Review article

Progress of clinical translation of handheld and semi-handheld photoacoustic imaging

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ABSTRACT

Photoacoustic imaging (PAI), featuring rich contrast, high spatial/temporal resolution and deep penetration, is one of the fastest-growing biomedical imaging technology over the last decade. To date, numbers of handheld and semi-handheld photoacoustic imaging devices have been reported with corresponding potential clinical applications. Here, we summarize emerged handheld and semi-handheld systems in terms of photoacoustic computed tomography (PACT), optoacoustic mesoscopy (OAMes), and photoacoustic microscopy (PAM). We will discuss each modality in three aspects: laser delivery, scanning protocol, and acoustic detection. Besides new technical developments, we also review the associated clinical studies, and the advantages/disadvantages of these new techniques. In the end, we propose the challenges and perspectives of miniaturized PAI in the future.

1. Introduction

Photoacoustic imaging (PAI) has emerged as one of the fastest growing biomedical imaging techniques in the last decade [1,2]. Briefly, as the delivery of a pulsed/modulated laser beam to the biological tissue, exogenous or endogenous chromophores will absorb the photons and convert the photon energy into heat. This conversion will induce rapid temperature change, generate thermoelastic expansion, and then radiate the acoustic wave out of the biological tissue, referred to as photoacoustic (PA) signal [3]. The principle of PAI shows that it is a hybrid imaging modality combining the advantages of both optics and ultrasound, such as rich endogenous contrast, high spatial resolution, and deep tissue penetration [1-5].

In terms of system configurations, PAI consists of two main sub-modalities: photoacoustic computed tomography (PACT) and photoacoustic microscopy (PAM) presented by Fig. 1(A) to (H) [3-5]. As shown in Fig. 1(A), PACT employs a full-field and uniformly distributed laser illumination to induce photoacoustic effect over the entire region of interest. The transducer with a high sensitivity and large directivity is preferred in PACT to receive PA signals over the entire imaging domain with a higher signal-to-noise-ratio (SNR) and less artifacts [6,7]. A full-view (360°) recording of PA signals by either scanning a single detector or employing a transducer array forms the map of PA sources using mathematical reconstruction methods, such as delay-and-sum, filtered back projection, time-reversal algorithm, and model-based reconstruction methods [8-12]. Comparing with PACT, PAM can provide a higher spatial resolution by employing higher frequency ultrasound transducer or focusing the excited laser beam, referred to as acoustic resolution photoacoustic microscopy (AR-PAM) and optical resolution photoacoustic microscopy (OR-PAM), respectively [13-15]. AR-PAMs use full-field illumination or weakly focused light to improve the energy density. A focused ultrasonic transducer (UST) is usually used to detect PA signals, and the acoustic focus of UST is smaller than the area illuminated by the pulsed laser beam as shown in Fig. 1(B) (Transmitted illumination, the light illumination and ultrasound detection are distributed on both sides of the target). Hence the lateral resolution of AR-PAM is determined by the acoustic focus. On the contrary, in OR-PAM (Fig. 1(C)), the excited laser focus is smaller than the acoustic focus by using an optical objective/scan lens (Fig. 1(C)). Hence, comparing with AR-PAM, OR-PAM is able to achieve a higher lateral resolution by sacrificing the penetration depth. Since the transmitted illumination is difficult to image thick samples, epi-illumination OR-PAM (Fig. 1(C-2), the light illumination and ultrasound detection are distributed on the same side of the target) is more convenient in...
biomedical and clinical studies. Apart from AR-PAM and OR-PAM, optoacoustic mesoscopy (OAMes) has been described as a bridge between PACT and PAM by introducing the concept of ‘virtual detector’ [16,17]. In OAMes, the focal point of the ultrasound detector is assumed to be a virtual detector with a small effective collection area but a higher sensitivity compared with the conventional UST (Fig. 1(D)). Although there are no clear boundaries between PACT, PAM, and OAMes, we can still categorize them with specific imaging features as shown in Fig. 1(I).

PACT employs high energy lasers with a pulse energy of tens of millijoules to cover the entire field of view (FOV). The pulsed width of PACT laser is usually tens to hundreds of nanoseconds and the pulse repetition rate is usually 10–100 Hz [1,2]. The wide pulsed duration causes a low-frequency PA excitation. By using the UST or UST arrays in the MHz scale, PACT achieves spatial resolutions of hundreds of microns and a centimeter-scale penetration, enabling non-invasive imaging of deep tissues as shown in Fig. 1(E) [3]. PAM prefers a pulsed laser with a high repetition rate of a few hundred kilohertz, and the pulsed duration is below 20 ns in general. The UST with a high center frequency and wide bandwidth from tens to hundreds of megahertz is required. Optical/acoustic focus induces a high spatial resolution around but a superficial imaging depth compared with PACT. By using advanced techniques such as objective lenses with high numerical apertures, optical detection with a ultrawide bandwidth, and super-resolution imaging strategies, PAMs are able to capture single capillary and visualize microvasculature both in animals and human beings as shown in Fig. 1(F) and (G) [18,19].

