Compact and Low-Cost Acoustic-Resolution Photoacoustic Microscopy Based on Delta Configuration Actuator

by

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Abstract

Photoacoustic (PA) Imaging is an emerging biomedical imaging modality based on the laser-generated ultrasound. The method has unique advantages in providing microvessel structure visualization, neuroimaging, and functional imaging provided by its physical principle. Photoacoustic microscopy (PAM) is one of the PA imaging instruments which provides high resolution and contrast imaging of a near-field target. Relying on the acoustic focusing, Acoustic-resolution PAM (AR-PAM) is capable of reaching a sub-centimeter of penetration depth with sub-millimeter resolution and is optimized for tissue samples and small animals. However, the state-of-art AR-PAMs are large in size and expensive in cost, which hinders its democratization. There are previous researches conducted on reducing the cost by introducing a low-cost optical source or ultrasound acquisition device. Few research has investigated the possibility of modification on actuator design. The total system cost should be further reduced by substituting the translation stage while maintaining the imaging quality. In this research, a delta configuration actuation is introduced to the AR-PAM. The delta-configuration actuation adapted from a low-cost off-the-shelf 3D printer has been implemented in the design. An economical PAM system that integrates the combination of hardware and software enhancement is designed and tested in this research. With the software approach, advanced beamforming methods such as Delay-and-Sum with Coherence Factor (DAS+CF) and Delay-Multiply-and-Sum (DMAS) algorithms are applied to obtaining the high-resolution PA image through 3D reconstruction. The preliminary phantom study demonstrated the applicability of low-cost delta configuration actuators for AR-PAM imaging. The simulation
study shows the beamforming algorithms has capability to remove the device precision error and increasing the tolerance. The research suggests that the 3D reconstruction algorithms significantly improve the resolution and contrast of the image quality.
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Chapter 1

Photoacoustic Imaging

1.1 Introduction to Photoacoustic Imaging

Photoacoustic (PA) imaging, also called optoacoustic or thermoacoustic imaging, is an emerging biomedical imaging modality. The PA imaging is based on the use of laser-generated ultrasound developed from the mid-1990s. The imaging modality relying on the photoacoustic effect which is firstly reported by Alexander Graham Bell in 1880. Bell observed the generation of sound owing to the absorption of modulated sunlight [15]. The PA effort refers to the generation of the acoustic waves by the absorption of electromagnetic (EM) energy, such as optical or radio-frequency waves.

Over the years, there are various of studies conducted on the photoacoustic techniques [16–27], in many scientific branches such as physics, chemistry, biology, medicine, and engineering. Starting from mid-1990s, the photoacoustic imaging in biomedical applications has been researched and developed [28–34].

Photoacoustic imaging can be considered as an ultrasound-mediated electromagnetic (EM) imaging or an ultrasound imaging with enhanced contrast [35]. In a PA
imaging, tissue and optical contrast agent absorbs a short EM pulse and a thermoelastic expansion is generated. The spatial distribution of the acoustic transient pressure inside the tissue that acts as the initial source for the acoustic waves is simultaneously excited. The acoustic waves travel to the surface of the tissue and detected by ultrasound receivers with various time delays. The initial acoustic source will be mapped by measuring the time of arrival with known speed of sound and shows the position of the energy absorption.

In biomedical photoacoustic imaging, visible and near-infrared (NIR) wavelength spectrum is commonly used. NIR provided the greatest depth penetration that reaches several centimeters. The specific tissue chromophores such as hemoglobin, melanin, water, or lipids absorb the optical excitation and produce a small temperature rise (less than 0.1K), which results in the emission of low-amplitude acoustic waves. Hence, the PA imaging shows the optical absorption and scattering properties of the tissue. This specification of PA imaging makes this modality more suitable for imaging the microvascular, which is difficult to visualize with ultrasound imaging. At the same time, many specific tissue chromophores have spectral dependence of optical absorption as shown in Figure 1.1 [1].

For instance, the presence of lipid deposits in atheromatous plaques can be revealed by choosing an excitation wavelength coincident with the lipid absorption peak at 1210 nm. The spectroscopic nature of the PA effect can be further exploited to quantify the concentrations of specific chromophores via their spectral signatures to provide physiological parameters. The spectroscopic measurement of blood oxygen saturation (sO2) is also achievable by this nature of PA imaging. By acquiring images at multiple wavelengths and applying a spectroscopic analysis, oxyhemoglobin (HbO2) and deoxyhemoglobin (HHb) can be quantified based on the known spectroscopic difference and imaging the blood sO2 level. [35].
Figure 1.1: Absorption coefficient spectra of endogenous tissue chromophores. (Oxyhaemoglobin (HbO2), red line; deoxyhaemoglobin (HHb), blue line; water, black line; (80% by volume in tissue), lipid(a), brown line; (20% by volume in tissue), lipid(b), pink line; melanin, black dashed line; Collagen, green line; and elastin yellow line.) Therefore, by tuning the wavelength of the laser, the image contrast can be enhanced [1]

The capability of mapping the micro-vascular structure and obtain the oxygenation and flow characteristic of the blood makes the PA imaging suitable for clinical research on imaging tumors, the function of the vessel, and other diseases. There are researches focusing on different aspect of PA imaging such as neuroimaging [36], molecular imaging [37], and microvascular imaging [38].

1.2 Introduction to Microscopy

Photoacoustic imaging can be mainly categorized as PA tomography (PAT) and PA microscopy (PAM). The PAM is firstly introduced by [39], [3], which is a technique that the PA image is collected by mechanically scanning. The image is then
formed directly from sets of acquired A-lines based on the known position information without reconstruction required as in PAT. The PAM provides a high resolution and contrast image at a desired penetration depth range depending on the configuration.

Comparing to conventional optical microscopies such as confocal, two-photon microscopy, or optical coherence tomography (OCT), PAM has many advantages over those imaging modality [2]. First, PAM has depth penetration capability because of the usage of ultrasound to generate imaging. Second, the PAM image contains functional and structural information of the tissue with high sensitivity. Third, PAM does not require optical sectioning to obtain 3D volume data. PAM can achieve a spatial resolution from sub-micrometer to sub-millimeter at maximum imaging depth ranging from a hundred micrometers to a millimeters [40], [41].

PAM maximizes its detection sensitivity by confocally aligning its optical illumination and acoustic detection. The axial resolution of PAM is primarily determined by the imaging depth and the frequency response of the ultrasonic transducer. The combined point spread function of the dual focuses defines its lateral resolution. Therefore, the PA microscopy systems have two main categories depending on its focus type, acoustic resolution PA microscopy (AR-PAM), and optical-resolution PA microscopy (OR-PAM).

