lines data, the reconstruction time using the modified delay-and-sum reconstruction algorithm was around ~ 43 min using the desktop configuration mentioned earlier. Reconstruction time can be reduced to ~ 21 min if 400 A-lines data are used. The reconstruction time can further be reduced if a larger grid size is used during reconstruction. For example, with 400 A-lines data and 0.2

mm/pixel grid size (instead of 0.1 mm/pixel used in all our work), the reconstruction time is only ~ 2.5 min. In case of the traditional reconstruction method, the reconstructions were very fast [~ 23 s for 800 A-lines data, and ~ 12 s for 400 A-lines data for 0.1 mm/pixel grid size]. Due to the nature of the modified algorithm, where at each UST location, the data were backprojected from many tiny section of the detector surface, the reconstruction time was longer than the standard delay-and-sum algorithm. However, this could be improved even further and made real time if reconstruction is done in C/C++ instead of MATLAB, as inherently MATLAB is not optimum for running loops. Another way to improve the reconstruction time is the use of graphics processor units (GPU).

2.4 Conclusion

By using a modified delay-and-sum reconstruction algorithm, the tangential resolution of PAT was improved more than threefold for both flat as well as cylindrically focused USTs. We have shown both simulation results and experimental validation that the modified reconstruction was effective. Three types of phantoms (both numerical and experimental) were used, namely point target, large circular object, and an N-shaped blood vessel phantom. In all three different types of phantoms, we observed improvements in terms of tangential resolution and SNR was maintained. Therefore, without using any external negative acoustic lens on the surface of the transducer, we can improve the tangential resolution without compromising the high SNR values.

Chapter 3

Compact PAT scanner using acoustic reflector²

3.1 Introduction

In a PAT, various types of transducers, such as single- element UST [121], linear arrays [122, 123], semicircular array [124], and circular array transducers [125] can be used to acquire the PA signals from the target object. In conventional PAT scanner, as only one SUT is used to rotate around the sample, the imaging speed is slow (several minutes) compared to array transducers (few seconds to few milliseconds depending on the array configuration) in which multiple detectors are used. However, array transducers are very expensive and require multichannel parallel amplification and data acquisition systems, whereas the single-element USTs are cheap and require single amplifier and single channel data acquisition systems. Recently, single-element transducers were used for high-speed PAT imaging using pulsed laser diode (PLD) based PAT [120, 126] and slip-ring based PAT [108] systems. However, the single-element transducers are inconvenient to use as they need a larger radius to scan around the sample and, hence, a larger water tanks.

²Part of the work presented in this chapter has been published in *Journal of Biomedical Optics*. **S. K. Kalva**, and M. Pramanik, "Use of acoustic reflector to make a compact photoacoustic tomography system," *Journal of Biomedical Optics* 22(2), 026009 (2017).

For deep tissue imaging, the PA signal is collected by positioning the SUT in orthogonal mode (perpendicular to the laser irradiation and facing toward the center in a circular scanning geometry) [127]. The sample and the SUT are placed in a water bath for better acoustic coupling. Due to the length of the transducer body and part of the connecting cable [Fig. 3.1(a)], the SUT needs more space to rotate around the sample. To house this type of SUT and part of connecting cable, a large water tank is required, which, in turn, occupies a large space. Also, there is an increased load on the motor as the scanning radius of the SUT is large. One way to reduce the scanning radius is to use a custom-made SUT of shorter length that occupies less space. Typically, such custom-made detectors increase the cost of the overall system and may not be easily available. To overcome such limitations, instead of positioning the UST in the horizontal direction, we proposed positioning it in the vertical direction. The generated PA waves from the sample were directed toward the detector using an acoustic reflector. The surface of the acoustic reflector was made of stainless steel. This acoustic reflector was attached to the transducer body with the reflector surface at 45 degree to the detector surface [Fig. 3.1(a)]. Thus, this configuration brought down the water tank size significantly and reduced the scanning radius. Since the scanning radius was reduced, the load on the stepper motor also got reduced. Hence, the overall PAT system size was reduced, which helped in designing a more compact and portable PAT system.

In this chapter, it had been shown that nearly similar images were obtained using an ultrasound transducer with reflector (USTR/SUTR) compared to that of a conventional UST/SUT. Three different types of phantom imaging were used for demonstration: (a)

point sources (pencil leads of 0.5 mm diameter), (b) horse hair (150 μm thickness) phantom arranged in a triangular shape, and (c) “N” shaped LDPE tubes of 0.38 mm diameter filled with rat blood mimicking the blood vessels embedded inside chicken breast tissue and *in vivo* rat brain imaging. A simple delay-and-sum reconstruction algorithm has been used to reconstruct the PA images [117, 121].

3.2 Materials and Methods

3.2.1 Photoacoustic tomography imaging system

Figure 3.1: (a) Images of conventional ultrasound transducer (UST), UST with acoustic reflector (USTR), acoustic reflector. (b) Schematic diagram of the PAT imaging system used in this work. R/A/F: Receiver, amplifier and filter for photoacoustic signal. DAQ: Data Acquisition Card, P1, P2 and P3 are coated prisms, L1: Plano concave lens, Rotating disc (connected to UST and controlled by a stepper motor), AM: Anaesthesia Machine, WT: Water Tank. The UST and sample are immersed in water bath for better acoustic coupling. (c) Point source phantom made using 0.5 mm diameter pencil leads (one at the centre and 4 other placed at ~ 1 cm from the centre). (d) Triangular shaped horse hair phantom (150 μm thickness). (e) ‘N’ shaped blood vessel phantom made using low density polyethylene (LDPE) tubes of 0.38 mm diameter embedded inside chicken breast tissue layer. On top of this layer, a 5 mm thick chicken breast tissue was placed [inset in Fig. 3.1(e)].

The PAT imaging system used is shown in Fig. 3.1(b). Here, a Q-switched Nd:YAG laser (Continuum, Surelite Ex) delivers laser pulses of 10 Hz at 532 nm wavelength (5 ns pulse width). Three right-angle uncoated prisms (PS911, Thorlabs) (not shown in

the schematic) and one uncoated planoconcave lens L1 (LC1715, Thorlabs) were used to deliver the laser pulses from the Nd:YAG laser to the sample. The laser energy density was maintained at $\sim 9 \text{ mJ/cm}^2$ on the sample surface, which is much lower than the ANSI safety limit [119] of 20 mJ/cm^2 at 400 to 700 nm. For point source and hair phantoms, a 532 nm wavelength laser was used. In biological tissue, the light penetration is greater in the NIR wavelength region, so an optical parametric oscillator (OPO) laser (Continuum, Surelite) pumped by the Q-switched (532 nm) laser was used as the light excitation source for “N” shaped blood vessel mimicking phantom and *in vivo* rat brain imaging. The OPO laser was set to deliver laser pulses of 680 nm wavelength to the sample. Three right-angle coated prisms P1, P2, and P3 (PS908L-B, Thorlabs) and one uncoated planoconcave lens L1 [LC1715, Thorlabs; Fig. 3.1(b)] were used to deliver the laser pulses from OPO to the sample. The laser energy density was maintained at $\sim 6 \text{ mJ/cm}^2$ on the sample surface. A flat UST (Olympus NDT, V306-SU), a flat USTR by attaching an acoustic reflector (F102, Olympus NDT) with the flat UST, a focused UST (Olympus NDT, V306-SU-NK, CF = 1.90 in.), and a focused USTR by attaching the same acoustic reflector to the focused UST were used to acquire the PA signal. The UST had a 2.25 MHz central frequency with $\sim 70\%$ nominal bandwidth and 13 mm diameter detector surface area. Figure 8(a) shows the flat UST, the acoustic reflector, and the acoustic reflector attached with the flat UST. Each UST acquires the PA data by moving around the sample in full 360 degree in circular geometry. In all the experiments, the PA signals were collected continuously for 480 s with UST at a rotational speed of 0.75 degree/s. The PA signal acquisition and saving was performed similar to description in section 2.2.3 in chapter 2.

3.2.2 Phantom experiments

Figures 3.1(c) – 3.1(e) show the phantoms used for the experiments. The first phantom was a point source object made using five pencil leads of 0.5 mm diameter held using a pipette and adhered to an acrylic slab [Fig. 3.1(c)]. One pencil lead was placed at the scanning center, and four other pencil leads were placed at a distance of ~ 1 cm from the scanning center. Figure 3.1(d) shows the second phantom made of horse hair of 150 μm thickness in triangular shape glued to the tips of pipettes and adhered to an acrylic slab. Each side of the triangle is ~ 1 cm. The third phantom was the blood vessel phantom made by LDPE tubes of 0.38 mm diameter filled with rat blood. These tubes were embedded inside a layer of chicken breast tissue in the shape of “N” [Fig. 3.1(e)]. Another chicken breast tissue layer of 5 mm thickness was placed on top [Fig. 3.1(e) inset]. All these phantoms were placed inside a water bath for better acoustic coupling.

