Freehand scanning photoacoustic microscopy with simultaneous localization and mapping

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Optical-resolution photoacoustic microscopy offers high-resolution, label-free hemodynamic and functional imaging to many biomedical applications. However, long-standing technical barriers, such as limited field of view, bulky scanning probes, and slow imaging speed, have limited the application of optical-resolution photoacoustic microscopy. Here, we present freehand scanning photoacoustic microscopy (FS-PAM) that can flexibly image various anatomical sites. We develop a compact handheld photoacoustic probe to acquire 3D images with high speed, and great flexibility. The high scanning speed not only enables video camera mode imaging but also allows for the first implementation of simultaneous localization and mapping (SLAM) in photoacoustic microscopy. We demonstrate fast in vivo imaging of some mouse organs, and human oral mucosa. The high imaging speed greatly reduces motion artifacts and distortions from tissue moving, breathing, and unintended handshaking. We demonstrate small-lesion localization in a large region of the brain. FS-PAM offers a flexible high-speed imaging tool with an extendable field of view, enabling more biomedical imaging applications.

1. Introduction

In vivo imaging of hemoglobin concentration and oxygen saturation offers key information for disease diagnosis and assessment. For example, physicians often need to assess tissue functionality at many anatomical sites or to localize lesions in a large region, such as identifying the tumor boundaries or micro-strokes [1–3]. Optical-resolution photoacoustic microscopy (OR-PAM) offers high-resolution label-free hemodynamic, functional, and molecular imaging with high sensitivity and is promising for many biomedical applications [3–12].

However, OR-PAM faces several technical challenges [13–16]. First, the field of view is small [17,18]. Second, many OR-PAM systems use bulky scanners and are not conveniently applicable to many anatomical sites. Although a few handheld OR-PAM techniques have been developed for flexible operation, they are limited by either slow speed or a small field of view [19,20]. Third, most OR-PAM systems are not fast enough [21]. Rapid scanners, such as MEMS mirrors, polygon scanners, and galvo mirrors, have been used in high-speed OR-PAM. They may suffer from either bulky probes or a small field of view [20–25]. The state-of-the-art OR-PAM still cannot achieve a large field of view, high flexibility, and fast speed in one system [18,19,26–29], which, however, is urgently needed in biomedical imaging.

To bridge this gap, we present freehand scanning photoacoustic microscopy (FS-PAM) with high C-scan rate and expandable field of view. We develop a compact handheld photoacoustic probe that can freely image various anatomical sites. A miniature hybrid resonant-galvo scanner is developed for fast dual-axis scanning. The miniature motor enables a larger scanning range in the slow axis than a traditional dual-axis MEMS scanner [29,32], offering a wide field of view mode. In this mode, FS-PAM can scan over 1.7 × 5 mm² at 2 Hz. The resonant mirror allows the miniature motor to scan at high speed, offering video camera mode imaging. In this mode, the B-scan and C-scan rates can reach 1288 Hz and 10 Hz respectively, and the high scanning speed can reduce motion artifacts from handshaking or breathing. Taking advantage of the high scanning speed, we can move the probe to expand the field of view, named as simultaneous localization and mapping (SLAM) imaging. To the best of our knowledge, this is the first implementation of SLAM-mode photoacoustic imaging. FS-PAM has an unlimited field of view, great flexibility, and high speed in one system. We demonstrate imaging of many organs, such as the mouse skin, liver, kidney, intestine, heart, and brain. We also image human oral mucosa. In addition, we validate the localization of small hemorrhages in a stroke model.

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2. Method

We build a dual-wavelength (532 and 558 nm) nanosecond pulsed laser (up to 1-MHz pulse repetition rate) for photoacoustic excitation \cite{16,21} (see Notes 1 of Supplement 1). The dual-wavelength laser beam is connected to a handheld probe via a single-mode fiber. As shown in Fig. 1(a), the handheld probe consists of a set of optical lenses, an ultrasound transducer, an optical/acoustic beam combiner, an acoustic lens, an aluminum-coated mirror, and a hybrid resonant-galvo scanner. Other details of the photoacoustic probe are presented in Notes 2 of Supplement 1. The 3D model of the imaging probe (see Fig. S2 of Supplement 1) shows the hybrid resonant-galvo scanner, consisting of a compact resonant mirror and a miniature galvo scanner, steers the optical and acoustic beams in two axes. The resonant mirror is made of an aluminum-coated reflector, a flexible-hinge frame, a pair of magnets, and a driving coil. Table. S1 of Supplement 1 shows the parameters of these components. The flexible-hinge frame is fabricated from BoPET by laser cutting. The two magnets are glued to the rotating reflector. The coil generates a periodic magnetic field to drive the rotating reflector at 1288 Hz (see Notes 3 of Supplement 1) \cite{30–34}. The resonant mirror with the driving coil is mounted to the shaft of a miniature galvo scanner for slow-axis scanning. Fig. 1(b) shows a photograph of the handheld probe. The hybrid scanner has a scanning range of \(~\)1.7 mm in the fast axis and 0–5 mm in the slow axis. Because the resonant mirror and the galvo scanner are independently driven, the interaction force between them is minimized. The size of the handheld probe is 5.9 × 3.0 × 4.4 cm³, and the total weight is \(~\)158 g. The resolution (6.2 µm for lateral resolution and 39 µm for axial resolution) and imaging depth (\(~\)0.9 mm) of the handheld probe is characterized in Notes 4 of Supplement 1.