Optical resolution PAM (OR-PAM) refers to the microscopy in which optical focus is tighter than acoustic focus. In this configuration, The lateral resolution is mainly defined by its optical confinement, as equation 1.1.

\[
R_{\text{Lateral},O} = 0.51 \cdot \frac{\lambda_O}{N_{A_O}}
\]  
(1.1)

where \(\lambda_O\) is the optical wavelength and \(N_{A_O}\) is the numerical aperture of the optical
objective lens. The constant 0.51 reflects the full width at half maximum (FWHM) of the optical focal spot in light intensity. OR-PAM systems can typically reach a lateral resolution of 0.2 to 10 μm [42]. The PA signal generates from the area with a dimension of the focused laser beam. Thus a lateral resolution of a few microns can be achieved with a depth range [43–51]. However, in OR-PAM, a maximum depth penetration is approximately 1 mm because of the spreading and distortion of the excitation laser beam. The tissue optical scattering prevents the tight laser focus being maintained beyond this depth [1].

Acoustic resolution PAM (AR-PAM) describes the system with a single focus transducer translating or rotating to map the PA signals [3,4,39,52–57]. The optical energy delivers around the focal point of the transducer. Light is usually weakly focused by conical reflection or fiber bundles which do not serve to localization and thus not influence the spatial resolution. Unlike the PAT, the weak focus of the optical laser reduces the laser energy requirement. In the AR-PAM system, both transducer and the excitation beam are moving together at the surface of the samples mechanically, generating and collecting PA signal to acquire a 3D image. At each step of imaging, an A-line with a depth profile of the absorbed energy is collected with the known lateral coordinates. Then it maps to a 3D volume greyscale image directly. Because of the acoustic scattering is much weaker beyond the optical diffusion limit, the AR-PAM can scan deeper targets comparing to the OP-PAM. The lateral resolution of AR-PAM is determined as equation 1.2

$$R_{Lateral,A} = 0.71 \cdot \frac{\lambda_A}{NA_A}$$

(1.2)

where $\lambda_A$ is the central wavelength of the photoacoustic wave and $NA_A$ is the numerical aperture of the ultrasound transducer. The constant 0.71 reflects the
Figure 1.2: Features of various PAM implementation types (PAM, photoacoustic microscopy; NA, numerical aperture; AR, acoustic resolution; and OR, optical resolution) [2]

FWHM of the acoustic focal spot in acoustic amplitude [42]. The maximum imaging depth of PAM is approximately proportional to its lateral resolution. So far, by varying the lateral resolution from 45um to 560 um, maximum imaging depths from 3 mm to 4 cm have been achieved by various AR-PAM systems.

There are also researches on investigating different optical and acoustic focal arrangement, their properties, and imaging quality [2]. As shown in Figure 1.2. The research suggests that with the second type (dark-field illumination [39,58]) and third type (Coaxial configuration based on an Opto-ultrasound beam combiner [51]) are able to achieve a depth penetration. However with the third type, approximately only a depth of 1.2 mm in live animals is reachable. On the other hand, with dark-field illumination (the second type), a whole body, internal organs, and deeper vessels imaging are acquirable.
1.3 Acoustic-Resolution PAM Designing

The acoustic-resolution photoacoustic microscopy usually provides a depth penetration of several millimeters depending on the configuration, which is not reachable by OR-PAM. Most of the existing AR-PAM uses the design of the dark-field illumination method to align its acoustic and optical focal path. The dark-field illumination can be further divided into two categories, mirror reflection, and fiber illumination.

The mirror reflection design [3, 4] is shown as Figure 1.3, 1.4. The optical fiber delivers the laser energy to the microscopy. The laser beam is focused on a conical lens, which passes the focal point and aligned to an optical condenser. The angles of the beam are well adjusted where the optical focal point is aligned around the acoustic focus point after reflected by the mirror. Another approach is to illuminate the sample directly by laser bundles [5] [6] [59]. The optical fiber bundle is fixed around the acoustic transducer, with an adjusted angle where the area around the acoustic focal point is illuminated at maximum optical density. This approach reduces the loads on the sensing tip by avoiding moving the complicated optical components while scanning. The design is shown in Figure 1.5 and Figure 1.6.

Mostly all the state-of-art acoustic-resolution photoacoustic microscopy is designed to move the bundled optical components with the transducer while scanning the sample by Cartesian linear translation stages. The linear translation stages (shown in Figure 1.7 [7]) provides high precision motorized motion at level of several micrometers. The device guarantees the accuracy of the localization sensing of the scanning tip, which leads to a higher image resolution. However, the bulky translation stages limit the compact-ability of the photoacoustic microscopy.
1.4 Problem Statement

To date, lots of development in PAM are focusing on improving the speed, image quality, and enlarging the depth penetration range [60–62]. However, most PAM
systems are still limited to table-top form because of the expensive lasers, ultrasound transducers, and the complex geometries that aligning the acoustic and optical focusing. On the other hand, the complexity of the PAM system leads to the high cost of the device that preventing the technology from being used more popularly.
Reducing the cost of building a PAM is critical for this emerging technology. Many researches are investigating reducing cost by introducing a low-cost optical source, especially in OR-PAM.

Although less frequently researched compared to the OR-PAMs, AR-PAM systems have unique depth penetration capability. Few research has been conducted in this aspect. There are three main parts in most of the Acoustic-resolution Photoacoustic Microscopies: optical source, ultrasound signal acquisition device, and the actuation device. During the years, studies are focusing on reducing the cost by introducing a substitution solution to different parts.

Similar to OR-PAM, some researches have been done in reducing the cost of
the optical source in AR-PAM. In AR-PAM, previous researches investigate the possibility of using an overdriven CW laser diode [63]. The lower energy lower focused optical source brings down the cost at a certain level. Many researches provide solution by introducing NIR laser diode to reduce the cost of this part [64–69]. However, the depth penetration is limited at the same time. In the research of Dangi et. al. [70], a ring ultrasound transducer is introduced to develop wearable photoacoustic microscopy.

While previous studies have focused on replacing laser sources or data acquisition devices, bulky and costly high precision linear translation stages are used to actuate the sensor to build a 3D PAM image. Although a Cartesian linear translation stage provides higher motion precision, it usually costs over 3,000 USD [7]. Depending on the increment of the degree-of-freedom (DOF) and required loading capacity, the cost can increase further. Here, we focused on substituting these linear translation stages. In this research, a new actuation configuration is introduced to demonstrate the possibility of further reducing the cost to build a PAM while maintaining the acquired image quality. To introduce a lower-cost actuator in PAM, a lightweight scanning tip is required so that the loading capacity is not critical to the actuator. With a new design of the scanning tip that aligning transducer and the optical path, a new method of PAM actuation can be achieved.