3.2.3 *In vivo* experiments

In vivo animal experiments were conducted using healthy female rats weighing ~ 120 g. The rats were procured from InVivos Pte. Ltd., Singapore. Animal experiments were performed according to the approved guidelines and regulations by the institutional Animal Care and Use committee of Nanyang Technological University, Singapore (Animal Protocol Number ARF-SBS/NIE-A0263). The animals were anesthetized using a cocktail of Ketamine (85 mg/kg) and Xylazine (15 mg/kg) injected intraperitoneally (dosage of 0.2 ml/100 g). Before the animals were placed in the PAT scanner, the hair on the head was trimmed and then epilated using hair removal cream. A breathing mask covering the mouth and nose of the animal was used to deliver an anesthesia mixture

of 0.75% isoflurane and O_2 (1.2 L/min) during PAT imaging. The animal was placed in a sitting position on its abdomen using a custom-made animal holder. Surgical tapes were used to hold the animal in its position. The animal along with the animal holder was mounted on a translational stage during the experiment. The translational stage was used to adjust the height of the animal to adjust the scan plane of the brain. After the data acquisition, animals were euthanized by intraperitoneal injection of Valabarb (sodium pentobarbitone 300 mg/ml).

3.2.4 Comparison of reconstructed images

To study the quality of the images obtained using an acoustic reflector, SNR was calculated for both flat and focused transducers and compared with SNR levels of the images obtained using unfocused and focused transducers without an acoustic reflector. The SNR was calculated as described in chapter 2, section 2.2.3. For point source phantom, PA amplitude was calculated for the point 3 in Fig. 3.1(c), and, in all the other cases, PA amplitude was calculated as the average of the PA amplitude of the whole target object. The noise was taken from the background where there was no object located.

3.3 Results and Discussions

3.3.1 Phantom experimental results

Figure 3.2 shows the reconstructed PA images of point source phantom. Figure 3.2(a) shows the reconstructed PAT images of the point targets using a flat SUT, and Fig.

Figure 3.2: Cross-sectional reconstructed PAT images of the point source phantom. (a) Reconstructed PAT image obtained using flat SUT, (b) Reconstructed PAT image obtained through flat SUTR, (c) Reconstructed PAT image obtained using focused SUT, (d) Reconstructed PAT image obtained through focused SUTR. Corresponding colour bars are shown for images of flat and focused transducers. Scale bar is shown in (a) for all the images.

3.2(b) shows the reconstructed PA images using a flat SUTR. Figures 3.2(c) and 3.2(d) show the reconstructed PA images obtained using a cylindrically focused SUT and a cylindrically focused SUTR, respectively. To study the quality of the images, the SNR was calculated (for point target 3). The SNR value with the flat SUT was 54.9 dB and with the flat SUTR was 50.56 dB. There was an $\sim 8\%$ reduction in the SNR with the flat SUTR. Corresponding SNR values for the focused SUT and the focused SUTR were calculated to be 48.18 and 47.76 dB, respectively. There was an $\sim 0.8\%$ degradation in the SNR with the focused SUTR.

Figure 3.3: Cross-sectional reconstructed PAT images of triangular shaped hair phantom. (a) Reconstructed PAT image for flat SUT, (b) Reconstructed PAT image for flat SUTR, (c, d) Reconstructed PAT images for focused SUT and focused SUTR, respectively. Corresponding colour bars are shown for flat and focused transducers. Scale bar is shown in (a) for all the images.

Next, a study was conducted using a horse hair triangular phantom. The hair was $150\ \mu\text{m}$ thick. Each side was $\sim 1\ \text{cm}$ in length. Figure 3.3(a) shows the reconstructed PAT image of the hair phantom using a flat SUT, and Fig. 3.3(b) shows the reconstructed PA image using a flat SUTR. Corresponding SNR values for these images were calculated to be 47.44 and 47.56 dB, respectively. SNR levels were maintained for flat SUTR as well as those of flat SUT. Figures 3.3(c) and 3.3(d) show the reconstructed PA images using focused transducers without and with an acoustic reflector, respectively. For these images, SNR values were computed to be 48.8 and 45.85 dB, respectively. The SNR was $\sim 6\%$ lower using a cylindrically focused transducer with an acoustic reflector

compared to its counterpart.

Figure 3.4: Cross-sectional reconstructed PAT images of ‘N’ shaped blood vessel phantom embedded inside chicken breast tissue. (a) Reconstructed PAT image for flat SUT, (b) Reconstructed PAT image obtained using flat SUTR, (c) Cross-sectional reconstructed PAT image for focused SUT, (d) Cross-sectional reconstructed PAT image obtained using focused SUTR. Corresponding colour bars are shown for the images of flat and cylindrically focused transducers. Scale bar is shown in (a) for all the images.

Next, an “N” shaped blood vessel phantom (LDPE tubes of 0.38 mm diameter) of $\sim 1 \text{ cm} \times 1 \text{ cm}$ dimensions embedded inside a chicken breast tissue layer was studied. A layer of 5 mm thick chicken breast tissue was placed on top of this layer. The reconstructed PAT images for unfocused SUT and unfocused SUTR are shown in Figs. 3.4(a) and 3.4(b), respectively. The corresponding SNR levels for these images were computed to be 24.61 and 24.32 dB, respectively. Figures 3.4(c) and 3.4(d) show cross-sectional reconstructed PA images obtained through a cylindrically focused transducer

without and with acoustic reflector, respectively. The SNR values were calculated to be 30.56 and 31.01 dB, respectively. The SNR was $\sim 1.5\%$ higher using a focused SUTR than a focused UT.

3.3.2 *In vivo* experimental results

Figure 3.5: Reconstructed cross-sectional PAT images of rat brain. (a) Reconstructed PAT image for flat SUT, (b) Reconstructed cross-sectional PAT image for flat SUTR, (c) Cross-sectional reconstructed PAT image obtained using focused SUT, (d) Reconstructed cross-sectional PAT images obtained using focused SUTR. SS: Sagittal Sinus, TS: Transverse Sinus. Yellow arrows point to the SS, TS. Corresponding color bars are shown for unfocused and cylindrically focused transducers. Scale bar is shown in (a) for all the images. (e) Rat brain after trimming and removal of hair on the head region before PAT imaging. (f) Rat brain after cutting open the top skin layer of the head region after the PAT imaging is completed.

Next, a study was conducted on small animals to demonstrate the feasibility of using

an SUTR to obtain similar quality images to that of a conventional SUT. As described earlier, anesthetized healthy female rats were used for this study. Figure 3.5 shows the cross-sectional reconstructed PAT images of the animal brain. The images obtained for flat SUT without and with acoustic reflector are shown in Figs. 3.5(a) and 3.5(b), respectively. Figure 3.5(c) shows the cross-sectional reconstructed PA image of the focused SUT, and Fig. 3.5(d) shows the cross-sectional reconstructed PA image of the focused SUTR. In all four images, sagittal sinus (SS) and transverse sinuses (TS) were clearly visible (yellow arrows). Figure 3.5(e) shows the image of the animal head before taking PAT images, and Fig. 3.5(f) shows the image of the animal brain after cutting open the top layer of skin on the head after the image acquisition was completed. To study the quality of the images, the SNR was calculated. The SNR was 22.76 dB (flat SUT) and 18.66 dB (flat SUTR). There was an $\sim 18\%$ reduction in the SNR levels with the usage of an acoustic reflector. The SNR for the images obtained with the focused SUT was 26.61 dB and that for the focused SUTR was 31.63 dB. There was a $\sim 19\%$ enhancement in the SNR levels using the focused transducer with an acoustic reflector compared to that of the images from the focused transducer without an acoustic reflector.

The ultrasound waves generated from the sample fall at 45 degree to the water and acoustic reflector (stainless steel) interface. At this incidence angle, due to the total internal reflection phenomenon, the ultrasound waves are entirely reflected back into the water medium. These reflected waves are detected by the SUT. As discussed above, the SNR was maintained in many cases, but there was a $\sim (1 \text{ to } 19)\%$ enhancement or degradation in the SNR levels for a few cases. This was due to the small difference

$\sim(2 \text{ to } 3)$ mm in the distance at which the SUT and SUTR were placed from the scanning center. The amplitude of the ultrasound signal detected by the SUT is inversely proportional to the square of the distance from the source (target object) to the detector surface, so the small difference in the distances results in SNR variations. If the scanning radii were kept exactly the same for the SUT and SUTR, then SNR levels in these cases would also have been similar. Visually, the quality of the PAT images obtained using an acoustic reflector augmented to the conventional SUTs was nearly similar to that of the PAT images obtained using conventional transducers of both flat and focused SUT.

