PHOTOACOUSTIC IMAGING AND ITS APPLICATION TO CONGENITAL BRAIN DISEASES

By

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To my Mom and my Dad
And all who have been supportive to me in my life
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>8</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>9</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>11</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>13</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>15</td>
</tr>
<tr>
<td>Fetal and Neonatal Brain Imaging Techniques</td>
<td>15</td>
</tr>
<tr>
<td>Overview of Photoacoustic Imaging</td>
<td>17</td>
</tr>
<tr>
<td>Dissertation Outline</td>
<td>24</td>
</tr>
<tr>
<td>2 FAST 3D MULTISPECTRAL COMPACT MOBILE PHOTOACUSTIC TOMOGRAPHY SYSTEM</td>
<td>26</td>
</tr>
<tr>
<td>Motivation</td>
<td>26</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>27</td>
</tr>
<tr>
<td>Ultrasound Transducer Array Probe Design</td>
<td>28</td>
</tr>
<tr>
<td>Light Excitation and Illumination</td>
<td>32</td>
</tr>
<tr>
<td>Data Acquisition</td>
<td>33</td>
</tr>
<tr>
<td>Life Supporting System</td>
<td>34</td>
</tr>
<tr>
<td>Temperature control and monitoring</td>
<td>34</td>
</tr>
<tr>
<td>Breathing mask for anesthesia and air delivery</td>
<td>35</td>
</tr>
<tr>
<td>3D Compact Mobile PAT System Information and System Optimization</td>
<td>36</td>
</tr>
<tr>
<td>System information</td>
<td>36</td>
</tr>
<tr>
<td>System optimization</td>
<td>37</td>
</tr>
<tr>
<td>Phantom and In-vivo Animal Experiments Demonstration</td>
<td>39</td>
</tr>
<tr>
<td>Phantom Experiment Evaluation</td>
<td>39</td>
</tr>
<tr>
<td>Animal Experiment Demonstration</td>
<td>39</td>
</tr>
<tr>
<td>Conclusion</td>
<td>40</td>
</tr>
<tr>
<td>3 QUANTITATIVE PHOTOACUSTIC TOMOGRAPHY GPU ACCELERATION AND MESH OPTIMIZATION</td>
<td>43</td>
</tr>
<tr>
<td>Motivation</td>
<td>43</td>
</tr>
<tr>
<td>Material and Methods</td>
<td>44</td>
</tr>
<tr>
<td>Finite Element based q-PAT Reconstruction Algorithm</td>
<td>44</td>
</tr>
<tr>
<td>Forward model</td>
<td>44</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Neonatal GMH/IVH Model</td>
<td>93</td>
</tr>
<tr>
<td>Imaging System</td>
<td>94</td>
</tr>
<tr>
<td>Photoacoustic Imaging of Neonatal Brain Hemorrhage Progression</td>
<td>94</td>
</tr>
<tr>
<td>Image reconstruction and multispectral PAT of oxygen saturation evaluation</td>
<td>95</td>
</tr>
<tr>
<td>Assessment of hematoma area</td>
<td>95</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>96</td>
</tr>
<tr>
<td>Results</td>
<td>96</td>
</tr>
<tr>
<td>Discussion</td>
<td>97</td>
</tr>
<tr>
<td>Conclusion</td>
<td>99</td>
</tr>
<tr>
<td>CONCLUSION AND FUTURE WORK</td>
<td>103</td>
</tr>
<tr>
<td>Summary of Research</td>
<td>103</td>
</tr>
<tr>
<td>Future Directions</td>
<td>104</td>
</tr>
<tr>
<td>LIST OF REFERENCES</td>
<td>106</td>
</tr>
<tr>
<td>BIOGRAPHICAL SKETCH</td>
<td>119</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3-1</td>
<td>List of dual-mesh parameters used in mesh density and time interval evaluation</td>
</tr>
<tr>
<td>3-2</td>
<td>Performance comparison between GPU- and CPU-based reconstructions.</td>
</tr>
<tr>
<td>3-3</td>
<td>Reconstructed target size (mm) using different meshes.</td>
</tr>
<tr>
<td>5-1</td>
<td>Results of Friedman Test on all samples. Degree of the freedom is 7 for all</td>
</tr>
<tr>
<td></td>
<td>the tests.</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Schematics of Photoacoustic Effect</td>
<td>18</td>
</tr>
<tr>
<td>1-2</td>
<td>Schematics of PAM configurations.</td>
<td>20</td>
</tr>
<tr>
<td>1-3</td>
<td>Schematics of photoacoustic endoscopy configuration.</td>
<td>21</td>
</tr>
<tr>
<td>1-4</td>
<td>Schematics of PAT configurations.</td>
<td>21</td>
</tr>
<tr>
<td>1-5</td>
<td>Molar extinction spectra of HbO and HbR in the near-infrared region.</td>
<td>22</td>
</tr>
<tr>
<td>1-6</td>
<td>Absorption coefficient spectra of endogenous tissue chromophores.</td>
<td>23</td>
</tr>
<tr>
<td>2-1</td>
<td>Sensitivity fields of different array geometries.</td>
<td>29</td>
</tr>
<tr>
<td>2-2</td>
<td>Dimensional schematics of the transducer array and probe.</td>
<td>29</td>
</tr>
<tr>
<td>2-3</td>
<td>Acoustic field distributions for the transducer.</td>
<td>31</td>
</tr>
<tr>
<td>2-4</td>
<td>Testing results of the probe using reflection echo method.</td>
<td>31</td>
</tr>
<tr>
<td>2-5</td>
<td>Design of optical fiber bundle.</td>
<td>33</td>
</tr>
<tr>
<td>2-6</td>
<td>Schematics of the DAQ system.</td>
<td>34</td>
</tr>
<tr>
<td>2-7</td>
<td>Schematic of mouse breathing mask.</td>
<td>36</td>
</tr>
<tr>
<td>2-8</td>
<td>3D fast PAT system.</td>
<td>38</td>
</tr>
<tr>
<td>2-9</td>
<td>PAT image of phantom.</td>
<td>41</td>
</tr>
<tr>
<td>2-10</td>
<td>PAT images at different depth of neonatal mouse brain. The results are obtained using 532 nm laser pulse.</td>
<td>41</td>
</tr>
<tr>
<td>2-11</td>
<td>PAT images at different depth of neonatal mouse brain. The results are obtained using 750 nm laser pulse.</td>
<td>42</td>
</tr>
<tr>
<td>2-12</td>
<td>PAT images at same depth of neonatal mouse brain with different wavelengths.</td>
<td>42</td>
</tr>
<tr>
<td>3-1</td>
<td>Comparison of reconstructed absorption energy density images using GPU and CPU</td>
<td>55</td>
</tr>
<tr>
<td>3-2</td>
<td>Reconstructed optical absorption coefficient image.</td>
<td>56</td>
</tr>
<tr>
<td>3-3</td>
<td>Reconstructed optical absorption coefficient images using different dual-meshes.</td>
<td>56</td>
</tr>
</tbody>
</table>
3-4  Reconstructed optical absorption coefficient images using different meshes and time intervals. ................................................................. 57
3-5  Reconstructed optical absorption coefficient images. ........................................ 58
4-1  Photoacoustic images of phantom experiments........................................ 65
4-2  Photoacoustic images of in-vivo mouse brain. ........................................ 66
4-3  Schematic of fan-shaped scanning approach for PAT........................................ 68
4-4  Schematic of conventional DAS vs. virtual element reconstruction approach.... 69
4-5  Photoacoustic imaging of three targets embedded in the background phantom along a vertical line in depth direction. ............................................ 73
4-6  Photoacoustic imaging of three targets embedded in the phantom in a triangular shape. ............................................................................. 74
4-7  Photoacoustic imaging of two mice bearing PDX tumors.......................... 74
5-1  Photoacoustic imaging of mouse embryo.................................................. 86
5-2  Photoacoustic image and vessel diameter change of one embryo from the sham group. ............................................................................. 87
5-3  Photoacoustic image and vessel diameter change of one embryo from the EtOH group. ............................................................................. 88
5-4  Percentage change 40 mins after administration. ..................................... 89
5-5  Photoacoustic images and oxygen saturation change for one embryo from the EtOH group. ............................................................................. 89
6-1  Representative photoacoustic images of hematoma lesion progression with comparison to histology......................................................... 100
6-2  Quantitative assessment of hematoma lesion size progression................ 101
6-3  3D progression of NBH in the whole neonatal mouse Brain..................... 101
6-4  Quantitative data of change in lesion to brain size ratio and percentage variation of oxygen saturation overtime (n=3). ................................. 102
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>ADC</td>
<td>Analog-to-digital converter</td>
</tr>
<tr>
<td>AR-PAM</td>
<td>Acoustic resolution photoacoustic microscopy</td>
</tr>
<tr>
<td>BEC</td>
<td>Blood ethanol concentration</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CSR</td>
<td>Compressed sparse row</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CUDA</td>
<td>Common Unified Device Architecture</td>
</tr>
<tr>
<td>DAQ</td>
<td>Data acquisition system</td>
</tr>
<tr>
<td>DAS</td>
<td>Delay and sum</td>
</tr>
<tr>
<td>DOT</td>
<td>Diffuse optical tomography</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FASD</td>
<td>Fetal alcohol spectrum disorders</td>
</tr>
<tr>
<td>FEM</td>
<td>Finite element method</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>GD</td>
<td>Gestation day</td>
</tr>
<tr>
<td>GMH</td>
<td>Germinal matrix hemorrhage</td>
</tr>
<tr>
<td>GPU</td>
<td>Graphic processing unit</td>
</tr>
<tr>
<td>HbO</td>
<td>Oxyhemoglobin</td>
</tr>
<tr>
<td>HbR</td>
<td>Deoxyhemoglobin</td>
</tr>
<tr>
<td>HbT</td>
<td>Hemoglobin concentration</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>IVH</td>
<td>Intraventricular hemorrhage</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>micro-CT</td>
<td>Micro-computed tomography</td>
</tr>
<tr>
<td>micro-MRA</td>
<td>Magnetic resonance angiography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NBH</td>
<td>Neonatal brain hemorrhage</td>
</tr>
<tr>
<td>OOI</td>
<td>Object of interest</td>
</tr>
<tr>
<td>OPO</td>
<td>Optical parametric oscillator</td>
</tr>
<tr>
<td>OR-PAM</td>
<td>Optical resolution photoacoustic microscopy</td>
</tr>
<tr>
<td>PAI</td>
<td>Photoacoustic imaging</td>
</tr>
<tr>
<td>PAM</td>
<td>Photoacoustic microscopy</td>
</tr>
<tr>
<td>PAE</td>
<td>Prenatal ethanol exposure</td>
</tr>
<tr>
<td>PZT</td>
<td>Lead zirconate titanate</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>$sO_2$</td>
<td>Oxygen Saturation</td>
</tr>
<tr>
<td>TD q-PAT</td>
<td>Time domain quantitative photoacoustic tomography</td>
</tr>
</tbody>
</table>
PHOTOACOUSTIC IMAGING AND ITS APPLICATION TO CONGENITAL BRAIN DISEASES

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Photoacoustic Imaging (PAI) which combines high optical contrast and high acoustic resolution, has unique advantages in vascular and hemodynamic imaging. It can offer high spatial and temporal resolution at deep imaging depth. In addition to structural vasculature imaging, when coupled with the photon transport model, multispectral PAI can recover important physiological information, such as hemoglobin concentration, oxygen saturation, metabolic rates, cerebral blood flow, and blood perfusion. In the past two decades, PAI has been through rapid advancements in hardware. Now, it has come to a stage of applications in preclinical and clinical studies, where the unique advantages of PAI can be beneficial.

Vascular and hemodynamic changes in the fetal and neonatal brain are essential for studying the development of congenital brain diseases. The primary challenge that precludes the rapid advancements of these studies is the lack of high-resolution hemodynamic imaging techniques that would be suitable for imaging the fetal brain of small lab animal species. Therefore, this dissertation is focused on developing photoacoustic imaging techniques that are suitable for non-invasive in-utero dynamic functional imaging of the fetal brain with high spatial and temporal resolution and deep imaging depth. This technique has great value for laboratory
research on fetal congenital brain diseases and is possible to be ultimately adapted to high-resolution imaging and characterization of fetal cerebral circulation in humans.

A fast 3D multispectral compact and mobile photoacoustic tomography (PAT) system were developed for high resolution non-invasive functional dynamic imaging of fetal brain circulations. For the first time, high-resolution fetal vasculature, blood perfusion, and oxygen saturation dynamics were imaged using PAT. The potential of PAT in congenital brain disease study has been successfully demonstrated using disease models. In addition to the system hardware development, the time-domain quantitative PAT (TD-q-PAT) algorithm has been significantly improved in computational speed and imaging quality, which makes functional imaging with TDq-PAT feasible for preclinical and clinical applications. Moreover, novel photoacoustic imaging approaches with new image reconstruction algorithms have been developed to realize high resolution and contrast imaging in deep tissue with fewer detectors, which has the potential to be translated to human fetal or neonatal brain imaging in the future.
CHAPTER 1
INTRODUCTION

Fetal and Neonatal Brain Imaging Techniques

Understanding the physiopathology is essential for the detection and early intervention of congenital brain diseases. Neuroimaging techniques for fetal and neonatal brain are critical tools for studying the physiopathology of congenital brain diseases. Brain vasculature and blood perfusion alterations are expected to compromise the delivery of nutrients and the removal of metabolites from neurons. Such alterations can result in hindered neuronal development and function. In addition, the nerve cells rely on a sufficient supply of oxygen. Alterations in fetal brain oxygen supply can lead to birth defects and neurodevelopmental delay in neonates. Thus, these alterations can be one of the most common mechanisms for many congenital brain diseases, and imaging the changes of vasculature, perfusion and oxygen metabolism in fetal/neonatal brain is critical for understanding the physiopathology, as well as for the detection and early intervention.

One of the most common methods for studying congenital diseases is by phenotyping mouse embryos and neonates, as rodents like mice provide an ideal model for studying human brain diseases. However, currently one of the major obstacles that preclude the rapid advancement of the studies on fetal cerebral circulation is the lack of high-resolution imaging techniques that would be suitable for imaging of small lab animal species. Up-to-date, various imaging techniques have been used for vasculature/hemodynamic imaging, including micro magnetic resonance angiography (micro-MRA), Doppler ultrasound imaging, micro-computed tomography (micro-CT) and optical coherence tomography (OCT) [1-6]. Micro-MRA provides high-resolution vascular imaging but uses contrast agents and is costly. Doppler ultrasound is widely used in embryo vascular imaging; however, its resolution and contrast are limited. Micro-
computed tomography offers inexpensive high-resolution imaging, but due to the use of ionizing radiation and exogenous contrast media, it is limited to ex-vivo imaging. Among the existing techniques for fetal vascular imaging, OCT provides the best resolution and can image microvasculature. However, the major limitation is its very limited imaging depth (a few hundred micrometers), making it impossible for non-invasive in-vivo imaging of the embryo in small animals. For imaging the embryo of a mouse, an incision must be performed to expose the embryo for OCT imaging [3, 6]. Such an intervention could affect the maternal-fetal circulation and cause occlusion in fetal blood vessels [3]. In addition, even with an incision, OCT is only able to image the superficial vasculature and cannot image the whole embryo or embryos located deep in uterus. Exposing the embryo could also cause dehydration artifacts as the study of fetal hemodynamic changes often requires a long observation period [3]. Another major limitation is the lack of real-time imaging. As the scanning is needed for OCT, monitoring hemodynamics and neural activities in real-time are not possible. Despite a recent publication stating that real-time OCT is possible, its feasibility should still be questionable as it requires several seconds for a 2-D B-scan [7]. Moreover, due to its long acquisition time (seconds to minutes), even using a forceps to fix the embryo would not eliminate enough bulk motion to avoid significant motion artifacts [3]. Another challenge is to perform the functional imaging to quantitatively assess physiological parameters, such as oxy- and deoxy-hemoglobin concentrations, cerebral blood flow (CBF), oxygen saturation, and metabolic rates, which are critical in the development of congenital brain diseases. Functional MRI is able to monitor brain functional activity on a large scale, however, its spatial and temporal resolutions are low [8]. Functional micro Doppler ultrasound can obtain the change in blood flow, however, it cannot image the change in oxygen level, which represents the earliest reaction to the change of neural activity [9, 10]. In addition,
understanding the correlation between vasculature change and hemodynamic changes will further elucidate the pathophysiology of congenital brain defects. Thus, an imaging technique that can provide both high-resolution vasculature and hemodynamic imaging is desired.

Photoacoustic Imaging (PAI), which takes the advantages of the high acoustic resolution and high optical contrast, offers a promising solution to these problems and shows great potential in fetal/neonatal brain imaging for studying the congenital brain diseases.