OAMes bridges the gap between PACT and PAM by employing UST with a bandwidth from 10 to 200 MHz, thus leading to a compromising penetration (0–10 mm) and spatial resolution (10–100 μm) between PACT and PAM. Similar to PACT, a reconstruction algorithm is required in OAMes since the transducer captures the PA signals from the entire illumination area. Similar to PACT, the frame rate of OAMes can be improved with transducer array. However, it is challenging to manufacture transducer arrays with a center frequency and bandwidth over 100 MHz. Besides, the spherical focused geometry is not applicable to the detection elements and the limited active detection area is also against the optimization of OAMes performance [49]. Hence, OAMes, especially raster scanning OAMes (RSOM) prefers a single UST instead of transducer arrays with a high center frequency, ultra-wide bandwidth, and large active detection area. Due to the noninvasiveness, high resolution, and label-free imaging of blood vessels within a moderate penetration depth, OAMes is considered as a potential approach especially in quantifying skin morphology and inflammatory landmarks as shown in Fig. 1(H) [20].

Over the last two decades, PAI has been used in numerous studies such as whole body imaging of small animals [3,21,22], in vivo vascular imaging in various organs of rodents and primates [23,24], and studying neurovascular coupling [25,26]. In addition, potential clinical applications include angiogenesis and vascular distribution inside/around tumors [27,28], retinal vasculature associated with diabetic retinopathy [29,30], intestinal diseases such as Crohn’s disease and mesenteric venous thrombosis [31,32], vascular lipid plaque [33,34], and breast cancer [27,35–37] as shown in Table 1. Although PAI has multi-scale and multi-parametric imaging capability with various advanced features, early-stage PAI systems suffer from several limitations. For example, it is hard to simultaneously satisfy the portable design of system and high image performance such as large FOV, fast image speed,
Table 1
Specifications of typical handheld and semi-handheld PAI systems. SLN, sentinel lymph nodes. NM, did not mention in the article. MMF, multimode fiber. SMF, single mode fiber. UST, ultrasonic transducer. L, lateral resolution. A, axial resolution. FOV, field of view.

| Clinical applications | Volume | Laser delivery | Wavelength | Pulse energy | Delivery device | Scanning protocol | Scanning device | Acoustic detection | Imaging performance |
|-----------------------|--------|----------------|------------|--------------|----------------|-------------------|-----------------|-------------------|-------------------|
|                       |        | Pulse Repetition Frequency |           |              |                |                  |                 |                   |                   |
|                       |        | Scanning protocol |             |              |                |                  |                 |                   |                   |
|                       |        | Acoustic detection |             |              |                |                  |                 |                   |                   |
|                       |        | Imaging performance |             |              |                |                  |                 |                   |                   |

Part 1 PACT

[65] Imaging guided SLN biopsy, human SNL imaging

SLN biopsy, imaging of human finger and wrist

Dorsalis pedis artery, tibialis posterior artery, Crohn’s disease, thyroid lobes, muscle, breast

Breast imaging, thyroid lobes, human skin, human extremities

SLN biopsy, monitoring of photothermal therapy

Human arm vasculature, imaging guided SNL biopsy

Dorsalis pedis artery, fingertip, human skin

Human skin

Nailfold microvasculature

Nailfold microvasculature

Melanoma

Oral imaging, human skin

Oral imaging, cancer monitoring

Part 2 OAMes

Part 3 PAM

(continued on next page)
Table 1

| Laser delivery | Scanning protocol | Acoustic detection | Imaging performance |
|----------------|------------------|--------------------|---------------------|
| Wavelength     | Pulses/energy    | Scanning device    | Imaging resolution |
|                |                  | mechanism          | Resolution          |
|                |                  |                    | Penetration          |
|                |                  |                   | Depth               |
| Volume         |                  |                   |                      |
|                |                  |                   |                      |

**Clinical applications**

- Oral imaging, multi-organ (sample surface)
- Oral imaging, animal (sample surface)
- Human skin, mole (sample surface)
- Human skin, mole (sample surface)
- Human skin, mole (sample surface)
- Melanoma, oral (sample surface)

**Volume**

- 22.3 mm × 20 mm × 50 mm
- 60 mm × 15 mm × 80 mm
- 17 mm × 15 mm × 31 mm
- 102.4 mm × 102.4 mm

**Resolution**

- 2-2 mm² in lateral
- 18.2 μm in lateral
- 26.4 μm in lateral
- 5.0 μm in lateral

**Penetration**

- 3.8 mm
- 10.5 μm
- 26 μm
- 5.0 μm

**Depth**

- 3.2 Hz
- 4 Hz
- 2.5 Hz
- 2 Hz

**Transducer**

- Flat
- Electrothermal
- Electrostatic
- Electromagnetic

**Acoustic detection**

- UST
- MEMS
- UST
- MEMS

**Scanning device**

- Flat
- Raster
- Flat
- Flat

**Delivery device**

- 80 μJ
- 90 μJ × 800 μJ in L
- 18 mJ/cm² × 2 μm in L
- 8.6 mJ/cm² × 3 μm in L

**Diameter**

- 2.4 mm
- 12 mm
- 5.0 mm
- 3.8 mm

**Frequency**

- 10 MHz
- 50 MHz
- 10 MHz
- 50 MHz

**Bandwidth**

- 10 MHz
- 50 MHz
- NM
- NM

**Resolution**

- 4 Hz
- 10 MHz
- NM
- NM

**Penetration**

- 2.4 mm
- 30 μm
- 5.0 μm
- 1.5 mm

**Volume**

- 15 mm³
- 80 mm³
- 200 mm³
- 102.4 mm³

**Applications**

- Oral imaging, multi-organ (sample surface)
- Oral imaging, animal (sample surface)
- Human skin, mole (sample surface)
- Human skin, mole (sample surface)
- Human skin, mole (sample surface)
- Melanoma, oral (sample surface)