For better understanding of the quality of the PAT images using an acoustic reflector, the resolution of the PAT system was compared for all four transducers. The resolution was calculated using full-width at half-maximum from the point spread function of the hair phantom images. For flat SUT and flat SUTR, the resolution was 301 and 281 μm , respectively. For focused SUT and focused SUTR, it was computed to be 294 and 269 μm , respectively. This resolution matches well with the theoretically expected resolution of 250 μm for a 2.25 MHz SUT, so the use of acoustic reflector did not affect the spatial resolution of the PAT system. The frequency response of the transducers with and without the reflector was also compared. As expected, we did not see any degradation of the transducer bandwidth with or without the reflector. The frequency response for all four transducers was calculated by taking Fourier transforms of the A-line PA signal (averaged six times) from the hair phantom. The bandwidth was calculated from the frequency response. The bandwidth for flat SUT, flat SUTR, focused SUT, and focused SUTR was found to be 2.29, 2.24, 2.46, and 2.44 MHz, respectively.

Hence, by using the acoustic reflector augmented to the conventional SUT surface at 45° , we used the transducer in the vertical plane instead of the horizontal plane. Therefore, the transducer rotated in a smaller circle around the sample. This vertical configuration lead to a size reduction of the water tank used for housing the transducer as well as the target object (the length and width of the tank will be reduced by ~ 11.5 cm: this included the length of the SUT and partial length of the connecting cable). This reduced the volume of the PAT system. Also, the load on the motor that rotated the SUT decreased as the torque required to rotate the SUTR decreases. Hence, the overall PAT system size was minimized. Together with a PLD-based PAT system, this configuration of SUTR will make the entire PAT system quite compact and hopefully translation to clinical applications will be easier. As the use of the acoustic reflector did not affect the SNR levels or the spatial resolution (and the bandwidth of the detectors) of the PAT system, this acoustic reflector can be used with any PAT system in which single-element USTs with circular scanning geometry are used. This will reduce the scanning radius and will make the PAT imaging system more compact in size. The SUTR-based PAT system can find applications in small animal brain imaging, breast cancer imaging, etc.

3.4 Conclusion

By attaching an acoustic reflector made of stainless steel to a conventional single-element UST, we obtained nearly similar quality PAT images as that of the conventional single-element UST for both flat and cylindrically focused UST. Phantoms as well as *in vivo* results confirmed that this vertical configuration of the UST was helpful for making a compact PAT scanner.

Chapter 4

Calibrating scanning radius in a multi-SUT based PAT system³

4.1 Introduction

A single-element ultrasound transducer rotates in a full 360° around the sample with a scan time ranging from several minutes to a few seconds to acquire the data, depending on the repetition rate of the pulsed laser (10–100 Hz in the case of a Q-switched Nd:YAG laser [128] and 7 KHz in the case of a state-of-the-art PLD laser [129–131]). This inhibits real-time monitoring of biological processes occurring in a fraction of a second. To circumvent this limitation, the state-of-the-art PACT system uses a circular ring array transducer offering 50 Hz frame rate (0.02 s per frame) [85]. However, these full-ring array transducers are custom made and need complex back-end parallel signal amplifiers and digitizer electronics, making them complex and expensive. Hence, SUTs are still preferred due to easy availability at a very low price. To achieve the frame rate as that of the ring array transducer PACT system, multiple SUTs can be employed. The schematic of the multi-SUT based PAT system is shown in Fig. 4.2(a). The

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configuration of these SUTs is different from that of the full-ring circular array-based PAT system (Fig. 1 in Ref. [76]). In the full-ring circular-array-based PACT system, the scanning radius of the transducer is fixed, and it need not rotate around the sample. In the multi-SUT based PAT system, the transducers can be used at any desired radius, depending on the sample size, and each transducer needs to rotate around the sample, partially depending on the number of SUTs used. Integrating a high-frame-rate PLD laser with cheap, multiple SUTs for PA data acquisition will find applications in monitoring neurofunctional activities such as epilepsy, and in characterization of pharmacokinetic, biodistribution profiles in the development process of drugs or contrast agents in small animal study. Fast imaging applications of this system would also include reducing motion artifacts by respiration or heartbeat in human as well as small animal studies.

In the multi-SUT PAT system, if N numbers of SUTs are used, then each transducer needs to rotate only $360/N$ degrees around the sample. This improves the data acquisition time N -fold. One major limitation in using these several SUTs is that experimentally it is very difficult to place all transducers exactly at the same distance from the scanning center. Each SUT rotates in concentric circles with a slight difference in scanning radius, resulting in an experimental error of approximately 1–3 mm. In the full-ring circular array transducer, this problem does not exist, as all the transducer array elements are exactly at the same radius from the scanning center. Usually a simple delay-and-sum method implementing a back-projection algorithm is used for reconstructing cross-sectional PA images in the PACT system. In this method, we need to input the scanning radius for reconstructing the PA image, which is conventionally cali-

brated by trial-and-error method. For circular ring array transducer and SUT PACT systems, only one reconstruction radius is needed, whereas, in a multi-SUT PAT system, an individual reconstruction radius for each SUT is needed because if a single reconstruction radius is used for the combined PA data from all SUTs, the reconstructed PA image is distorted. This distortion increases as the number of transducers used increases. The target image can be perfectly reconstructed by using the corresponding radius of each SUT. The conventional trial-and-error method to find out these scanning radii is inefficient and time consuming, as it involves human training to assess the quality of the reconstructed images. This difficulty increases further in the case of a multi-SUT PAT system compared to full-ring array transducer and single-SUT PAT systems. To overcome these limitations, there is a need to automate the process of finding the scanning radius for each SUT in a PACT system with circular scanning geometry.

In this chapter, a simple calibration technique has been proposed to automatically find the scanning radius for each SUT. The importance of using different scanning radii for different transducers in a multi-SUT PACT system also has been demonstrated. Three different numerical phantoms (point source, Derenzo, and blood vessel network) and an experimental point target phantom were used to show the efficacy of the proposed method. The Pearson correlation (PC) coefficient (PCC) metric was used to compare the quality of the reconstructed images for numerical phantoms, and SNR was used for experimental phantoms.

Figure 4.1: (a) Schematic diagram of k-wave simulation geometry in MATLAB. 900 x 900 pixels (0.1 mm/pixel) were used in all simulations. SUT: single-element ultrasound transducer. (b) Quantifying the scanning radius using a point source “P” placed at (550,550) pixel location in the simulation geometry. r_1 is nearest distance between the point source “P” and SUT. r_2 is farthest distance between the point source ‘P’ and SUT. (c) Point source numerical phantom. Five points located one at the scanning center and remaining four at 1 cm away from the center. (d) Derenzo numerical phantom. (e) Blood vessel network numerical phantom.

4.2 Methods

4.2.1 Numerical Simulations

All numerical simulations were done using the k-wave toolbox [118] in MATLAB. The simulation geometry used is shown in Fig. 4.1(a). The computational grid consists of 900 x 900 pixels (0.1 mm/pixel) with a perfectly matched boundary layer. Table 1 shows the different parameters used for the transducers SUT1 to SUT8 to generate the PA data. We have used 2.25 MHz center frequency transducers with 13 mm diameter. To simulate a real-time scenario where the transducers in a multi-SUT PACT system rotate in concentric circles around the sample with a slight difference in the radius, we have simulated the PA data with different scanning radii, as well as different bandwidths,

SNR levels, and sensitivity for each transducer, as shown in Table 4.1. The numerical phantoms used were a point source phantom consisting of one point target at the scanning center and the remaining four at 1 cm from the scanning center [Fig. 4.1(c)], Derenzo phantom [Fig. 4.1(d)], and blood vessel network phantom [Fig. 4.1(e)]. In the case of the 2-SUT PACT system, SUT1 and SUT2 (each 200 detecting locations) were used for generating simulated PA data; similarly for the 4-SUT PACT system, SUT1, SUT2, SUT3, and SUT4 (each 100 detecting locations) were used, and for the 8-SUT PACT system, SUT1 to SUT8 (each 50 detecting locations) were used. The simulated PA data were generated with a time step size of 40 ns and a total of 1100 time steps for the point source phantom and 1350 time steps for Derenzo and blood vessel network phantoms. The reconstructed images in all these scenarios were compared with that of single-SUT acquiring the PA data at 400 locations around the sample in 360°. A modified delay-and-sum algorithm was used for all the reconstructed PA images [121].