**Overview of Photoacoustic Imaging**

Developing an imaging technology that takes full advantage of the characteristics of optical imaging and achieves higher imaging resolution in deeper biological tissues, has always been the goal that researchers pursue. Photoacoustic imaging breaks the limit of the spatial resolution of optical imaging in deep tissues, making high-resolution deep tissue imaging possible. Laser-induced PAI is based on the photoacoustic effect, in which, tissue absorbs light energy to generate the ultrasound wave through the photo-thermal effect. PAI combines the advantages of both optical imaging and ultrasound imaging. The contrast of PAI comes from the difference in the light absorption coefficients of different biological tissues, and as it detects the laser-induced ultrasound signals, it can provide high acoustic resolution. In addition, the attenuation of ultrasound is much less than the light attenuation at the same imaging depth, thus, PAI offers high-resolution imaging ability at a much deeper depth.

The photoacoustic effect consists of two phenomena: the photo-thermal effect and the thermoelastic effect. The photo-thermal effect is based on the atomic electron transition. Electrons in atoms and molecules vibrate at its specific natural frequency. When light irradiates the tissue, electrons will absorb the energy of the incident light wave which has the same frequency as the natural frequency of these electrons and reaches higher energy levels. The absorbed light energy will be released when the electrons transit back to lower energy levels.
causing the thermal effect. Tissues consisting of different atoms and molecules have different natural frequencies, thus, they will selectively absorb light with their resonant frequencies.

Another essential phenomenon is the time-variant thermoelastic expansion. When using the short-pulsed laser to irradiate biological tissues, the local temperature of the tissue rises rapidly leading to expansion, and then the temperature drops causing the tissue to shrink. The acoustic wave is generated by this phenomenon. The photoacoustic effect is shown in Figure 1-1.

![Figure 1-1. Schematics of Photoacoustic Effect.](image)

The acoustic pressure $p(r, t)$ rises after thermal expansion. Based on Newton's equation of motion, continuity equation, and thermoelasticity equation, the photoacoustic wave equation can be derived as:

$$p(r)∇\left(\frac{1}{\rho(r)}∇p(r, t)\right) - \frac{1}{v_s^2(r)} \frac{∂^2 p(r, t)}{∂t^2} = -\frac{β}{C_p} \frac{∂H(r, t)}{∂t} = -\frac{μ_aβ}{C_p} \frac{∂I(r, t)}{∂t} \quad (1-1)$$

where $v_s$ is the sound velocity, $H$ is absorbed energy density, $I$ is the time and space-dependent light intensity, $β$ is thermal expansion coefficient, $C_p$ is the specific heat capacity, $μ_a$ is the optical absorption coefficient of the medium.

For homogenous elastic media, Equation 1-1 can be described as:
\[ \nabla^2 p(r, t) - \frac{1}{v_0^2} \frac{\partial^2 p(r, t)}{\partial t^2} = -\frac{\mu_a \beta}{C_p} \frac{\partial I(r, t)}{\partial t} \]  

Equation 1-2 is the general time-domain photoacoustic wave equation. If the sound velocity remains constant in the media, then Equation 1-2 can be expressed as:

\[ \nabla^2 p(r, t) - \frac{1}{v_0^2} \frac{\partial^2 p(r, t)}{\partial t^2} = -\frac{\mu_a \beta}{C_p} \frac{\partial I(r, t)}{\partial t} \]  

The initial photoacoustic pressure is given as:

\[ p_0(r, t) = -\mu_a \frac{\beta v_0^2}{C_p} F(\vec{r}) \]  

F(\vec{r}) is the local fluence. From Equation 1-3, we can see that the acoustic pressure changes with the light absorption coefficient \( \mu_a \), therefore, PAI can offer the optical contrast. By utilizing different wavelengths of light, different tissue chromophores with different absorption coefficients can be identified.

The first photoacoustic effect was reported by Alexander G. Bell in 1880 [11]. However, due to the lack of well-developed sensors and laser technologies, this discovery didn’t draw enough attention from the researchers at that time. The interest in the photoacoustic effect was revived after Mark L. Veingerov discovered the application of the photoacoustic effect on measuring the concentration of carbon dioxide in 1938 [11]. Since the 1960s, the technology of laser had been developed, resulting in development in many research areas including photoacoustic technology. Applications based on photoacoustic effect had started to be developed in science and industry during the 1970s and 1980s. The application of photoacoustic effect in biomedical imaging emerged in the 1990s [12-14][33-35]. Robert A. Kruger began to investigate the application of photoacoustic tomography in biomedical imaging for the first time in 1994, and since then PAI had been developed rapidly. The first in-vivo photoacoustic imaging
appeared in 2003 [15, 16]. Lihong V. Wang and his team reported photoacoustic tomography of the in-vivo mouse brain. As an emerging imaging technique, PAI has gone through a major development in the past two decades. Various applications of PAI have been developed from micro- to macroscale and with imaging depth from millimeters to centimeters. In general, based on different applications, PAI can be classified into three categories: photoacoustic microscopy (PAM), photoacoustic endoscopy (PAE) and photoacoustic tomography (PAT).

There are two major kinds of PAM, which are optical-resolution PAM (OR-PAM) and acoustic resolution PAM (AR-PAM). PAM was first developed in 2005 [17] and had been applied for high-resolution vascular imaging. Figure 1-2 shows a configuration of PAM. Raster scanning is required to acquire a 2D or 3D image. High-frequency focused ultrasound transducer is used in AR-PAM and focused laser beam is used in OR-PAM to realize high spatial resolution. The reported lateral resolution and axial resolution for AR-PAM are about 45 μm and 15 μm, respectively [18]. For OR-PAM, the lateral resolution and axial resolutions are 2.14 μm and 10 μm, respectively [19]. Imaging depth for PAM is very limited. For AR-PAM, the imaging depth is up to 3mm, and for OR-PAM, it is only a few hundred micrometers [20]. Thus, the application of PAM has been limited mostly to superficial vasculature imaging.

Figure 1-2. Schematics of PAM configurations. A) configuration of AR-PAM; B) configuration of OR-PAM.
Photoacoustic endoscopy is a specific imaging application for intravascular and intestine imaging [21, 22]. Figure 1-3 shows an example of the configuration of photoacoustic endoscopy. The size of the ultrasound transducer is usually very small, and the optical fiber, ultrasound transducer, and micro-motors are integrated into the catheter. Similar to PAM, scanning is usually required in photoacoustic endoscopy.

Figure 1-3. Schematics of photoacoustic endoscopy configuration.

PAT has been developed for various applications from the in-vivo animal model to pre-clinical human imaging. Figure 1-4 shows the simple configuration of PAT. The object for imaging is irradiated by a pulsed laser. A single ultrasound transducer can be used to scan around the object, or an ultrasound array can be used to acquire the image without scanning. Unfocused ultrasound transducers or cylindrically focused transducers are employed in different PAT applications. Inverse reconstruction algorithms like delay and sum, finite element method (FEM), etc. are used to reconstruct the PAT images in 2D or 3D [12].

Figure 1-4. Schematics of PAT configurations. A) configuration of single transducer-based PAT; B) configuration of array-based PAT.
In addition to single-wavelength PAT imaging, multispectral functional PAT can be realized by using fast-tuning laser and ultrasound transducer array. Due to the different light absorption coefficient of different tissue chromophores, multiple wavelengths can be used to retrieve the map of different chromophores. For instance, based on the molar extinction coefficient spectra shown in Figure 1-5, deoxyhemoglobin (HbR) and oxyhemoglobin (HbO) can be identified using wavelength like 720 nm and 850 nm, at which HbR and HbO are more sensitive, respectively.

![Molar extinction spectra of HbO and HbR in the near-infrared region.](image)

Figure 1-5. Molar extinction spectra of HbO and HbR in the near-infrared region.

As a novel imaging technique, PAT is ideal for high-resolution in-vivo imaging of vasculature as hemoglobin itself provides the strongest photoacoustic signal for imaging in the visible and near-infrared regions (Figure 1-6) [23-30]. It can image the vasculature of the entire adult mouse brain with a resolution of at least 100μm (up to 4cm in depth) and offers clear imaging of superficial microvasculature with a resolution comparable to OCT [23, 26, 31, 32]. Super-resolution photoacoustic tomography was newly reported to have a 25μm resolution in deep adult mouse brain vascular imaging [33-35]. Recently, it has been demonstrated that PAT offers a more accurate vessel representation on the macro- and micro scale in comparison to ultrasound and Doppler ultrasound imaging [36, 37]. In addition, when coupled with a photon
transport model, PAT can quantitatively assess physiological parameters, such as oxy- and deoxy-hemoglobin concentrations, cerebral blood flow (CBF), oxygen saturation, and metabolic rates [23, 26, 28-30, 38-42]. These quantitative functional parameters can provide additional important information for monitoring fetal vasculature development, hemodynamic alterations, and cardiovascular functions. Moreover, real-time imaging ability was realized in PAT using transducer array with frame rates ranging from 6 fps to 100 fps [43-52].

Figure 1-6. Absorption coefficient spectra of endogenous tissue chromophores [20].

The technology of PAT has gone through rapid development in the last 20 years. Lihong V. Wang and his team were the first to apply an ultrasound transducer array for fast photoacoustic imaging. They have greatly expanded the research field of PAT and raised its global influence [53]. P. C Beard and his group developed the PAT technology based on the Fabry-Perot polymer film sensing interferometer[54]. Vasilis Ntziachristos and Daniel Razansky have improved the PAT hardware to realize multispectral 3D rea-time volumetric imaging and advanced its application to multiscale imaging in human, neuroimaging, molecular imaging, etc [55, 56]. Huabei Jiang and his team developed the quantitative PAT (q-PAT) based on the
radiative transfer equation coupled with the Helmholtz photoacoustic wave equation, which allows for accurate recovery of the absolute absorption coefficient of heterogeneous media [57, 58]. Besides, He also realized the great potential of PAT in epilepsy and arthritis researches. They creatively applied PAT and q-PAT to study the hemodynamics in epilepsy and the development of inflammation in arthritis and make significant progress in these applications[59, 60]. Many researches have made remarkable successes in this field and their work has greatly advanced the technology and the applications of PAT in cancer imaging, neuroimaging, human vascular imaging, whole-body imaging of small animals.

**Dissertation Outline**

As developing a high-resolution in-vivo imaging technique for phenotyping embryos and neonates of small animals is desired, this dissertation is focused on advancing PAT technique to image, monitor and quantitatively evaluate the vasculature and hemodynamics of fetal and neonatal brain, and apply it to study the mechanisms of congenital brain diseases using murine models. Chapter 2 focuses on the development of the fast 3D multispectral photoacoustic tomography system for non-invasively monitoring the hemodynamic and vascular changes in the fetal brain located in the deep uterus. Chapter 3 presents the quantitative photoacoustic tomography I improved for quantitatively assessing the critical physiological parameters in the development of congenital brain diseases. Chapter 4 presents the work of developing novel photoacoustic imaging approaches in deep tissue which has potential for human neonatal brain imaging, including the development of novel imaging approaches and reconstruction algorithms. Chapter 5 focuses on the application of PAT in fetal alcohol study, including photoacoustic imaging of dynamic vascular changes of the fetal brain blood vessel caused by acute prenatal ethanol exposure, and quantitative photoacoustic tomography of hemodynamic changes of fetal brain blood vessels. Chapter 6 focuses on the application of PAT in neonatal brain hemorrhage
study. The injury progression in the whole neonatal brain was monitored and evaluated using PAT. Chapter 7 summarizes the study of this thesis and proposes future works.
CHAPTER 2
FAST 3D MULTISPECTRAL COMPACT MOBILE PHOTOACOUSTIC TOMOGRAPHY SYSTEM

Motivation

Neuroimaging of fetus and neonates requires non-invasive, high spatial and temporal resolution and deep imaging depth. Current imaging techniques used for fetal or neonatal imaging, such as MRI, ultrasound, and OCT, have many limitations. The temporal resolution for MRI is usually low and it requires exogenous contrast agents for angiography. Ultrasound imaging has limited contrast. OCT offers a high resolution, but the imaging depth is limited to hundreds of micrometers [1-6].

Photoacoustic tomography can provide high-resolution in-vivo imaging with an imaging depth of several centimeters and is ideal for imaging vasculatures without exogenous contrast agents contrast [23-25, 33-35]. Besides, it offers the functional imaging ability to recover physiological parameters such as oxygen saturation, blood perfusion, metabolic rates, etc. [23, 26, 28-30, 38-42]. These advantages make PAT very suitable for fetal/neonatal cerebral hemodynamics and vasculature imaging. Thus, developing a PAT system that can provide real-time, multispectral, multi-scale, dynamic imaging in deep tissue is highly desirable. In addition, most current PAT systems are bulky with laser and mechanical systems fixed to the optical table. This makes it difficult to transport the PAT system to the clinical room or different sites for different studies.

In this work, a fast 3D multispectral compact and mobile photoacoustic system that can be used for real-time non-invasive vascular and hemodynamic imaging of the fetal brain located in the deep uterus has been developed. The customized cylindrically focused ultrasound array was designed with optimized parameters to achieve the best sensitivity, bandwidth, and tomographic resolution. A large field of view (> 40 mm in diameter) was achieved. PAT usually
requires averaging of data to obtain a good image quality; however, this would significantly reduce the speed of acquisition, making it unsuitable for dynamic imaging. To realize fast acquisition without additional averaging operation and maintain a good imaging quality at the same time, this system was optimized to improve the signal to noise ratio (SNR). This system can provide good image quality with a single pulse. Based on the 20Hz fast tuning OPO laser, the frame rate is 20fps. This fast acquisition can also eliminate the motion artifacts due to respiration, heartbeat, etc. without additional interference with the animal or post-processing for removing motion artifacts, which is beneficial in many applications. The fast tuning OPO laser in this system makes it feasible to perform multispectral functional dynamic imaging. A customized optical fiber bundle was designed to allow homogenous light delivery on a large region of interest. The conventional PAT system design which places the animal under the water tank with coupling media like ultrasound gel used between the interface causes many problems and is not suitable for imaging the embryos of small animals. To avoid these problems, in this system, the animal is immersed in water with a life-supporting system designed to keep the animal alive and breathe freely underwater. The 3D fast PAT system demonstrated here can offer high speed, high resolution, functional dynamic imaging of small animal fetus, thereby providing a very powerful tool for studying congenital diseases. This technique has great value for laboratory research on fetal cerebral circulation and is possible to be ultimately adapted to high-resolution imaging and characterization of fetal cerebral circulation in humans.

**Materials and Methods**

The major works of the system design will be discussed in this section, including 1) design of cylindrically focused ultrasound transducer array probe; 2) light excitation and illumination; 3) data acquisition; 4) life-supporting system; 5) System optimization.
Ultrasound Transducer Array Probe Design

The ultrasound transducer is based on piezoelectric materials, which can excite and receive the ultrasound wave through the piezo-electric effect \([\text{\ldots}}\). There are many kinds of piezoelectric materials. For our application of photoacoustic imaging, instead of considering both the excitation and receiving of the ultrasound, only the ultrasound detection will be our interest. Ceramics and polymers are the traditionally used materials for ultrasound detection. Ceramics like lead zirconate titanate (PZT) have great sensitivity and dynamic range, and polymers have broad frequency bandwidth. As there is a tradeoff between sensitivity and bandwidth, when designing the ultrasound transducer, both need to be considered. The goal is to achieve the best sensitivity in the region of interest (ROI) while keeping good bandwidth. For traditional ultrasonography arrays, PZT is usually the material that is used for the transducers. However, ceramics has limited flexibility and bandwidth. It is not easy to be shaped and massive backing for achieving broader bandwidth is not feasible in an array probe since the space is limited. Hence, another kind of material called piezocomposite, which consists of thin ceramic rods embedded in a flexible polymer, has been developed \([61]\). It combines higher sensitivity of ceramics and broader bandwidth and flexibility of polymers. In our application, a kind of piezocomposite material is used to fabricate the cylindrically focused ultrasound transducer array.

Other than the material of the transducers, the geometry of the array can also affect the imaging quality. There are mainly two kinds of geometries: linear array and circularly curved array. Figure 2-1 shows the simulation of the ultrasound field distribution of linear and circularly curved array \([62]\). A semicircle \((180^\circ)\) geometry is shown in Figure 2-1 A and a standard linear ultrasound array geometry with a limited view of \(53^\circ\) is shown in Figure 2-1 B. From the results, we see that the sensitivity field of the linear array is much more distorted for the linear array
comparing to the sensitivity field of the circular array. Besides, the linear array is way less sensitive than the circular array. This is due to the fact that the sensitivity field of each element in the linear array does not overlap as much as it does in a circular geometry. Thus, to achieve a better image quality, a circularly curved array will be used as the array geometry of our probe.

![Sensitivity fields of different array geometries. A) semicircular array geometry; B) a standard linear ultrasound array](image)

**Figure 2-1.** Sensitivity fields of different array geometries. A) semicircular array geometry; B) a standard linear ultrasound array [62].