**PACT systems utilizing linear, curved, and spherical transducer arrays as well as the associated potential clinical applications.**

**2. Progress in handheld and semi-handheld PACT**

PACT owns a centimeter-scale penetration depth, a regular spatial resolution from tens of to hundreds of microns, and functional/metabolic imaging capability (average ~3 cm, Table 1, part 1) [3]. By injection of exogenous chromophore and localization, super-resolution PACT can even achieve nanoscale spatial resolution [50]. The conventional setups for PACT usually employ a single-element transducer to capture the time-resolved PA signals and a motorized rotator to scan the transducer in a full-view (360°) circular orbit. [51]. In conventional PACTs, it is preferred to capture PA signals with more detection angles and carry out averaging to eliminate the background noise, leading to time consuming and bulky size. Hence, it is difficult to achieve compact design and high temporal resolution, which are quite essential in both clinical and fundamental applications (For example, a system with a pulsed repetition rate of 100 Hz and a scanning step of 1° will cost 3.6 s for one image, while the array-based system can capture with a frame rate of 100 Hz). To accelerate imaging speed of PACT, full-ring transducer arrays are always introduced to replace circular scanning as shown in Fig. 1(A) [3,35,52,53]. By using transducer arrays, PACT can capture PA signals in different views for each single laser pulse with a cost of detection bandwidth (single element transducer: up to hundreds of MHz; transducer array: tens of MHz), leading to a higher temporal resolution (10–200 Hz, or frames per second) and a lower spatial resolution. Full-ring-array-based PACT can receive PA signals over a view of 360° to eliminate artifacts and improve SNR, which has been extensively applied in detecting breast cancerous lesions [35,53], evaluating human joint diseases [52,54,55], and visualizing vasculatures in human extremities [56]. However, due to the configuration of full-ring transducer arrays, it is hard to design handheld or semi-handheld devices. In contrast, transducer arrays with linear (Fig. 2(A)), curved (Fig. 2(B)), spherical (Fig. 2(C)), and planar (Fig. 2(D)) shapes are widely used in handheld platforms in conjunction with laser delivery based on optical fibers or fiber bundles. In this section, we focus on various handheld PACT systems utilizing linear, curved, and spherical transducer arrays as well as the associated potential clinical applications.
2.1. Linear transducer array

Medical ultrasonography (US), one of the most important clinical imaging modalities, usually uses an ultrasound transducer array to generate ultrasound pulses and receive echoes in real-time. There are many types of transducer arrays used for different scenarios, such as linear arrays for imaging superficial small organs, convex arrays for abdominal imaging, and cavity arrays for endoscopic inspection. Based on the principle of PAI, it is easy to integrate PACT into clinical US platform by providing additional laser delivery systems. This integration makes PACT compact and portable for clinical applications. Fig. 2(A) presents a typical PACT system based on a linear array of medical US, in which optical fibers/fiber bundles are usually employed to deliver laser energy to the sample. Various handheld PACTs have been proposed based on this configuration [57–63]. Most of reported PA-US systems used dark-field illumination configurations since the piezoelectric transducer is opaque (Fig. 2(A) left). It is a convenient way to deliver the oblique incidence laser pulses to the imaging plane using optical fibers or fiber bundles from the side of the transducer array. By using dark-field illumination, PAI can avoid the strong signals from the surface and enhance the SNR of deep vessels. However, the laser energy is confined in a specific depth within the sample. Thus, bright-field illumination is proposed to extend the depth of field (Fig. 2(A) right). Wang et al. developed a handheld PACT system based on a linear array of medical US (CL15-7, Philips) with a linear array [64]. The array and optical delivery fiber bundles are assembled with a right angle, an optical transparent acoustic reflector was mounted at an angle of 45° in the center to merge the light and ultrasound beam. Song et al. proposed a handheld dual-modality PA/US system using the strategy shown in Fig. 2(E) [65]. A linear array with 128 elements and a center frequency of 7 MHz was employed for ultrasonic detection. An optical system consisting of a multi-mode fiber, two cylindrical lenses, and a polymethyl methacrylate optical/acoustic coupler was used to deliver the laser pulses with a pulse repetition rate of 20 Hz to the samples. With the specific illumination strategy, the handheld probe can resolve blood vessels located at a varied depth from 0.1 mm to ~20 mm. In vivo imaging of Indocyanine green (ICG) labeled sentinel lymph nodes of rats demonstrated the clinical potential of this probe as shown in Fig. 2(E-2). By selecting the optimal excitation wavelength of ICG, PACT clearly showed the positions and morphologies of the target. With additional structural information provided by US imaging, the handheld system was applied to guide the biopsies of sentinel lymph nodes using co-registered real-time PA/US images. In addition to optical fibers/fiber bundles, an alternative way to excite PA signals in handheld design is to use laser diodes (LD) or laser-emitting diodes (LED) [66, 67]. Compared with Optical Parametric Oscillator, dye-based lasers, or Nd:YAG lasers, LD and LED are much smaller and lighter with a higher pulse repetition rate. Steenbergen et al. developed a handheld PA/US system by integrating medical US and LDs with a pulse energy of 0.56 mJ and a repetition rate of 10 kHz [66]. Xia et al. proposed a study of human fingers with a commercial LED-based PA/US system (AcousticX, PreXion) [67]. The system employed a 128-element linear array and four LED arrays with a pulse energy of 200 µJ and a high repetition rate of up to 4 kHz. Although the progress of LD/LED-based PACT is exciting, it is still challenging to increase the output energy, shorten the pulse duration and improve the beam quality of LD/LED without sacrificing the portability.