This proposed PAM system is desired to serve as a research platform scanning biological samples and small animals in the future photoacoustic research. The platform requires a millimeters-level depth range and sub-millimeters resolution with flexible hardware and software configurations. Photoacoustic microscopy usually requires less light energy comparing to the full-body illumination tomography because of the optical focus. An AR-PAM provides a deeper scanning range that is difficult for an OR-PAM to reach. Therefore, an AR-PAM is more suitable for this
desired purpose. At the same time, AR-PAM can provide a larger designing flexibility on the scanning tip instead of moving heavy optical focus components. It is referred for implementing a new actuation configuration.

Here, we propose a delta-configuration actuation AR-PAM, which is adapted from a low-cost commercially available off-the-shelf 3D printer to miniaturize the size of the system and to drastically reduce the cost. The mechanism allows rapid and accurate 3-D transnational positioning with easily programmable motion control provided by the 3D printer. We introduce an economical PAM system that integrates the unique sensor actuation hardware and the software approach of obtaining the high-resolution image through 3D reconstruction. The sensor head is composed of a single element transducer and optical fibers to deliver pulsed light. The sensor is moved under delta configuration in a horizontal motion pattern covering the area of interest with a minimum step size of 0.1 mm. The Coherence-weighted delay-and-sum (DAS) and delay-multiply-and-sum (DMAS) reconstruction algorithms are applied to the recorded RF data to dynamically focus the target for all depths as well as reducing the motion error affect in the image scanning. A tube phantom study is conducted to test the image quality of the system. A simulation study proves the beamforming algorithm is able to reduce the motion error effect.
Chapter 2

Hardware Design and Control

2.1 System Architecture

This chapter introduces the design and control of the proposed Acoustic-Resolution Photoacoustic Microscopy (AR-PAM). The proposed AR-PAM is desired to provide a sub-millimeters resolution with a relatively small size tip that allows minimal contact with the medium materials during the scanning. The system is aimed to serve as a research platform in the future to scan the small animals and biological tissue samples with both depth and resolution flexibility. The system introduces a new control kinematic to the PAM which is delta-configuration actuation. It replaces the Cartesian linear translation stages to move the transducer. This approach addresses the problem of linear translation stages in the previous design. Meanwhile, delta-configuration has a unique advantage that the actuators are away from the scanning tip, which minimizes the size and loading on the tip. Further, it reduces the torque required for the stepper and lowers the cost. Both size and the cost to build a PA microscopy can be reduced with this kinematic approach while maintaining the precision of the motion.
This proposed AR-PAM system (shown in Figure 2.1) contains multiple parts from optical, signal collection and processing, to robotics controlling. Different components are communicating to and controlled by the host workstation with specific protocols, as shown in Figure 2.2. In our setup, the host workstation uses Verasonics Vantage Host Controller which communicating to Verasonics Vantage 128 Research Ultrasound system, Quantel Q-smart 450 laser, and customized Monoprice MP Mini Delta 3D Printer that serves as the actuator. The entire system is operating under the Matlab environment including the functions of laser control, data collection, signal processing, and the motion control of the actuator. The prolific sub-200 USD delta 3-D printer market proves a reasonable motion precision delta configuration can be mass-produced cheaply, which rationalizes the usage of this mechanism in microscopy design. Also, a programmable motion platform allows a flexible scanning trajectory, which is desired by prototyping and research [71].
Figure 2.2: System Architecture of the proposed AR-PAM

The proposed AR-PAM is scanning the target sample with two steps. The first step is collecting the RF photoacoustic data with a certain scanning pattern. After the data is collected, the RF photoacoustic data is software processed with filters and beamforming algorithms to reconstruct high-resolution 3D photoacoustic images. Given the fact that a low-cost of the 3D printer might compromise the motion precision, the beamforming enhancement is capable of canceling this error within a certain range. A simulation study is conducted on this aspect which will be discussed in the result section.

Since this project is designed toward open-source application, the system setup with different components should work similarly with the communication protocols modified appropriately. In this chapter, the specific of the components that we are using will be introduced for future guidance.

The host workstation in this setup is the Verasonics Vantage Host Controller, which is designed to provide the optimal performance with the ultrasound system.
The machine has a custom BIOS, installed Verasonics software including HAL, VSX, firmware and drivers; PCI express host adapter, MATLAB software, and over 500 example scripts [72]. The controller is running Windows 10 operating system with Intel Xeon W-2155 CPU, 32GB RAM, and NVIDIA Quadro P2000 graphic card to provide computational power.

For collecting the photoacoustic signal, a single-element ultrasound transducer (ndtXducer) is used on the Verasonics Vantage 128 Research Ultrasound system in this project. The transducer has a nominal frequency of 20.0 MHz and a focal length of 0.5 inches (12.4 mm) with an element dimension of 0.25 inches (6.2 mm). It is capable of both transmitting and receiving a signal which enables the system to collect both ultrasound and photoacoustic imaging of the desired target. The Vantage 128 ultrasound system has 128 independent transmitting and receiving channels, which is able to receive real-time RF data from each channel at 0.5 - 27 Mhz and transmit at 0.5 MHz to 20 Mhz on its standard frequency configuration. The redundant configuration allows future add-ons to this system. The received data transfer to host computer via 8 PCI express lanes with transfer rate up to 6.6 GB/sec [72].

To generate optical energy, a pump laser (Quantel Q-smart 450) is introduced in this project. The machine can shoot a 532 nm wavelength laser at a repetition rate at 20 Hz. The laser beam has an energy of 200 mJ with divergence less than 0.5 mrad according to the datasheet [73]. The machine is also compatible with an optical parametric oscillator (OPO) that allows future research which requires changing laser wavelength.

To move the single element transducer, an off-the-shelf 3D printer (Monoprice MP Mini Delta 3D Printer) is customized on both hardware and software levels. The printer provides a scan area of 100 mm diameter with 120 mm height at a minimum
step size of 50 um according to the manual [74]. The 3D printing nozzle has been removed and replaced by the microscopy tip containing optical fibers and a single element transducer. On the software level, the controller of the printer has been switched to receive g-code commands in real-time through serial communication from the host workstation.

2.2 Delta Configuration Platform

Delta configuration mechanism is a 3 degrees-of-freedom (DOF) parallel structure that allows 3 natural translations X, Y, and Z. The delta robot design was first introduced and patented in 1985 named DELTA [75]. The controlling method of the mechanism is also called "Linear Delta", which intends the motion is realized with linear actuation (Figure 2.3). The linear delta has two main categories, horizontal and vertical as shown in Figure 2.4.

