Toward whole-body quantitative photoacoustic tomography of small-animals with multi-angle light-sheet illuminations

YIHAN WANG,1 JIE HE,1 JIAO LI,1,2,4 TONG LU,1 YONG LI,3 WENJUAN MA,3 LIMIN ZHANG,1,2 ZHONGXING ZHOU,1,2 HUIJUAN ZHAO,1,2 AND FENG GAO1,2,5

1College of Precision Instrument and Optoelectronics Engineering, Tianjin University, Tianjin 300072, China
2Tianjin Key Laboratory of Biomedical Detecting Techniques and Instruments, Tianjin 300072, China
3Cancer Institute and Hospital, Tianjin Medical University, Tianjin 300060, China
4jiaoli@tju.edu.cn
5gaofeng@tju.edu.cn

Abstract: Several attempts to achieve the quantitative photoacoustic tomography (q-PAT) have been investigated using point sources or a single-angle wide-field illumination. However, these schemes normally suffer from low signal-to-noise ratio (SNR) or poor quantification in imaging applications on large-size domains, due to the limitation of ANSI-safety incidence and incompleteness in the data acquisition. We herein present a q-PAT implementation that uses multi-angle light-sheet illuminations and calibrated recovering-and-averaging iterations. The scheme can obtain more complete information on the intrinsic absorption from the multi-angle illumination mode, and collect SNR-boosted photoacoustic signals in the selected planes from the wide-field light-sheet excitation. Therefore, the sliced absorption maps over whole body of small-animals can be recovered in a measurement-flexible, noise-robust and computation-economic way. The proposed approach is validated by phantom, ex vivo and in vivo experiments, exhibiting promising performances in image fidelity and quantitative accuracy for practical applications.

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1. Introduction

Photoacoustic tomography (PAT) has the unique capabilities to provide high optical absorption contrast and excellent spatial resolution for soft tissue imaging [1–5]. Its applications have included clinical and preclinical studies in breast and skin cancer, vascular diseases, as well as small animal imaging [6–8]. However, the conventional PAT (c-PAT) still faces major challenges of providing quantitative optical information about tissues, because its output is the distribution of the absorbed light energy density or initial acoustic pressure $P_0$, i.e., the product of the absorption coefficient and the photon fluence within the irradiated regions. If physiologically and pathologically relevant optical properties could be quantitatively estimated from c-PAT, the research significance and practical potential of PAT in clinical and biomedical studies will be dramatically enhanced. Thus, there is a pressing need to develop quantitative PAT (q-PAT), mainly focusing on quantifying the distribution of optical properties inside tissues from the c-PAT results [9, 10]. Accordingly, q-PAT can also accurately obtain concentrations of endogenous and exogenous chromophores (hemoglobin, melanin, fluorescence agent, nanoparticle, etc) based on their spectral specificity and multi-wavelength measurements, so that the morphological, functional and molecular information of biological tissues can be simultaneously provided [11–14].

Currently, q-PAT is still in the development stage, generally including two steps: the first step is to recover the map of the light absorbed energy density or $P_0$, which has been extensively studied as the acoustic inverse problem or the c-PAT process; the second step is a more challenging and non-trivial optical inverse problem known as the q-PAT process, for reconstructing the distribution of the absorption coefficients from $P_0$ by introducing a forward model of light transport. Although some reconstruction theories of q-PAT have been investigated [10], its practical application is still an open problem due to the greater requirements for the signal-to-noise ratio (SNR) of photoacoustic (PA) signals, the stability and robustness of the reconstruction method. The accuracy of quantitative results is also related to the match of the reconstructed $P_0$ with the absorbed energy density calculated by the optical forward model.

To meet these requirements, some strategies for optimizing the measurement setups and the reconstruction methods need to be profoundly studied. (1) Excitation source settings: To simplify the light transport modelling process, some theoretical studies of q-PAT tend to set the point sources in their numerical experiments [15]. However, in practical applications, the point source or sources may cause low SNR of PA signals under the request of ANSI safety limit for biological tissues, especially for deep tissue imaging. The wide-field illumination, instead of the point source or sources, can be employed to improve the SNR of measurement and then offer more reliable reconstructed $P_0$ as the input of the q-PAT reconstruction [16–21]. For example, Li et al. [16], Liu et al. [17] and Shan et al. [18] have obtained reliable experimental results in mapping the optical absorption coefficients using the wide beam irradiating from the top of object. Nevertheless, this illumination mode is not suitable for cross-sectional imaging of small-animal torso. Jetzfellner et al. have shown a high-fidelity quantitative result of a tissue-mimicking phantom using the whole-angle illumination from the side surface [19]. Brochu et al. have obtained some quantitative results from in vivo experimental data, using a commercial multispectral optoacoustic tomography (MSOT) system, which provides a homogeneous, 360-degree illumination by a multi-arm fiber bundle [20]. (2) Illumination modes: The single-illumination utilizes an illumination approach, where the incident light comes from either a fixed direction or fixed directions, or the single source is configured without different spatial modulations [22]. While, by use of illumination sources with various directions or spatial modulations, the multiple-illumination (MI) mode is able to attain more comprehensive and complete information on the intrinsic absorption of the targets, thus also alleviating non-uniqueness and ill-condition of the optical inverse problem [15, 23–25]. Zemp et al. have applied the MI setting in theoretical researches and proposed both non-iterative and iterative solutions for the q-PAT reconstruction. The studies have
demonstrated that the iterative strategy has higher robustness and stability in the reconstruction process [15, 24]. Pulkkinen et al. have realized the MI mode through spatially modulated illumination patterns at one side of the target [26]. Compared with the MI mode of multi-direction illuminations, this approach is not able to image the part deep inside the tissue. (3) Model calibration: For absolute quantitative reconstruction of optical properties, the match between the experimental reconstructed result in the c-PAT process and its optical calculating model in the q-PAT process is an important problem to solve, because the reconstructed $P_0$ images are not typically recovered as absolute quantities. Thus, a solution should be proposed to calibrate $P_0$ before the q-PAT reconstruction, such as using a reference phantom with known optical properties [19].