2.2. Curved transducer array

Although the linear-array-based PACT can improve the temporal resolution by avoiding mechanical scanning of transducer in conventional PACTs, it has several inherent limitations such as radial artifacts...
and morphological distortions due to the limited detecting views [41]. Compared to the linear array, the cylindrically focused curved array is able to cover more detecting views and eliminate artifacts/distortions, which make curved array more suitable for clinical application such as imaging of deep subcutaneous tissues [31,68–71]. Ntziachristos et al. developed the first handheld multi-spectral optoacoustic tomography (MSOT) system with a cylindrically focused curved array [68]. The ultrasound array has a center frequency of 8 MHz, a total number of 128 elements with a focal length of 2 cm, and a fan-shaped view of 135°. Similar to previous PA/US systems and handheld MOST, the laser pulses were delivered by the optical fiber bundles positioned at both sides of the transducer array. The MSOT systems realizes ultrafast PA-US imaging by utilizing a laser with a pulsed repetition rate of 50 Hz and achieves multispectral functional imaging via a fast-tunable pulsed laser. In addition, they proposed various special handheld MOST systems and mathematical algorithms for specific clinical and fundamental applications, such as muscle oxygenation monitoring [69], in vivo imaging of human thyroid [70], imaging of blood vessels in human foot [71], and assessment of Crohn’s disease [31]. Fig. 2(F) shows a procedure of human dorsalis pedis artery imaging with a handheld MOST reported in 2016 [71]. Fig. 2(F-2) presents the MSOT PA image and contrastive duplex US images of the vasculature on a foot, which shows the dorsalis pedis artery. Furthermore, to combine the advantages of linear array with extended FOV and curved array with a better imaging performance, Razansky et al. proposed a hybrid-array-based optoacoustic and ultrasound system [72]. The array consists of a linear array with 128
elements in the center and two concave segments with total 128 elements in the sides of linear array, resulting a large FOV of $40 \times 40 \text{mm}^2$ and a high resolution of $\sim 110 \mu m$.

2.3. Spherical transducer array

By using the one-dimensional (1D) transducer array, the portable PACT is only able to recover cross-sectional images in real-time, referred to as B-scan. Additional scanning is required to form a 3D image. Compared with 1D arrays, spherical arrays enable real-time volumetric imaging [73]. Razansky et al. firstly implemented a spherical array with 256 elements in a handheld PACT. The elements with a center frequency of 4 MHz, a bandwidth of 100%, were disposed on a spherical surface with a radius of 40 mm, covering an angle of 90° [74, 75]. The laser pulses were delivered via a fiber bundle through the hole in the center of the array. A transparent polyethylene membrane was used to protect the transducer surface, and the gap was filled with the coupling medium for acoustic propagation. A series of clinical and fundamental studies have been proposed including human skin imaging [75], visualization of vasculature in human extremities [76], breast cancer detection [77], and evaluation of thyroid lobes [78]. Fig. 2(G) shows a diagram of human thyroid lobes imaged with a handheld PACT based on the spherical array (2019) [78]. A pulsed laser with a tunable wavelength range of 680–950 nm and a high pulsed repetition rate of 100 Hz was employed in this system. To ensure the laser safety, the repetition rate was finally set to 10 Hz. Fig. 2(G–2) shows a maximum amplitude projection (MAP) image of the carotid artery bifurcation of a volunteer. Although the spherical array covers more views compared with linear/curved array, in addition to the limited FOV, it is difficult to form a homogeneous illumination pattern on the sample surface. Moreover, spherical array is not a standard probe in medical US and the applicable scenario is limited [73]. One of the potential scenarios might be volumetric visualization of superficial vascular and chromophores in soft tissue, such as dermatology, subcutaneous fat and lymphatic tissue, and breast.