![Figure 2.3: Linear Delta design from the US Patent [8]](image-url)
The research suggests that compared to the rotational mechanism, the linear mechanism allows a large travel space Z direction. At the same time, its dominant advantage of good sensitivity, that guarantees the motion precision and resolution [9]. On the other hand, the effort of the actuator is closest to the tool efforts with a linear delta mechanism. This advantages makes the design suitable for machining application and those applications that requires stiffness [76] [77].

Within the family of the vertical linear delta, the platform is actuated by three linear motion (prismatic joints) which is realized by rotational motors attached to lead screws or belt drive systems. The mechanism is widely used with measuring machine (Renishaw equator), pick&place robotics (Adept Robot), and 3D printer (Figure 2.5).

There are many methods for solving the inverse kinematics of the linear delta mechanism. Using Cartesian coordinates or geometric relations is the classic way to achieve this. In this section, inverse kinematics using Cartesian coordinates [13] will be presented. Figure 2.6 shows the schematic and the symbol representations.

\[ \vec{a}_{r,i} + \vec{S}_i + \vec{d}_i = \vec{P} + \vec{R}_i \]  

(2.1)

Figure 2.4: Horizontal and vertical Linear Delta design [9]
in Equation (2.1),

\[
\begin{bmatrix}
\bar{x} \\
\bar{y} \\
\bar{z}
\end{bmatrix}
= \begin{bmatrix}
x \\
y \\
z
\end{bmatrix}
+ \begin{bmatrix}
x \\
y \\
z
\end{bmatrix}
+ \begin{bmatrix}
Rcos\theta_i \\
Rcos\theta_i \\
0
\end{bmatrix}
+ \begin{bmatrix}
dx \\
dy \\
dz
\end{bmatrix}
+ \begin{bmatrix}
0 \\
0 \\
Si
\end{bmatrix}
= \begin{bmatrix}
x \\
y \\
z
\end{bmatrix}
+ \begin{bmatrix}
rcos\alpha_i \\
rcoa\alpha_i \\
0
\end{bmatrix}
,\quad \text{and } i = 1, 2, 3.
\]

Substitute the components to Equation 2.1 then,

\[
\begin{bmatrix}
Rcos\theta_i \\
Rcos\theta_i \\
0
\end{bmatrix}
+ \begin{bmatrix}
0 \\
0 \\
Si
\end{bmatrix}
+ \begin{bmatrix}
dx \\
dy \\
dz
\end{bmatrix}
= \begin{bmatrix}
x \\
y \\
z
\end{bmatrix}
+ \begin{bmatrix}
rcoa\alpha_i \\
rcoa\alpha_i \\
0
\end{bmatrix}
,\quad \text{and } i = 1, 2, 3.
\]

Three equations can be delivered from Equation 2.2.

\[
dx = x + r\cos\alpha_i - R\cos\theta_i
\]

\[
dy = y + r\sin\alpha_i - R\sin\theta_i
\]

\[
dz = z - Si
\]

By taking the square, we can cancel out the direction of the rod because its length
is constant.

\[ d_x^2 + d_y^2 + d_z^2 = (x + r \cos \alpha_i - R \cos \theta_i)^2 + (y + r \sin \alpha_i - R \sin \theta_i)^2 + (z - S_i)^2 \] (2.4)

\[ S_i^2 - 2zS_i + C^2 + z^2 - d_i^2 = 0 \] (2.5)

where

\[ C^2 = (x + r \cos \alpha_i - R \cos \theta_i)^2 + (y + r \sin \alpha_i - R \sin \theta_i)^2 \] (2.6)

The slider distance can be calculated in Equation 2.7 that,

\[ S_i = z \mp \sqrt{z^2 - V} \] (2.7)

\[ V = C^2 + z^2 - d_i^2 \] (2.8)

Two different real solutions are calculated by Equation 2.7.
A customized delta-configuration 3D printer allows an easily programmable motion trajectory that is desired by the PAM. In our system setup, the controller board on the customized 3D printer receives the g-code command by real-time serial communication. The g-code command contains the motion control information. The controller calculates the inverse kinematic with its specification and drives the motor. The communication protocols will be discussed in a later section.

2.3 Optical Components

![Figure 2.7: Dual beam laser beam splitter sketch](image)

To deliver the optical energy to the scanning target, the optical path has been designed to optimize the illumination of the target. The laser beam generated by the pump laser has a wavelength of 532 nm. To make sure the target absorbing the optical energy evenly and avoiding the illumination shadow, the laser beam is split and delivered from two sides of the target. Further, because of the maximum laser energy emitting to tissue without causing damage is limited. It is difficult to provide enough optical energy without adjusting the optical angle and focal distance.

The laser beam splitting optical path is designed as shown in Figure 2.7 [5]. A beamsplitter, two lens, and two fiber holders are required. The beamsplitter is
Figure 2.8: Dual beam splitter optical path

aligned with the output beam from the laser, which divided the single beam into two. Then the two beams are individually fed into the lens. The fiber holder is set to align the fiber end right at the focal point of the lens, where the split laser beam could be focused and feed into the optical fiber. Figure 2.8 shows the beam coming out from the pump laser being split and feeds into the optical fibers.

The end-effector of the PAM is also designed so that the target can be illuminated optimally. The part is designed and 3D printed for properly mounting on the 3D printer platform, as well as aligning the transducer with the optical fibers as shown in Figure 2.9. The part is designed to be able to hold a maximum of four optical fibers to illuminate the target evenly. The fibers are fixed with the steel tube so
that the angles are properly adjusted. The angles of the fiber are set so that the center-line of the laser beam is focusing at the focal distance of the transducer as shown in Figure 2.10. With this approach, the target located at the depth of focus will receive the maximum optical energy to generate strong thermoelastic expansion.
The green corns in the figure indicate the laser illumination.

The side view of the printed scanning tip is shown in Figure 2.11. The fibers pass through the steel tube and set properly at the angle to illuminate the transducer focal point.

### 2.4 System Synchronization

To map the accurate depth of the received thermoelastic expansion signal by the single element transducer, laser illumination must be excited at appropriate timing. The process requires time synchronization of the transducer collecting time and the laser transmitting time so that a precise signal traveling time can be measured. The laser is exciting at a maximum rate of 20 Hz as introduced in the previous section. The ultrasound system has a higher frequency. The system synchronization pipeline is designed as shown in Figure 2.12.