To quantitatively map the distribution of local optical absorption coefficients from the c-PAT results and enhance the applicability of q-PAT in more practical cases, we herein develop a q-PAT imaging implementation combining the wide-field light-sheet excitation and multi-angle illumination mode. The former enables boosting SNR of $P_0$ images from the specific-size light-sheet illumination and guarantees the excitation in a certain imaging plane without loss of modelling simplicity of the photon fluence. The latter can provide more comprehensive and complete information on the intrinsic absorption from the multi-angle pattern. Moreover, a calibration scheme to match the experimental $P_0$ image with its optical calculating model is also proposed to support the absolutely quantitative reconstruction of optical absorption coefficients. The sliced absorption maps are stably recovered adopting an iterative process with multi-angle recovering-and-averaging, in which the estimated absorption map in one iteration step has synthesized the reconstruction results from each illumination angle. The proposed approach is experimentally validated by a self-built PAT system. The phantom experimental result shows the quantitative accuracy of this q-PAT approach. The ex vivo and in vivo experiments demonstrate the improvement of image fidelity and recovery of the optical absorption coefficient in practical applications compared to c-PAT.

2. Methods

2.1 Experimental setup

A layout of the experimental setup is shown in Fig. 1. The optical excitation is sourced from a pulsed Nd:YAG laser (Nimma-600, Beamtech Optronics, China), generating 6 ns pulses at 532 nm with a repetition rate of 10 Hz. The beam light is via a lens system including a beam expander, a collimator and a variable-slit aperture, to create uniform light-sheet illumination with a width of 30 mm and a thickness of 2 mm. The light is delivered to a certain imaging plane with an incident energy density lower than the ANSI limit of 20 mJ/cm². The cylindrical imaging chamber, made of a thin transparent polyethylene film, is positioned along the axis of rotation, the z axis, by connecting it to the rotation stage *1 (RSA60, Zolix, China). The diameter of the imaging chamber is the same as the width of the light sheet, where the corresponding circular cross-sectional region of the imaging chamber is considered as the slice to be reconstructed. To achieve multi-angle scanning, the rotation stage *2 (RAK200, Zolix, China) is employed to carry a transducer with a central frequency of 3.5 MHz (V380, Olympus NDT), cylindrically focusing on the illumination plane. Two stages collectively complete the whole scanning process to acquire the PA signals from the multi-angle illuminations. The received PA signals are then amplified by a 50-dB amplifier (PREAMP2-D, US Ultratek), and digitized by a data acquisition card with a sampling rate of 75 MHz (PCI8552, ART Technology, China). The whole PAT measurement is synchronized by the pulsed Nd:YAG laser. To ensure a stable speed of sound in the water, especially for in vivo experiments, the heating elements are placed in the water tank to maintain a constant temperature of 33 °C.
2.2 Measurement process

Figure 2 shows the multi-angle PAT measuring process. The multi-angle illuminations are achieved by sequentially rotating the rotation stage *1 at a certain interval, \( i.e., \frac{360}{I}^{\circ} \), where \( I \) is the total number of incidence angles. At the #1 illumination angle, the transducer taken by rotation stage *2 scans the target circularly from 90° to 270° at an angular interval of 2° in one imaging plane. At the #i \((i \in [1, I])\) illumination angle, measuring positions of the transducer are same as the ones in the #1 illumination angle, shown as the small white spots in Fig. 2. At the end of one-plane multi-angle measurements, the target could be shifted at a vertical displacement by the lifting stage (PSA200-11-Z, Zolix, China) for choosing different imaging planes.

![Figure 2. Schematic of the multi-angle PAT measuring process.](image)
2.3 Iterative reconstruction with multi-angle recovering-and-averaging

Figure 3 demonstrates the framework of the iterative reconstruction approach with multi-angle recovering-and-averaging.