4.2.2 Calibration Method

In a circular scanning geometry, a single transducer collects a time-resolved PA signal known as A-line at each sensor location (here 400 sensor locations) around the sample in full 360 degree. For an arbitrary point source “P” in the imaging region as shown in Fig. 4.1(b), the A-line acquired at each sensor location has a peak amplitude observed at a particular time step. From these time steps where peak amplitude is observed for each sensor location, the distance between the point source “P” and the transducer can be calculated as:

$$distance(mm) = timestep * (c/f_s) \quad (4.1)$$

where c is the speed of sound in water (1.5 mm/ μ s), and f_s is the sampling frequency (25 MHz). At one of the transducer locations, the point source will be nearest to the transducer (say r_1) and at another location diametrically opposite, the point source will be farthest from the transducer (say r_2) [Fig. 4.1(b)]. Hence, reconstruction radius (r) for a SUT can be calculated as:

$$r = \frac{(r_1 + r_2)}{2} \quad (4.2)$$

Table 4.1: Transducer Specifications

Transducer #	Radius (mm)	Bandwidth (%)	SNR (dB)	Sensitivity (%)
SUT1	40	70	40	100
SUT2	41	68	31	97
SUT3	37	72	25	95
SUT4	43	74	37	94
SUT5	39	66	34	93
SUT6	42	71	26	96
SUT7	38	69	28	92
SUT8	40	73	32	91

We have written a code in MATLAB to implement this method. To quantify the scanning radius for the transducers SUT1–SUT8, we considered a point source “P” at (550,550) pixel location in the simulation grid. All transducers collect the A-lines around the sample in full 360° . For each transducer, the reconstruction radius is calibrated by using this proposed technique and found to be 40.02 mm, 41.04 mm, 37.11 mm, 43.02 mm, 39 mm, 42.12 mm, 38.13 mm, and 40.02 mm for SUT1 to SUT8, respectively. These calibrated radii were perfectly matching (negligible error of 0.05% – 0.34%) with the ideal radius of each SUT, as shown in Table 4.1. This radius calibration for each SUT in the multi-SUT-based PAT/PACT system needs to be done only once. Later, for any kind of phantom, the initial pressure rise distribution can be reconstructed from the acquired PA data with the same calibrated radius of each SUT.

4.2.3 Experimental Method

Figure 4.2: (a) Schematic diagram of multi-SUT PACT system. SM, stepper motor; SMP, stepper motor pulley unit; CRP, circular rotating plate; P1, P2, P3, uncoated right angle prisms; L1, plano-concave lens; SUT1-SUT4, single-element ultrasound transducers; AMP, amplifier; DAQ, data acquisition card. (b) Single point target for calibrating scanning radius. (c) Five-point target phantom.

Figure 4.2(a) shows the experimental schematic diagram. The same Nd:YAG laser and the external optics (prisms/lens) were used as previously described in chapter 2, section 2.2.3. Four SUTs [SUT1–SUT4 as shown in Fig. 4.2(a)] of same specifications as mentioned in chapter 2, section 2.2.3 were used for PA data acquisition. Initially, after mounting the transducers, the scanning radius for each transducer was found using our proposed calibration method with the help of a pencil lead (0.5 mm diameter) acting as the point source [Fig. 4.2(b)]. The pencil lead was placed at an arbitrary position in the scanning region. The PA signals were acquired in a full circle around the point target at a rotational speed of 0.75 degree/s for 480 s. The acquired A-lines were aver-

aged into 100 A-lines. The scanning radius for each transducer was found from these A-lines using the proposed algorithm. Respective reconstruction radii for SUT1–SUT4 were calibrated to be 71.79 mm, 72.99 mm, 71.16 mm, and 69.12 mm. To validate our proposed method experimentally, we have used the same five-point target phantom [Fig. 3.1(c), also shown in Fig. 4.2(c)] as described in chapter 3, section 3.2.1. The quality of the reconstructed images obtained using multi-SUT PACT systems for $N = 2$ (each SUT collects 200 A-lines) and $N = 4$ (each SUT collects 100 A-lines) was compared with that of single-SUT PACT system (400 A-lines) using SNR metric. The SUTs were made to rotate at a rotation speed of 1.5 degree/s in all these scenarios ($N = 1, 2$, and 4). The acquired PA signals were recorded inside a Windows operating desktop (Intel Xeon, 3.7 GHz 64-bit processor, 16 GB RAM) using a DAQ card (Spectrum, M2i.4932-exp) after amplifying using low noise amplifiers by 48 dB (Mini-circuits, ZFL-500LN-BNC). All the PA data were acquired at 25 MHz sampling rate. A TTL sync signal from the laser was used to synchronize the DAQ.

4.2.4 Comparison of Reconstructed Images

The degree of correlation between the target phantom and the reconstructed image was evaluated using the PC coefficient defined as:

$$PC = \frac{cov(t_i, t_r)}{\sigma(t_i)\sigma(t_r)} \quad (4.3)$$

where t_i is target initial pressure rise distribution, t_r is target reconstructed initial pressure distribution, σ is the standard deviation, and cov is the covariance.

For experimental point target phantom images, SNR was used as a comparison metric for the quality of the images. SNR for each reconstructed PA image was computed as described in chapter 2, section 2.2.3.

4.3 Results and Discussion

Figure 4.3 shows the reconstructed cross-sectional PA images of five point targets' numerical phantom. Figure 4.3(a) shows the reconstructed PA image obtained using SUT1 for a full 360^0 rotation. Figures 4.3(b) – 4.3(d) show the reconstructed PA images obtained for 2-SUT PACT system configuration (using SUT1 and SUT2). In this configuration, each transducer collects the PA data around the sample for 180^0 rotation. Figure 4.3(b) is the reconstructed PA image obtained using SUT1 from 0^0 to 180^0 around the target phantom. Figures 4.3(c) and 4.3(d) show the reconstructed PA images for the combined PA data from SUT1 and SUT2 without and with radius compensation, respectively. Similarly, Figs. 4.3(e) – 4.3(g) and 4.3(h) – 4.3(j) represent the reconstructed PA images for 4-SUT PACT and 8-SUT PACT system configurations, respectively. In Figs. 4.3(b), 4.3(e), and 4.3(h), the target object information was lost, as the data collected around the sample for one SUT are limited (180^0 , 90^0 , 45^0) as shown by the red arrow marks. The point targets were not reconstructed properly in Fig. 4.3(e) as compared to Fig. 4.3(a), whereas point targets 1 and 5 were not visible in Fig. 4.3(h). This is the limited view problem [132, 133], where the transducer does not collect the information around the sample in full 360^0 . This limited view problem can be overcome by employing multiple SUTs to cover around the sample in full 360^0 . Reconstructing the PA data collected from all transducers in each of the 2-, 4-, and 8-SUT configurations by using a single reconstruction radius, the shape of the target phantom

Figure 4.3: Reconstructed PA images for point target numerical phantom using modified delay-and-sum reconstruction algorithm (a) for single-SUT PACT configuration (360° rotation), (b) – (d) for 2-SUT PACT configuration (each SUT - 180° rotation), (e) – (g) for 4-SUT PACT configuration (each SUT - 90° rotation), and (h) – (j) for 8-SUT PACT configuration (each SUT- 45° rotation). (b) Reconstructed image for PA data obtained using SUT1, (c) reconstructed image for combined PA data obtained using SUT1 and SUT2 without radius compensation, (d) reconstructed image for combined PA data obtained using SUT1 and SUT2 with radius compensation, (e) reconstructed image for PA data obtained using SUT1, (f) reconstructed image for combined PA data obtained using SUT1 to SUT4 without radius compensation, (g) reconstructed image for combined PA data obtained using SUT1 to SUT4 with radius compensation, (h) reconstructed image for PA data obtained using SUT1, (i) reconstructed image for combined PA data obtained using SUT1 to SUT8 without radius compensation, and (j) reconstructed image for combined PA data obtained using SUT1 to SUT8 with radius compensation. PC coefficients are shown at the bottom in each image. Color bar is shown for all images on the left. Scale bar is shown in (a).

looks distorted [Figs. 4.3(c), 4.3(f), and 4.3(i)]. This distortion in shape was high when more numbers of SUTs were used. By compensating the reconstruction radius, i.e., by using a corresponding reconstruction radius for each SUT (calculated using the calibration method proposed earlier), the shape of the target phantom can be preserved [Figs. 4.3(d), 4.3(g), and 4.3(j)]. These reconstructed images were similar in quality as that of the reconstructed image obtained using SUT1 in the case of a single-SUT PACT scenario. To compare the quality of the reconstructed images, PCCs were calculated for the point target phantom. From Table 4.2, it can be observed that the PCC values for multi-SUT PACT scenarios (0.19 for 2-SUTs, 0.19 for 4-SUTs, and 0.134 for 8-SUTs) are quite close to that of the PCC value of 0.19 for single-SUT PACT configuration.