The laser emission has two stages. The first step is the energy pump getting
Figure 2.12: Laser synchronization pipeline.

ready which is called flashlamp in our setup. Then the Q-switch excites the laser and generates a pulsed laser beam. To synchronize the laser with the data collection, the triggering signal built-in is utilized.

When the pump is ready on the laser, a triggering signal is sent to the ultrasound system (step 1 in the figure), which indicates the laser is ready to shoot. The ultrasound system waits for the signal to continue. Then the process will simultaneously send a triggering out signal (step 2-1) to excite the laser, along with start collecting the ultrasound signal (step 2-2). There is a fixed delay period during step 2-1 and 2-2. The laser machine shoots the laser beam after another constant delay in 40 ns. Then the photoacoustic signal is collected with the sensor, and the system will continue for the next round.

The fixed delay during the process is measured and automatically fixed in the collected RF data. The function of transmitting and receiving on the single element transducer provides the system capability to scan the ultrasound imaging and photoacoustic imaging at the same time. Given the physical principle behind two imaging modality, the traveling time of the photoacoustic signal from the same
target should be lying exactly at the half time of the ultrasound signal if perfectly synchronized.

By this approach, with several rounds of calibration, the fixed delay in the synchronization pipeline is measured as 70 samples in the collected photoacoustic RF data.

### 2.5 Scanning Control

The single element transducer moves with its aligned optical fibers by the delta configuration actuator. The specific control method of the 3D printer using a g-code simplifies the system. G-code is a widely used computer numerical control (CNC) programming language. It is mainly used in computer-aided manufacturing to control automated machine tools. G-code instructions are provided to a CAM controller that includes information on where to move, how fast to move, and what path to follow [78]. The controller calculates lower-level motor control parameters with the machine’s specific kinematic. G-code is widely used in controlling the 3D printers, generated by the software to follow the sliced shape of the CAD model.

The modified delta-configuration 3D printer is controlled by the g-code as well. The controller receives the g-code that contains the information about the goal position and velocity, calculates the inverse kinematic, and controls the motors. By utilizing the Matlab serial communication function, it is possible to send the g-code command to the printer controller in real-time. The motion control software is embedded within the synchronized controlling program. The transducer is moved to the desired scanning position, performing photoacoustic signal collection and moved along the designed trajectory until finishing scan the entire target area.

The actuator is able to perform the scanning at the X-Y plane with a maximum
area of 100 mm diameter. The minimum step size of 50 um, which is the minimum distance between two collected RF data. The trajectory of scanning can be defined based on needs. The height (Z) of the scanning trajectory plane is depending on the height of the sample target and the container.

In our system setup, a 20 mm x 20 mm square area in the X-Y plane of the actuator is designed as an area-of-interest for photoacoustic imaging scanning. The planar scanning simplified the depth mapping of the target as well as maintaining a lateral resolution at the same depth. The step size of our system is set to be 200 um on both x, y-direction. Initially, the transducer moves from it’s resting position to the starting point of the trajectory. The trajectory of the transducer is designed as shown in Figure 2.13. The transducer moves along the X-axis from its starting point and performs a move-stop-scan routine. After 100 steps (20 mm) are scanned, the transducer moves back to the starting position and moves one step (200 um) on Y-axis. The motion pattern repeats until it reaches the 100 steps (20 mm) on Y direction. The single-direction scanning pattern is designed to avoid systematic
error in localization caused by the mechanical structure such as gears and belts.

At each step of the scanning trajectory, the transducer stops, and multiple photoacoustic signals are collected. Unlike ultrasound imaging, the thermoelastic expansion in PA imaging is weaker than the reflected ultrasound wave. This leads to the signal collected by the transducer is small in amplitude. With the noise in the signal introduced by a different source, the signal-to-noise ratio (SNR) is small. In some cases, the target PA signal is weaker than the noise being collected. A common technique to achieve noise reduction is to collect multiple frames of data at the same spot. Due to the fact that most of the noise is randomly generated, an averaging filter can significantly reduce the noise. In our setup, the averaging filter size is set to be 10. At each position of the transducer, 10 individual RF data are collected.
Chapter 3

Image Reconstruction

3.1 Beamforming in Ultrasound and Photoacoustic Imaging

In photoacoustic microscopy, a one-dimensional PA signal (A-line) is acquired in each shot, with information on energy absorption property mapping to the time arrival. Through the transducer scanning along with the two transverse directions, a volume PA image can be obtained. In acoustic-resolution PAM, the axial resolution depends on the numerical aperture and the bandwidth of the transducer. The lateral resolution depends on the focal of the single element transducer. However, with the depth penetration capability of AR-PAM, the image quality in the out-of-focus region is significantly degraded.

To solve the problem, Zhang et al. [79] proposed a method to maintain a constant US focal depth from the skin surface based on the pre-known skin profile. The downside of the method is the slow acquisition speed and the reduction of lateral resolution in the off-focus region. Delay-and-Sum (DAS) is the most basic and common beamforming algorithm because of simplicity [80]. Liao et al. [81] proposed
a synthetic aperture focusing technique (SAFT) with a virtual detector (VD) that expanding the focal region of the microscopy. SAFT is commonly used in radar and ultrasound researches. By regarding a US transducers traveling distance as an effective aperture, new A-lines are obtained by linearly combining appropriately delayed scan-line signals. By implementing coherence factor (CF) [82–84], 1-D SAFT [85], two-dimensional (2-D) SAFT [86], and adaptive SAFT along the arbitrary vessel direction [87] have been investigated. Minimum variance (MV) is another adaptive beamforming algorithm that weights the signals and reduces the effect of the off-axis signal introduced by Nguyen et al. [88]. Park et al. combine the MV with the coherence factor in PAI [89]. A short-lag spatial coherence beamformer is used to enhance the contrast of the PAI by Bell et al. [90]. Bandaru et al. introduces the delay and standard deviation beamforming algorithm with conventional ultrasound imaging [91]. A more improved version of the SAFT based on a delay-multiple-and-sum (DMAS) has been demonstrated by Park et al [14].

In this research, the Delay-and-Sum algorithm is implemented to the obtained photoacoustic image as a baseline of reconstruction. Then the coherence factor (DAS+CF) and Delay-multiply-and-sum (DMAS) will be introduced.

### 3.2 Delay-and-Sum Algorithm with Coherence Factor in Imaging

In the Delay-and-Sum algorithm, the acoustic focal point from the single element transducer is considered as a virtual detector (VD) as shown in Figure 3.1 ((a) Schematic diagram of a virtual detector (VD) concept for SAFT. (b) Geometry of the VD to find a time delay for SAFT. z, depth of the synthesized point; zf , depth of the VD; r , depth (axial) distance; and r 0, distance from the VD to the synthesized
point.) [14, 81]. A certain angular extension form the VD is assumed to be able to detect the PA signal. While scanning, the PA radiation fields of the consecutive VDs overlap, and the VDs at adjacent positions detect the signal generated from the overlapped field repeatedly.