The optical model of $P_0$ in the q-PAT process is expressed as

$$P_0(r) = \Gamma \mu_a(r) \Phi[r, \mu_a(r), \mu'_a(r)],$$

where $\Phi(r)$ is the distribution of photon density varying over spatial position $r$, which is a function of the absorption coefficient $\mu_a$ and the reduced scattering coefficient $\mu'_a$. The Grüneisen parameter $\Gamma$ is considered to be spatially constant. In this work, we focus on using the proposed q-PAT strategy to recover the sliced absorption maps in a robust and computation-economic way. An appropriate calibration has been applied to reconstructed $P_0$ images, in order to eliminate the effect from scaling factors between the experimental recovered $P_0$ and the calculated $P_0$ from the selected optical forward model, which may be related to many factors, such as $\Gamma$, acoustic attenuation, and characteristic parameters of the experimental setup. The experimental recovered $P_0$ and the calculated $P_0$ of a reference phantom with the known optical properties are adopted in the calibration process for effectively estimating the scaling factors. Moreover, although the $P_0$ image of a certain plane can be obtained by using the cylindrically focused transducer, the photon density of $\Phi(r)$ contained in $P_0$ is generated from a three-dimensional (3-D) photon migration model. Thus, in order to accurately calculate the optical forward model using a simplified two-dimensional (2-
D) framework, the calibration process should also be used to render the forward optical model of $P_0$ more suitable for the sliced q-PAT reconstruction. We introduce a calibrated 2-D reconstruction scheme proposed by Schweiger and Arridge, which has been widely used in diffuse optical tomography (DOT) reconstruction [29–32]. The whole calibration process is described as follows, and the feasibility of the calibrated 2-D reconstruction scheme will be discussed in section 4.

We first carry out the same measuring and recovering process on the target and a reference phantom, respectively. Then, for the $i$-th incidence angle, we calculate the calibration coefficient matrix $C(i) = [C(i, r), C(i, r_2), \cdots, C(i, r_y)]$ to calibrate the reconstructed task $P_0$ image $P_0^{*}(i) = [P_0^{*}(i, r), P_0^{*}(i, r_2), \cdots, P_0^{*}(i, r_y)]$, with $N$ being the total number of the pixels in each image. The element in $C(i)$ is

\[
C(i, r_n) = P_0^{ref}(i, r_n) / \hat{P}_0^{ref, 2D}(i, r_n) \quad (n = 1, 2, \cdots, N),
\]

with $r_n$ being the spatial position of the $n$-th pixel; $P_0^{ref}(i, r_n)$ and $\hat{P}_0^{ref, 2D}(i, r_n)$ belong to the experimental reconstructed reference $P_0$ image $P_0^{ref}(i)$ and the optical forward model $\hat{P}_0^{*}(i)$ calculated in a 2-D framework, respectively. We finally get the calibrated task $P_0$ image $\hat{P}_0^{*}(i) = [\hat{P}_0^{*}(i, r), \hat{P}_0^{*}(i, r_2), \cdots, \hat{P}_0^{*}(i, r_y)]$, where

\[
\hat{P}_0^{*}(i, r_n) = P_0^{*}(i, r_n) / C(i, r_n).
\]

Thereafter, the following q-PAT reconstruction can be performed in a 2-D framework.

The reference phantom is prepared as follows: to simulate the optical scattering and absorption, Intralipid (10%) and India ink with different concentrations have been used [33], and the reference phantom is fabricated to have the similar optical scattering and absorption as the background region of the imaging object. In the case of bio-tissue imaging, the absorption coefficient of the reference phantom is set to $\mu_{a, ref} = 0.01 \text{mm}^{-1}$, and the reduced scattering coefficient is set to $\mu'_{s, ref} = 1 \text{mm}^{-1}$, which is a typical scattering of the biological tissue. Finally, 2% agar powder with low gelling temperature (36°C, A9414, SIGMA) is used to solidify the whole mixed solution.

For the sliced q-PAT reconstruction, we propose an iterative calibration approach with multi-angle recovering-and-averaging, which is an extension to the fixed-point iteration strategy used by both Cox et al. [22] and Jetzelflner et al. [19]. The reconstruction is largely affected by $\Phi(r)$, which, due to the calibration aforementioned, is effectively obtained by solving the 2-D diffusion equation (DE) with the Robin boundary condition [34]. We focus on reconstructing the heterogeneous distribution of $\mu_a$, and the distribution of $\mu'_s$ is set to be known and homogeneous during the reconstruction. The finite element method (FEM) framework is used to obtain numerical solutions. The proposed iterative approach is further detailed in the following form of pseudo codes.
Algorithm 1. Iterative reconstruction with multi-angle recovering-and-averaging

Input: Calibrated $P_0$ images $\hat{P}_{0}^{ik}(i) (i \in [1, I])$ at multiple illumination angles.