2.4. 2D planar array

Besides the limited FOV and the challenge for light delivery, a membrane-sealed chamber filled with coupling medium is necessary in spherical-array-based handheld PACT as shown in Fig. 2(F). A practical solution is 2D planar array as shown in Fig. 2(D) [79–82]. Wang et al. proposed a handheld PACT based on a clinical planar array (iU22, Philips Healthcare), demonstrated the clinical potential using in vivo imaging guided the biopsy of the sentinel lymph nodes in mice [79]. Similar to linear-array-base PACT, LDs and LEDs have been implemented in planar-array-based PACT to achieve compact design and high-speed imaging. Zheng et al. proposed a compact PACT integrating LDs and a planar array [81]. Three compact continuous-wave LDs with wavelengths of 450, 638, and 808 nm were used instead of conventional optical fiber to achieve multi-spectral coherent frequency-domain PAI. The weight was significantly reduced to 21 g compared with conventional optical fiber to achieve multi-spectral coherent frequency-domain PAI. The weight was significantly reduced to 21 g compared with conventional optical fiber to achieve multi-spectral coherent frequency-domain PAI. The weight was significantly reduced to 21 g compared with conventional optical fiber to achieve multi-spectral coherent frequency-domain PAI.
states, detection, all-optical PAI has a better spatial resolution but sacrifices detected PA signal in each point is contributed by all the chromophores. Bandwidth of 65% was employed to detect PA signal. Fig. 2 (H-2) shows ence tomography (OCT) to provide both functional and morphological information of the biological tissues. Customized diffuser serves as the acoustic delay line for acoustic detection. A two-dimensional planar array with 72 piezoelectric elements, an optical window, a center frequency of 2.25 MHz, and a bandwidth of 65 % was employed to detect PA signal. Fig. 2 (H-2) shows the 3D rendering of human blood vessels.

Apart from conventional PAI based on piezoelectric transducers, some novel optical methods have been proposed to detect PA signals such as Fabry–Perot [83,84], a kinectic probe [85], and micro-ring resonators [86,87]. Fig. 3 (A) shows the principles of acoustic detection in all-optical PACT system based on Fabry–Perot chamber [83]. The excitation light passed through the Fabry–Perot detector to achieve full-field illumination of the chromophores inside the sample without any attenuation due to the transparency of the detector to the excitation wavelength. PA signals induced by the excitation light propagate to the tissue-detector interface, result in the change of the Fabry–Perot cavity due to the vibration, and thus lead to the variation of interference intensity of the probe laser beam. By scanning the probe beam two-dimensionally in the surface of the chamber, a volumetric PA image can be reconstructed. Although the probe beam is scanned in a form of point-by-point, which is similar to the scanning mechanism of PAM, the detected PA signal in each point is contributed by all the chromophores in the imaging domain. Hence, the Fabry–Perot acts as a two-dimensional planar array in this configuration and a reconstruction algorithm similar to conventional PACTs is required. By using optical detection, all-optical PAI has a better spatial resolution but sacrifices penetration depth because of the tight aperture and wide detection bandwidth (Table 1, part 1, lines 7) compared with PACT based on piezoelectric devices (Table 1, part 1, lines 1–6). It is noted that Beard et al. have proposed a series of PACT prototypes based on Fabry–Perot sensors [88–92]. Fig. 3 (B) presents a semi-handheld all-optical PACT system, which is mounted on an articulated arm to provide multi-degree freedom for accessing the dorsalis pedis artery and fingertip in different states, i.e. stimulation in terms of immersion in cold (left hand panels) and warm (right hand panels) water (Fig. 3 (C)). Using the articulated probe, the system can facilitate the imaging examination of different regions of interest over the entire human body to assist the clinical diagnosis [92]. Since the Fabry–Perot sensor is transparent, the developed all-optical PACT systems are easily combined with optical coherence tomography (OCT) to provide both functional and morphological information of the biological tissues [90,91].

3. Progress in handheld and semi-handheld RSOM

OAMs aims to balance the penetration depth and resolution, focuses on potential applications with a depth ranging from 0 to 10 mm and a spatial resolution of tens of microns compared with PACT [49]. Fig. 4 (A) illustrates the imaging strategy of raster scanning RSOM. Optical fibers or fiber bundles are usually used to deliver the laser pulses and form broad beam epi-illumination. The absorbers located close to and outside of the acoustic focus are recovered with a synthetic aperture focusing technique termed as ‘virtual detector’ [93,94]. In RSOM, the resolution is determined by the acoustic diffraction. Therefore, an ultra-wideband UST with a bandwidth of 100–200 MHz is generally employed to achieve a high spatial resolution. Compared with PACT (~ 3 cm imaging depth and ~200 μm resolution, Table 1, part 1) and PAM (~1 mm imaging depth and ~5 μm lateral resolution, Table 1, part 1), RSOM provides a compromise spatial resolution and penetration depth, which is suitable for high resolution imaging of vasculature in deep tissues (4 mm imaging depth and spatial resolution of ~15 μm, Table 1, part 2) [16,95].

Ntziaichristos et al. have proposed series of OAMes and RSOM studies in clinical applications, such as skin imaging of healthy volunteers [16], monitoring of vascular-targeted therapies [96], label-free visualization of psoriasis [20], and nailfold microvascular structure assessment [47]. Fig. 4 (B) shows a semi-handheld RSOM system assisting with an articulated arm reported in 2017 [20]. A spherically focused transducer is used to deliver the laser beam with a repetition rate of 2 kHz. Fig. 4 (C) and (D) present a potential clinical application of the semi-handheld RSOM. Due to the capability of high resolution in dermis, RSOM can reveal the structural differences between psoriatic (Fig. 4 (C)) and normal (Fig. 4 (D)) skin. In 2018, a similar semi-handheld RSOM system was developed using a center frequency of 55 MHz. With a lower center frequency and ultra-wide bandwidth from 10 to 120 MHz, the proposed RSOM provides a deeper penetration depth and a higher SNR without sacrificing the spatial resolution [96].
4. Progress in handheld and semi-handheld PAM