With DAS algorithm, equation 3.1 combining delayed scan-line signals to a new A-line.

$$y_{DAS}(t) = \sum_{i=1}^{N} s_i(t - \Delta t_i),$$

(3.1)

In Equation 3.1, $s_i(t)$ is the acquired zero-mean signal at the $i$'th scan. $N$ is the synthesized number of scan-line that is determined by the angular extent of the radiation field. $\Delta t_i$ is calculated as Equation 3.2 which is the time delay to the received signal at scan $i$.

$$\Delta t_i = sign(z - z_f) \cdot \frac{r - r'}{c},$$

(3.2)
c is the speed of sound, z is the depth of the synthesized point, and \( z_f \) is the VD depth, \( r \) and \( r' \) are the axial distance and the distance from synthesized point to the VD.

By introducing the coherence factor (CF) as a weighting coefficient, the image quality can be further improved. If the target occurs in the scanning direction that is off-axis, the delayed signals are no longer in phase. Then the information from the off-axis target is dominating the SAFT applied signals rather than the synthesized direction target in the case. The CF is defined as Equation 3.3.

\[
CF(t) = \frac{\left| \sum_{i=1}^{N} s_i(t) \right|^2}{N \sum_{i=1}^{N} |s_i(t)|^2} \quad (0 \leq CF(t) \leq 1), \tag{3.3}
\]

When the calculated CF = 1, it means that the amplitude of the output should be maintained because of the strong coherence. On the other limit, if CF = 0, the output should be decreased due to incoherence. The CF-weighted DAS output is calculated as Equation 3.4

\[
y_{DAS,CF}(t) = CF(t) \cdot y_{DAS}(t) \tag{3.4}
\]

### 3.3 Delay-Multiply-and-Sum Algorithm in Imaging

Although the Delay-and-Sum algorithm (Equation 3.1) is simple and robust in the radar and ultrasound fields, the method has a limited resolution and poor off-axis interference rejection. Those factors cause broad image clutter to the enhanced PA image. Another method to solve the problem is the Delay-Multiply-and-Sum (DMAS) algorithm [14]. The DMAS method reinforces the signal components from
the direction of interest by combining the delayed scan-line signals. Unlike the DAS algorithm, the delayed signals are combinatorially coupled and multiplied before the summation, as shown in Equation 3.5.

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

Because of the dimensional squared and distortion, the Equation 3.5 needs to be modified with an additional step. A new equivalent scan-line signal \( \hat{s}_{ij} \) is calculated by Equation 3.6 for a signed geometric mean to the coupled scan-line signals.

\[ \hat{s}_{ij}(t) = \text{sign}[s_i(t)s_j(t)] \cdot \sqrt{|s_i(t)s_j(t)|} \quad \text{for} \quad i \leq i < j \leq N. \]  

With this additional geometry mean process, the dimension of the \( \hat{s}_{ij}(t) \) equals to the \( s_i(t) \) with the sign kept. The new A-line output then is calculated as Equation 3.7.

\[ y_{DMAS}(t) = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \hat{s}_{ij}(t) \]  

Note that the direct current (DC) and harmonic components appear in the spectrum of \( y_{DMAS}(t) \). The product of the signals in the time domain is equal to the convolution of the spectra of those two in the frequency domain according to the functional relationship between the two domains. Since \( s_i(t) \) and \( s_j(t) \) have similar frequency ranges, the new components centered at the zero frequency and the harmonic frequency appear in the spectrum after the coupling operation. A band-pass (BP) filter should be applied to the signal to remove the DC component while keeping the harmonic signals. Therefore, the final result should be calculated as Equation 3.8.

\[ y_{F\cdot DMAS}(t) = h_{BP}(t) \ast y_{DMAS}(t) \]
In Figure 3.2, the block diagram shows the entire process to acquire the DMAS signal from the original A-line signals.

Figure 3.2: Block diagram of a modified DMAS [14]
Chapter 4

Results

4.1 Image Resolution - Step Error Analysis

To investigate the acquired PA image lateral resolution with the actuator motion accuracy, a simulation is conducted in the Matlab K-Wave package. K-Wave is a Matlab toolbox for the time-domain acoustic wave simulation commonly used in simulating photoacoustic signals.

In this experiment, a 2-D scanning is simulated with a length of 10mm. The point sensors are placed in the surface line with 200um distance apart from each other to simulate the microscopy scanning. To simulate the mechanical error introduced by the actuator, a random position error with a controlled standard deviation is applied to the sensor position. Then the collected RF data are reconstructed by the conventional Delay-and-Sum beamforming algorithm without knowing the sensor placing error. A point target with 20 um diameters is placed at the centerline of the sensors at the depth of 8 mm. The simulation is shown in Figure 4.1 (note that the sensors in figure is lower than pixel resolution so not all sensors are visible)

With ideal sensor placement (error SD = 0 um), the acquired PA image enhanced
Figure 4.1: K-Wave simulation setup.

Figure 4.2: K-Wave simulation result when sensor placement is ideal by conventional DAS beamforming is shown in Figure 4.2. The lateral FWHM is 0.3911 mm.

As the sensor placement error increases to 50 um, the result enhanced by DAS is shown in Figure 4.3. The FWHM is measured as 0.4046 mm. Further, in Figure 4.4, the curve between the FWHM of DAS enhanced point target with respect to the increasing standard deviation of sensor placement error is plotted.
Figure 4.3: K-Wave simulation result when error SD = 50 um

Figure 4.4: Simulation lateral FWHM vs. sensor placement error standard deviation

4.2 Noise Reduction

As we discussed in the previous part, the photoacoustic signal detected by the single element transducer is relatively weak comparing to the reflected ultrasound signal in a similar setup. On the other hand, there are noises generated by multiple sources. Mainly the noises are generated by the median material, reflections, and electromagnetic noise from the circuits. Most of those noises are random noise, which is able to be filtered.
Figure 4.5: (a) An example of collected RF data with a target PA signal at 500 depth. (b) Processed RF signal with 10 window size average filter.

In our setup, an average filter with a window size of 10 is designed for collecting RF data. As shown in Figure 4.5 (a), the 10 raw RF data contains significant noise. The target PA signal is locating around the depth of 500 samples, which is overlaying by the noises. With the average filter applied, the filtered signal is appearing as shown in Figure 4.5 (b). The target clearly appears with the noise being reduced.