Initialize: The total number of incidence angles $I$; the initial optical properties $\mu_{0}^a = \mu_{\text{ref}}$ and $\mu'_{0} = \mu'_{\text{ref}}$; the stopping criteria $\varepsilon = 1.5 \varepsilon - 1$; the maximum number of iterations $K = 100$; the regularization parameter $\sigma = 1 \varepsilon - 2$; the number of current iteration $k = 1$.

While $k \leq K$
  
  For $i$ from 1 to $I$:
    Calculate $\Phi^k(i)$ using the forward model.
    Calculate $\mu^k_s(i) = \sum_{n=1}^{N} (\hat{P}_{0}^{ik}(i, r_n) - \Phi^k(i, r_n))$ for the $i$-th incidence angle, where $\sigma = \Phi^k(i, r_n) + \mu^k_s(i)$. (4)
  
  End for

 Calculate $\mu^k_a = \frac{1}{I} \sum_{i=1}^{I} \mu^k_s(i)$. (5)

 Calculate the averaged error $E^k = \frac{1}{I} \sum_{i=1}^{I} \left( \frac{\sum_{n=1}^{N} (\hat{P}_{0}^{ik}(i, r_n) - \Phi^k(i, r_n))^2}{\sum_{n=1}^{N} (\Phi^k(i, r_n))^2} \right)$. (6)

 If $E^k \leq \varepsilon$
  Return $\mu^k_a$ as the final result and end iteration.
 Else
  Update the number of current iteration $k = k + 1$
  Update $\mu^k_a = \mu^k_a$ for next iteration.
 End if

End while

Output: the distribution of the absorption coefficients $\mu^k_a$.

It is worth noting that the regularization parameter $\sigma$ here is set to ensure long-term convergence of the algorithm, and the same FEM mesh is adopted for the reconstruction of each incidence angle. Moreover, for different incidence angles, calculating steps of $\Phi^k(i)$ and $\mu^k_s(i)$ in the proposed iterative approach can be implemented simultaneously using a parallel computing strategy to greatly reduce the calculation time.

3. Experiments

3.1 Phantom experiment

To investigate the performances of the proposed approach, we have conducted the phantom experiment with three targets mimicking the different organs of the mouse. The experimental agar phantom is made in the same way as the reference phantom mentioned above, and the structure of the phantom is illustrated in Fig. 4. The small-animal-sized cylindrical phantom, with a radius of $R = 15$ mm and a height of $H = 50$ mm, is embedded with three cylindrical targets (*1, *2, and *3) having different radii ($r_1 = 3.5$ mm, $r_2 = r_3 = 2.5$ mm) and the same height of $H_a = 30$ mm located at $(d_1 = 5$ mm, $\theta_1 = 90^\circ)$, $(d_2 = 6$ mm, $\theta_2 = 225^\circ)$ and $(d_3 = 6$ mm, $\theta_3 = 315^\circ)$, respectively. The PAT measurement is acquired in the imaging plane at a distance of $H_i = 15$ mm from top surface of the phantom. To verify the quantitative validity in
the case of low contrasts between the targets and the background, the absorption coefficients are set relatively close. Table 1 lists the absorption and reduced scattering coefficients of the targets and the background in the phantom experiment.

| Region | μa/μs (mm⁻¹) |
|--------|--------------|
| *1     | 0.015/1      |
| *2     | 0.030/1      |
| *3     | 0.020/1      |
| background | 0.010/1  |

We have carried out the multi-angle PAT measurements with the total number of the incidence angles \( I = 4 \), and the reconstructed \( P_0 \) images from each incidence are shown in Fig. 5(a). The multi-angle c-PAT result, defined as \( \frac{1}{I} \sum_{i=1}^{I} P_0(i) \), is also shown in Fig. 5(b). It can be observed from Fig. 5(a) that, for each single-angle c-PAT result, not all the targets can be acquired at the same time and the intensity distribution of each \( P_0 \) image is not uniform. These because that in the case of the excitation source illuminating the target boundary, the distribution of \( \Phi_r \) in the near-source region is higher than that in the deep region. For comparison, as shown in Fig. 5(b), the multi-angle scheme exhibits better image quality, and it evidently demonstrates that the information on the intrinsic absorption is obtained more completely. However, although the multi-angle c-PAT result in Fig. 5(b) suffices to provide appropriate light-absorbing structures of the targets with great spatial resolution, the map of absorption coefficients still cannot be achieved accurately.

Figure 6 illustrates the comparison between the exact absorption image and the q-PAT results with different illumination angles for the phantom experiment. Figure 6(b)–6(d) depict 4-angle, 6-angle and 8-angle q-PAT results respectively, of which the corresponding profiles are exhibited in Fig. 6(e)–6(h) along the blue dashed lines drawn on the exact absorption image shown in Fig. 6(a). The results show that the absorption images reconstructed by the proposed multi-angle q-PAT scheme can offer more satisfactory image fidelity, structural
precision, and quantitative performance compared to the c-PAT results. Moreover, 6- and 8angle results produce greater image fidelity than 4-angle result. For instance, the whole boundary of region *1 in 4-angle q-PAT result couldn’t be clearly observed in Fig. 6(b), whereas it can be easily extracted from 6- and 8-angle reconstructed images.