Another type of numerical phantom used was the Derenzo phantom [Fig. 4.1(d)]. It consists of various sized circular target objects at various locations. Figure 4 shows reconstructed PA images. Figure 4.4(a) shows the reconstructed PA image of the Derenzo phantom obtained using SUT1 for a full 360° . Figures 4.4(b) – 4.4(d) show the reconstructed PA images obtained for a 2-SUT PACT scenario. The reconstructed PA image obtained using SUT1 from 0° to 180° around the sample is shown in Fig. 4.4(b). The reconstructed PA images for the combined PA data from SUT1 and SUT2 without and with radius compensation are shown in Figs. 4.4(c) and 4.4(d), respectively. Similarly, the reconstructed PA images for 4-SUT PACT and 8-SUT PACT system configurations are represented in Figs. 4.4(e) – 4.4(g) and 4.4(h) – 4.4(j), respectively. As the acquired PA data for each SUT were only 180° , 90° , and 45° in the case of 2-, 4-, and 8-SUT scenarios, a limited view problem is posed. Hence, the target object information contains small artifacts [red arrow marks in Fig. 4.4(b)]. All the circular objects were not

Figure 4.4: Reconstructed PA images for Derenzo numerical phantom using modified delay-and-sum reconstruction algorithm (a) for single-SUT PACT configuration (360° rotation), (b) – (d) for 2-SUT PACT configuration (each SUT - 180° rotation), (e) – (g) for 4-SUT PACT configuration (each SUT - 90° rotation), and (h) – (j) for 8-SUT PACT configuration (each SUT - 45° rotation). (b) Reconstructed image for PA data obtained using SUT1, (c) reconstructed image for combined PA data obtained using SUT1 and SUT2 without radius compensation, (d) reconstructed image for combined PA data obtained using SUT1 and SUT2 with radius compensation, (e) reconstructed image for PA data obtained using SUT1, (f) reconstructed image for combined PA data obtained using SUT1 to SUT4 without radius compensation, (g) reconstructed image for combined PA data obtained using SUT1 to SUT4 with radius compensation, (h) reconstructed image for PA data obtained using SUT1, (i) reconstructed image for combined PA data obtained using SUT1 – SUT8 without radius compensation, and (j) reconstructed image for combined PA data obtained using SUT1–SUT8 with radius compensation. PC coefficients are shown at the bottom in each image. Color bar is shown for all images on the left. Scale bar is shown in (a).

reconstructed in Figs. 4.4(e) and 4.4(h) (red arrow marks) as compared to Fig. 4.4(a). This limited view problem can be avoided by using multiple SUTs. Cross-sectional reconstructed PA images using a single reconstruction radius for all transducers in each of the 2-, 4-, and 8-SUT configurations are shown in Figs. 4.4(c), 4.4(f), and 4.4(i). This distortion in shape was high in the case of 8-SUT compared to 4-SUT and 2-SUT scenarios. The shape of the circular objects can be preserved [Figs. 4.4(d), 4.4(g), and 4.4(j)] by using an individual reconstruction radius for each SUT (obtained using the calibration method proposed earlier). PCCs were calculated for all the reconstructed PA images of the Derenzo numerical phantom (Table 4.2). The PCC values for multi-SUT PACT scenarios were 0.59 for 2-SUTs, 0.62 for 4-SUTs, and 0.58 for 8-SUTs PACT system scenarios. These values matched with that of the PCC value of 0.59 in the case of single-SUT PACT configuration.

Table 4.2: PCC values of reconstructed initial pressure rise distribution^a

	Point source phantom			Derenzo phantom			Blood vessel phantom		
S	0.19			0.59			0.46		
M	A	B	C	A	B	C	A	B	C
2-SUTs	0.14	0.10	0.19	0.43	0.33	0.59	0.38	0.28	0.44
4-SUTs	0.04	0.02	0.19	0.23	0.12	0.62	0.11	0.05	0.46
8-SUTs	0.001	0.005	0.134	0.15	0.11	0.58	0.03	0.03	0.40

^aS, Single-SUT PACT system; M, Multi-SUT PACT system; A, SUT1; B, all SUTs (using single reconstruction radius); C, all SUTs (using individual reconstruction radius obtained from the calibration method).

Next, a complex blood vessel network numerical phantom [Fig. 4.1(e)] was used to show the efficacy of the proposed algorithm. Figure 5 shows reconstructed PA images for the blood vessel network phantom. Figure 4.5(a) shows the reconstructed PA image

obtained using SUT1 for a full 360° rotation. Figures 4.5(b) – 4.5(d) show the reconstructed PA images for 2-SUT PACT system configuration. The reconstructed PA image obtained using SUT1 from 0° to 180° around the sample is shown in Fig. 4.5(b). The reconstructed PA images without and with radius compensation for the combined PA data from SUT1 and SUT2 are shown in Figs. 4.5(c) and 4.5(d), respectively. Similarly, the reconstructed PA images for 4-SUT PACT system configuration are represented in Figs. 4.5(e) – 4.5(g) and for the 8-SUT PACT system scenario are represented in Figs. 4.5(h) – 4.5(j). Due to the acquired PA data by each SUT being limited for 180° , 90° , and 45° in the case of 2-, 4-, and 8-SUT scenarios, the target object information was lost partially, as shown in Figs. 4.5(b), 4.5(e), and 4.5(h). The entire blood vessel network was not reconstructed in Figs. 4.5(e) and 4.5(h) (red arrow marks) as compared to Fig. 4.5(a). This problem can be avoided by using multiple SUTs. During reconstruction, when a single reconstruction radius is used for all transducers in each of the 2-, 4-, and 8-SUT configurations, the shape of the network gets distorted, as shown in Figs. 4.5(c), 4.5(f), and 4.5(i). This distortion in shape was high in the case of 8-SUT and 4-SUT compared to 2-SUT system scenarios. The shape of the target object can be retained [Figs. 4.5(d), 4.5(g), and 4.5(j)] by employing an individual calibrated reconstruction radius computed for single point source “P” [Fig. 4.1(b)] using the proposed algorithm for each SUT. To study the quality of the reconstructed images, PCC values were calculated, as shown in Table 4.2. The PCC values for multi-SUT PACT scenarios were 0.44 for 2-SUT, 0.46 for 4-SUT, and 0.40 for 8-SUT PACT system scenarios. These values were approximately similar to that of the PCC (0.46) for the single-SUT PACT system scenario.

Figure 4.5: Reconstructed PA images for blood vessel network numerical phantom using modified delay-and-sum reconstruction algorithm (a) for single-SUT PACT configuration (360° rotation), (b) – (d) for 2-SUT PACT configuration (each SUT - 180° rotation), (e) – (g) for 4-SUT PACT configuration (each SUT - 90° rotation, and) (h) – (j) for 8-SUT PACT configuration (each SUT - 45° rotation). (b) Reconstructed image for PA data obtained using SUT1 (c) reconstructed image for combined PA data obtained using SUT1 and SUT2 without radius compensation, (d) reconstructed image for combined PA data obtained using SUT1 and SUT2 with radius compensation, (e) reconstructed image for PA data obtained using SUT1, (f) reconstructed image for combined PA data obtained using SUT1 to SUT4 without radius compensation, (g) reconstructed image for combined PA data obtained using SUT1 to SUT4 with radius compensation, (h) reconstructed image for PA data obtained using SUT1, (i) reconstructed image for combined PA data obtained using SUT1 – SUT8 without radius compensation, and (j) reconstructed image for combined PA data obtained using SUT1 – SUT8 with radius compensation. PC coefficients are shown at the bottom in each image. Color bar is shown for all images on the left. Scale bar is shown in (a).

Next, to validate our proposed calibration method experimentally, we have used a pencil lead phantom [Fig. 4.2(c)]. The reconstructed PA images are shown in Fig. 4.6. Figure 4.6(a) shows the reconstructed cross-sectional PA image obtained using SUT1 for a full circular scan (single-SUT scenario). For the 2-SUT scenario, Figs. 4.6(b) – 4.6(d) represent the reconstructed PA images. The reconstructed PA image obtained using SUT1 from 0^0 to 180^0 around the sample is shown in Fig. 4.6(b). Figures 4.6(c) and 4.6(d) show the reconstructed PA images for the combined PA data from SUT1 and SUT2 without and with radius compensation, respectively. Similarly, Figs. 4.6(e) – 4.6(g) represent the reconstructed PA images for 4-SUT PACT system configuration. Due to the rotation of the transducers for only 180^0 and 90^0 in the case of 2- and 4-SUT scenarios, the point targets were not perfectly reconstructed in Figs. 4.6(b) and 4.6(e), as that in Fig. 4.6(a). This is due to the limited view problem, which can be overcome by using multiple SUTs. The reconstructed images in Figs. 4.6(c) and 4.6(f) look distorted, as they are reconstructed without any radius compensation. This distortion is high for the 4-SUT scenario compared to the 2-SUT scenario. By using an individual reconstruction radius (computed for a single point target [Fig. 4.2(b)] using the proposed calibration method), the point targets were perfectly reconstructed [Figs. 4.6(d) and 4.6(g)], as that in Fig. 4.6(a). SNR values obtained for reconstructed images in the case of 2-SUT and 4-SUT PACT system configurations were 34.69 dB and 35.06 dB, which were similar to that of SNR (34.88 dB) for single-SUT PACT system configuration.