The developed hybrid resonant-galvo scanner enables the wide field

Fig. 1. (a) Schematic of freehand scanning photoacoustic probe. A mini-galvo scanner and a resonant mirror rotate the confocally aligned optical and acoustic beams in two directions for high-speed imaging. AL, acoustic lens; CL, correction lens; CM, Collimator; DAQ, data acquisition; MT, galvo motor; OBJ, objective; RM, resonant mirror; UT, ultrasonic transducer. (b) Photograph of the handheld probe. (c) Diagram of SLAM imaging mode. Multiple partially overlapped PA images are stitched together to break the limit on the field of view.
of view mode, and video camera mode. In the video camera mode, we can extend the field of view with a SLAM algorithm and freehand scanning, as shown in Fig. 1(c). 5–10-Hz C-scan rate enables real-time imaging with reduced distortions in freehand scanning. We first calibrate the scanning trajectory to reduce image distortions. Then, we compute the translation, rotation, and scaling between images as follows. We extract the coordinates of the same features in two consecutive images using the scale-invariant feature transform (SIFT) and speeded-up robust features (SURF) methods. The feature points are used to determine an “affine” transformation matrix, which can transfer the two images to the same coordinates. Repeating this operation, we can stitch multiple partially overlapped images [35–39] into a large image. The field of view of the stitched image is determined by the freehand-scanning, rather than one C-scan, and thus can be greatly enlarged. Detailed steps for trajectory calibration and image stitching can be seen in Notes 5&6 of Supplement 1.

3. Results and discussion

FS-PAM can work in either a wide field of view mode or a video camera mode. In the wide field of view mode, FS-PAM uses the fast hybrid scanner to image the sample at a wide field of view in the slow axis. The portable handheld probe enables flexible fast imaging of various tissues. In the video camera mode, FS-PAM employs freehand scanning to acquire images larger than a single C-scan. Table. S2 of supplement 1 shows the summary of experimental parameters. All the animal and human experiments are approved by the animal and human ethical committee of the City University of Hong Kong.

3.1. Wide field of view mode imaging of mouse and human

With the hybrid scanner, the scanning range of the slow axis which is driven by a miniature stepper motor is adjustable, enabling a wide field of view mode. FS-PAM can acquire high-speed dual-wavelength images in this mode. The microvessel and oxygen saturation (sO$_2$) [4-6,40] in the mouse ear are imaged. The 532 and 558-nm pulse energies are 100 nJ and 90 nJ respectively. When the optical beam is focused 0.5-mm below the skin surface, the maximal permissible pulse energy is ~278 nJ, higher than the pulse energies used in the in vivo experiments. The laser pulse repetition rate (PRR) is 500 kHz for each wavelength. In the fast axis, the B-scan rate is 1288 Hz, the scanning range is 1.7 mm, and the average step size is ~7.8 µm. The step size in the slow axis is ~4.3 µm. The C-scan rate reaches 2 Hz with a FOV of ~1.7 × 5 mm$^2$. We continuously monitor the mouse ear for 2 min. Fig. 2(a) and 2(b) are representative vascular and sO$_2$ images whose scanning trajectories are calibrated (Notes 6). At high imaging speed, we can observe blood flowing in some vessels (see Video 1). As shown in Fig. 2(c), we also image the skin on the back of the mouse. Fig. 2(d) shows the 3D vasculature image. These results show that FS-PAM can acquire high-speed in vivo images with high resolution, great sensitivity, and good stability.