Another commonly used filter is also applied to these RF data which is the median filter. The filtered signal is shown in Figure 4.6. The target also clearly appears and the noise is reduced.
To investigate how the SNR improves with the increment of the filter window size in the averaging filter, a group of RF data containing the PA target signal is marked. The raw RF data are combined with all possible combinations for the size of each window. Then the filtered results are averaged for evaluating the SNR improvement with respect to the window size. Figure 4.7 shows the relationship between the SNR with the increasing window size of the averaging filter. The SNR of increasing from 42.41 dB to 51.31 dB with 10 window size filter applied.

Figure 4.7: The relationship between signal-to-noise ratio (SNR) with the size of the average filter window size.
4.3 Tube Phantom Study

The proposed acoustic-resolution photoacoustic microscopy (AR-PAM) system is tested with tube phantom. The phantom study is conducted to investigate the photoacoustic imaging quality with the target at a different depth, as well as the image contrast and resolution enhancement with different image reconstruction algorithms applied.

The tube phantom is designed as shown in Figure 4.8. There are two different kinds of tubes filled with different materials. The ink tube is filling with India ink with an inner diameter of 0.25 mm. The water tubes are set as negative control. The phantom is placed in the water for scanning. The area-of-interest is 2 mm by 2 mm square as described in the previous section.

An ultrasound imaging scanning is conducted with the PAM as a comparison group (Figure 4.9). The showing result is enhanced by Delay-and-Sum (DAS) beamforming algorithm for higher contrast and resolution. Because of the different physi-
Figure 4.9: The ultrasound imaging of the phantom.

cal principles behind ultrasound imaging and photoacoustic imaging, the water tube is visible in the ultrasound image. The ultrasound scanning demonstrates the functionality of the system in motion control, data collection, signal processing, and image reconstruction. The ultrasound imaging result is set as the baseline of the later photoacoustic imaging.

Two rounds of photoacoustic imaging of the phantom are collected with two different depth set to compare the depth penetration of the PAM. The focal distance of the single element transducer is 0.5 inches (12.4 mm). The depth of the phantom is set at 14.2 mm and 19.3 mm under the transducer.

Table 4.1: Image Evaluation of 14.2 mm Depth Scanning

<table>
<thead>
<tr>
<th></th>
<th>SNR (dB)</th>
<th>FWHM (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-reconstruction</td>
<td>47.0418</td>
<td>0.6029</td>
</tr>
<tr>
<td>DAS</td>
<td>70.3786</td>
<td>0.3497</td>
</tr>
<tr>
<td>DAS+CF</td>
<td>102.3261</td>
<td>0.2177</td>
</tr>
<tr>
<td>DMAS</td>
<td>101.8412</td>
<td>0.2207</td>
</tr>
</tbody>
</table>
Figure 4.10: PAM results for depth 14.2 mm phantom. Pre-reconstruction, DAS, and DMAS reconstruction with corresponding cross-section images when \( x = 10 \) mm are presented.

The collected photoacoustic signals are post-processed with different beamforming algorithms. The images are reconstructed in 3D space for generating high-quality images visible from different view-angle. The Delay-and-Sum (DAS), Delay-and-Sum with Coherence Factor (DAS+CF), and Delay-Multiply-and-Sum (DMAS) reconstruction algorithms are implemented in 3D in the research.

The photoacoustic image and reconstructed results are shown below. In Figure 4.10, the round 1 scanning result is demonstrated. The first row displays the maximum-intensity-projection (MIP) image on the X-Y plane of the 3D PA image. On the second row, the Y-Z plane slice images of the cross-section at \( x = 10 \) mm are shown. From left to right, each column represents the image from pre-reconstruction,
DAS, DAS+CF, and DMAS reconstruction images.

Table 4.2: Image Evaluation of 19.3 mm Depth Scanning

<table>
<thead>
<tr>
<th></th>
<th>SNR (dB)</th>
<th>FWHM (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-reconstruction</td>
<td>4.8078</td>
<td>7.8086</td>
</tr>
<tr>
<td>DAS</td>
<td>42.1936</td>
<td>0.9344</td>
</tr>
<tr>
<td>DAS+CF</td>
<td>59.0670</td>
<td>0.2383</td>
</tr>
<tr>
<td>DMAS</td>
<td>86.4929</td>
<td>0.3968</td>
</tr>
</tbody>
</table>

In Table 4.1, the signal-to-noise ratio (SNR) and the Full-Width-at-Half-Maximum (FWHM) quantification under different reconstruction algorithms are listed. In Figure 4.11, and Table 4.2, the result of the round 2 PA scanning is shown with the same layout from the first round scanning.
Figure 4.11: PAM results for depth 19.3 mm phantom. Pre-reconstruction, DAS, and DMAS reconstruction with corresponding cross-section images when $x = 10$ mm are presented.
Chapter 5

Discussion

The phantom study demonstrates the capability of the AR-PAM system on multiple levels. On hardware, system stability and repeatability to scanning the target are tested. The ultrasound imaging collected by the platform with minor system modification proves the hardware design. The acquired photoacoustic images before reconstruction suggest the proposed delta configuration actuation provides enough precision in motion and localization. At the same time, the study proves the working of system architecture and the synchronization pipeline.

Comparing with the Cartesian configuration actuator, the Cartesian configuration has easy kinematic for understanding. And it excels in horizontal precision, that the lateral motion resolution does not change with the position. However, the tip of a Cartesian mechanism is heavier and bulky. On the other hand, delta-configuration has all the actuators on the frame structure. As a result, the tip is much lighter without any motion drive device attached. This weight reduction leads to reduced inertia which makes the mechanism able to respond quickly at the end of a movement while retaining its accuracy. At the same time, with an overall simplified construction and fewer parts used in delta-configuration, the maintenance and costs
are lower. Therefore, a delta configuration suits this compact and low-resolution microscopy designing better.

With the simulation of 2-D scanning, the effect of the mechanical error in moving the transducer on the PA image lateral resolution is investigated. With a 50um linear error, which is the minimum step size as the datasheet of the customized 3D printer used in this project, the image lateral resolution is reduced by 0.135 mm (3.45%). This result proves the feasibility of the low-cost delta-configuration 3D printer for the microscopy application. Further, the result of the simulation demonstrates the beamforming algorithm is capable to cancel certain level of the image distortion caused by the positioning error of the actuator. With the beamforming, the image lateral resolution can maintain within the desired range even with the sensor placement error equal to the minimum designing step size.