High temporal resolution is always a priority for better diagnosis and continual monitoring of any biological changes happening inside the body. Researchers are always

Figure 4.6: Reconstructed PA images for point target phantom (made of five pencil leads of 0.5 mm diameter, one at the scanning center and remaining at ~ 1 cm from the scanning center) using modified delay-and-sum reconstruction algorithm (a) for single-SUT PACT configuration (360° rotation), (b) – (d) for 2-SUT PACT configuration (each SUT - 180° rotation), and (e) – (g) for 4-SUT PACT configuration (each SUT - 90° rotation). (b) Reconstructed image for PA data obtained using SUT1, (c) reconstructed image for combined PA data obtained using SUT1 and SUT2 without radius compensation, (d) reconstructed image for combined PA data obtained using SUT1 and SUT2 with radius compensation, (e) reconstructed image for PA data obtained using SUT1, (f) reconstructed image for combined PA data obtained using SUT1 to SUT4 without radius compensation, and (g) reconstructed image for combined PA data obtained using SUT1 to SUT4 with radius. SNR values are shown at the bottom in each image. Color bar is shown for all images on the left. Scale bar is shown in (a).

trying to improve the acquisition speed for PA imaging. Multiple SUTs for a circular scanning PACT system are more effective and economic in improving the temporal resolution. For instance, when a single SUT takes “ t ” s to acquire the PA data in full 360° around the sample, N numbers of SUTs take only “ t/N ” s. Therefore, we can save “ $t * (N - 1)/N$ ” s for each frame. Instead of acquiring one frame in “ t ” s with one SUT, we can acquire N frames in the same time using N -SUTs, thereby increasing the temporal resolution N -fold. The state-of-the-art PLD-PAT system [120] using a single SUT demonstrated imaging speed up to 0.33 Hz frame rate (3 s for one frame). By using 8-SUTs, imaging speed can be improved to 2.67 Hz frame rate (0.375 s for each frame), which is eight times the imaging speed obtained using the single SUT-based PLD-PAT system [120]. Hence, we can save 2.625 s for each frame using the 8-SUT-based PLD-PAT system.

In addition to the temporal resolution, maintaining the quality of images is also equally important. Using a simple delay-and-sum reconstruction algorithm requires the scanning radius parameter for optimal reconstruction of PA images. The conventional trial-and-error method to find the scanning radius for each SUT is ineffective and user dependent. In this work, we have calibrated the reconstruction radius using the proposed simple algorithm. This avoided the human errors in finding the optimum reconstruction radius for multiple SUTs. This calibration needs to be done only once for any transducer configuration. The efficiency of the proposed algorithm was demonstrated for a multi-SUT PACT system ($N = 2, 4$, and 8) using three numerical phantoms and a five-point target phantom. From the PCC values obtained for all the numerical phantoms and the SNR values for the experimental phantom, the quality of the reconstructed

images was maintained for the multi-SUT PACT system scenario as that of single-SUT PACT system scenario. Also, the time required to reconstruct the cross-sectional PA images obtained using the multi-SUT PACT system were similar to that of the PA images obtained using the single-SUT PACT system in the case of all three numerical and experimental phantom data. Since the calibration method also depends on the velocity of ultrasound in the medium, we believe the calibration will work when the medium is kept the same (water). If the medium is changed, we may have to do re-calibration.

4.4 Conclusion

The scanning radius is as an important parameter in a delay-and-sum reconstruction algorithm to obtain an optimally reconstructed cross-sectional PA image. We have come up with an algorithm to calibrate the scanning radius for a SUT in circular scanning geometry. This circumvented the limitations in finding the reconstruction radius using conventional trial-and-error method. This was very helpful when multiple SUTs were used in a circular scanning PACT system, as each PA datum from an individual SUT needed to be reconstructed with its own corresponding radius. We have shown the effectiveness of the proposed method using three numerical phantoms (point source, Derenzo, blood vessel) in the case of 2-, 4-, and 8-SUT PAT system configurations and a point source phantom in the case of 2 and 4-SUT PAT system configurations.

Chapter 5

Desktop PLD-PAT-G2 system⁴

5.1 Introduction

Dynamic imaging is needed for monitoring of pharmacokinetic, biodistribution of exogenous contrast agents, blood-velocity imaging, oxygen saturation imaging, small animal neurofunctional imaging, etc [134]. For high-speed imaging, customized full-ring array transducers can be used, e.g., recently a state-of-the-art single-impulse PACT system at 50 Hz frame rate was reported [85]. But these custom-made array transducers require complex back-end parallel signal amplifiers and digitizer electronics, which in turn increase the cost, making it challenging for clinical translation. In recent years, pulsed laser diodes [120, 135–140] or light emitting diodes (LEDs) [141–147] are being used for PA imaging due to their compact size (no need of optical table), low cost, and higher pulse-repetition rate (KHz) for real-time imaging compared to conventional Nd:YAG/OPO lasers. Although LED light sources have been explored for high-frame rate PA imaging, still there are some limitations for them to be effectively translated into clinics. Some of the disadvantages of the LED light source are they deliver low per pulse energy in the order of few μJ , light penetration depth is very low inside tis-

⁴Part of the work presented in this chapter has been published in *Optics Letters*.
S. K. Kalva, P. K. Upputuri, and M. Pramanik, “High-speed, low-cost, pulsed-laser-diode-based second-generation desktop photoacoustic tomography system,” *Optics Letters* 44(1), 81-84 (2019).

sue, and have limited wavelength tunability. All these limit the LED light source to be used in clinical applications. But PLDs deliver higher per pulse energy up to few mJ, penetration depth is also high (up to few cm) compared to LEDs. Recently compact PLD light source that can deliver up to four wavelengths has been developed, which is very helpful for functional imaging. Hence PLDs light sources are preferred to LED light source for preclinical and clinical PA imaging applications. A PLD-based PAT imaging system was successfully demonstrated for brain imaging [148], high-frame rate imaging using array transducers [130], rheumatology [139], and the diagnosis of cardiovascular disease [149]. A first-generation portable PLD-PAT system was developed by mounting the PLD inside the PAT scanner and using one SUT [120], and *in vivo* imaging was demonstrated in 5 s scan time [148]. As demonstrated in chapter 3, instead of using the SUT horizontally, an SUTR was used in vertical direction, which reduced the scanning radius and thereby the size of the PAT scanner [128]. To improve the frame rate further, instead of using one SUT, multiple SUTs can be used [108, 150], as demonstrated in chapter 4. By consolidating all these techniques, we developed a second-generation PLD-based desktop PAT (PLD-PAT-G2) system using a PLD laser inside the scanner and eight SUTRs. We have characterized the spatial resolution and imaging depth of this system, and also demonstrated its dynamic imaging ability using uptake and clearance process of ICG *in vivo* rat brain vasculature.

5.2 PLD-PAT-G2 system

The schematic of the PLD-PAT-G2 system (with photograph) is shown in Figs. 5.1(a) – 5.1(c). A PLD laser (QD-Q1924-ILO-WATER) capable of delivering 2000 pulses per second of ~ 107 ns pulse width at ~ 816 nm wavelength onto the sample was used. The

Figure 5.1: (a) Schematic of the PLD-PAT-G2 system; LDU, laser-driving unit; DAQ, data acquisition card; AMP, amplifier; SM, stepper motor; AM, anesthesia machine; PLD, pulsed laser diode; SUTR, acoustic-reflector-based ultrasound transducer. (b) Closer view of the transducers and laser illumination. (c) Photograph of the system. (d) Photograph of the horse-hair phantom. (e) LDPE tubes filled with ICG and blood on chicken breast tissue. (f) Stacked layers of chicken tissue.

PLD laser was controlled by a laser driving unit (LDU) consisting of a water cooling system, a 12 V power supply (Votcraft, PPS-11810), a variable power supply (EA-PS 8160-04 T) to change the laser power, and a function generator (RIGOL DG1022) to control the pulse repetition rate (up to 2 KHz). The output laser power after the diffuser was 6.8 W at 2 KHz pulse repetition rate. The laser energy density was maintained to be 0.17 mJ/cm^2 , well below the ANSI safety limit [119] for a scan time of 0.5 s (1.58 mJ/cm^2). The generated PA waves were acquired by eight unfocused SUTRs (SUT: V309-SU/U8423013, augmented with 45° acoustic reflector: F102, Olympus NDT) of 5 MHz central frequency with 70% fractional bandwidth and 13 mm active area

diameter. All eight SUTRs were placed around the sample at 45° intervals in a circle. To acquire the PA data in full circle, each transducer needs to rotate 45° around the target object. For comparison purpose, we also collected the data for one full rotation (360°) of one SUTR around the target object. Three different scan speeds of 30 deg/s, 45 deg/s, and 90 deg/s were used for comparison between the PA data obtained using eight SUTRs and one SUTR. Similar amplifiers, DAQ card and desktop configuration were used for acquiring and saving the PA data as described in chapter 4, section 4.2.3. For better comparison purpose, PA data for all the cases were averaged into 400 A-lines before reconstruction. In this work, all the reconstructions were done using a simple delay-and-sum reconstruction method.