![Dimensional schematics of the transducer array and probe. A) dimensional schematic of transducer array; B) dimensional schematic of transducer element; C) dimensional schematic of the probe; Ra: radius of array; Rse: radius of single element; L: element length; P: pitch of each element; W: element width](image)

**Figure 2-2.** Dimensional schematics of the transducer array and probe. A) dimensional schematic of transducer array; B) dimensional schematic of transducer element; C) dimensional schematic of the probe; Ra: radius of array; Rse: radius of single element; L: element length; P: pitch of each element; W: element width.
The dimensional schematics of the cylindrically focused ultrasound transducer array is shown in Figure 2-2. The array \(L=20\text{mm}, W=0.7\text{mm}, P=0.77\text{mm}, Ra=65\text{mm}, Rse=58\text{mm}\) has 256 elements. The element height \(L\) and the radius of every single element \(Rse\) define the focal parameter of the transducer array. The radius of the array \(Ra\) and angle \(\Theta\) determine the distribution of sensitivity and elevational focus. The pitch \(P\) is the summation of the element width \(W\) and space interval between elements. The pitch is 0.77mm, allowing 256 elements in 174°. The parameters of the curved transducer array were optimized for imaging fetus in the deep uterus. Simulation of the ultrasound field distribution of the array was used to evaluate and optimize the array parameters. The ultrasound field distribution of the array is shown in Figure 2-3. In order to improve the sensitivity of the probe while expanding the field of view (FOV), we reduced the center frequency to 4.4MHz. By increasing the radius of the array \(Ra\), reducing the element width \(W\), and lowering the center frequency, a large \(FOV(>40\text{mm in diameter})\) is achieved. The sensitivity is also related to the element size, as the element width is reduced, to ensure a good sensitivity of each element, we increased the element height \(L\). With the transducer center frequency, \(Ra\) and \(L\) determined, we optimized the radius of each single element \(Rse\) to obtain the optimal tomographic resolution. The tomographic resolution within 40mm diameter ROI is about 1.2 mm. To obtain the optimal sensitivity and bandwidth, in addition to choosing the piezocomposite material to fabricate the transducer, specific backing material was used to improve the receiving bandwidth and sensitivity. The testing results (Figure 2-4) shows that our array probe has a broad relative bandwidth of 113%. The probe is fabricated by Japan Probe. With good sensitivity, broad bandwidth, and large FOV, this probe can provide great imaging quality in deep tissue, which makes it suitable for imaging embryos in the uterus of small animals.
Figure 2-3. Acoustic field distributions for the transducer. A) acoustic filed distribution for the transducer array; B) acoustic field distribution for a single element; C) side-view of acoustic field distribution for the transducer array; D) Profile of the distribution shown in (a) along z.

Figure 2-4. Testing results of the probe using reflection echo method. A) representative channel echo; B) Fast Fourier Transform of the signal.
**Light Excitation and Illumination**

Based on the light absorption coefficient spectra of hemoglobin (Figure 1-6), Nd:YAG laser which excites 532nm wavelength laser pulses gives great imaging quality for vasculature imaging, however, the penetration depth is too shallow at such short wavelength. It has been proved that the near-infrared region is suitable to image vasculatures in the deep tissue, as it has deeper penetration depth in biological tissue and good light absorption by hemoglobin. Laser with an optical parametric oscillator (OPO), which can provide wavelengths in the near-infrared region, needs to be applied in this system. To realize real-time multispectral PAT imaging, a laser with fast tuning ability is also required. Therefore, this system employs a tunable OPO laser system with fast tuning at each laser pulse (OPOTEK, PhocusMobile, wavelength range: 690–950 nm, 1200–2600 nm; repetition rate: 20Hz, peak energy 60 mJ @ 750 nm, pulse width 5–7 ns, beam diameter 6mm). The laser beam is coupled into an optical fiber bundle through the interlock ports. In addition, the laser has a built-in energy meter that monitors OPO pulse energy in real-time and provides feedback for harmonics auto-optimization and logs pulse energy for data normalization in multispectral PAT imaging. Thus, no additional optical path and optical power meter are needed, which eliminates the use of the optical table in the PAT system. The 20Hz repetition rate enables a 20 fps frame rate for single-wavelength imaging of vasculature and 10 fps frame rate for multispectral imaging of hemodynamics. Moreover, this laser system is compact and transportable, which is suitable for our mobile PAT system.

Light illumination has a critical impact on image quality. To achieve a homogenous light delivery over a large ROI, a customized optical fiber bundle is designed and fabricated. It is optimized for 690 nm to 950 nm wavelength. Figure 2-5 shows the design of the optical fiber bundle. The fiber bundler a fused input end and is split into two output ends with rectangular aperture (25 mm × 1 mm), allowing more homogenous rectangular illumination over a whole
mouse fetus. We also have a high energy fiber bundler with one round-shaped output for traditional round illumination geometry, which is commonly used in PAT mouse brain imaging.

Figure 2-5. Design of optical fiber bundle.

Data Acquisition

The data acquisition system (DAQ) mainly contains two parts: the pre-amplifier and the analog-to-digital converter (ADC). For the pre-amplifier, the important factors to be considered are the gain, bandwidth, and noise-suppressing. For the ADC, the critical factors are sampling rate, resolution, trigger/frame rate, and bandwidth. Another aspect needs to be considered for the DAQ system in our application is the compactness.

We want to achieve real-time PAT imaging ability with all the 256-channel photoacoustic data collected and recorded simultaneously. Therefore, with Photo Sound Technologies, we designed a new 256-channel DAQ system that has the desired parameters for our PAT system (Figure 2-6).
This DAQ system collects 256 channel PAT data simultaneously. The maximum data acquisition length per frame for each channel is 4096, which covers the whole detectable region of our probe. The collected signal will be transmitted to the computer via the USB 3.0 interface in real-time. The sampling rate is 40MSPS, and resolution: 12-bit. The ADC is continuous with no buffering, which enables fast data acquisition up to 50 fps. Each ADC channel has its integrated voltage-controlled amplifier with digitally controllable gain ranging from 40-91dB. As the amplifier and ADC are integrated into a small case (27.5 cm × 18cm × 5.99 cm), this DAQ system is very compact. The analog bandwidth is 75 kHz to 12.5MHz. For each laser pulse, a trigger will be sent to the DAQ system to acquire 256-channel PAT data, thus, a PAT image can be formed by each laser pulse.

![Figure 2-6. Schematics of the DAQ system.](image)

**Life Supporting System**

**Temperature control and monitoring**

To perform in-vivo animal experiments using PAT, a life-supporting system needs to be added. In our system, the animal is placed underwater for PAT imaging. To keep the body temperature of the animal, feed-back controlled heating sticks are used for heating and
maintaining the temperature of the water. However, the temperature of the water will still have small fluctuations. As the change in temperature will also affect the sound velocity and the PAT image reconstruction algorithm like Delay and Sum is very sensitive to the change of sound velocity, monitoring the temperature of the water is demanded. A thermoelectric thermometer (National Instruments, USB-TC01) with a J-type thermocouple was employed in the system to continuously monitor the temperature. The logged temperature data will be used for PAT image reconstruction using a sound velocity calibrated reconstruction algorithm.

**Breathing mask for anesthesia and air delivery**

To immerse the mouse entirely in water for PAT imaging of embryo, a breathing mask is needed to let the mouse breathe freely underwater. Currently, there is no commercially available waterproof breathing mask for small animals. Here, a fully sealed breathing mask was designed and fabricated for the mouse to breathe underwater. The schematics of the mask are shown in Figure 2-7. It consists of a transparent resin case 1, silicone rubber seal 2, nylon tooth bar 3, silicone rubber ring 4, and nylon sealing nut 5. There is an anti-slip structure 6 on the air inlet, mounting holes 7 are used to fix the mask on the mouse holder. To realize the hermeticity of the mask, the following designs were made: 1. To solve the problem that the current conical mask cannot fit the mouse face completely, a 3D laser scanner was used to obtain the spatial coordinate data of the mouse face contour, and reverse engineering software was employed to make the model for fabricating the mask. 2. To solve the problem of the difference between mice having different weights, the silicone rubber seal 2 was designed, which has the inner surface completely fitted the mouse face. 3. To achieve the hermeticity between the interface of the mask and mouse face, the tooth bar 3 was designed, which is used to fix the mask to the mouse and to serve as the outlet of air. Firstly, the mouse incisors are fixed in the hole 9, and then the tooth bar will be pulled back until the mouse face completely fits the silicone seal 2. At this time, sealing
nut 5 will be tightened, and the inside surface 8 will squeeze the silicone rubber ring 4 to completely seal the mask.

Figure 2-7. Schematic of mouse breathing mask. 1. a transparent resin case, 2. silicone rubber seal, 3. nylon tooth bar, 4. silicone rubber ring, 5. nylon sealing nut. 6. anti-slip structure, 7. mounting holes, 8. curved inside surface, 9. fixing hole.

3D Compact Mobile PAT System Information and System Optimization

System information

The schematics and photo of the system are shown in Figure 2-8. The laser beam generated by a portable fast-tuning OPO laser (OPOptek Inc, Phocus Mobile; wavelength range: 690–950 nm; 1200–2600 nm; repetition rate: 20Hz) is coupled into a custom-made optic fiber bundle through interlocked ports. The photoacoustic signal is detected by a cylindrically focused transducer array probe having 256 elements (Japan Probe, array radius: 65mm; central frequency: 4.4 MHz; bandwidth 113%, FOV > 40 mm in diameter) coupled with a custom-made 256-channel amplifier/DAQ system (Photo Sound Technologies, sampling rate: 40MSPS;
resolution: 12-bit; frame rate: 50Hz; adjustable gain: 40-91dB). The collected data is transferred to a computer via a USB 3.0 interface in real-time. The transducer array was mounted on rotational and translational motors to allow imaging at different angles and depths and was immersed in deionized water for acoustic coupling. The animal holder was mounted onto a moving stage so that the position of the mouse was able to be easily adjusted to place the embryo in the imaging center. This design allowed 3D imaging of the entire mouse embryo. The LabVIEW program was implemented to control the acquisition of PAT data and temperature data simultaneously, as well as recording the laser output energy.

**System optimization**

The system was optimized to improve the SNR to realize fast acquisition without additional averaging operation. The system provides a good image quality with a single pulse. Based on the 20Hz fast tuning OPO laser, the frame rate of single-wavelength imaging is 20 fps, and 10fps for multispectral (two wavelengths) imaging. A higher frame rate can be achieved by using a laser with a higher repetition rate. This fast acquisition can eliminate the motion artifacts due to breathing, heartbeat, etc. without additional interference with the animal or post-processing for removing motion artifacts.

To reduce the electrical noise, several improvements were made to the system design: 1. The ultrasonic transducer array was designed to have good noise shielding performance. 2. The customized DAQ system integrates the amplifier and ADC to reduce the noise induced by the interference between the amplifier and acquisition system. 3. To reduce the noise induced by the connection interface between the ultrasound transducer array and DAQ system, a third-party ITT cannon QLC260 connector with customized pin arrangement, which provides excellent noise shielding performance, was applied in this system. 4. The amplifier is very sensitive to the noise
induced by the power supply. To reduce this noise, the power supply with the best shielding performance was employed. By the big margin, the power supply with the best shielding performance is Mean WELL HEP-xxx-12A series (xxx = 100, 150, 240).

To reduce the acoustic noise, isolation layers (polyamide material) was used between the transducer and the cantilever. The acoustic impedance of the polyamide \( (2 \text{ kg/m}^2\text{s} \times 10^6) \) is much less than the material of cantilever \( (45.40 \text{ kg/m}^2\text{s} \times 10^6) \). According to Snell’s law, about 84% of acoustic noise can be blocked. In addition, electric noise from the rotator can be blocked by the isolation layer. Vibration isolation pads integrated on the adjustable bracket legs and between the water tank and the bracket can effectively block the acoustic noise coming from ground and water.

Figure 2-8. 3D fast PAT system. A) schematics of the 3D fast PAT system; B) photograph of the PAT system (Photo courtesy of author); AP: amplifier, BM: breathing mask, DAQ: data acquisition, HT: heater, LM: linear translation step motor, MH: mouse holder, MS: moving stage, RM: rotator step motor, TM: thermoelectric thermometer, UT: ultrasonic transducer array.
**Phantom and In-vivo Animal Experiments Demonstration**

**Phantom Experiment Evaluation**

The system was first evaluated using a phantom experiment. Two human hairs were embedded horizontally in the tissue-mimicking phantom media, which was made of 2% Agar, India ink, and TiO$_2$. The absorption coefficient of the background media was 0.007 mm$^{-1}$ and the scattering coefficient was 1.0 mm$^{-1}$. The light wavelength used to excite PAT signals was 750 nm. The collected PAT data were reconstructed using the Delay and Sum algorithm, which is the most commonly used reconstruction algorithm in PAT [12, 63]. The reconstructed image is generated by a single laser pulse, without additional averaging. The results are shown in Figure 2-9. Reconstructed target size was evaluated using full width half maximum (FWHM). It shows that the target can be clearly recovered. The resolution of the system is around 113μm.

**Animal Experiment Demonstration**

Neonatal CD-1 mouse was used in the experiment. The mouse was anesthetized using isoflurane in oxygen. 4% isoflurane was used during induction and 1-2.5% for maintenance through the whole imaging session. The mouse was placed on a 3D printed mouse holder. 3D PAT imaging of the whole mouse brain was performed. 532 nm and 750 nm wavelengths were used to generate PAT signals. The tomographic images at different depths acquired using 532 nm and 750 nm are shown in Figure 2-10 and Figure 2-11, respectively. All the images are reconstructed using single pulse data without averaging. The results show that the small blood vessels and brain structures in the neonatal mouse brain can be clearly recovered using our system. Figure 2-12 shows the PAT images obtained using 532 nm and 750 nm at the same imaging depth in deep brain tissue. The result shows that 750 nm can recover more details in deeper tissue, while 532 nm provides good imaging of vasculatures at a shallower depth.
Combining different wavelengths, the image of the whole neonatal mouse brain can be better recovered.

**Conclusion**

In summary, we have developed a Fast 3D multispectral compact and mobile PAT system, which can be used for real-time non-invasive vascular and hemodynamic imaging of the fetal brain in the deep uterus. The parameters of the transducer array were designed to realize the optimal sensitivity, bandwidth and resolution and a large field of view. The customized fiber bundle can provide homogenous light delivery to the fetus. In addition, through careful design, the SNR of the system was improved, which enables the system to provide good image quality using a single laser pulse. Thus, fast acquisition can be achieved. The frame rate is 20 fps for PAT imaging, and 10 fps for two-wavelength functional imaging. The system is designed to be compact and mobile without the use of an external light path or the bulky optical table, making it easy to transport to different sites. The phantom and in-vivo experiments have shown that this system can offer a high-resolution tomographic image of the small blood vessel and brain structures, as well as deep imaging depth. The system delivers high speed, high resolution, deep-depth, functional dynamic imaging of fetuses and neonates of small animals, thereby providing a very powerful tool for phenotyping embryos and studying the development of congenital diseases.
Figure 2-9. PAT image of phantom experiments. A) PAT image of two parallelly placed human hair. B) intensity profile along the red dashed line.

Figure 2-10. PAT images at different depth of neonatal mouse brain. The results are obtained using 532 nm laser pulse.
Figure 2-11. PAT images at different depth of neonatal mouse brain. The results are obtained using 750 nm laser pulse.

Figure 2-12. PAT images at same depth of neonatal mouse brain with different wavelengths. A) PAT image obtained using 532 nm; B) PAT image obtained using 750 nm.
CHAPTER 3
QUANTITATIVE PHOTOACOUSTIC TOMOGRAPHY GPU ACCELERATION AND MESH OPTIMIZATION

Motivation

Functional imaging to quantitatively assess physiological parameters is important for congenital brain disease study. As an emerging imaging technique, PAT has demonstrated its ability of high-resolution imaging ability in deep tissue. In addition, using multispectral imaging, PAT can provide functional ability to monitor brain activities. Among various PAT approaches, the finite-element-based quantitative photoacoustic tomography (qPAT) is particularly powerful, as it can accurately recover the absolute optical absorption coefficient of tissue, which can be used to recover important functional parameters, including hemoglobin concentration, oxygen saturation, and metabolic rate [57, 64-67].

Compared to FEM-based frequency-domain qPAT (FD-qPAT) [68], TD-qPAT approach has been demonstrated to provide better image quality with a much more accurately recovered target size and fewer artifacts [57]. Nevertheless, a primary challenge is that the time-domain approach is extremely computationally demanding, which usually requires several hours to reconstruct an image with a typical mesh size for in vivo breast imaging. Hence, it is unrealistic for the TD-qPAT to be used in any clinical applications, where a large amount of data often needs to be processed in a limited time scale.