Featuring high resolution and rich contrast, PAM is able to provide structural, functional, and metabolic information of biological tissues [97,98]. Traditional PAMs usually use bulky motor stages to realize 2D scanning, making it difficult to achieve small, light, and fast imaging devices [99]. Various PAMs have been proposed by adopting different optimized strategies in laser delivery, scanning protocol, and acoustic detection. Among all the improvements, scanning protocol is the key of the strategy for PAM miniaturization. In this section, we will discuss handheld and semi-handheld PAM based on several novel scanning devices [100–115]. PAMs scanning with galvanometer scanners adopting two scanning mechanisms: raster scanning and rotatory scanning, have been discussed firstly. Raster-scanning-based PAMs have realized handheld photoacoustic imaging of animal and human [100–102]. In contrast, rotatory-scanning-based PAMs enables the development of the semi-handheld PAMs with a large FOV, a high temporal resolution, elimination of relative motion between the imaging interface and the samples for both animal and human studies [103–105]. In addition to galvanometer, three types of MEMS scanner based on electrothermal [106–112], electrostatic [113] and electromagnetic [114,101–115] effects have been applied in the development of handheld PAMs with ultracompact design.

4.1. Scanning with galvanometer scanner

By utilizing 2D galvanometer scanners, there are two major scanning mechanisms: raster scanning mechanism (Fig. 5(A)) and rotatory scanning mechanism (Fig. 5(B)). In PAM, a laser-induced 1D depth-resolved signal (A-line) will be captured by a transducer at each point of the scanning path. Then, a 2D cross-sectional image (B-scan) can be obtained by 1D scanning of the imaging interface or the sample. Subsequently, 3D images can be finally formed by stacking multiple B-scan slices. Therefore, a 2D motorized stage is required in conventional PAMs. Unfortunately, the fast and accurate 2D mechanical scanning is time consuming and costly. In addition, there exists relative motion between the imaging probe and the samples [99]. Utilizing 2D galvanometers or MEMS scanners can realize pure optical raster scanning and has the potential to overcome these limitations [116,117]. However, this method will sacrifice part of sensitivity and SNR due to the use of flat transducers serving as acoustic detectors. A PAMs employed water-immersion galvanometer or MEMS scanner combing with focused UST to scan laser and acoustic focus. However, the damping of the water will limit the scanning speed. Rotatory scanning mechanism proposed by Xi et al. has the potential to solve these problems [118]. As shown in Fig. 5(B), each scanning point represents a depth-resolved signal (red line). When the optical focus scans along the optical scanning trace (green line), PA signals are generated along this line and detected by a cylindrically focused transducer to form a B-scan image. To realize planar scanning and 3D imaging, the optical scanning trace rotates 180° around the center of the imaging domain at a given angle step. Meanwhile, the UST synchronously rotates to achieve the overlap with the corresponding optical scanning trace. Compared with the raster scanning mechanism, rotatory scanning has a better balance between speed and sensitivity, a larger FOV (as shown in Table 1, part 3), and is free of relative motion between the imaging probe and the samples.

4.1.1. Raster-scanning-based PAM

Based on the raster scanning mechanism, several miniaturized imaging prototypes have been developed [100–102]. Wang et al. developed a handheld AR-PAM system consisting of a 25 MHz transducer, a two-dimensional translation stage, and laser delivery fiber bundles [101]. A Q-switched pulsed laser with a wavelength of 532 nm and a pulsed repetition rate of 10 Hz was employed to induce PA signals in melanoma. However, due to the relatively low spatial resolution (230 μm in lateral and 59 μm in axial), it is more appropriate to be classified as mesoscopy rather than microscopy (Table 1, part 3). Zemp et al., for the first time, developed a handheld real-time optical resolution photoacoustic microscope (HH-ORPAM) [100]. This design has successfully realized a handheld probe with a footprint of 4 cm × 6 cm and a weight of ~500 g. In addition, HH-ORPAM employs a high-speed optical raster scanning by utilizing an image-guided fiber bundle (~800 μm) consisting of 30,000 individual single mode fibers, a pulsed laser with a tunable repetition rate of up to 600 kHz, and a 2D galvanometer scanning in the proximal port of the fiber bundle. It is exciting that this HH-ORPAM features integration and miniaturization. However, the use of fiber bundles is costly and suffers from a limited FOV and spatial resolution.

Yang et al. built a handheld photoacoustic microscope with an adjustable light focus [102]. Fig. 5(C) shows the detailed devices used in the probe, including a fiber collimator, a fast 2D galvanometer scanner, a plan achromatic objective, a XYZ-direction stages, transparent glass sheet tilted with an angle of 45° and a flat responsive UST (effective aperture: 5 mm; center frequency: 15 MHz). In the design, the fast 2D galvanometer scanner along with the raster scanning of light spot with a B-scan image speed of 25 frames per second, and the adjustment stage optimizes the optical focus to achieve the best imaging performance. The probe is able to detect subcutaneous micro-vessels in human skin, with a fast image speed, a high spatial resolution and a deep penetration depth. In detail, it takes 16 s to obtain a 2 × 2 mm² MAP image, with a lateral resolution of ~8.9 μm and an imaging depth of ~2.4 mm. Fig. 5(D) and (E) present a volumetric rendering of a human lower lip and in vivo imaging of subcutaneous blood vessels of a human wrist, respectively. In the photograph, the normal skin area (blue dash box: A) and a vascular nevus area (red dash box: B) are marked. The subsequent MAP images display subcutaneous vascular in area A and morphological information in area B. Several post-processed MAP images of area B with side-view, depth-coded and edge-extracted projection show the detail information of the nevus.