To quantitatively assess the reconstruction results, the averaged regional reconstructed values have been calculated to evaluate the reconstruction accuracy and the root mean square error (RMSE) in each region has been obtained to analyze the regional uniformity of the reconstructed maps of absorption coefficients. In this experiment, all of the reconstructions are executed on the same FEM mesh, and the map of the exact absorption coefficients is also described on the same mesh. The image can be divided into the different target regions and the background region. We define $M^{(R)}$ as the total number of the nodes in exact region $R$, $X^{(R)}_{\text{exact}} (m)$ and $X^{(R)}_{\text{recon}} (m)$ as the exact and reconstructed absorption coefficient on the $m$-th node in exact region $R$, respectively. Thus, the averaged regional reconstructed value of region $R$ can be defined as $\frac{1}{M^{(R)}} \sum_{m=1}^{M^{(R)}} X^{(R)}_{\text{recon}} (m)$. Table 2 lists the averaged regional absorption coefficients reconstructed by 4-angle, 6-angle and 8-angle q-PAT strategies. It can be analyzed from the table that, the reconstructed values are close to the exact ones and the maximum relative error of the reconstruction is 17.33% coming from region *1 by 4-angle reconstruction. The rest of the relative errors are all less than 10%, which demonstrates the validity and accuracy of the multi-angle q-PAT approach. It can still be seen from the table that, the reconstructed values in the same region by different reconstructions are similar. In contrast, as depicted in Fig. 6, the uniformity in the same region have obvious differences by using 4-angle, 6-angle and 8-angle q-PAT strategies, which can be assessed by RMSE in each region, as shown in Fig. 7.

![Fig. 6](image_url)

Fig. 6. (a) Exact absorption image of the phantom. q-PAT results with different illumination angles for the phantom experiment in Section 3.1: (b) 4-angle (c) 6-angle and (d) 8-angle results. Profiles of the reconstructed absorption coefficient images along (e) Line 1, (f) Line 2, (g) Line 3 and (h) Line 4.
Table 2. Averaged Regional Absorption Coefficients Reconstructed by 4-angle, 6-angle and 8-angle q-PAT Strategies

| Region | \( \mu_a (\text{mm}^{-1}) \) | Exact | 4-angle | 6-angle | 8-angle |
|--------|-----------------|-------|--------|--------|--------|
| *1     | 0.015           | 0.0124| 0.0132 | 0.0135 |
| *2     | 0.030           | 0.0280| 0.0281 | 0.0291 |
| *3     | 0.020           | 0.0194| 0.0184 | 0.0181 |
| background | 0.010   | 0.0107| 0.0094 | 0.0091 |

The RMSE in exact region \( R \) is defined as

\[
\text{RMSE}(R) = \sqrt{\sum_{m=1}^{M_R^X} (X_{\text{recon}}(m) - X_{\text{exact}}(m))^2 / M^X_R}. \tag{7}
\]

It can be found from Fig. 7 that, for the entire target regions, 6- and 8-angle reconstructions have the smaller RMSE compared with 4-angle scheme. In region *2, the RMSE of 8-angle reconstruction is only 49% of that in 4-angle reconstruction. The relatively large value of RMSE in one region indicates the highly nonuniform distribution of the reconstructed values. Compared with 4-angle reconstruction, 8-angle q-PAT strategy showcases the better performances in image fidelity and uniformity in each of the same target region. Furthermore, to reach the stopping condition, the numbers of iterations of 4-angle, 6-angle and 8-angle reconstructions are 11, 7 and 6, respectively. It explains that increasing the number of illumination angles, the stopping condition can be reached quickly.

![Fig. 7. RMSE of the reconstructed absorption coefficients for different regions by 4-angle, 6-angle and 8-angle q-PAT strategies.](image)

3.2 Ex vivo experiments with biological tissue

To further validate the feasibility of the proposed q-PAT approach with multi-angle light-sheet illuminations for biomedical applications, the ex vivo experiments are conducted on porcine tissues. The first experiment is designed to assess the feasibility of the proposed method in recovering absorption map of a selected layer with the measured data of this slice. The second experiment is to validate its feasibility in realizing 3-D imaging by stacking the absorption maps at multi-layers, reconstructed from the measured data of different slices separately.