Oral mucosa is the mucous membrane lining the inside of the mouth. Oral cancers and other mucosal diseases show early symptoms in the oral mucosa [41]. Furthermore, some systemic diseases are also manifested in the oral mucosa, and some oral manifestations can be used as the basis or clues for the diagnosis of systemic diseases. Oral mucosal diseases related to autoimmunity are common in clinical practice, such as lupus erythematosus and pemphigus [42,43]. We demonstrate that...
FS-PAM can be used in the imaging of the human oral mucosa (Fig. 2(e)). We use two optical wavelengths to image the microvessels and oxygen saturation. Both the 532 nm and 558 nm light have a pulse energy of 80 nJ. The PRR is 250 kHz. The average step size in the fast axis is ~8.6 µm.

Other experimental parameters are the same as that of the previous mouse experiment. Fig. 2(f) shows the sO2 image of a volunteer’s healthy oral mucosa. The experimental results demonstrate that FS-PAM can reveal the microvessel details (also see Fig. S7 in supplement 1 and Video 2). Similarly to the oral mucosa, many internal organs’ superficial layers are full of microvessels that can be imaged by FS-PAM during surgery.

3.2. Video-camera-mode imaging of the internal organs

FS-PAM can be used in the video camera mode to assess the internal organs in surgery. Multiple exposed organs of the ICR mice (8-weeks old) are examined. To minimize motion artifacts, we use a 4.6-Hz C-scan rate to image the tissues over 1.7 × 2 mm². The step size is ~4.3 µm in the fast axis and ~7.8 µm in the slow axis. The curved scanning trajectory is calibrated (see Fig. S8 of Supplement 1). The A-line rate is 500 kHz for each wavelength. The pulse energy is 70–80 nJ. Mice are anesthetized with inhaled isoflurane at 1.5 mL/min. The abdomen skin is opened to expose the internal organs as shown in Fig. 3(a).

As shown in Fig. 3(b)-(e), we image the microvasculature and oxygen saturation (sO2) [17,21,38] in the intestine, stomach, kidney, and liver. Although breathing and hand motion exist, the fast system can acquire high-resolution images with minimal motion artifacts or distortions. With the high imaging speed, we can examine a large area on different organs and locate regions of interest. In Video 3, the total freehand scanning area is ~30 times larger than one C-scan and can be further expanded. These results demonstrate that FS-PAM can rapidly acquire high-resolution images of the vessel microstructure and sO2 with an expandable larger field of view, minimal motion artifacts, and a high signal-to-noise ratio of up to 29 dB.

3.3. High-speed imaging of the heart

Heart functional information is useful for heart surgeries. Here, we use FS-PAM to monitor the heart wall in a failure process. The C-scan rate is 4.6 Hz over a 1.7 × 2-mm² area. The step size is ~4.3 µm for the fast axis and ~7.8 µm for the slow axis. The pulse energy (532 nm and 558 nm wavelength) is 70–80 nJ. To expose the heart, we open the rib cage of the mouse under anesthesia.

The fast C-scan rate enables dynamic imaging of the dying process. As shown in Fig. 4(a), we can clearly observe the microvessels and oxygenation change in heart beating (see Video 4). As shown in Fig. 4(a), the vessel “Va” is an artery that can be identified according to the initial sO2 map. The position of point “p” at a fork of the vessel “Va” is determined from the fast imaging and plotted in Fig. 4(b). Here, the position of point “p” is determined from the distance from the point to the bottom right corner of the image.

We capture the blood loss process by continuously imaging the heart wall for ~22 s. The vessel “Va” in Fig. 4(c) shows reduced and eventually ceased blood flow (see Video 5). The sO2 in the vessel “Va” and the nearby region decreases fast as shown in Fig. 4(d). Fig. 4(e) shows the change in the average PA amplitude and sO2 in the region of interest. The average PA amplitude fluctuates in the first 7 s and then rapidly decreases. The average sO2 decreases from the beginning and reaches an extremely low level (0.2–0.3) starting from ~6 s. This result indicates that lung failure and the low oxygen content is an important factor in this heart failure event.

3.4. Video-camera-mode imaging with SLAM function

A single C-scan may not satisfy the application requirement due to the limited field of view, The developed resonant-galvo scanner allows the FS-PAM to enlarge the imaging region. We can freehand scan a large region of interest and stitch multiple partially overlapped images together, which is named as a SLAM mode. This approach can expand the field of view to a large freehand scanning range. The leaf phantom imaging experiment shows that the stitched PA images can match the...
real structures (see Fig. S9 in Supplement 1).