To effectively reduce the computational time needed for TD-qPAT, the use of a graphic processing unit (GPU) appears to be a natural choice. In medical imaging, where it usually requires massive computation, GPU has been successfully applied in different imaging techniques including magnetic resonance imaging (MRI), computed tomography (CT), ultrasound imaging, and diffuse optical tomography (DOT) for image reconstruction and analysis
In this work, we implemented an accelerated FEM-based TD-qPAT approach using GPU for high-performance parallel computing. The accelerated GPU-based TD-qPAT also allows us to study some important issues previously impractical to study, including the optimization of mesh density/meshing scheme (spatial sampling rate) and time interval (temporal sampling rate) required for TD-qPAT reconstruction [67, 73]. We demonstrate these GPU-based improvements with phantom experimental data.

**Material and Methods**

**Finite Element based q-PAT Reconstruction Algorithm**

Our TD-qPAT method has two steps: (1) obtaining the map of absorbed optical energy density by iteratively solving the Helmholtz-like photoacoustic wave equation using finite element method, and (2) recovering the map of the absorbed coefficient from the absorbed energy density by solving the radiative transfer equation [58]. The first step is the time-consuming part that needs to be accelerated. In this Chapter, we will discuss the acceleration and optimization of the first step of TD-qPAT.

**Forward model**

The TD-qPAT algorithm is based on the finite element solution to the following time domain photoacoustic wave equation with the first-order absorbing boundary conditions:

\[
\nabla^2 p(r, t) - \frac{1}{v_0^2} \frac{\partial^2 p(r, t)}{\partial t^2} = -\frac{\Phi(r) \beta}{C_p} \frac{\partial J(t)}{\partial t}
\]  

(3-1)

\[
\nabla p \cdot \hat{n} = -\frac{1}{v_0} \frac{\partial p}{\partial t} - \frac{p}{2r}
\]  

(3-2)
where $p$ is the pressure wave, $v_0$ is the speed of the acoustic wave in the medium, $\beta$ is the thermal expansion coefficient, $C_p$ is the specific heat capacity, $\Phi = \mu_a H(r)$ is the absorbed energy density, and we assume that $J(t) = \delta(t - t_0)$ in this study. Expanding $p$ and $\Phi$ as the sum of coefficients multiplied by a set of basis function: $p = \sum p_j \psi_j; \Phi = \sum \Phi_k \psi_k; \hat{n}$ is the unit normal vector.

After the finite element discretization, Equation 3-1 can be written as:

$$[K]\{p\} + [C]\{\dot{p}\} + [M]\{\ddot{p}\} = \{B\}$$

(3-3)

where the elements of matrix $[K]$, $[C]$, $[M]$ and column vector $\{B\}$ are:

$$K_{ij} = \int_S \nabla \psi_i \cdot \nabla \psi_j dS + \frac{1}{2r} \oint_{\psi_i} \psi_i \psi_j dl;$$

$$C_{ij} = \frac{1}{v_0} \int_i \psi_i \psi_j dl;$$

$$M_{ij} = \frac{1}{v_0^2} \int_S \psi_i \psi_j dS;$$

$$B_i = \frac{\beta}{C_p} \int_S \psi_i \left( \sum_k \psi_k \Phi_k \right) dS \frac{\partial J}{\partial t};$$

The Newmark’s time stepping scheme is used here [72], and we can obtain the following formula:

$$[A]\{p\}_{t+\Delta t} = \{b\}$$

(3-4)

where the elements of matrix $[A]$ and column vector $\{b\}$ are:

$$[A] = [K] + \frac{1}{\alpha \Delta t^2} [M] + \frac{\delta}{\alpha \Delta t} [C];$$
\[ \{ b \} = \{ B \} t + \Delta t + [ M ] \{ a \} t + [ C ] \{ b \} t; \]
\[ \{ a \} t = \frac{1}{\alpha \Delta t^2} \{ p \} t + \frac{1}{\alpha \Delta t} \{ \dot{p} \} t - \left( 1 - \frac{1}{2\alpha} \right) \{ \ddot{p} \} t; \]
\[ \{ b \} t = \frac{\delta}{\alpha \Delta t} \{ p \} t - \left( 1 - \frac{\delta}{\alpha} \right) \{ \dot{p} \} t - \left( 1 - \frac{\delta}{2\alpha} \right) \Delta t \{ \ddot{p} \} t; \]

And the derivatives of \( p \) at the subsequent instant are:
\[ \{ \ddot{p} \} t + \Delta t \Delta t = \frac{1}{\alpha \Delta t^2} \{ p \} t + \Delta t \Delta t - \frac{1}{\alpha \Delta t} \{ \dot{p} \} t - \left( 1 - \frac{1}{2\alpha} \right) \{ \ddot{p} \} t; \]
\[ \{ \dot{p} \} t = \{ \dot{p} \} t + \Delta t (1 - \delta) \{ \ddot{p} \} t + \delta \Delta t \{ \ddot{p} \} t + \Delta t \Delta t; \]

Here \( \Delta t \) is the time interval, \( \alpha \) and \( \delta \) are time-stepping parameters. In our work, we determined \( \delta = 0.5 \) and \( \alpha = 0.25 \). When \( \delta \geq 0.5 \), and \( \alpha \geq 0.25(0.5 + \delta)^2 \), the Newmark algorithm preserves unconditional stability, which means that \( \Delta t \) does not affect the solution [73]. At the initial time, \( p_0 = \dot{p}_0 = \ddot{p}_0 = 0 \). Matrix \([ A] \) is a sparse \( N \times N \) matrix, where \( N \) is the number of fine mesh nodes; \( \{ b \} \) and \( \{ p \} \) are column vector. Thus, the problem of solving Equation 3-1 in the forward problem becomes solving the sparse linear system of Equation 3-4.

**Inverse model**

In order to form an image from the presumably uniform initial guess of the absorbed energy density distribution, the least-squares minimization: \( F(p, \Phi) = \sum_{j=1}^{M} (p_j^o - p_j^c)^2 \) was employed to update \( \Phi \) from its initial value, where \( p_j^o \) and \( p_j^c \) are the observed and computed values of the acoustic pressure at \( M \) boundary locations. Using iterative Newton’s method, we attained the following equation:
\[ (J_t^T J_t + \lambda I) \Delta \chi = J_t^T (p_t^o - p_t^c) \]

(3-6)

where \( \Delta \chi \) is the update vector for \( \Phi \); \( \lambda = 0.8 \) is the regularization parameter determined by Tikhonov and Marquardt regularization schemes; \( I \) is the identity matrix; \( p_t^o = \)
\( (p_1^0, p_2^0, p_3^0, \ldots, p_M^0)^T \); \( p_i^c = (p_1^c, p_2^c, p_3^c, \ldots, p_M^c)^T \); and \( J \) is the Jacobian matrix consisting of \( \partial p/\partial \Phi \) at \( M \) boundary locations. The Jacobian matrix is formed with submatrix for \( n \) time steps.

Through the iterative solution of Equation 3-6 and Equation 3-1, we can update the absorption energy density \( \Phi \) by minimizing the sum of least square error of the computed and measured acoustic data on the boundary measurement sites.

**Acceleration of the TD-qPAT based on Graphics Processing Unit (GPU)**

Based on the intrinsic nature of a high degree of locality for each element in our FEM TD-qPAT algorithm, massive parallelization can be realized to achieve high computational speed. We implemented a GPU-based parallelized code with NVIDIA’s Common Unified Device Architecture (CUDA), which provides a unified hardware and software solution for parallel computing. In this work, we implemented the GPU-accelerated code using the C programming language based on the linear algebra library cuSPARSE, cuBLAS, and the CULA dense library, which provide the linear algebra operations on matrix and vectors for sparse and dense linear systems.

Based on the forward model discussed above, two procedures would be carried out many times during the computation: assembling the vector \( b \) in Equation 3-4 and solving the linear matrix equation. The matrix \( A \) in Equation 3-4 is highly sparse, thus, methods for solving a sparse matrix linear system can be applied here. In this work, we implemented an iterative conjugate gradient solver with preconditioning. In general, the convergence of an iterative method highly depends on the spectrum of the coefficient matrix \( A \) and can be greatly improved by using a preconditioner, which modifies the coefficient matrix to reduce the steps for convergence. The compressed sparse row (CSR) format with low memory requirements was applied to reduce the required memory and accelerate the sparse matrix-vector multiplication for the conjugate gradient method. A single-precision solver was used to reduce storage
requirements and to improve computational speed. A set of linear algebra operations needed in
the algorithm was produced with the application of the cuSPARSE and cuBLAS library.

In the inverse model, the Hessian matrix, which is obtained by dense matrix
multiplication $J^TJ$, was computed using a dense matrix multiplication solver culaDeviceSgemm
from the CULA dense library. To solve the dense linear system of Equation 3-6, we used a direct
solver with Cholesky decomposition. A device interface was applied in order to manually
transfer data and reduce unnecessary data exchange between CPU and GPU. Tasks involved in
the forward and inverse models that do not require a significant amount of time, such as
generating global matrix A, CSR format transformation, and assembly of RHS, were also
parallelized with customized codes. Fast shared memory was used to avoid accessing global
memory directly. Page-lock memory was used for achieving higher bandwidth between host and
device.

One of the most time-consuming and memory-intensive procedures is the computation of
the Jacobian matrix for solving Equation 3-6 in the inverse model [68]. Thus, the parallelization
of this module is critical for accelerating the TD-qPAT algorithm. Based on the high locality for
calculation of $\partial p/\partial \Phi$, a significant speed up can be achieved using a parallelized code. CUDA
offers the two-level hierarchy: grid and thread blocks. A thread block is an array of threads that
cooperate with each other. Thread blocks provide coarse-grained scalable parallelism and can be
executed independently, allowing scalability to GPUs with different numbers of processor cores.
In the block level, CUDA uses threads for fine-grained parallelism, by sharing data through
shared memory and synchronizing their executions. In our parallelized code, the assembly of the
Jacobean matrix was divided into many blocks, which were executed simultaneously. In
In addition, we needed to use several kernels sequentially to calculate all $N_0$ columns of the Jacobian matrix. Each kernel determined a subset of $N_0$ nodes for all rows.

The implemented GPU-accelerated code was validated using three different sets of phantom data with one, two, and four targets having different absorption coefficients. The reconstructed images of absorption energy density were compared with that using our existing unparalleled CPU-based code. Computation times taken on CPU and GPU with different mesh densities were evaluated.

**Impact of Mesh Density and Time Interval on Reconstructed Image Quality**

In our TD-qPAT algorithm, a dual-mesh scheme was applied for fast yet accurate inverse computation [74]. The fine mesh was used in the forward model for accurate calculation of wave propagation, while the coarse mesh was employed in the inverse model for parameter recovery. In the FEM-based reconstruction algorithm, mesh density (spatial sampling rate) used to discretize the problem domain of interest is critical, and largely determines the resolution and background artifacts of the reconstructed images, as well as the computational cost. Thus, understanding the impact of fine mesh and coarse mesh density on the image quality is necessary for optimal image reconstruction using our TD-qPAT algorithm. Taking advantage of our accelerated algorithm, we were able to perform massive computations in a short amount of time. In this work, we computed the phantom data with a set of dual-mesh combinations with different fine mesh and coarse mesh densities. The reconstructed absorption coefficient images are evaluated. The parameters for the dual meshes used are listed in Table 3-1.

In addition, the time interval (temporal sampling rate) chosen for the image reconstruction using the Newmark time-stepping scheme, with respect to the mesh density (spatial sampling rate), has a significant impact on the reconstructed image quality. Here we investigated a set of time intervals with dual meshes having different mesh densities.
Adaptive Meshing

To achieve further improvement in image quality and computational efficiency, an adaptive meshing scheme is adopted. The mesh adaptation is achieved by locally refining the region of heterogeneity [74]. Adaptive mesh refinement provides fine mesh resolution in the target region and reduces mesh nodes in other regions to improve image quality and computational economy while preserving solution stability. Reconstructed image quality and computation time are both evaluated and compared with the conventional uniform meshing scheme.

Phantom Experiment Results

Three phantom experiments were conducted. In the first experiment, we embedded two targets with a diameter of 2 mm and an absorption coefficient of 0.05 mm$^{-1}$ each in a 51 mm diameter solid background phantom, consisting of TiO2 and India ink. Agar powder was used for solidifying the phantom solution. The background absorption coefficient was 0.01 mm$^{-1}$. In the second experiment, one target of 2 mm diameter with an absorption coefficient of 0.032 mm$^{-1}$ was embedded in the background phantom. In the third experiment, we embedded four targets with different absorption coefficients: 0.08, 0.08, 0.07, and 0.045 mm$^{-1}$, respectively, in the background phantom. Each target had a size of 1 mm in diameter. PAT images in 2D were formed using our GPU-based FEM reconstruction algorithm.

Evaluation of GPU-Accelerated TD-qPAT

We validated the GPU-accelerated code with the first two sets of phantom experimental data using different meshes and compared the reconstructions with our existing CPU-based code. All the images obtained are the results of three iterations. Figure 3-1 shows the reconstructed absorbed energy density images for the two experimental cases with Mesh 1 (Table 3-1). From the results, we can see that all the targets can be clearly reconstructed with the correct position
and target size. The reconstructed images obtained with GPU-accelerated code are visually identical to the images obtained from the CPU-based code. Table 3-2 lists the computation times taken by CPU and GPU codes per iteration with respect to different mesh sizes, and corresponding speed-ups (i.e., the ratio between computation times of GPU and CPU). All the data was computed with a high-end GPU: NVIDIA TITAN X, and CPU: i5-4460, 3.2 GHz. A numerical comparison of the relative difference between the CPU- and GPU-based reconstructed results was conducted with the following equation:

\[
E = \frac{1}{N} \sum_{i=1}^{N} \left| \frac{R_{\text{gpu},i} - R_{\text{cpu},i}}{R_{\text{cpu},i}} \right| \times 100\% \quad (3-7)
\]

We note that the error in all cases was less than 0.1%, asserting that our single-precision GPU-based code provides sufficient accuracy. It shows that GPU computing offers a significant improvement in computation time compared to CPU computing. For a smaller mesh pair of 1525 coarse mesh nodes and 5977 fine mesh nodes, it took only 2 min to generate one full set of images using the GPU-based code, while the CPU-based code took 161 min. For a larger mesh pair of 1525 coarse mesh nodes and 23,665 fine mesh nodes, it took only 8 min for the GPU-based code, while it required 1088.6 min for the CPU-based code to reconstruct one full set of images. The acceleration ratio increased with increasing mesh size. The speed-up using an NVIDIA TITAN X GPU increased from 80.5-fold to 134.4-fold as the node number of fine mesh changed from 5977 to 23,665. It indicates that the parallel computing code provides an advantage of dealing with a larger mesh.
Evaluation of Impacts of Mesh Density and Time Interval on Reconstructed Image Quality

Impact of coarse and fine mesh density on image quality

The experimental data of the phantom with four targets having different absorption coefficients (Case 3) was reconstructed using dual meshes with different mesh densities (Table 3-1). The reconstructed optical absorption coefficient images were evaluated both qualitatively and quantitatively. Figure 3-2 shows the reconstructed optical absorption coefficient image and the quantitative profiles along lines crossing the centers of the four targets using Mesh 3. From Figure 3-2, the four targets were clearly reconstructed with accurate size, position, and optical properties. Figure 3-3 presents the reconstructed absorption coefficient images and the quantitative optical property profiles using dual meshes with different fine mesh and coarse mesh densities. Table 3-3 lists the reconstructed target size using different meshes estimated by calculating the full width half maximum of the absorption property profiles. The actual target size was 1 mm in diameter.

Impact of time interval on image quality

Different time intervals (from 80 to 400 ns) were used to reconstruct phantom data (Case 1) using dual meshes with different mesh densities. Figure 3-4 presents the reconstructed optical absorption coefficient images with different time intervals using Mesh 1 and Mesh 4.

Evaluation of Adaptive Mesh

The experimental data of phantom Case 3 was also processed to evaluate the adaptive meshing-based reconstruction. Figure 3-5 shows the reconstructed optical absorption coefficient images and quantitative profiles of four targets using both the adaptive meshing and uniform meshing. In this case, the adaptive meshing-based reconstruction provided the same image quality and accuracy of optical absorption properties as the uniform meshing, while the
computation time taken by the adaptive meshing-based reconstruction was 4.6 min, a significant improvement over the uniform meshing-based reconstruction, which required 12 min.

**Discussion**

As discussed above, the primary limitation for the FEM-based TD-qPAT is the massive computational cost. In our previous breast cancer study using TD-qPAT, it took about several hours on average to reconstruct one set of data. Due to the high computational cost, it is thus unrealistic to apply this powerful functional imaging tool to any clinical studies with a reasonable number of subjects. With the dramatic improvement in computation time using our parallelization approach, it now takes only a few minutes to obtain one set of images. Thus, applying this quantitative photoacoustic tomography to clinical studies like breast cancer detection becomes feasible. In addition, by taking advantage of this accelerated algorithm, we can perform optimization associated with the mesh sizes/meshing schemes for improved quality of image reconstruction.