3.2.1 Single-layer reconstruction

The biological sample is designed as a sandwich shape, as shown in Fig. 8(a). The layer having two small pieces of porcine liver tissue is sandwiched between the top and bottom layers composed of porcine tenderloin tissue. The porcine liver tissue has larger absorption...
coefficient than tenderloin tissue, used to simulate the lesion target. The whole sample with dimensions (length × width × height) of 18 mm × 20 mm × 14 mm is placed into the imaging chamber. Then the remaining space between the sample and chamber is filled with the agar mixture with the same optical properties as the reference phantom. The 8-angle PAT measurements are obtained in the imaging plane close to the interlayer of the ex vivo sample. Figure 8(b) and 8(c) depict the reconstructed results of multi-angle c-PAT and q-PAT, respectively. As shown in Fig. 8(b), the multi-angle c-PAT result has bright regions close to the boundaries of the sample and internal targets, but barely visible tissue structures in the central region of the sample. In contrast, the multi-angle q-PAT result shows the distribution of absorption coefficients with greater image performances in image fidelity, structural precision of the targets and the background tissue, as well as relative quantitative contrast. We finally obtain that the absorption contrast of $\mu_a^{\text{tenderloin}}/\mu_a^{\text{liver}}$ is about 1.56.

3.2.2 Multiple-layer reconstruction

The geometric sketch and photographs of the second experimental sample are illustrated in Fig. 9. The cylindrical sample has a radius of 15 mm and a height of $H_F = 20$ mm, embedded with a piece of cuboid-shaped porcine liver tissue with a length of $H_L = 7.2$ mm and a thickness of $H_T = 4$ mm located at $H_I \approx 7$ mm from the top surface of the sample. The background of the sample is the agar mixture with the optical properties $\mu_s = 0.1$ mm$^{-1}$ and $\mu_s' = 1$ mm$^{-1}$. The reference phantom with the same size of the cylindrical sample and the same optical properties is also fabricated. A series of cross-sectional 8-angle PAT measurements are sequentially acquired from $z = 15$ mm to $z = 7.5$ mm with the z-axial interval of 0.5 mm. The measurement range is $H_S = 7.5$ mm, which means measurements from a total of 16 layers are obtained.

Figure 10(a) depicts the six selected q-PAT reconstructed images of different layers (S1–S6), marked in Fig. 9(a). As shown in Fig. 10(b), the 3-D result is achieved by stacking and interpolating recovered 16-layer absorption maps along the z-axis within the measurement range.
range of Hs. Figure 10(c) showcases the absorption map in the xz-plane cutting along the dotted lines in Fig. 10(a). The q-PAT results show the satisfactory image fidelity, uniformity and structural precision. The whole boundary of the target region can be clearly observed in S3 and S4, which are the layers across the target area. Moreover, the reconstructed image sequences clearly show the changes along the z-axis. It indicates that the selected cylindrically focused transducer can provide an effective z-axis spatial resolution. Figure 10(b) also reveals the feasibility of realizing 3-D imaging by recovering and stacking the sliced absorption maps.

Some quantitative indexes are listed in Table 3, including averaged regional absorption coefficients and full width at half maximum (FWHM) along the dotted lines in the images of S2, S3, S4, S5, and the xz slice, as shown in Fig. 10. The interactive seeded region growing segmentation method is adopted to select the target region [35].

| Layer | $\mu_a$ (mm$^{-1}$) | FWHM (mm) |
|-------|---------------------|------------|
| target (literature*: 0.7) | background (exact: 0.1) | (exact: 7.2 for S1–S6) | (exact: 4 for xz slice) |
| S1    | —                   | 0.1008     | —                      |
| S2    | 0.1184              | 0.0815     | 8.0526                 |
| S3    | 0.2525              | 0.0885     | 7.8729                 |
| S4    | 0.2496              | 0.0865     | 7.7221                 |
| S5    | 0.1208              | 0.0826     | 9.8791                 |
| S6    | —                   | 0.0903     | —                      |
| xz slice | 0.2421               | 0.0869     | 6.6667                 |

* The optical parameters in healthy porcine liver are determined in the native state and after thermal coagulation using a double integrating sphere system in the wavelength range of 400 ± 2400 nm [36].

It can be analyzed from the table that, the reconstructed absorption coefficients of the tissue sample in layers across the target (S3 and S4) are lower than that in Ref [36], but they are in the same order of magnitude. The differences may be caused by the individual variations of the samples and the different methods of sample processing. Nevertheless, the reconstructed results between S3 and S4 layers only have minor differences. Furthermore, the recovered background from different layers show the satisfactory and reliable results compared to the exact values. For the calculated FWHM, the relative errors of the target
layers (S3 and S4) are 9.346% and 7.251%, respectively. It is observed that, the reasonable resolution in xy-plane can be obtained by the proposed q-PAT approach, while the spatial resolution in xz-plane has a larger relative error. This is because that the crosstalk between different layers exists and is caused by the selected cylindrically focused transducer with the ~6 dB pulse-echo beam width (BW) of 1.3 mm, which may also lead to the reconstructed artifacts of the target existing in S2 and S6.