We demonstrate the SLAM imaging in the brain. To reduce image distortions, we use a 10-Hz C-scan rate to examine different regions in the brain cortex. The C-scan range is \(1.7 \times 1.3 \text{ mm}^2\). Fig. 5(a) shows seven representative \(sO_2\) images of the brain. Consecutive frames have sufficient overlapped features and no obvious distortions from freehand scanning. As shown in Fig. 5(b), the images can be stitched together to reconstruct a large image (see Video 6 and Notes 5 of Supplement 1). We highlight the boundary of the reconstructed image with yellow lines in Fig. 5(b). The combined area is \(~8.3\) times of one C-scan area. The vessels labeled as “1”, “2”, and “3” in the PA image match the corresponding visible vessels in the brain photograph.

We further localize a small lesion in a hemorrhagic stroke model. The C-scan rate is 4.6 Hz, and one C-scan area is \(1.5 \times 2 \text{ mm}^2\). As shown in Fig. 5(c), we use a focused high-power laser beam to induce a small hemorrhage spot in the brain. To locate the lesion in photoacoustic imaging, we scan the handheld probe over a large region and construct a large image (see Video 7). The stitched image is shown in Fig. 5(c). The lesion and its surrounding vessels can be identified in the reconstructed image. The field of view of the reconstructed image is \(~13\) times larger than one C-scan. Fig. 5(c) shows that the main vessels labeled as “1”, “2”, and “3” in the PA image match the corresponding visible vessels in the brain photograph. The results show that FS-PAM can enlarge the field of view without sacrificing imaging quality.

FS-PAM has several advantages to achieve the expandable field of view. First, the handheld probe is compact, lightweight, and suitable for
freehand scanning. Second, the hybrid scanner offers fast 2D scanning over a relatively wide field of view, which ensures the raw photoacoustic images have sufficient overlapped features. Third, the hybrid scanner maintains the optical and acoustic alignment, isolates mechanical coupling between the fast and slow axes, and thus does not sacrifice the imaging quality. This is also of great importance for robust image stitching. The light resonant mirror allows the motor to swing it at a high speed over a large range. This offers an efficient drive method that enables either a wide field of view or a high C-scan rate. Last but not least, the high-resolution images have dense microvessels in the overlapped regions, which offer abundant features for image stitching. In the expandable field of view mode, the freehand scanning speed is limited by the C-scan area and frame rate. To have sufficient overlapped features and minimize image distortion, the handheld probe needs to be scanned slower than the slow axis of the hybrid scanner. The reported slow axis can scan at 9–13 mm/s.

It is worth mentioning that the expandable field of view is affected by the hand-controlled moving stability. The current field of view and the scanning speed can be further improved. The scanning range of the fast axis can be increased by adjusting the hinge parameters of the resonant mirror. This may sacrifice the scanning speed of the resonant mirror, but the deteriorated B-scan rate can be compensated for with computational methods, such as deep-learning-based interpolation algorithms [10,44].

To summarize, relying on the developed hybrid scanner and interpolation method, we can further increase the field of view and the C-scan rate. The hybrid resonant-galvo scanner makes it possible to achieve a large field of view and real-time imaging in a compact PA probe. The demonstrated expandable field of view offers a new imaging ability for

Fig. 5. (a) Representative sO₂ images of the mouse brain. The scale bar is 320 µm. (b) SLAM image of the brain sO₂. (c) Localization of mini-hemorrhagic spot in the mouse brain. The boundaries of the SLAM areas are labeled with yellow lines. The scale bars in (b) and (c) are 1 mm.
handheld PAM.

4. Conclusion

We report FS-PAM that can operate in either the wide field of view mode or video camera mode. The video camera mode imaging is enabled by a handheld photoacoustic probe and a hybrid scanner. In the hybrid scanner, a 1288-Hz resonant mirror and a miniature galvo scanner offer high-speed 2D scanning in a few-millimeters-sized field of view. The decoupled fast and slow axes achieve a wide field of view scanning at a high C-scan rate. The compact handheld probe can be freely moved to various anatomical sites. We demonstrate in vivo microvascular and oxygen saturation imaging of multiple internal organs in an area of ~30 times larger than one C-scan. The handheld high imaging speed with uncompromised resolution and sensitivity enables in vivo imaging of changes in blood perfusion and oxygen saturation. The fast-imaging ability effectively reduces artifacts caused by hand motion or breathing. The video camera mode enables real-time imaging of different anatomical sites. SLAM imaging is developed on top of free-hand scanning and feature-based image stitching. FS-PAM can break the limit on anatomical sites. SLAM imaging is developed on top of free-hand scan

Data Availability

Acknowledgments

Appendix A. Supporting information

Declaration of Competing Interest

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