4.1.2. Rotatory-scanning-based PAM

In raster-scanning-based PAM, a spherical focused UST is usually used to obtain the best SNR. To achieve three-dimensional imaging, a motorized translation stage is required to scan the imaging probe. The scanning process introduces relative motion between the imaging probe and the sample. In order to eliminate the relative motion and reduce the volume size, it is common to use flat responsive transducers instead of the spherically focused transducer and scan the laser beam with a two-dimensional galvanometer. This scanning mechanism is suffering from a low SNR [103–105].

Xi et al. developed the first portable/semi-handheld OR-PAM system based on a rotatory scanning mechanism [103,104]. Fig. 5(F) shows the schematic of the system, which employed a high repetition ratio Q-switched pulsed laser (up to 20,000 Hz), a fast 2D galvanometric scanner, and a scan lens to realize the fast-optical rotatory scanning. In detection, a motorized rotator equipped with a customized accelerating gear group was used to rotate a cylindrically focused transducer (center frequency: 15 MHz; aperture size: 12.7 mm; bandwidth: 75 %) to achieve confocal configuration of the optical/ acoustic foci. This approach provides a large FOV of up to 8.4 mm in diameter and a depth of field (DOF) of 1.5 mm with a lateral resolution of 10.4 μm and an axial resolution of 90 μm. Compared with the traditional raster-scanning-based OR-PAM systems, the portable design based on rotatory scanning can realize quick automatic adjustment of placement for various image positions, with subjects in a comfortable posture. Furthermore, it also capable of multi-scale imaging of small, middle size animals and humans, representing the flexibility for different scenarios. Fig. 5(G) and (H) show the photoacoustic imaging results of a rabbit ear, rabbit eyes, and human oral lips. It is the first time for OR-PAM to track the recovery progress of an oral ulcer. All of these results demonstrate the universal applications of this approach. In addition to portable OR-PAM, Xi et al. further developed a dual modality system integrating the portable
OR-PAM and a rotary scanning spectral-domain OCT [105]. OR-PAM and spectral-domain OCT provide hemoglobin and structural alternations of an ulcer in human lip, respectively, demonstrating the clinical potential of oral inspection.

4.2. Scanning with MEMS scanner

Although the use of galvanometer scanners is able to partially reduce the volume of OR-PAMs, the size of existing galvanometer scanners prevents the further miniaturization, limiting the applications of handheld PAM. With the rapid development of MEMS devices, there are three major types of MEMS scanners used to achieve further optimization of handheld OR-PAMs: electromagnetic, electrostatic and electrothermal scanners [48]. Electrothermal MEMS scanners are usually fabricated by biaxially-oriented polyethylene (BOPET) film and soft lithography of polydimethylsiloxane (PDMS) [119–121]. The MEMS mirror is actuated by controlled electromagnetic forces induced by four pairs of Neodymium permanent magnets and homemade electromagnets. Different from electromagnetic MEMS, electrostatic MEMS scanners are usually fabricated with single-crystal silicon wafers. Four electrostatic bidirectional rotators driven by a steady-state analog actuation voltage result in a steady-stage analog angle of rotation of the MEMS mirror in the center [113]. The electrothermal MEMS scanner is mainly driven by four electrothermal bimorph-based actuators fabricated from a lateral-shift-free large-vertical-displacement (LSF-LVD) design [106, 109,122]. Compared to the electromagnetic and electrostatic MEMS, electrothermal MEMS owns the merits of compact size, low cost, and large deflection angles, and low driven voltage, which is more suitable to develop imaging device for clinical applications.

4.2.1. Electrothermal MEMS scanners

MEMS scanner was firstly implemented in PAI by Jiang et al. [123]. An electrothermal MEMS scanner was employed to scan the laser beam across a ring-shaped polyvinylidene fluoride (PVDF) UST. Further optimization studies were carried out by employing customized piezo-electric transducer [124], multi-modality system integrating diffuse optical tomography [125], human skin imaging and tumor visualization. Then, Jiang et al. and Xi et al. developed a series of photoacoustic endoscopy (PAE) based on the electrothermal MEMS scanners [126, 127]. Although the volume of the handheld PAM is slightly bigger than PAE, it has better performance in terms of both spatial resolution and FOV. Xi et al. integrated all optical, acoustic, and mechanical components into a cubic probe with a volume of $22 \times 30 \times 13$ mm$^3$ and a weight of 20 g (Fig. 6(A)), which is the lightest in Table 1 [106]. There are three key components in the probe: a two-dimensional MEMS scanner, a high NA achromatic lens and a 10 MHz flat UST. The probe can provide a frame rate of 0.2 Hz, a FOV of $2 \times 2$ mm$^2$, a lateral resolution of 3.8 $\mu$m and an axial resolution of 104 $\mu$m. It has been successfully applied to carry out imaging studies of the animal organs and human oral cavity. Fig. 6(B) shows the capillary loops of a human lip inside various tissues including upper lip, lower lip, pterygomandibular fold, back surface of the tongue, and gum. Subsequently, Xi et al. proposed a dual-modality handheld microscope integrating OR-PAM and OCT systems [107]. In this dual-modality imaging probe, a cold mirror was used to allow the merge of PA and OCT light beams. In addition, two adjustable fiber collimators (350–700 nm and 650–1050 nm) were used to realize co-axial alignment of the PA and OCT foci. By further optimizing the imaging system, Xi et al. developed an ultra-compact PAM probe with a weight of ~8 g and a diameter of ~13 mm [111]. In the probe, an optical rotary joint and a customized electrical slip ring were integrated to delivery excitation light and transmit electrical signals. By utilizing a special design, the probe achieves a high axial resolution of 2.25 $\mu$m, and a faster frame rate of 0.1 Hz. Due to the light weight and high resolution, the probe can be mounted on the rat head to monitor the cerebral hemodynamics.