5.3 Results and Discussions

5.3.1 Phantom experiments

Figure 2 shows the reconstructed cross-sectional PA images of a triangular shaped horse-hair phantom of $150\ \mu\text{m}$ thickness and a side length of $\sim 8\ \text{mm}$ [Fig. 5.1(d)]. Figures 5.2(a) – 5.2(c) show the reconstructed images for the PA data obtained around 360° using one SUTR for different scan times of 4 s, 8 s, and 12 s, respectively. Figures 5.2(d) – 5.2(f) represent the reconstructed images for the PA data obtained using eight SUTRs rotating 45° around the sample for scan times of (4/8) s, (8/8) s, and (12/8) s, respectively. The quality of the images obtained using eight SUTRs was compared with that of images obtained using one SUTR using SNR metric. SNR was calculated for each reconstructed image as described in chapter 2, section 2.2.3. The reconstructed images obtained for 0.5 s, 1 s, and 1.5 s scan time were similar to that of the recon-

structed images obtained for 4 s, 8 s, and 12 s scan time, respectively, in terms of SNR values (shown on each image) and the shapes of the target objects were also similar. Hence, the highest frame rate of 2 Hz (0.5 s per frame) can be obtained using the PLD-PAT-G2 system. At this imaging speed, we do not see any noticeable artifact (blur due to the high scan speed [120]) in the reconstructed images. We also have another artifact due to the use of eight USTs versus one UST (especially in the angles of the triangle), which we have not corrected in this work. The resolution of this system was calculated from the FWHM of the point spread function (obtained from the hair phantom image) and was found to be $\sim 165 \mu\text{m}$. Use of multiple SUTRs did not compromise the system resolution or SNR.

Figure 5.2: Reconstructed PLD-PAT-G2 images of triangular shaped horse-hair phantom. (a) – (c) Reconstructed images obtained using 1-SUTR for different acquisition times of 4 s, 8 s, and 12 s, respectively. (d) – (f) Reconstructed images obtained using 8-SUTRs for acquisition times of 0.5 s, 1 s, and 1.5 s, respectively. SNR values are shown on each image.

Next, a study was conducted to determine the imaging depth of the PLD-PAT-G2 system. Two LDPE tubes, one filled with ICG (12633-25 mg, SigmaAldrich) and the other filled with rat blood, were placed on chicken breast tissue, as shown in Fig. 5.1(e).

ICG was prepared with 323 μM concentration to have absorption peak around 800 nm. Layers of chicken breast tissue were stacked on top of the LDPE tubes to image ICG and blood at different depths of 1 cm, 2 cm, and 3 cm [Fig. 5.1(f)]. PA data were acquired at different depths for all scan times of 12 s, 8 s, 4 s using one SUTR and 1.5 s, 1 s, 0.5 s (only these images are shown here for brevity) using eight SUTRs. The comparison of SNR for ICG and blood at different depths for all the scan times can be observed in Fig. 5.3(a). The reconstructed cross-sectional PA images of ICG and blood for 0.5 s acquisition scan at different depths of 1 cm, 2 cm, and 3 cm can be seen in Figs. 5.3(b), 5.3(c), and 5.3(d), respectively. ICG had higher contrast and SNR compared to blood at all depths. We can successfully image at 3 cm deep inside the tissue (*in vitro*) using this PLD-PAT-G2 system.

Figure 5.3: Deep-tissue imaging of ICG and blood tubes embedded inside chicken breast tissue using the 8 SUTR PLD-PAT-G2 system. (a) Signal-to-noise ratio of ICG and blood at different depths for various scan times. (b) – (d) PAT images at 1 cm, 2 cm, and 3 cm depths (scan time 0.5 s).

5.3.2 *In vivo* experiments

Next, *in vivo* brain imaging was carried out on healthy female rats weighing $\sim(80-100)$ gm purchased from InVivos Pte. Ltd., Singapore. Standard animal protocol was followed for *in vivo* experiments as described in chapter 3, section 3.2.3. Figures 5.4(a) – 5.4(c) show the reconstructed PA images using one SUTR rotating around 360° for different scan times of 12 s, 8 s, and 4 s, respectively. Figures 5.4(d) – 5.4(f) represent the reconstructed PA images using eight SUTRs rotating around 45° for different scan times of 1.5 s, 1 s, and 0.5 s, respectively. Figure 5.4(g) shows the photograph of the rat head before taking PAT images. Figure 5.4(h) is the photograph taken after removing the skin layer on the head once PAT imaging was finished. The PAT images obtained for 0.5 s, 1 s, and 1.5 s scan times were similar to those of the PAT images obtained for 4 s, 8 s, and 12 s scan times in terms of SNR values (shown on each image, SNR was calculated using the signal from the SS region and background noise from the region away from the whole brain). Also, the SS, TS, and cerebral veins (CV) can be clearly seen in the images obtained from the 45° rotation of eight SUTRs for 0.5 s, 1 s, and 1.5 s and are similar to images obtained for 360° rotation of one SUTR for 4 s, 8 s, and 12 s, respectively.

Next, the high-speed *in vivo* dynamic imaging ability of the PLD-PAT-G2 system was demonstrated at 0.5 s acquisition scan time. We had conducted a study to visualize the hemodynamic changes in the cortical vasculature of rat brain due to the fast uptake and clearance process of ICG ($323 \mu\text{M}$ conc.). ICG was administered ($0.3 \text{ mL}/100 \text{ gm}$) into the tail vein of the healthy female rat weighing $\sim(80-100)$ gm. Before administering ICG, pre-injection baseline PAT images were obtained. After injecting ICG, for

Figure 5.4: *In vivo* PLD-PAT-G2 imaging of cortical vasculature of rat. (a) – (c) PAT images obtained using a single SUTR for different acquisition times of 12 s, 8 s, and 4 s, respectively. (d) – (f) PAT images obtained using 8-SUTRs for acquisition times of 1.5 s, 1 s, and 0.5 s, respectively. SS, sagittal sinus; TS, transverse sinus; CV, cerebral veins. SNR values are shown on each figure. (g) Photograph of the rat head before PA imaging. (h) Photograph of the animal brain, the top skin layer on the head cut open after the completion of PA imaging. The yellow arrows in (a) point to the corresponding vascular structures in (h).

the first 5 min, the PA data were continuously obtained at $\sim(0.9\text{--}1.1)$ s time intervals (scan and saving the data; reconstruction was done later) and later at 2 min intervals until the next 5 min and at 5 min intervals for the next 25 min. Due to the increase in optical absorption by ICG at 816 nm wavelength, the average PA signal in the superior SS started to increase and subsequently decrease over time, as can be seen in Fig. 5.5. A polynomial fit with a degree of 15 was used to model the data in Fig. 5.5. A similar trend was observed on multiple rats. This trend was similar to the one reported earlier in Ref. [148, 151]. The uptake of ICG was maximum at ~ 4.35 min post-injection, and the clearance process of the dye was monitored for 35 min post-injection.

Figure 5.5: Pharmacokinetics of ICG in rat brain showing the uptake and clearance over 35 min post-injection time. Red arrow shows the ICG injection time into the tail vein.

The current imaging speed can be further improved by using a higher torque motor and/or by using more SUTRs (this will increase the cost of the overall system). However, with further reduction in scan time, there may be artifacts introduced in the PAT image, which can be corrected by a more efficient reconstruction algorithm. Although the circular ring-arraybased PAT system offers real-time imaging, it is custom-made.

Hence, the cost is high (array transducers and the back-end electronics cost several hundred thousand dollars). In addition, ring-array transducers have fixed central frequency, bandwidth, and radius, whereas SUTRs offer flexibility, can be placed at a desired location, and are readily available in the market at a low price (few hundred dollars) with various frequency and bandwidth options. Overall, the PLD-PAT-G2 system costs $\sim(5-10)\%$ of the full ring-array-based Nd:YAG/OPO PAT system. However, PLD poses a few limitations that need attention and improvement in the future, *e.g.*, (a) low per-pulse energy and (b) wavelength tunability, although multiple wavelength PLDs are available, but only with a few wavelength options [136,152,153]. More wavelength options for PLD will lead to better functional and spectroscopic imaging. In spite of these drawbacks, due to its compactness, portability, high speed, and low cost, the proposed PLD-PAT-G2 system will find applications in neurofunctional activities, characterization of pharmacokinetic and biodistribution profiles in the development process of drugs, or any other exogenous contrast agents in small animal studies.

5.4 Conclusion

We have successfully demonstrated an ultra-compact, portable, affordable desktop PAT imaging system with the use of a PLD that renders high temporal and spatial resolutions, making it more suitable for real-time imaging. The system was capable of imaging cross-sectional PA images in 0.5 s (the fastest *in vivo* imaging system with multiple single-element UST reported so far). The cross-sectional *in vivo* brain images were obtained in 0.5 s (10 times faster than the first-generation PLD-PAT system [148]). There was no compromise on image quality (in terms of SNR) or resolution or imaging depth.