3.3 *In vivo* mouse experiment

We further demonstrate the *in vivo* applicability of the developed multi-angle q-PAT approach by imaging a 7-week-old male healthy KM mouse with ~29 g weight. The experimental procedures in this study are reviewed and approved by the subcommittee on research animal care at Tianjin Medical University Cancer Institute & Hospital. Before the measurements, the mouse is anesthetized with 10% chloral hydrate, and fixed in the cylindrical imaging chamber, as shown in Fig. 11(j). The remaining space of the imaging chamber is filled with the mixture solution of Intralipid (10%) and India ink, having the same optical properties as the reference phantom. The head of the mouse is sticking out of the matching liquid to keep its normal breathing. A series of cross-sectional PAT measurements are sequentially acquired from thoracic to abdominal regions of the mouse with 1.5 mm z-axial interval. To reveal finer structures and obtain more complete information on the internal absorption of the lab mouse, the number of incidence angles in each imaging plane is set to 15. To qualitatively assess the imaging performances, three selected cross sections owing rich
anatomical contents are exhibited in Fig. 11, along the dotted lines 1, 2, and 3 in Fig. 11(j). The reconstructed results of multi-angle c-PAT (Fig. 11(a-c)) and q-PAT (Fig. 11(d-f)) are shown in the first and second columns, respectively. The photographs of cryoslices at the roughly corresponding positions are also depicted in the third column of Fig. 11.

All the multi-angle c-PAT results display indistinct central regions of cross sections due to the heterogeneous distribution of photon density. For example, the inner boundary of the right lung (RL) cannot be seen clearly in Fig. 11(a), while it can be found from the q-PAT result in Fig. 11(d). In addition, multi-angle q-PAT results obviously present the better performances in image fidelity and uniformity. As depicted in Fig. 11(d), 11(e), and 11(f), the significant regions related with main anatomical structures, such as the lungs, the liver (LV), the spinal cord (SC) and the stomach (SM), are revealed clearly. Furthermore, some fine structures can also be captured, such as several major intrahepatic vessels (VS) in Fig. 11(e) and the peritoneal vessels in Fig. 11(f). All of the above indicate that the multi-angle q-PAT approach enables providing effectively reconstructed absorption images to recognize the physiological features in vivo.

4. Discussion and conclusions

To enhance the applicability of q-PAT in mapping the distribution of local optical absorption coefficients in practical applications, we have developed a q-PAT implementation using a multi-angle illumination mode with wide-field light-sheet excitation sources and an iterative calibration process with multi-angle recovering-and-averaging. Experimental validations have been performed on a self-built PAT imaging system.

To assess the imaging performances of the proposed approach and its quantitative accuracy in recovering the absorption coefficient, the small-animal-sized phantom experiment has been conducted. As shown in Fig. 5(a), the reliable imaging performance in a wide near-source region, illustrates that the wide-field light-sheet excitation enables boosting SNR of $P_0$ images even for the single-angle c-PAT reconstruction. The multi-angle c-PAT result in Fig. 5(b) clearly showing all the targets demonstrates that the multi-angle illumination mode can attain more comprehensive and complete information on the intrinsic absorption of the sample. Accordingly, the multi-angle wide-field illumination mode is able to alleviate non-uniqueness of the optical inverse problem and improve the convergence in the q-PAT reconstruction, compared with the whole-angle circular illumination mode, which can also obtain the reconstructed $P_0$ images with high SNR [19]. In Fig. 6, the accurate quantitative absorption images have been obtained by cooperating with an iterative calibration process of multi-angle recovering-and-averaging approach.

To further validate the accuracy of the calibration process in Eq. (3), a simulation investigation of the calibrated 2-D reconstruction scheme is performed using cylindrical phantoms, as illustrated in Fig. 12(a) and 12(b). Both the task and reference phantoms have the same geometry, with a radius of $R = 15$ mm and a height of $H_L = 20$ mm, as well as the same background optical properties of $(\mu_a = 0.01 \text{mm}^{-1}, \mu_s' = 1 \text{mm}^{-1})$ and $(\mu_{a_{\text{ref}}} = 0.01 \text{mm}^{-1}, \mu_{s_{\text{ref}}} = 1 \text{mm}^{-1})$. The task phantom is embedded with a cylindrical target at $(d = 7$ mm, $\theta = 0^\circ)$, with a radius of $r = 2.5$ mm, a height of $H_T = 5$ mm, and the optical properties of $(\mu_a = 0.1 \text{mm}^{-1}, \mu_s' = 1 \text{mm}^{-1})$. To simulate the actual experimental conditions, we set $H_L = 2$ mm as the thickness of the uniform light-sheet source, and $BW = 1.3$ mm as the beam width of the cylindrically focused transducer. The PAT measurement is acquired in the middle layer of the target. Figure 12(c) show the FEM mesh of the cylindrical phantom, generated for calculating the simulated measurements by a 3-D optical forward model. A real 2-D phantom with the same geometry as the one in Fig. 12(a) is also constructed, and its FEM mesh is exhibited in Fig. 12(d). Without loss of generality, only one incidence angle case is chosen for the analysis.
The reconstructed $P_0$ in the imaging plane (BW) is simulated by