From Figure 3-2, we can see that the absorption coefficient image can be reconstructed with accurate target size, shape, position, and optical property. In the dual-mesh scheme, the coarse mesh is for parameter recovery and the fine mesh is for accurate calculation of pressure wave propagation. As shown in Figure 3-3, as the fine mesh density increased, the artifacts reduced in the images. This is more obvious when comparing it with low mesh density. For fine mesh with high density, the improvement is not noticeable. This is due to the reason that accurate calculation of pressure files requires a sufficient fine mesh density, while the finite number of known boundary data limits the use of overly fine mesh size. When the coarse mesh density was decreased, the reconstructed target shape and size became inaccurate, even if the fine mesh density was high. As the optical property files are defined on the coarse mesh, it is reasonable that the coarse mesh density would have more impact on the resolution of the reconstructed
image. As there is a trade-off between the image reconstruction and computation time, we need to optimize the dual-mesh combination to achieve fast yet accurate reconstruction. Based on the results computed with different coarse and fine mesh densities, the best result considering time and accuracy is achieved using a coarse mesh size of over 3600 nodes, and a fine mesh size of over 14,000 nodes for a 5 cm diameter problem domain, which costs a computation time of about 12 min for the GPU algorithm.

Another factor critical for achieving high-quality image reconstruction is the time interval (temporal sampling rate). Figure 3-4 presents the reconstructed absorption coefficient images with different time intervals using fine meshes of 5977 nodes and 23,665 nodes for comparison. From Fig. 5, we observed that for higher mesh density (spatial sampling rate), it required a higher temporal sampling rate or smaller time interval to achieve high image quality. For lower mesh density, the requirement for temporal sampling rate was not as high as for a finer mesh. This is reasonable because the least needed time step $\Delta t$ is related to the time that the fastest wave needs to propagate between the successive nodes of the mesh. Thus, the higher the mesh density is, the smaller the time step is required. To achieve an optimal image reconstruction, one needs to choose an optimal time interval for given mesh size.

In order to further optimize the meshing and improve image reconstruction, an adaptive mesh refinement scheme was studied. Figure 3-5 A and B present the reconstructed optical property images using adaptive and uniform meshes, respectively. The comparison of quantitative profiles for the four reconstructed targets is shown in Figure 3-5 C-F. Adaptive meshing was achieved by locally refining the heterogeneity, which resulted in high mesh density in the target region while reducing the total mesh nodes needed for computation. The results
show that adaptive meshing significantly reduced the computation time (from 14 to 4.6 min) while preserving the same accurate reconstruction.

**Conclusion**

In summary, we have developed a GPU accelerated FEM-based TD-qPAT approach along with optimization of image reconstruction, and successfully demonstrated its performance. The computation time was dramatically reduced, and image quality was significantly improved. The work presented here suggests that the GPU-accelerated TD-qPAT can be a promising powerful functional imaging tool for clinical studies with a significant number of subjects.

![Comparison of reconstructed absorption energy density images using GPU and CPU. A) and C) results using GPU; B) and D) results using CPU; A) and B) for Case 1; C) and D) for Case 2.](image-url)
Figure 3-2. Reconstructed optical absorption coefficient image. A) and quantitative profile B) for phantom data case 3, using Mesh 3.

Figure 3-3. Reconstructed optical absorption coefficient images using different dual-meshes. A) Mesh 1 (1525-5977); B) Mesh 4 (1525-23665); C) Mesh 2 (2457-9705); D) Mesh 3 (3627-14325); E)-G) are comparison of absorption coefficient profiles: E) Mesh 1 and Mesh 4 for target 1; F) Mesh 3 and Mesh 4 for target 3; G) Mesh 1 and Mesh 3 for target 1.
Figure 3-4. Reconstructed optical absorption coefficient images using Mesh 1 (1525-5977). A)-C) and Mesh 4 (1525_23665); D)-F) with different time intervals: A), D): 350 ns; B), E): 150 ns; C), F): 60 ns.
Figure 3-5. Reconstructed optical absorption coefficient images. A) adaptive meshing; B) uniform meshing. C)-F): quantitative profiles along transects crossing the centers of the four targets.

Table 3-1. List of dual-mesh parameters used in mesh density and time interval evaluation.

<table>
<thead>
<tr>
<th>Dual-mesh</th>
<th>Node number of coarse</th>
<th>Node number of fine mesh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesh 1</td>
<td>1525</td>
<td>5977</td>
</tr>
<tr>
<td>Mesh 2</td>
<td>2457</td>
<td>9705</td>
</tr>
<tr>
<td>Mesh 3</td>
<td>3627</td>
<td>14325</td>
</tr>
<tr>
<td>Mesh 4</td>
<td>1525</td>
<td>23665</td>
</tr>
<tr>
<td>Mesh 5</td>
<td>5977</td>
<td>23665</td>
</tr>
</tbody>
</table>
Table 3-2. Performance comparison between GPU- and CPU-based reconstructions.

<table>
<thead>
<tr>
<th>Dual-mesh pair #</th>
<th>GPU (mins)</th>
<th>CPU (mins)</th>
<th>Speedup</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1525_5977</td>
<td>2.1</td>
<td>169.1</td>
<td>80.5</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>2457_9705</td>
<td>6.3</td>
<td>580.2</td>
<td>92.1</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>3627_14325</td>
<td>12</td>
<td>1280</td>
<td>106.6</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>1525_23665</td>
<td>8.1</td>
<td>1088.6</td>
<td>134.3</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>5977_23665</td>
<td>31</td>
<td>42663</td>
<td>137.6</td>
<td>&lt;0.1%</td>
</tr>
</tbody>
</table>

Table 3-3. Reconstructed target size (mm) using different meshes.

<table>
<thead>
<tr>
<th>Mesh #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target 1</td>
<td>2.02</td>
<td>1.30</td>
<td>1.06</td>
<td>1.80</td>
<td>1.00</td>
</tr>
<tr>
<td>Target 2</td>
<td>1.98</td>
<td>1.35</td>
<td>1.01</td>
<td>2.01</td>
<td>1.00</td>
</tr>
<tr>
<td>Target 3</td>
<td>2.00</td>
<td>1.27</td>
<td>1.07</td>
<td>1.98</td>
<td>1.03</td>
</tr>
<tr>
<td>Target 4</td>
<td>2.04</td>
<td>1.40</td>
<td>1.08</td>
<td>2.02</td>
<td>1.05</td>
</tr>
</tbody>
</table>
CHAPTER 4
NOVEL PHOTOACOUSTIC IMAGING APPROACHES

Novel Imaging Reconstruction Algorithm

Photoacoustic imaging (PAI) is one of the fastest-growing biomedical optical imaging techniques capable of providing nonionizing and noninvasive imaging of tissue absorption with high spatial resolution and has been demonstrated in biological and clinical applications [75]. Among all the imaging techniques, PAI has the unique advantages of high optical absorption contrast and ultrasonic spatial resolution, as well as real-time, non-invasive functional imaging ability. It has great potential in various clinical and research applications, such as brain imaging [28, 76, 77], cancer detection [78, 79], arthritis diagnosis [80, 81] and pathological analysis [77]. However, the image quality largely depends on the signal to noise ratio (SNR), and reconstruction algorithm. In deep tissue imaging, like in the neonatal brain, the SNR is very low due to the strong attenuation of brain tissue. To improve image quality, a large number of detectors is required. It is not very feasible due to the high cost of detectors and multi-channel DAQ system. Scanning can be applied instead of physically increasing the detector numbers, however, it sacrifices the temporal resolution, which significantly limits its applications in functional dynamic brain imaging.

In our previous study, we developed an array system with 256 detectors and optimized the system to improve SNR (Chapter 2). We have demonstrated its ability in fetal brain imaging in the uterus using a murine prenatal alcohol exposure model (Chapter 5). However, the cost of the system is high due to a large number of elements and multi-channel DAQ. In addition, the sensitivity of the ultrasound transducer depends on the size of the elements, to ensemble a large number of detectors, it is hard to minimize the size of the probe. Especially in 3D imaging probe which requires a large number of detector elements to obtain good image quality, reducing
detector number is very demanding. The high-frequency PA signal decreases fast as it propagates in water or tissue, the larger size of the probe would increase the distance between the source and detectors, resulting in reduced image quality. Therefore, a new approach to improve the image quality in terms of resolution and contrast, as well as to reduce the number of detectors for deeper imaging depth is highly desirable.

In addition to improving system design, the PAI image quality also greatly depends on the reconstruction algorithm. Delay-and-sum (DAS) beamforming method is the most commonly used algorithm for PAI image reconstruction. The DAS sums the PA signals based on the time delays calculated by the distance between the source and detectors. The DAS has the advantage of simplicity, however, it has some drawbacks, such as low resolution and low contrast. In addition, it requires a large number of detectors to reduce the artifacts induced by the algorithm. Efforts have been made to improve the DAS algorithm. Delay-multiply-and-sum (DMAS) was developed in 2015 by Matrone et al. [82], which improved the image quality to some extent. In this study, we developed a new reconstruction approach based on DAS, DMAS and by taking account of the contribution of virtual elements of the entire detector aperture. We validated the algorithm with phantom and in-vivo animal experiments. The results showed a great improvement of image quality with a significantly reduced number of detectors.

Materials and Methods

The DAS beamforming can be expressed as follows:

\[ S_{DAS}(t) = \sum_{i=1}^{N} s_i(t + \Delta t_i) \tag{4-1} \]

Where \( S_{DAS} \) is the output of the DAS, and \( s_i(t + \Delta t_i) \) is the signal detected by the ith detector with the corresponding time delay \( \Delta t_i \). In DMAS, to after the time-shifting of the
received signals as in DAS, the signals are combinatorially coupled and multiplied. The DMAS can be defined as follows:

\[ S_{DMAS}(t) = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} s_i(t)s_j(t) \]  

(4-2)

It can be further modified as:

\[ \hat{s}_{ij}(t) = \text{sign} \left( s_i(t)s_j(t) \right) \cdot \sqrt{|s_i(t)s_j(t)|} \]  

\[ S_{DMAS}(t) = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \hat{s}_{ij}(t) = \sum_{n=1}^{(N^2-N)/2} \hat{s}_n(t) \]  

(4-4)

where \( S_{DMAS}(t) \) is the output of DMAS; \( N \) is the number of detectors, and there is \( (N^2-N)/2 \) multiplications; \( s_i(t) \) is the delayed PA signal received by the \( i \)th detector. This operation is an auto-correlation function of the received aperture [82].

In this study, to improve the DAS, the virtual element algorithm is developed. In the conventional DAS algorithm, the initial pressure rise can be solved in the time domain by back-projection of the received pressure wave. It is known that the photoacoustic signal collected by a transducer is the integral of the acoustic wave pressure over the entire active surface of the transducer [83]. Instead of taking the transducer as a point detector like in DAS and DMAS, in this virtual element approach, we divide receiving aperture into a set of virtual elements. The virtual signal for each virtual element can be expressed as

\[ s_{kl}(t_{kl}) = s_k(t_{kl}) \frac{S_l}{S} \]  

(4-5)

where \( s_{kl}(t) \) is the collected acoustic signal at virtual element \( I \) for the \( k \)th detector; \( t_{kl} = \frac{|\vec{r}-\vec{r}_{kl}|}{c} \) is the time for the initial pressure wave at position \( \vec{r} \) to reach virtual element \( I \) at position \( \vec{r}_{kl} \) at
scanning position \( k \); \( s_k \) is the signal collected by the physical element (i.e., the transducer); \( S_i \) is the size of the virtual element \( i \); \( S \) is the active size of the physical element;

The distance between two adjacent virtual elements is defined as \( \lambda/4 \), where \( \lambda \) is the acoustic wavelength in the water at the center frequency of the transducer. The initial pressure \( p_0(\vec{r}) \) at position \( \vec{r} \) is reconstructed by back-projection of the virtual signals received by all the virtual elements, which can be expressed as

\[
S_{\text{Virtual}} = \sum_{k=1}^{N} \sum_{i=1}^{n} s_{ki} (\vec{r}, t_{ki})
\]

where \( n \) is the number of virtual elements in a detector, and \( N \) is the total number of detectors.

To further eliminate the artifacts and noise, we applied the idea of the auto-correlation function of the received aperture into the virtual element algorithm. The received aperture is divided into virtual elements and the signal and autocorrelated. The algorithm can be expressed as follows:

\[
s_{ij, \text{virtual}} = \text{sign}(s_{i, \text{virtual}}) \sqrt{|s_{i, \text{virtual}} \ast s_{j, \text{virtual}}|}
\]

\[
S_{\text{Virtual}}(t) = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} s_{ij, \text{virtual}}(t)
\]

The PA signal contains both strong positive and negative signals. If the multiplication involves two negative values, then the sign would be negated, which makes it unable to differentiate strong positive and negative signals. In other to remain the sign of \( s_{i, \text{virtual}} \), in this algorithm, instead of using the sign of the multiplication, we use the sign of the \( s_{i, \text{virtual}} \), which retains the dimensionality of the input signals without losing its sign.

To further improve the focusing quality, a coherence factor (CF) can be applied in the reconstruction approach. The signal coherence is defined as
Results and Discussion

To validate the new algorithm, phantom and in-vivo experiments were conducted. Three sets of phantom experiments were conducted using human hair embedded in tissue-mimicking phantom media: 1) 5 hair vertically inserted in the phantom media, 2) one hair horizontally embedded in the phantom media, 3) two hairs horizontally embedded in the phantom media. 750 nm laser pulse was used to excite the PA signals. The phantom was circularly scanned by a 3.5 MHz ultrasound transducer. Figure 4-1A-C shows the results of the first phantom experiments. Figure 4-1 A is the DAS results reconstructed using 10 detector signals, B is the image reconstructed by the new algorithm using 10 detector signals, C is the intensity profile along the dashed line, the blue profile is DAS results using 10 detectors, the red profile is the results of the new algorithm using 10 detectors, the green profile is the DAS result using 360 detectors. The hair size is estimated using the full-width-half-maximum (FWHM). The FWHM for DAS (10 detectors), DAS (360 detectors) and the new algorithm (10 detectors) are 0.1949mm, 0.1693mm, and 0.1204mm. Figure 4-1 D and E are results for experiment 2 and 3. D is the results reconstructed using DAS with 10 detectors, and E is the results reconstructed by the new algorithm with 10 detectors. The artifacts in DAS image are due to the insufficient number of detectors and transducer aperture. The artifacts are significantly reduced in the new algorithm which takes account of the entire receiving aperture. Comparing to DAS, the results showed a significant improvement in image quality in term of resolution and contrast. Usually, a circular PAT scan requires more than 120 detecting positions to obtain sufficient imaging quality. The results showed a substantial reduction in detector number. To further validate this algorithm, we
conducted in-vivo animal experiments. Two mouse brains with imaged and the results are shown in Figure 4-2. A and D are DAS results reconstructed using 360 detectors for mouse 1 and 2, respectively. B and E are DAS results using 60 detectors and C and F are results of the new algorithm using 60 detectors. Comparing to the DAS results with a limited number of detectors, the new algorithm can better recover the small blood vessels and reduce the artifacts. It can provide comparable image quality with a significantly reduced detector number.

Figure 4-1. Photoacoustic images of phantom experiments. A), B) PAT images of five vertically placed hairs reconstructed using DAS and new virtual element algorithm, respectively; C) intensity profile along the red dashed line in A and B; D) DAS results of phantom with one and two horizontally placed hairs; E) Results of new virtual element algorithm for phantom with one and two horizontally placed hairs.
Conclusion

We developed a reconstruction algorithm that can be used for improving the image quality and reducing the number of detectors. PAI of deep brain imaging requires a large number of detectors, which increases the economic burden and system complicity. The new approach can provide comparable image quality with a limited number of detectors, which has great potential in applications like deep brain imaging. It also has a potential application in minimizing detector numbers in a 3D imaging probe.
Fan-shaped Scanning Approach for minimized Photoacoustic Tomography

To date, there are two major challenges that preclude the advancement of PAT imaging in human brain imaging which are the limited FOV and penetration depth. The conventional linear or circularly scanning based PAT provides a very small effective imaging region or field of view (FOV). In addition, as the skull has a significant impact on ultrasound propagation [84, 85], it is very hard to use conventional PAT to image the human brain. For human neonatal brain imaging, the anterior fontanelle of the infant skull provides an opportunity for PAT imaging with less impact of the skull. However, the conventional circularly or linear scanning is not very suitable, as the PA signal has to penetrate through the skull to reach the detectors. Therefore, developing a new imaging approach that minimizes the impact of the skull is desired. In this study, we proposed a novel scanning approach based on the fun-shaped scanning of a single transducer at one or two discrete positions. Another challenge is that there is not an effective image reconstruction algorithm that is readily available. For example, delay and sum (DAS), which is the most commonly used reconstruction algorithm, is typically implemented for the configuration of linear or circular scanning PAT, and not suitable for a fan-shaped scanning PAT. We applied the new reconstruction algorithm (discussed in the previous section) to realize our new scanning approach. We demonstrated this approach using phantom and animal experiments.