The spatial resolution was $\sim 165\ \mu\text{m}$ (with 5 MHz unfocused UST). Due to the low frequency ultrasound detection in PAT (typically 1 – 10 MHz), the longer pulse width of the PLD (107 ns) does not affect spatial resolution. By using a PLD with low pulse width and high-frequency UST, spatial resolution can be further improved. Using this PLD-PAT-G2 system, it was also shown that an imaging depth of 3 cm can be achieved (*in vitro*). The potentiality of this system for dynamic *in vivo* imaging was demonstrated by monitoring a fast uptake and clearance process of ICG in the cortical vasculature.

Chapter 6

Conclusion and future directions

6.1 Conclusion

This dissertation presented the practicality of improving tangential resolution, effectiveness of acoustic reflector in making the PAT circular scanner compact, calibrating the reconstruction radius for multiple single-element UST based PAT system and finally by combining all these individual improvements together with compact pulsed laser diode to build a low-cost, desktop model PLD based PAT system for high speed tomographic imaging.

Conventional PAT system had the limitations of bulky, low repetition rate Nd:YAG laser, low-scanning speed with a single SUT and degraded tangential resolution for the images reconstructed using simple delay-and-sum algorithm. Our final aim was to overcome these challenges by building a low-cost, portable, high-speed desktop PAT imaging system which gives better quality images in terms of spatial (tangential) resolution.

Initially, we had successfully improved the tangential resolution using a modified

delay-and-sum algorithm technique which was validated for various shaped (point targets, large circular objects, N-shaped blood vessel network) numerical as well as experimental phantom data. We achieved more than three-fold improvement in tangential resolution for both flat (non-focused) and cylindrically focused SUTs with improved high SNR levels. Our algorithmic method can be used as an alternate method for improving the tangential resolution with other advantages compared to various other techniques like using a negative acoustic lens attached to the transducer detector surface or using a virtual point detector as they have difficulties. Apart from improving the tangential resolution, we have also shown that using this modified delay-and-sum reconstruction algorithm we can preserve the shape of the target objects. This is very much needed for effective diagnosis and treatment purposes. This reconstruction technique can also be effectively used for thermoacoustic tomography imaging.

Later, we reduced the size of the PAT scanner by using an acoustic reflector augmented at 45° to the conventional SUT: SUTR. This compactness was achieved by placing the SUTR in vertical plane instead of placing SUT in conventional horizontal plane. This vertical placement saved a lot of space occupied by the combined transducer body and connecting cable in horizontal plane (~ 11.5 cm). There by, whole volume of the PAT scanner got minimized and hence load on the motor also reduced as the torque required to rotate the transducer decreased. Using this SUTR we achieved similar quality images in terms of SNR as that of the images obtained using SUT in case of various shaped (point targets, triangle shaped object, *in vitro* N-shaped blood vessel network) phantoms and *in vivo* rat cortical imaging without any compromise in the spatial resolution. This acoustic reflector can be effectively used with any type of single-element

USTs (flat as well as focused) for circular scanning based PAT systems to make them compact by reducing the scanning radius required by the transducer.

Further, to increase the imaging speed, we have used multiple transducers and proposed a simple calibration method to obtain the reconstruction radii of multiple SUTs in a circular scanning PAT system and validated it for various shaped numerical (point targets, derenzo, blood vessels network) and experimental (point targets) phantoms data. Our calibration method was very important when multiple SUTs are used in a circular scanning PAT system as individual PA datum from each SUT needs to be reconstructed with corresponding transducer scanning radius for obtaining promising PA reconstructed images. This methods overcame the limitations in finding scanning radius using conventional user-dependent trial-and-error method. The efficacy of the proposed method was demonstrated for multiple-SUTs: 2, 4, and 8 SUTs for numerical phantoms and 2, 4 SUTs for experimental phantom and obtained similar quality images in terms of PCC values (numerical phantoms) and SNR values (experimental phantom) compared to that of corresponding 1-SUT images. Usage of 'N' number of single-element transducers in circular scanning PAT system reduced the scanning time by N-fold.

Then by combining all the above techniques we built an affordable, compact desktop model second generation PLD-PAT system (PLD-PAT-G2) for high speed tomographic imaging applications by using a compact, portable, low-cost, high pulse repetition rate PLD along with multiple SUTRs (eight). We obtained similar quality images using 8-SUTRs rotating in 45^0 for different scan speeds of 1.5 s, 1 s, and 0.5 s as that of images obtained using 1-SUTR rotating in 360^0 for scan speeds of 12 s, 8 s, and 4

s respectively for both phantom (triangular shaped horse hair) and *in vivo* data. We achieved high temporal resolution of 0.5 s (state-of-the-art *in vivo* high speed imaging using multiple single-element transducers) and high spatial resolution of 165 μm using this system. Using this PLD-PAT-G2 system, we successfully imaged 3 cm deep inside the chicken breast tissue. The high-speed dynamic imaging capability of this system was demonstrated in visualizing the pharmacokinetics (wash-in and wash-out) of the ICG dye inside cortical vasculature of rats.

6.2 Future directions

There are certain limitations using this PLD-PAT-G2 system that needs to be overcome in terms of imaging speed, calibration techniques for reconstruction radii of transducers, and functional imaging applications. The current imaging speed (0.5 s) is limited by the stepper motor (torque) and also the number of transducers (8 SUTRs) used. In the future, we propose to use a more robust stepper motor that can deliver higher torque and more number of SUTRs (~ 16 SUTRs) in order to improve the imaging speed further from half a second down to the range of few milliseconds. With this higher imaging speed, we shall be able to monitor respiratory motion and/or heartbeat in *in vivo* small animals. By using continuous scanning method for such high-speed imaging, there might be artefacts (undesired blurring of the target object and curved lines) introduced in the reconstructed PAT image. To overcome this drawback we need highly sophisticated stepper motors that can provide us the position of the motor during the motion of the transducer in order to correct the artifacts in the reconstructed images obtained using delay-and-sum algorithm or we need to explore alternate reconstruction techniques.

Also, the proposed calibration technique for finding the reconstruction radius/scanning radius of the transducer depends on the speed of sound (Eq. 4.1). This speed of sound depends on the type of coupling medium (water/mineral oil etc.) and the type of biological tissue (imaging object) used. If either of the coupling medium and/or biological tissue (imaging object) is changed, then we need to redo the calibration. Due to this limitation, every time we change the imaging object (for eg., rat) all the SUTRs need to be rotated at least once in 360° to obtain the corresponding scanning radii and thereafter 45° PA data can be reconstructed by using these scanning radii. In future, we propose to use fiducial markers to co-register the reconstructed images from all the SUTRs' PA datum thereby we can avoid 360° scanning. This will help to image any object with different medium configurations.

The current PLD laser delivers only single wavelength thereby limiting the PA imaging to visualize only structural information. In future, we propose to use PLD laser that can deliver multiple wavelengths that helps in functional imaging applications such as to map oxyhemoglobin and deoxyhemoglobin concentrations and SO_2 levels. Although there are currently PLD lasers available that can deliver multiple wavelengths but there are only few wavelength options. For visualizing better functional neuronal activities and for spectroscopic imaging applications, we propose to use PLD laser with wavelength tunability option.

Stroke is the second largest cause of death worldwide. It is a neurologic deficit occurring due to the abnormal cerebral blood circulation. Ischemic stroke is the most prevalent with an occurrence possibility of 88% and intracerebral hemorrhage of 9%

and subarachnoid hemorrhage of 3% possibility in humans [154]. Women are most likely prone to stroke (11%) compared to men (9%) and it is especially more prevalent in younger age groups [155]. Hence there is a strong need to understand the pathophysiology of these diseases. Therefore, we propose to use our high-speed PLD-PAT-G2 system for visualizing the disease progression of ischemic stroke in *in vivo* animal models and also to monitor the effectiveness of drugs in curing the stroke. Also, we further propose to use this imaging system for monitoring neurofunctional activities such as epilepsy, and for characterizing pharmacokinetic, biodistribution profiles in the development process of drugs or contrast agents in small animal study.

List of publications

Peer reviewed journals

1. **S. K. Kalva**, and M. Pramanik, "Experimental validation of tangential resolution improvement in photoacoustic tomography using a modified delay-and-sum reconstruction algorithm," *Journal of Biomedical Optics* 21(8), 086011 (2016).
2. **S. K. Kalva**, and M. Pramanik, "Use of acoustic reflector to make compact photoacoustic tomography system," *Journal of Biomedical Optics* 22(2), 026009 (2017).
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 14. N. Awasthi, K. R. Prabhakar, **S. K. Kalva**, M. Pramanik, R. V. Babu, P. K. Yalavarthy, "PA-Fuse: A Deep Supervised Approach for Fusion of Photoacoustic Images with Distinct Reconstruction Characteristics," *Biomedical Optics Express* 10(5), 2227-2243 (2019).
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1. **S. K. Kalva**, and M. Pramanik, "Modified delay-and-sum reconstruction algorithm to improve tangential resolution in photoacoustic tomography," *Proc. SPIE* 10064, 100643W (2017).
2. **S. K. Kalva**, and M. Pramanik, "Compact photoacoustic tomography system," *Proc. SPIE* 10064, 100643X (2017).
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