The experiment shows the averaging filter which is applied during the data acquiring significantly reduces the noise of the collected frame signal. Signal-to-noise ratio (SNR) is one of the most commonly used parameters for evaluating the signal. It compares the level of the desired target to the background in signal power. A higher ratio indicates a higher contrast of the signal. As Figure 4.7 shows, the average SNR of a single frame RF data with the tube target at depth of 14.2 mm is 42.41 dB. With the filter data size increases, the SNR has a logarithmic increment to 51.31 dB when the size is ten. By comparing the median filter to averaging filter, the lateral filter shows a better performance in SNR increment.

To acquire a high-quality PA image, the proposed AR-PAM system processes the collected PA image by software after the scanning. With the beamforming technique, the PA image quality is further enhanced. In the experiment, there are three algorithms applied individually to the acquired signals, Delay-and-Sum (DAS), Delay-and-Sum with Coherence Factor (DAS+CF), and Delay-Multiply-and-Sum
(DMAS). Delay-and-Sum is a commonly used beamforming technique. The other two methods are more advanced algorithms that enhance image quality further. The DAS results are shown in the result as a baseline of reconstruction improvement.

In this study, two specifications of the image are measured for quantifying the imaging quality. The image contrast and resolution are quantified. The image contrast is quantified by SNR as introduced. To evaluate the lateral resolution of the acquired data, full width at half maximum (FWHM) is measured. FWHM is an expression of the extent of function given by the difference between the two extreme values of the independent variable at which the dependent variable is equal to half of its maximum value, which defines the resolution of the acquired image. Comparing the PA image with the collected ultrasound image, the ink tubes are appearing at the same location, while the water tubes do not. The comparison suits the initial hypothesis and demonstrates the correctness of the received PA image.

As the Table 4.1 shows, when the target is at the depth of 14.2 mm, the DAS reconstruction improves the image in both contrast and resolution from pre-reconstruction (SNR: 47.0418 dB, FWHM: 0.6029 mm) to (SNR: 70.3786 dB, FWHM: 0.3497 mm). With the advanced reconstruction algorithms, the image quality is enhanced to DAS+CF (SNR: 102.3261 dB, FWHM: 0.2177 mm) and DMAS (SNR: 101.8412 dB, FWHM: 0.2207 mm). Both algorithms result in a similar improvement in the image, with a slightly leads by DAS+CF algorithms. With the target depth increasing to 19.3 mm (Table 4.2), the pre-reconstruction data is fuzzy (SNR: 4.8078 dB, FWHM: 7.8086 mm). However, with the reconstruction enhancement, the image quality improves to DAS (SNR: 42.1936 dB, FWHM: 0.9344 mm). Comparing between DAS+CF (SNR: 59.0670 dB, FWHM: 0.2383 mm) and DMAS (SNR: 86.4929 dB, FWHM: 0.3968 mm), the first method provides higher resolution and the lateral method provides a better increment on the imaging contrast when the
target depth is large.

On the system implementation level, the customized delta-configuration 3D printer costs 180 USD [74]. The customized scanning tip is 3D printed which costs can be ignored. The entire motion controller and actuators are already included with the off-the-shelf 3D printer, and there is no extra cost to connect the controller to the host machine by utilizing the Matlab serial communication package. The experiment successfully proves the low-cost solution to PA microscopy by introducing the delta-configuration actuation.

However, there are some limitations to this AR-PAM system at the current stage. First, the system synchronization limits the scanning time significantly. A scanning round of a square area of 20 mm (100,000 frames in total, step size = 0.1 mm, average filter data size = 10) takes approximately 70 minutes. The AR-PAM is running at an average scanning rate of around 25Hz. Comparing to the ultrasound scanning round (the ultrasound system is not synchronized with the laser), the scanning of the same data size takes around 25 minutes, which average scanning rate is approximately 65Hz. The main reason for this speed drop is causing by system synchronization. The laser machine emitting laser energy at a maximum rate of 20 Hz. Counting in the synchronizing delay, an average of 25 Hz rate is reasonable.

Second, image reconstruction requires intensive computational power. The quantification below is measured at the high-configured workstation (CPU: AMD Ryzen threadripper 3970x 32-core processor, Memory: 125.7 Gb, GPU: TITAN RTX) with Matlab parallel CPU computing. With the data size of 10,000 frames, Delay-and-Sum (DAS) reconstruction takes about 3 minutes. Similarly, Delay-and-Sum with Coherence Factor reconstruction takes approximately 4 minutes. The Delay-multiple-and-Sum (DMAS) required the most computation process, which takes approximately 25 minutes. An optimized reconstruction algorithm or parameters
that generate similar image quality can be investigated for future research.

Third, the depth penetration of the AR-PAM is limited. The experiment indicates a 5 mm depth is able to be achieved with reconstruction enhancement. However, the image quality is significantly reduced comparing to a shallower depth. The main reason is the light spreading and distortion. With the AR-PAM design, the optical focal point is aligned with the acoustic focus point. The optical energy beyond this depth is weaker. By adjusting the design of the scanning tip and increasing the optical focal distance, the problem should be solved at a certain level. Although the issue of optical shadowing does not appear during this study, the current scanning tip design allows another two fibers mounting from the side. With another two sets of beam splitters, the laser beam can be split into four beams, and the preventing the shadow issue.

Finally, the AR-PAM has only tested with the phantom study. A continuous scanning study with biomedical materials and living animals is required for the future study. The next stage of this research should be focused on design the mounting mechanism for scanning different phantom on this AR-PAM.
Chapter 6

Conclusion

In this research, an economical delta-configuration actuation acoustic-resolution photoacoustic microscopy (AR-PAM) system is introduced and tested. The combination of the hardware and software approach to obtain a high-resolution PA image is proved to be working.

The research answers to the question of the possibility of substituting the expensive and bulky linear translation stages commonly used in the state-of-art AR-PAMs. The unique characteristic of the linear delta mechanism gives the system stability and repeatability in motion control. By adopting a low-cost commercially available off-the-shelf 3D printer with unique PA optical and acoustic aligning design to the tip, the size and the cost of the system is miniaturized.

This research also investigates the PA image reconstruction in 3D volume space. Two advanced PA image enhancement methods (delay-multiply-and-sum, and coherence weighting delay-and-sum) are applied to the system. Compared with the original pre-processed PA image and the enhanced image with the conventional method (delay-and-sum), the enhanced method provides significant improvement to the image quality. The phantom study demonstrates the high-quality PA image
obtained by the system with its hardware and software integration.

The future work of this research should be focusing on solving the existing limitation and applying the system into different working scenarios. The control and reconstructing program could be optimized to reduce the computational power requirement and time duration. Minor designing upgrades could be done to increase the scanning range in depth. And with continues research, more biomedical materials should be tested with this system.
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