Fan-Shaped Scanning Approach

The fan-shaped scanning approach is schematically shown in Figure 4-1. To demonstrate this approach, a single element acoustic transducer (Valpey Fisher, Hopkinton, Massachusetts), having an effective diameter of 6 mm with a center frequency of 1 MHz, was used as the detector to receive photoacoustic (PA) signal from a sample/target immersed in water upon absorption of light emitted by an OPO laser at 780 nm (Phocus Mobile, Opoteck, Carlsbad,
The transducer placed on a rotation stage (Newmark, California) revolved around the center of the transducer surface to realize the fan-shaped scanning and to detect the PA signals from different directions. The scanning covered an angle of 120° with an interval of 0.1 mm, providing a large FOV. It took 20 minutes to complete the scanning, which was limited by the rotating speed of the rotator used in this system.

We hypothesize that the number of detector positions will affect the resulting PAT image quality. Therefore, two scanning schemes (A and B) were performed and compared, in which scheme A had only one detector position while scheme B had two discrete detector positions with a separation of 4 mm. The PA signal was acquired by a data acquisition (DAQ) board (NI, 5152) controlled by a LabVIEW program.

Figure 4-3. Schematic of fan-shaped scanning approach for PAT. L stands for the distance between the transducer and the target.
Image Reconstruction

In the conventional DAS algorithm, the initial pressure rise can be solved in the time domain by back-projection of the received pressure wave. The transducer is considered as a point detector located at the center of the transducer surface. To reconstruct an image, the center of the transducer and the object of interest (OOI) must have relative displacement to provide a difference in delay time. However, in our fan-shaped scanning scheme for PAT, as the center of the transducer is fixed, there is no relative displacement between the transducer center and the OOI. Thus, the traditional DAS approach is not suitable for the fan-shaped scanning configuration.

It is known that the photoacoustic signal collected by a transducer is the integral of the acoustic wave pressure over the entire active surface of the transducer [83]. We applied our new imaging algorithm in this application for image reconstruction. The schematic is shown in Figure 4-4. As the transducer rotates, it provides the difference in delay time between the virtual elements, which allows the reconstruction of a photoacoustic image.

![Figure 4-4. Schematic of conventional DAS vs. virtual element reconstruction approach.](image)

Result and Discussion

To validate the fan-shaped scanning approach, two sets of phantom experiments were performed. Figure 4-3 A shows the photograph of the phantom with three targets (pencil leads,
0.5 mm in diameter each), in which the three pencil leads were embedded in phantom in a vertical line with a separation of 2 mm in depth for any two adjacent targets. The distance between the transducer and the top target was 7 mm. Figure 4-3 B presents the PAT images recovered using the conventional DAS, and Figure 4-3 C, D presents the virtual signal approach with scanning schemes A and B, respectively, where we see that the targets are recovered with entirely incorrect dimensions using the conventional DAS, that the targets can be detected with appreciable dimensions using the virtual signal approach with scanning scheme A, and that the targets can be reconstructed with accurate dimensions using the virtual signal approach with scanning scheme B. Figure 4-3D shows the intensity profiles across the center of each target along the white dashed lines shown in Figure 4-3 C. The full width at half maximum (FWHM) of these profiles was calculated to be 1.78, 1.57, and 2.42 mm for targets 1, 2 and 3, respectively, using scheme A, and 0.5638, 0.5428, and 0.5448 mm for targets 1, 2 and 3, respectively, using scheme B.

In the second phantom experiment, three pencil leads were embedded in the phantom in a triangular shape as shown in Figure 4-4 A. The distance between the transducer and the closest target is 14 mm. Figure 4-4 gives the PAT images for this phantom experiment. Again, we see that the virtual signal approach using scanning B provides the best quality of image (right, Figure 4C), while the targets are incorrectly recovered using the conventional DAS (Figure 4-4 B). Figure 4-3D shows the intensity profiles along the white dashed line across each target center. The FWHM of each recovered target was calculated to be 1.74, 2.21, and 2.64 mm for targets 1, 2 and 3, respectively, using scheme A, and 1.13, 1.56, 1.78 mm for targets 1, 2 and 3, respectively, using scheme B. We notice that the resolution is lower than that for the first experiment. This is because the targets were located farther away from the detector in the case of
the second experiment, resulting in lower signal-to-noise ratio. In other words, the spatial resolution based on this new fan-shaped scanning approach is related to the distance between the target and transducer, that is, the larger this distance and the poorer the spatial resolution. The reason is that when the target is closer to the transducer the signal from the target can be detected at more scanning angles/positions for more effective image reconstruction and that the signal to noise ratio is larger in this case. We also notice that for both the experiments, the conventional DAS cannot deliver a well-reconstructed image (Figures 4-3 B and 4-4 B). The new approach can provide a much better lateral resolution in both cases.

To further demonstrate the ability of the fan-shaped scanning approach, we conducted experiments using two mice bearing patient tissue-derived xenograft (PDX) tumor administrated with near-infrared (NIR) 830-ATFironoxidate nanoparticles (IONP) solution as contrast agent [86, 87]. The PAT images obtained for the two animals using scheme B are shown in Figure 4-5. We can see that the tumors are recovered clearly for both cases. The intensity profiles along the dashed lines across the tumor center are given in Figure 4-5 C, D, G, H. From these profiles, the sizes of the tumors were photoacoustically measured to be 6.06 and 4.65 mm, which are in good agreement with the actual tumor sizes. The results from both the phantom and animal experiments have demonstrated the advantages of this novel fan-shaped scanning approach for miniaturized PAT with a large FOV. Compared to the miniaturized transducer array-based PAT system [56, 88], our new approach would provide an inexpensive alternative as it avoids the use of costly transducer array and the associated multichannel data acquisition system. Meanwhile, the use of a larger transducer element in our approach would provide significantly better sensitivity and imaging depth. We note that the fan-shaped scanning of a single transducer certainly is relatively slow and offers a lower temporal resolution. This, however, can be
significantly improved by the combination of a single transducer with a fast scanning mechanism such as MEMS mirror scanning of the ultrasound beam.

**Conclusion**

In summary, we have described and explored a new scanning approach to realize miniaturized PAT along with a virtual element reconstruction algorithm. Several phantom and animal experiments were conducted, and the results obtained indicate that this fan-shaped scanning approach is capable of imaging small targets located in deep tissue. This work would produce at least the following three significant impacts: (a) A highly miniaturized handheld probe potentially to be used for imaging neonatal brain through the anterior fontanelle of the infant skull; (b) A large effective imaging region or FOV could be realized using the approach described, and (c) A high detector sensitivity and deep penetration depth could be obtained by utilizing one or two transducers having a relatively large size. In addition, another application of an endoscopic PAT system could be achieved using the fan-shaped scanning configuration.
Figure 4-5. Photoacoustic imaging of three targets embedded in the background phantom along a vertical line in depth direction. A) Photography of the phantom with three vertically arranged pencil leads (0.5mm diameter) (Photo courtesy of author). Recovered PA images by using conventional DAS B) and the virtual signal reconstruction approach C), respectively; D) Intensity profiles of the recovered three targets along the white dashed line across the center of each target shown in c. T1-T3 indicate targets 1-3.
Figure 4-6. Photoacoustic imaging of three targets embedded in the phantom in a triangular shape. A) Photography of the phantom with three pencil leads (0.5mm diameter) (Photo courtesy of author). Recovered PAT images using conventional DAS B) and the virtual signal reconstruction approach C), respectively; D) Intensity profiles of the three targets along the white line across the center of each target shown in c. T1-T3 indicate targets 1-3.

Figure 4-7. Photoacoustic imaging of two mice bearing PDX tumors. Photographs of tumor-bearing mice A), E) (Photo courtesy of author); PAT images of the tumors for the two mice B), F); Intensity profiles of the tumors along the dashed lines 1 and 2 shown in B) (C, D), and along the dashed lines 1 and 2 in F (G, H), respectively.
CHAPTER 5
PHOTOACUSOTIC IMAGING OF ACUTE PRENATAL ETHANOL EXPOSURE IN FETAL BRAIN

Motivation

The adverse effects of prenatal ethanol exposure (PEE) on the developing fetus lead to a spectrum of structural abnormalities, behavioral defects, and neurocognitive disabilities, which is termed fetal alcohol spectrum disorders (FASD) [89, 90]. In the United States, FASD represents the leading preventable cause of neurodevelopmental delay and birth defects [91]. FASD is estimated to affect at least 1% (40,000 births) of all births every year in the U.S [92]. Moreover, recent studies showed that 2-5% of younger school-age children in the U.S. have FASD [93, 94]. The prevalence of FASD ranges from 20 to 50 per 1,000 live births in worldwide studies of school-age children [95, 96]. In the United States, 1 in 10 pregnant women admits to ethanol (EtOH) consumption [97]. The risk for binge EtOH exposure is high during the first to the second trimester, which is also the critical period for neurogenesis and angiogenesis in the fetal brain [98, 99]. Unfortunately, the lifelong consequences of prenatal EtOH exposure are not readily curable and cause a harsh economic burden [100, 101]. It is widely believed that early detection of FASD and its subsequent intervention strategies are critical to allow the earliest and most effective intervention for both the mother and infant and to reduce the development of adverse consequences and associated costs [102].

Understanding the mechanisms of how maternal EtOH consumption affects the fetal brain can provide us with useful insights for early detection of FASD. Vasculature and blood perfusion alterations are expected to compromise the delivery of nutrients and the removal of metabolites from neurons. Thus, such alterations result in hindered neuronal development and function. Previously, major research efforts have mainly been focused on EtOH-induced neuronal damage in the fetal brain [103-108], with only a few studies that have aimed to
understand the role of cerebral perfusion and acute vasculature changes in FASD [1, 109, 110]. Moreover, understanding the correlation between cerebral perfusion and acute vasculature change will further elucidate the pathophysiology of FASD.

One of the primary challenges lies in the imaging technique. Suitable imaging techniques that can perform non-invasive high-resolution vascular and hemodynamics imaging in small animal models are highly demanded. Current imaging techniques that have been used in FASD are micro-MRA, micro-CT, ultrasound Doppler imaging, and OCT [1-6]. As discussed in Chapter 1, they all have many limitations in fetal vascular and hemodynamics imaging, such as using contrast agents, costly, low resolution, shallow imaging depth, or inability to perform non-invasive in-vivo imaging. Among these imaging techniques, OCT has the best performance in vascular imaging, however, its shallow imaging depth (hundreds of micrometers), significantly limits its application. It cannot non-invasively image embryo in the deep uterus. An incision must be done to expose the embryo and forceps are used to fix the embryo for imaging. This causes many problems, like affecting maternal-fetal, causing occlusion in fetal blood vessels, causing dehydration artifacts[25,28]. Even with the incision, OCT can only image the superficial vasculatures and is not able to image the whole embryo. Another limitation is the inability to perform real-time imaging as scanning is needed, which usually takes several minutes to finish a 2-D scan. This significantly limits its application in monitoring brain hemodynamics and neural activities in real-time. In addition, motion artifacts cannot be avoided and post-processing is required to reduce the artifacts [25].

Photoacoustic tomography (PAT) as an emerging imaging technique, provides an ideal solution to all these problems. PAT, which obtains images from laser pulse induced ultrasound signals through the photoacoustic effect, takes the advantages of the high acoustic resolution and
high optical contrast [23-25]. As hemoglobin itself provides the strongest photoacoustic signal for imaging in the visible and near-infrared regions, PAT is ideal for high-resolution in-vivo imaging of vasculature [23-30]. In addition, by using multiple wavelengths, functional imaging ability can be realized to obtain quantitative physiological parameters. These quantitative functional parameters can provide additional important information for monitoring fetal vasculature development, hemodynamic alterations, and cardiovascular functions caused by maternal EtOH exposure. To date, there is no reported study about in-vivo imaging of vasculature and hemodynamics in the murine fetal brain using PAT.

In this study, we explored the vascular dynamic changes in the murine fetal brain after binge-like maternal EtOH consumption using a real-time photoacoustic tomography system we developed for imaging embryo of small animals[77]. EtOH-induced relative changes of vessel diameter and density in the fetal brain were measured for the first time using PAT. A rapid decrease of vessel diameter and density was observed in the fetal brain within minutes after maternal EtOH consumption, indicating a decrease of blood perfusion in the fetal brain. Significant differences between PEE and sham groups indicated the drastic effect of EtOH on fetal brain vasculature and perfusion. In addition, reduction in oxygen saturation in the fetal brain blood vessel was observed, suggesting EtOH-induced fetal hypoxia, which could be a result of vasoconstriction and decreased blood perfusion. These results demonstrated the capability of PAT as a high resolution non-invasive in-vivo imaging technique for fetal vascular dynamics monitoring and evaluating in small animals.

**Materials and Methods**

**Real-time PAT System for Embryo Imaging**

The real-time multispectral photoacoustic imaging system was used in this study. The laser beam generated by a portable fast-tuning OPO laser (OPOtek Inc, Phocus Mobile,
Carlsbad; wavelength range: 690–950 nm; frequency: 20Hz) is coupled into a custom-made optic fiber bundle (CeramOptec GmbH, Germany) through interlocked ports. The photoacoustic signal is detected by a cylindrically focused transducer array having 256 elements (Japan Probe Co Ltd, Japan, array radius: 65mm; central frequency: 4.4 MHz; bandwidth 113%) coupled with a custom-made 256-channel amplifier/DAQ system (PhotoSound Technologies Inc, Houston; sampling rate: 40MSPS; resolution: 12-bit; frame rate: 50Hz; adjustable gain: 40-91dB). The collected signal was transferred to a computer via a USB 3.0 interface in real-time. Due to the 20Hz repetition rate of the laser, the frame rate of single wavelength PAT imaging was 20 fps, and the frame rate for multispectral PAT imaging using 700 nm and 850 nm was 10 fps in this study. One complete frame of data for a single wavelength was collected in 0.05 seconds. This fast acquisition time reduces the artifacts caused by maternal respirations, heartbeats, fetal movement, and all the other bulk movements. The transducer array was mounted on rotational and translational motors to allow imaging at different angles and depths and was immersed in deionized water for acoustic coupling. The animal holder was mounted onto a moving stage so that the position of the mouse was able to be easily adjusted to place the embryo in the imaging center. This design allowed 3D imaging of the entire mouse embryo. The water in the tank was heated to approximately 37 °C and the temperature was continuously monitored using a temperature input device (National Instruments, USB-TC01) with a J-type thermocouple. Temperature based speed of sound calibration was applied in the delay and sum reconstruction algorithm to avoid artifacts caused by the change of temperature. Air and anesthesia are delivered with the mask we designed to let the mouse breath freely underwater.
Animal Manipulation, EtOH Administration and Imaging Procedures

To study the change of fetal brain blood vessel diameter and density induced by maternal EtOH consumption, six second-trimester equivalent (gestation day GD.17) pregnant CD-1 mice (Charles River Laboratories, Inc. Wilmington, MA) were used in this study. Three mice were used in the EtOH group and three mice were used in the sham group. In addition, we conducted multispectral PAT imaging to obtain EtOH induced oxygen saturation change in the fetal brain blood vessel. Three mice were used in this multispectral study.

Animals were anesthetized by inhalation of isoflurane in oxygen (4% isoflurane during induction and 1-2.5% for maintenance through the whole imaging session). The abdominal hair of the mouse was removed before imaging. The mouse was imaged before the EtOH administration. After the initial imaging, the pregnant mouse was administrated with 20% EtOH (made from ACS grade, 190 proof EtOH) at a volume of 3g/kg through an intraperitoneal (IP) injection. The embryo was imaged every 5 minutes after EtOH administration for a 40-minute period. For the sham group, the equivalent amount of physiological saline was administrated through an IP injection, following the same imaging procedures. At the endpoint of the experiments, the mouse was euthanized by the IACUC approved injection overdose of 150mg/kg of pentobarbital.