$$P_0^* = \sum_{\{\text{ref}, \text{tsk}\}} a^*_\mu \Phi^*,$$

where $\Phi^*$ is calculated in a 3-D framework, and its distribution in the imaging plane of the reference phantom is shown in Fig. 13(a). The maps of $P_0^\text{ref}$ and $P_0^\text{tsk}$ are respectively illustrated in the left sides of Fig. 13(b) and 13(c). From Eq. (3), the calibrated task $P_0$ image can be obtained by

$$\hat{P}_0^\text{cal} = \frac{\hat{P}_{0,2D}^\text{tsk}}{P_0^\text{ref}} P_0^\text{tsk}.$$

The maps of $\hat{P}_{0,2D}^\text{tsk}$ and $\hat{P}_0^\text{cal}$ are respectively illustrated in the right sides of Fig. 13(b) and 13(c). For comparison, the optical model of the real 2-D task $P_0$ is calculated as $\hat{P}_{0,2D}^\text{tsk}$ shown in Fig. 13(d).

To verify the feasibility and accuracy of the calibration scheme, the relative errors between $\hat{P}_0^\text{cal}$ and $\hat{P}_{0,2D}^\text{tsk}$ are calculated. Moreover, to demonstrate the universal applicability of the calibration scheme, another reference phantom (with different optical properties from the first reference phantom) has also been used in the simulation experiment. All the results are exhibited in Fig. 14. The $P_0$ profiles along Line 1 and Line 2 in Fig. 12(d) are illustrated in Fig. 14(a) and 14(b), respectively. The results show the calibrated task $\hat{P}_0^\text{cal}$ and the real 2-D task $\hat{P}_{0,2D}^\text{tsk}$ have the good consistency, even when the optical properties of the reference phantom do not coincide with ones of the background in the task phantom. As shown in Fig. 14(c) and 14(d), all the relative errors between $\hat{P}_0^\text{cal}$ and $\hat{P}_{0,2D}^\text{tsk}$ are less than 6%, which indicates the feasibility of the calibrated 2-D reconstruction.
scheme. The experimental results in Fig. 6 have also shown its effectiveness in recovering sliced absorption maps.

![Image](image.png)

**Fig. 14. Simulation experiment in section 4. The $P_0$ profiles along (a) Line 1, and (b) Line 2. The relative errors along (c) Line 1, and (d) Line 2.**

Furthermore, the modelling simplicity of the photon fluence is guaranteed by the specific-size light-sheet illumination. The desirable results including quantitative assessments in Table 2 and Fig. 7, demonstrate the capability of the q-PAT approach to achieve the satisfactory results. Moreover, with the increasing number of incidence angles, the q-PAT results exhibit the better uniformity and the faster convergence speed during the reconstruction.

To investigate the validity of the proposed approach in biological applications, *ex vivo* and *in vivo* experiments have been performed on porcine tissues and a lab mouse. The *ex vivo* q-PAT results in Fig. 8 and Fig. 10 illustrate that the approach has the ability to quantitatively provide the absorption contrast ratio of soft tissues, which act as a significant index in differentiating the benign from the malignant lesions or staging malignant progression of tumors. Compared with the multi-angle c-PAT results, the *in vivo* q-PAT results in Fig. 11 have shown better performances in image uniformity and better clarity in resolving anatomical features, demonstrating the potential of the proposed method in revealing the physiological and functional information *in vivo*. It is worth noting that, the reduced scattering coefficient may affect the final quantification of absorption coefficient because of its influence on the distribution of photon density. The homogeneous distribution of reduced scattering coefficients with the typical scattering configuration of biological tissues has been used in the reconstruction of biological experiments. Then we have finally obtained the relative intensity distribution of the absorption coefficients or the absorption contrast ratio of tissues. In order to accurately estimate the scattering in biological tissues, multi-wavelength [37] or multi-modal strategies can be introduced, such as combining DOT and PAT, where the time-domain DOT has the ability to reconstruct the distribution of reduced scattering coefficients in an effective way [38, 39].

In conclusion, the experimental validations reveal that the proposed q-PAT implementation exhibits promising performances in image fidelity and quantitative accuracy for practical applications. Furthermore, we will keep exploring in both theoretical and
experimental aspects to improve the performances of mapping the optical properties in deep
tissues, such as adopting a more accurate photon migration model in the q-PAT process, e.g.
the Monte Carlo simulation. Besides, multispectral PAT applications in near-infrared window
should also be studied in the future work, such as estimating blood oxygen level in vivo [40],
and improving quantitative determination of the concentrations of chromophores using the q-
PAT scheme to fully consider the wavelength-dependent light fluence attenuation with depth
[12, 41].

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**Disclosures**
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