Image Reconstruction, Processing, and Quantification

Photoacoustic imaging of dynamic vascular changes induced by acute prenatal ethanol exposure

Photoacoustic signals were acquired at an isosbestic wavelength of 800nm, at which the relative PA signal change reflects the change of total hemoglobin concentration (HbT). The time for collecting data for each 2D image was 0.05 seconds. PAT images were reconstructed using a delay and sum reconstruction algorithm with a temperature-based speed of sound calibration. A
high-pass filter and a Hessian based filter was employed to enhance the blood vessels in the reconstructed 2D images [111]. The vessel diameter was calculated using the full width half maximum (FWHM) [112]. Vessel density was calculated in the selected brain region. The vessel density is defined as the ratio of vasculature area to the total selection area. Image J was used in this processing. The relative change over time was assessed, and the statistical significance of the change of vessel diameter over time was calculated using a nonparametric Friedman test. A Student’s t-test was performed to evaluate the statistical difference between sham and EtOH groups. A value of P<0.05 was considered statistically significant.

**Quantitative photoacoustic tomography of hemodynamic changes of fetal brain blood vessels**

Photoacoustic signals were acquired at two selected wavelengths: 700nm and 850nm, based on the molar extinction coefficient spectrum of HbO and HbR (Figure 5-5 A). It took 0.1s to collect one set of multispectral data. The temperature was adjusted and monitored by using a feed-back controlled heating stick and thermoelectric thermometer.

Offline data processing was performed using our accelerated q-PAT algorithm described in Chapter 3. The spectral results of absorption coefficient $\mu_a$ were further unmixed using a spectral fitting to obtain the distribution of HbO and HbR. Oxygen Saturation ($sO_2$) map was calculated as $sO_2 = C_{HbO}(x,y)/(C_{HbO}(x,y) + C_{HbR}(x,y))$. The spectral fitting method can be described as the following [113, 114]:

$$\mu_a(\lambda_i, x, y) = \varepsilon_{HbR}(\lambda_i)C_{HbR}(x, y) + \varepsilon_{HbO}(\lambda_i)C_{HbO}(x, y)$$

(5-1)

where $\mu_a(\lambda_i, x, y)$ is the reconstructed absorption coefficient at a specific wavelength $\lambda_i$, $\varepsilon_{HbR}(\lambda_i)$ and $\varepsilon_{HbO}(\lambda_i)$ are molar extinction coefficients of HbR and HbO, respectively. $C_{HbR}(x, y)$ and $C_{HbO}(x, y)$ are molar concentrations of HbR and HbO, respectively. By solving the linear equations of multiple wavelengths, $C_{HbR}(x, y)$ and $C_{HbO}(x, y)$ can be calculated.
Results

The PAT image of the fetal vasculature is shown in Figure 5-1 B. Different blood vessels in the brain region, as well as in other organs such as heart and liver, can be clearly identified in the image. The results of one embryo from the sham group are presented in Figure 5-2, and the results of one embryo from the EtOH group are presented in Figure 5-3. Data from all six embryos of six mice from sham and EtOH groups are shown in Figure 5-4. The data represent percentage changes of vessel diameter 40 minutes after EtOH administration to diameter prior to EtOH administration.

Figure 5-2 presents the results of one embryo in the sham group. Figure 5-2 A is the PAT image of the whole embryo vasculature. Figure 5-2 B and C are the images of the brain region before and after the administration of physiological saline respectively. Figure 5-2 D represents the percentage change of vessel diameter every 5 minutes after administration over a total of 40 minutes. The results show that the change in vessel diameter is not significant. Figure 5-2 E displays the percentage change of vessel density in the selected brain area over 40 minutes after EtOH administration. No significant change is observed in vessel density.

Figure 5-3 presents the results of one embryo in the EtOH group. Figure 5-3 A is the PAT image of the vasculature of the whole embryo. Figure 5-3 B is the image of the brain region before EtOH administration, and Figure 5-3 C is the brain region image 40 minutes after binge-like administration of EtOH. Figure 5-3 D shows the percentage change of vessel diameter every 5 minutes over 40 minutes after EtOH administration. A significant decrease in vessel diameter can be seen in the results. The median percentage decrease of vessel diameter is 31.23% at 40 minutes after EtOH administration. Figure 5-3 E shows the percentage change of vessel density in the selected blood vessel over 40 minutes post-administration. The blood perfusion decreased by 25.13% after 40 minutes.
The results of the Friedman test for all samples are shown in Table 5-1. From the results, we can observe the statistical significance in the change of vessel diameter over time in all three samples in the EtOH group, while no statistically significant change can be seen in the sham group. Figure 5-4 is the summary of results at 40 minutes post-administration for all samples. Figure 5-4 A displays the percentage change of vessel diameter, and Figure 5-4 B shows the percentage change of vessel density. A Student’s t-test on vessel diameter and vessel density change showed that compared to the sham group, the EtOH group had a statistically significant difference (P<0.001).

The hemodynamic change of oxygen saturation (sO₂) for one embryo in the EtOH group is shown in Figure 5-5. The average sO₂ of the vessel indicated in Figure 5-5 E, F was calculated every five minutes over 45 minutes after EtOH administration, and the percentage change over time is shown in Figure 5-5 D. The average sO₂ decreased 39.78% after 45 minutes post-administration of EtOH. In the other two EtOH-administrated mice, the average sO₂ decreased 31.93% and 25.30% after 45 minutes, indicating an EtOH induced hypoxia in fetal brain blood vessels.

**Discussion**

A real-time PAT system was built to study the vascular dynamic changes. The major and micro blood vessels of a mouse fetus were clearly imaged non-invasively. More importantly, hemodynamic responses to maternal EtOH consumption were observed within the blood vessels in the fetal brain. Our results show that there is a rapid decrease in fetal brain blood vessel diameter and vessel density in 40 minutes after maternal EtOH consumption, whereas no significant changes were observed in the sham groups. The resulting vasoconstriction in the fetal brain is hypothesized to correlate with the dose and concentration of the acute application of
EtOH [109, 115-121]. The decrease of the blood vessel density indicates the decrease of blood perfusion in the brain area [122, 123]. From Figure 5-3, we can see that the photoacoustic signal of blood vessels attenuated in 40 minutes after EtOH administration, which correlates with a decrease of hemoperfusion since the signal is mostly generated from the optical absorption of hemoglobin in blood vessels [122-124]. These results indicate that even a single binge-like maternal EtOH episode can cause a decrease in blood supply in the fetal brain. The second trimester of gestation is the critical period for fetal neurogenesis and brain angiogenesis. During this stage, the neuronal and vascular developments are initiated in the fetal brain. The maternal-fetal blood circulation for the delivery of nutrients and the removal of metabolites are essential for neuronal development. Decrease of blood supply in the fetal brain caused by maternal EtOH consumption could cause neurodevelopmental delay and retardation in neonates, which could result in lifelong consequences involving physical and intellectual disabilities.

In addition to the changes in vessel diameter and density, we observed a reduction of the oxygen saturation within the fetal brain blood vessel. EtOH affects the oxygen transport in the fetus by constricting placental, umbilical and fetal blood vessels [109, 119-121, 125]. Some previous studies found that EtOH could cause vasoconstriction by enhancing the release of prostaglandins locally [126], and endothelin synthesized in vascular walls of the placenta and umbilical cord [127]. The increased production of these substances could attenuate blood circulation to the tissue, and thus, causing decreased oxygen level [128]. Oxygen reduction could also initiate prostaglandins to be produced from arachidonic acid [129], and therefore, it further decreased the oxygen saturation, resulting in fetal hypoxia. Our results of the decreased vessel diameter, density, and the consequent oxygen reduction agree well with these previous findings. Hypoxia during the second trimester can cause a delay in cell proliferation, growth, or migration.
Organs like the brain are very sensitive to hypoxia and can be damaged dramatically by maternal EtOH exposure. The nerve cells rely on sufficient supply of oxygen and nutrients. Decreased oxygen supply can lead to reduced size of brain and decreased thickness of cortex, which are the most common brain abnormalities occurring in FASD neonate. Thus, EtOH-induced fetal hypoxia can be one of the common mechanisms of FASD.

Our study shows the advantages of PAT over other imaging modalities. The strong optical absorption of blood makes it an ideal technique for imaging blood vessels without using exogenous contrast agents like in MRA. The deep imaging depth of PAT allows it to image the whole embryo non-invasively, solving problems of limited imaging depth and the need for an incision, like in OCT. The real-time imaging ability of our PAT system can avoid the motion artifacts from breathing and other physical motion, which cannot be completely avoided in the techniques that require scanning or relatively long imaging time. The high resolution of PAT enables it to image small and micro-vessels, which cannot be produced by ultrasound imaging. In addition, multispectral PAT offers the ability of functional imaging of EtOH-induced oxygen saturation change in the fetus, which has essential impacts on fetal brain development [39, 40, 130-132]. PAT makes it possible to correlate the change in critical physiological parameters like oxygen saturation with the change in the vasculature, providing more insight into the pathophysiology of FASD.

In this study, a dose of 3g/kg EtOH with a concentration of 20% was administrated in the mother mouse as an acute exposure model, which resulted in a blood EtOH level representing a binge-like intoxication in humans [1, 133-135]. The blood ethanol concentration (BEC) increased drastically within the first 20 mins after IP injection of EtOH and continued to rise to its maximum at about 40 mins post-administration [21]. The dynamic vascular reaction to EtOH
in this study is consistent with the metabolism of EtOH measured in the previous studies. Previous studies have shown that the effect of EtOH is dose-dependent. Depending on the dose and concentration of EtOH administrated and the type of blood vessel, EtOH may cause vasodilatation or vasoconstriction [109, 115-118, 136]. In addition, the central and direct effects of EtOH on brain blood vessels may oppose each other, leading to contradictory experimental results [137]. Researchers also found that the prostaglandin levels are associated with the pattern of EtOH administration. Acute EtOH exposure can cause prostaglandin level to increase, resulting in vasoconstriction and fetal hypoxia, whereas chronic EtOH exposure has the opposite effect. Thus, it is necessary to investigate the effect of EtOH based on different concentrations, doses, and patterns of administration in future studies. Previous studies have shown that maternal binge-like EtOH exposure resulted in a drop in fetal cerebral blood flow (CBF), blood acceleration, and velocity-time integral in fetal cranial arteries [1, 138]. While our study shows a rapid decrease of fetal cranial blood vessel diameter and vessel perfusion, the relationship between these effects and reduction in blood flow velocity remains to be further investigated. Various studies have demonstrated the ability to assess the blood flow velocity using photoacoustic imaging [26, 42, 131, 139]. Our future studies will also investigate the relationship between blood flow velocity and the changes in vasculature and perfusion using photoacoustic imaging.

Conclusions

This study has demonstrated the feasibility of using PAT for evaluating rapid vasculature and perfusion changes in the fetal brain induced by acute maternal EtOH consumption using a murine second-trimester equivalent model. The results showed an immediate decrease in fetal brain blood vessel diameter and perfusion after EtOH administration, which persisted over 40 minutes. This drastic decrease is not observed in the sham groups. In addition, multispectral PAT
was conducted to obtain the EtOH-induced oxygen saturation change over time. A reduction in oxygen saturation was observed in the fetal brain blood vessel, which could be a result of decreased blood circulation and vasoconstriction. The results presented in this study demonstrate that PAT can be a very powerful tool that overcomes the limitations associated with other imaging techniques for visualizing fetal vasculature and hemodynamics to study the pathophysiology of FASD.

Figure 5-1. Photoacoustic imaging of mouse embryo. A) photograph of the embryo (Photo courtesy of author); B) In-vivo photoacoustic image of the fetal vasculature.
Figure 5-2. Photoacoustic image and vessel diameter change of one embryo from the sham group. A) Photoacoustic image of the whole embryo; B) Image of the brain area before physiological saline administration; C) Image of the brain area 40 mins after physiological saline administration; D) and E) Percentage change of diameter overtime of the blood vessel labeled in B) and C); Change of diameter was quantified at three different position along the blood vessel to ensure the consistency of the result.
Figure 5-3. Photoacoustic image and vessel diameter change of one embryo from the EtOH group. A) Photoacoustic image of the whole embryo; B) Image of the brain area before EtOH administration; C) Image of the brain area 40 mins after EtOH administration; D) and E) Percentage change of diameter overtime of the blood vessel labeled in B) and C); Change of diameter was quantified at three different position along the blood vessel to ensure the consistency of the result. The yellow arrow in B) shows small blood vessels that disappear in C).
Figure 5-4. Percentage change 40 mins after administration. The box is the interquartile range, whiskers are the outliers, and the red line is the median.

Figure 5-5. Photoacoustic images and oxygen saturation change for one embryo from the EtOH group. A) Molar extinction spectrum of HbO and HbR in the near-infrared spectrum. The multispectral data sets were acquired at the two wavelengths indicated in the graph; B) Photograph of the mouse fetus (Photo courtesy of author); C) Photoacoustic image of the fetus; D) Percentage change of oxygen saturation overtime for the blood vessel selected from E) and F); E) and F) are oxygen saturation images before and 45 mins after EtOH administration.
Table 5-1. Results of Friedman Test on all samples. Degree of the freedom is 7 for all the tests.

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CHAPTER 6
NEONATAL BRAIN HEMORRHAGE MONITORING AND EVALUATION USING PHOTOACOUSTIC TOMOGRAPHY

Background and Motivation

Neonatal Brain hemorrhage (NBH) is a major consequence of preterm birth, which is the most common neurological disorder in neonates. It occurs at a rate of 3.5 per 1000 live birth [140]. In the United States, approximately 12000 preterm infants are developing intraventricular hemorrhage (IVH) [141, 142]. NBH is typically caused by the rupture of immature blood vessels in the subependymal germinal region (i.e. germinal matrix hemorrhage (GMH)) with extension into the ventricle (i.e. intraventricular hemorrhage (IVH)) in the premature neonatal brain [143].

The reason of the rupture of blood vessel primarily lies in the intrinsic fragility of the germinal matrix vasculature and changes of cerebral blood flow [144]. NBH has become an important socio-economic problem due to the rise in preterm birth rates in the past two decades [144].

Infants who suffered NBH often develop hydrocephalus and consequential neurological deficits, such as seizure, cerebral palsy, cognitive or learning deficits [145-147].

Due to the inadequate clinical treatments, developing and testing therapeutic strategies to treat this disease is highly demanding [148, 149]. Understanding the mechanisms of NBH pathology such as its progression in brain tissue and its consequential neural dysfunction, by characterizing standardized animal models is essential for developing potential treatments.

The use of animal models is necessary due to the nature of the progression of injury study, the complexity of human subjects, as well as the requirement of controlled experimental conditions for evaluating potential treatments [150-152]. There are several animal species used for GMH/IVH model, including pig, dog, rabbit, sheep, rat, and mouse. Comparing to other animals, mouse or rats has various advantages, such as better neurodevelopmental similarities to preterm human infants, low expenses of reproduction, easiness of using and maintaining.
Moreover, there are extensive studies of the germinal matrix [153, 154] and neurobehavior developments in a mouse model [155]. Studies of the standardized animal model showed that human neonatal germinal matrix hemorrhage can be studied using newborn mice and rats [156]. Previous studies have shown that there are differences between neonatal and adult GMH/IVH [157]. It is demonstrated that compared to the mature brain, the damage of the neonatal mouse brain will be more severe after injection of blood, thrombin or plasminogen. In addition, it shows that compared to the adult brain, the premature brain is more sensitive to proteolytic plasma enzymes. Therefore, neonatal mouse model is required for studying the NBH due to the age-dependent brain responses in the GMH/IVH model.

Currently, imaging and characterizing NBH models primarily rely on traditional imaging techniques, such as magnetic resonance imaging (MRI), computed tomography (CT), and ultrasonography (US). However, there are some limitations in these techniques. MRI is very costly due to the requirement of the high magnetic field for high-resolution imaging of the neonatal mouse brain [158]. In addition, the acquisition time is usually long (seconds to minutes), and this low temporal resolution makes it unsuitable for dynamic imaging [159, 160]. CT employs ionizing radiation which limits its application in neonates and infants [161]. The US imaging can offer real-time imaging ability, however, its low acoustic specificity leads to low contrast [161]. To overcome these limitations, non-invasive, non-ionized imaging technique which offers a high spatial and temporal resolution, and high contrast is needed. As an emerging imaging technique, photoacoustic tomography (PAT) which combines high acoustic resolution and high optical contrast, provides an ideal solution.

As we discussed in Chapter 1, PAT offers a unique optical contrast, therefore, by choosing the suitable wavelength, different tissue chromophores can be recovered. As
hemoglobin is the endogenous contrast agent which provides the strongest PA signal in the near-infrared region, PAT is an ideal imaging tool for hemorrhage detection. In addition, PAT provides functional imaging ability, which assesses the critical pathophysiological parameters such as oxygen saturation ($sO_2$), hemoglobin concentrations (HbT), cerebral blood flow (CBF). These important parameters can be used to evaluate the grades of hemorrhage and monitor brain recovery. Moreover, PAT can offer real-time dynamic imaging to monitor the progression of the hemorrhage model, which will deliver valuable insights for understanding the physiopathology of a neonatal brain hemorrhage.

In this study, we monitor and evaluated the progression of the neonatal brain hemorrhage (IVH/GMH model) in the whole neonatal brain using PAT. The dynamic changes of the lesion area in the whole brain were assessed. The hemoglobin evolution process and oxygen saturation change of the hematoma were evaluated quantitatively. This study reports the first noninvasive assessment of the NBH progression in the whole brain using PAT. We demonstrated the potential of PAT as a powerful tool for studying the physiopathology of the neonatal brain hemorrhage.

**Materials and Methods**

**Neonatal GMH/IVH Model**

CD-1 mouse pups of postnatal day zero (P0) were anesthetized with 3% isoflurane in mixed air and oxygen. The pups are placed on a stereotaxic frame for collagenase injection. 0.3 units collagenase VII-S (Sigma, St Louis, MO) was injected in the neonatal mouse brain through a 33-gauge Hamilton syringe (0.210 mm outer diameter). The needle was remained in place for 5 minutes after injection to prevent “back-leakage”. Mice were injected of collagenase in the periventricular germinal matrix area (1 mm lateral, 0.6 mm behind the eye, 3 mm below the scalp surface).
Imaging System

The multispectral photoacoustic imaging system is shown in this study is shown schematically in Figure 6-1. In this system, a portable fast-tuning OPO laser Phocus Mobile, Opoteck, Carlsbad, California, wavelength 690-950 nm, frequency 20Hz) was used as the excitation source. The laser beam was coupled into a high energy fiber bundle optimized for near-infrared tuning ranges. The built-in energy meter monitors the OPO pulse energy in real-time and provides feedback for harmonics auto-optimization and logs pulse energy for data normalization. The delivered laser energy density to the animal was about 22 mJ/cm², which is far below the ANSI safety limit in the NIR region (100 mJ/cm²). A custom-built cylindrical focused ultrasound array probe (Japan Probe Co Ltd, Japan, 256 elements, array radius: 65 mm; central frequency: 4.4 MHz; bandwidth 113%) was used to detect the photoacoustic signals. Data were amplified and recorded with a custom-made 256-channel amplifier/DAQ system (PhotoSound Technologies Inc., Houston, Texas; sampling rate: 40MSPS; resolution: 12-bit; frame rate: 50 Hz; adjustable gain:40-91 dB), and transferred in real-time to a computer via a USB 3.0 interface. The water in the tank was kept around 37 °C and was continuously monitored through thermometer (National Instruments, USB-TC01) with a J-type thermocouple. The logged temperature data was used for PAT image reconstruction using a sound velocity calibrated delay and sum (DAS) algorithm to eliminate the artifacts induced by temperature changes.

Photoacoustic Imaging of Neonatal Brain Hemorrhage Progression

The in-vivo whole neonatal mouse brains were imaged noninvasively for two hours after the injection of collagenase. Tomographic images were acquired at every imaging planes in real-time, and the whole mouse brain was imaged through scanning the probe in the vertical direction. The mice were anesthetized with 3% isoflurane in mixed air and oxygen during the
whole imaging process. The animal experiments in this study were conducted in accordance with the research ethics board at the University of South Florida.

**Image reconstruction and multispectral PAT of oxygen saturation evaluation**

Images were reconstructed with the PAT data using a temperature calibrated delay and sum algorithm. For blood vessel and structure visualization, a bandpass filter was applied to enhance the contrast of the small structures. For quantitative evaluation, the original signals were used, to ensure accuracy. The evolution of pathophysiological parameters including oxygen saturation ($sO_2$) were imaged using multispectral PAT. Wavelengths of 720nm and 850nm which were fast tuned at each laser pulse were employed in this study. The frame rate of the two-wavelength 2D imaging was 10 fps. The multispectral data were unmixed using a spectral fitting method coupled with q-PAT to recover the $sO_2$ quantitatively. The details of this method were described in Chapter 3 and Chapter 5. Briefly, the spectral results of the absorption coefficient $\mu_a$ were recovered using q-PAT and then further unmixed using a spectral fitting to obtain the distribution of HbO and HbR. Oxygen Saturation ($sO_2$) map was calculated as $sO_2 = C_{HbO}(x,y) / (C_{HbO}(x,y) + C_{HbR}(x,y))$, and hemoglobin concentration is evaluated as $C_{HbT}(x,y) = C_{HbO}(x,y) + C_{HbR}(x,y)$. $C_{HbO}(x,y)$ and $C_{HbR}(x,y)$ are the concentration of oxy- and deoxyhemoglobin, respectively.

**Assessment of hematoma area**

The lesion size during the injury process was assessed quantitatively using the PAT data. Due to the irregular shape of the hematoma, MATLAB was used to evaluate the lesion area by subtracting the data of different time points during the hemorrhage from the data of the baseline. The light fluence was normalized. The images were registered before subtraction to eliminate the motion-induced error. Then the size of the lesion area was calculated by taking account of the
pixels that have intensity larger than half maximum. The ratio of the lesion area to the brain area was calculated by diving the pixel number of the lesion by the pixel number of the entire brain area.

**Statistical analysis**

The relative change of lesion size, and oxy- and deoxyhemoglobin change over time were calculated and statistical significance was analyzed using one-way ANOVA (p<0.05 was considered statistically significant).

**Results**

The representative tomographic image of the same imaging plane at different time points during the hemorrhage progression is shown in Figure 6-1. The lesion can be clearly seen in the PAT, and is in good agreement with its corresponding histological section. The yellow arrow indicates the hemorrhage lesion, and the red arrow indicates the needle hole. The red dashed line indicates the local ischemia. Figure 6-2 A shows the representative tomographic images of the lesion size progression. From these images, we can see that the hemorrhage started in the right periventricular region and gradually developed to the lateral and fourth ventricle. The dynamic change of lesion to brain size ratio is shown in Figure 6-2 B. It shows that the hematoma region increased with time. Figure 6-3 presents the 3D progression of NBH in the whole neonatal mouse Brain. Figure 6-3 A, C are representative 3D images at two different time points, and Figure 6-3 B, D are corresponding representative tomographic images at different depth of the whole brain. The progression of the lesion at different depths of the brain can be clearly seen in the PAT images. The data of the 3D lesion to brain size ratio in the whole neonatal mouse brain for three mice is shown in Figure 6-4 A. The data is presented as mean±SD. The statistical significance of the change of lesion to brain size ratio was observed in all three mice (P<0.05).
To further analyze the process of NBH. We recovered the dynamic change of oxy- and deoxyhemoglobin concentration in the hematoma area. We observed a decrease in oxygen saturation in the hematoma area. The percentage variation (SEM) of $sO_2$ overtime for this study (n=3) is shown in Figure 6-4 B. The $sO_2$ in lesion decreased by $4.978 \pm 1.135\%$. There is no statistical significance in $sO_2$ in the first 2 hours of NBH. However, we observed a larger decrease in $sO_2$ 48 hours post NBH. The $sO_2$ decreased by $25.741 \pm 11.722\%$.

Discussion

In this study, we investigated the progression of neonatal brain hemorrhage using the collagenase induced NBH model. The injection is in the right germinal matrix region. The needle hole can be clearly seen in the PAT image. The bleeding started from the germinal matrix and right lateral ventricle, then gradually progressed into the left lateral ventricle, followed by the fourth ventricle. The dynamic change of hematoma lesion size over time was monitored and assessed quantitatively. The progression of the NBH in the horizontal tomographic brain section and in 3D of the whole mouse brain are both evaluated. It shows that the process of hematoma growth can be clearly seen and evaluated. The results showed a GMH with intraventricular extension and ventricular enlargement, which indicated a grade IV (severe) GMH as defined by clinical imaging studies in premature infants [162]. According to the previous studies, the extent of bleeding is the essential factor that associates the most with morbidity and mortality [150, 163]. Grade I to II hemorrhage related to developmental disabilities and grade III to IV related to long-term complications like mental retardation and cerebral palsy [150]. The studies showed that there were about 85% of the survivors of NBH developed major cognitive dysfunction [164, 165]. Therefore, monitoring and quantitatively evaluating the process of bleeding progression and severity will give us more insights into the development of NBH and its complications.
The dynamic change of oxygen saturation in the hematoma area over time was investigated in this study. The results showed a small decrease in $sO_2$ during the first 2 hours of NBH. And a larger decrease after 48 hours post-hemorrhage. Clinically, IVH/GMH is induced by rupture of tiny arteries, resulting in hematoma formation or blood clot in the lesion core, and periphery tissue distortion. We observed that the change of $sO_2$ is varies at different stages of hemorrhage. At the beginning of the bleeding, HbO consists of a large portion of the hemoglobin, and with the progression of hematoma, the concentration of HbR increases, resulting in decreased oxygen saturation. IVH/GMH can induce hypoxia-ischemia, in addition, due to the lack of regional autoregulation in cerebral vasculature and insufficient vascularization in the periventricular region, the hypoxia-induced injury can be intensified. In the PAT images, the local ischemia in the periphery region of the hematoma can be clearly observed. The low optical absorption in the surrounding tissue indicates a reduction in cerebral blood flow during the acute phase of NBH. The hematoma can induce mechanical destruction like tearing of blood vessels in GM, and CBF may be compromised locally resulting in ischemic damage \[166\].

Oxygen metabolic and blood supply is essential for neurological development. The alteration of these factors can be an important mechanism of neurodevelopmental delay and deficits.

In this study, we investigated the NBH using injected collagenase into the germinal matrix of the neonatal mouse brain, which results in a grade IV hemorrhage as hematoma progressed into the ventricles. This is analogous to clinical imaging studies in premature human infants \[]. In this model, the blood vessels in the GM region are ruptured and the ependymal is broken, leading to blood filling the ventricles. Comparing to the IVH model using injected blood, this model exhibits a progressive and spontaneous progression of focal bleeding,
rebleeding and blood vessel rupture, which are comparable to GMH/IVH of human premature infants [166, 167].

This study has demonstrated the unique advantages of PAT over other imaging techniques in studying neonatal brain hemorrhage in a small animal model. Unlike ultrasound imaging, which has low contrast due to low acoustic specificity, PAT offers high specificity and contrast of hematoma, as strong light absorption of hemoglobin in the NIR region provides high optical contrast of hematoma to surrounding tissues without using any exogenous contrast agents. Comparing to MRI, PAT can provide high temporal and spatial resolution at a much lower cost. Comparing to optical imaging techniques like OCT, which has low imaging depth (several hundred micrometers) and cannot be used for whole-brain imaging, PAT provides much deeper imaging depth. Our study has demonstrated the capability of PAT for imaging and evaluating the hemorrhage in the whole neonatal mouse brain.

In addition to hematoma progression and oxygen metabolic, brain function alteration is a very important complication of NBH. Our future study will study the post-hemorrhage brain functional connectivity alteration using PAT [], and its correlation to the alteration of oxygen saturation, hematoma, and local ischemia.

**Conclusion**

In this study, for the first time, the progression of NBH was monitored and evaluated using PAT. The hematoma lesion progression has been monitored and assessed in the whole neonatal brain. Hematoma area and local ischemia of the surrounding tissues can be clearly identified. The dynamic change of hematoma to brain size ratio over time was evaluated. The dynamic change of oxygen saturation was recovered during the first 2 hours of hemorrhage and after 48 hours post hemorrhage. The results presented in this study demonstrated the great potential of PAT in studying the pathophysiology of neonatal hemorrhage.
Figure 6-1. Representative photoacoustic images of hematoma lesion progression with comparison to histology. Red arrow indicates the needle injection site, yellow arrow indicates the hematoma, and the red dashed lines indicate the local ischemia. Mouse brain histology (Photo courtesy of author). Atlas of mouse brain (Reprinted with permission from MBL Online, http://www.mbl.org/atlas232/atlas232_frame.html (March 10, 2020)).
Figure 6-2. Quantitative assessment of hematoma lesion size progression. A) representative tomographic images of the lesion size progression. B) the dynamic change of lesion to brain size ratio overtime.

Figure 6-3. 3D progression of NBH in the whole neonatal mouse Brain. A) and C) representative 3D image at different time points during hemorrhage. B) and D) corresponding representative tomographic images of different brain sections.
Figure 6-4. Quantitative data of change in lesion to brain size ratio and the percentage variation of oxygen saturation over time (n=3). A) dynamic change of lesion/brain size ratio in the whole brain over time. B) percentage variation of $sO_2$ overtime.
CHAPTER 7
CONCLUSION AND FUTURE WORK

Summary of Research

Current imaging techniques which are used in fetal brain vascular and hemodynamic imaging, such as Magnetic resonance angiography (micro-MRA), ultrasound Doppler imaging, micro-computed tomography (micro-CT), optical coherence tomography (OCT), is either suffering from low spatial/temporal resolution, shallow imaging depth, invasive procedure or use of ionizing radiation or exogenous contrast media. Photoacoustic imaging (PAI), which offers optical contrast with high resolution and deep imaging depth beyond the scale of pure optical imaging, provides an ideal solution to all these problems. Combined with the photon transport model, TD-qPAT can reconstruct the absolute absorption coefficient of tissue, which can be used to recover the physiological information like oxygen saturation, metabolic rate, blood perfusion, etc. Therefore, PAI has great potential in studying the development of congenital brain diseases. This dissertation presented the development of PAI techniques for non-invasive high-resolution functional dynamic imaging of fetal/neonatal brain vasculature and hemodynamics. The work includes the hardware development of a fast 3D compact and mobile PAT system for dynamic functional imaging of fetal/neonatal brain, which has great value for preclinical studies of congenital brain diseases and has the potential to be adapted to human fetal/neonatal brain imaging. In addition to hardware development, we significantly improve the quantitative TD-qPAT algorithm in computational speed and image quality, which makes preclinical and clinical applications feasible. Furthermore, we developed novel PAI approaches and reconstruction algorithm to achieve better image quality in deep tissue with fewer detectors. Lastly, for the first time, high-resolution dynamic change of fetal brain vasculature, blood perfusion, and oxygen saturation in an acute prenatal ethanol exposure (PEE) murine model was imaged with PAT,
which successfully demonstrated the potential of PAT in the study of congenital brain disease like fetal alcohol spectrum syndrome (FASD). Moreover, the murine model of germinal matrix hemorrhage (GMH) in neonatal cerebrum was studied using our PAT techniques. The progress of the GMH was imaged and quantitatively assessed during the hemorrhage. Functional imaging of oxygen saturation change in the neonatal brain was studied.

In summary, driven by the goal of developing powerful imaging tools for brain imaging of preclinical congenital brain disease studies, we developed hardware and algorithms to achieve fast high-resolution deep penetration depth functional photoacoustic imaging. Different fetal and neonatal brain disease models were investigated. The results demonstrated the value of PAT in the preclinical study of congenital brain diseases and showed promising potentials in translation to clinical application to humans.

**Future Directions**

This study can be continued for clinical translations of photoacoustic imaging techniques. The prototype we described in Chapter 4 has great potential in brain imaging of human infants. The next step will be developing a probe for clinical applications and conducting experiments to validate the feasibility of the technique for human neonatal brain imaging, including phantom experiments with a human skull, in-vivo animal experiments and clinical experiments in human infants. The novel reconstruction algorithm, which can significantly reduce the number of detectors, can be applied to develop a real-time 3D volumetric PAT probe with fewer detectors. This will substantially reduce the cost and system complexity.

Another work we can continue in the future is to translate the fast 3D photoacoustic system to clinical applications of fetal or neonatal brain imaging in humans. We will adapt the system to a portable handheld real-time PAT system, which can be easily transported between clinical and laboratory sites for different studies. A mechanical arm will be used to balance out
the weight of the probe or fix the probe at any positions and angles, to make it easier for clinicians to perform the imaging. Imaging data will be processed online using GPU high-performance computing to display the images in real-time.

Preclinical studies will also be continued. Taking the advantages of our fast multispectral PAT system, mechanisms of the development of FASD can be studied. For example, the change in fetal brain vessel diameter and oxygen saturation can be imaged simultaneously, therefore, correlations between the vasoconstriction/vasodilation and fetal hypoxia induced by acute prenatal ethanol exposure can be investigated. The correlations of the changes in blood flow velocity, perfusion, vessel diameter, and metabolic rates can be studies using our PAT system. Vascular and hemodynamic changes in different ethanol administration patterns and with different doses can also be studies. As PAT is a non-invasive technique, we can monitor the developmental changes from the early stage of gestation to neonates. These studies will advance our understandings of the physiopathology of FASD.
LIST OF REFERENCES


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BIOGRAPHICAL SKETCH

Tianqi Shan received her B.S. degree in the Department of Physics in Jilin University, China, in 2011. She received her M.S. degree in Department of Physics in Tulane University, US, in 2013. In fall 2014, she started to pursue her Ph.D. in the Department of Biomedical Engineering at University of Florida. Her research includes instrumentation and algorithm development in Photoacoustic Imaging, quantitative reconstruction algorithm, Photoacoustic Imaging of brain disease, Photoacoustic deep tissue imaging. She received her Ph.D. degree in May 2020.