4.2.2. Electrostatic MEMS scanner

In addition to the electrothermal MEMS, Yang et al. developed a handheld dual-view photoacoustic imaging pen with an electrostatic MEMS scanner [113]. As shown in Fig. 6(C), they integrate the electrostatic MEMS scanner and acoustic detector with a center frequency of 10 MHz into a handheld imaging pen, which is capable of performing forward and side-view imaging. The distal head of the imaging pen has a diameter of 12 mm, which can provide an FOV of 2.4 mm in diameter, an axial resolution of 137.4 $\mu$m, an imaging speed up to 0.25 Hz, and a lateral resolution of up to 18.2 $\mu$m in different modes. Benefiting from the flexible design, the adjustable detection, and the switchable image mode, the imaging pen can meet different requirements of clinical studies, making it more suitable for clinical examination of internal areas inside the human body. Fig. 6(D) presents in vivo imaging results in different regions in human oral cavity including upper lip, lower lip, sublingual, left wall of the oral cavity, right wall of the oral cavity, and soft palate. Besides, Yang et al. proposed a handheld OR-PAM laparoscopy system based on an electrothermal MEMS scanner, which is more appropriate to be designate as a PAE platform [112].

4.2.3. Electromagnetic MEMS scanner

Apart from electrothermal and electrostatic MEMS scanners, electromagnetic MEMS scanner has been widely used in PAM for its specific merit of stability of working in water. Wang et al. proposed an OR-PAM system using a 1D water immersible electromagnetic MEMS scanner [119]. In this system, a flat transducer with a center frequency of 50 MHz was equipped with an acoustic lens to enhance the sensitivity. A one-dimensional electromagnetic MEMS scanner was immersed in the water to reflect both laser pulses and acoustic waves. Hereafter, a series of OR-PAM systems have been developed based on electromagnetic MEMS scanners [119–121,128,129]. Wang et al. reported a handheld OR-PAM device with a volume of $80 \times 115 \times 150$ mm$^3$ [114]. In this probe, a two-axis electromagnetic MEMS mirror was used to achieve fast raster scanning, allowing a volumetric rate of 2 Hz over an imaging domain of 2.5 mm $\times$ 2.5 mm $\times$ 0.5 mm. In order to maximize the SNR, an optical-acoustic beam combiner was used to provide the acoustic and optical coaxial alignment. This probe was successfully applied to observe vasculature of animals and human skin.

In contrast, Kim et al. demonstrated a handheld OR-PAM with a diameter of 17 mm and a weight of 162 g (Fig. 6(E)) based on a similar water immersible two-axis electromagnetic MEMS scanner [115]. In addition, by using a 50 kHz pulsed laser, the probe can obtain PA images with a size of 700 $\times$ 700 pixels at a volumetric imaging speed of 0.05 Hz. The lateral and axial resolutions of the probe were measured as 12 $\mu$m and 30 $\mu$m, respectively. The entire system was integrated in a medical car similar to a medical US system, which is suitable for both animal studies and patient care. Fig. 6(F) shows three-dimensional imaging results of a human mole in different views.

5. Summary

In summary, we reviewed the progresses in handheld and semi-handheld PAI systems. The general idea has been discussed in three aspects: laser delivery, scanning protocol and acoustic detection. Specific imaging systems and corresponding applications have been evaluated and discussed in terms of FACT, OAMes, and PAM. Table 1 presents a comparison of the handheld and semi-handheld PAI platforms including laser delivery, scanning protocol, acoustic detection, and clinical applications. In terms of laser delivery, optical fibers and fiber bundles are standard methods to transmit laser energy as well as in conventional optical imaging such as OCT, diffuse optical tomography, and laser speckle contrast imaging [130–132]. There is a subtle difference between OR-PAM and other modalities. To achieve field illumination, PACT always employs a high-energy laser source and deliver the laser energy using multi-mode fibers or fiber bundles [65,71,78,82,92]. For OAMes and AR-PAM, high energy multi-mode
fibers or fiber bundles are also preferred to achieve a high SNR because the spatial resolution is totally determined by acoustic diffraction [20, 96, 101]. In contrast, the lateral resolution of OR-PAM depends on the optical diffraction limit, thus a single-mode fiber is better to generate a Gaussian beam [102, 103, 106, 113–115]. Another potential solution to miniaturize the volume of PAI system is employing LD or LED [67, 81, 133, 134]. Compared with conventional laser source used in PACT, LD and LED can provide ultra-compact design of optical delivery and high pulsed repetition rate (kHz). Although several groups have reported LD/LED-based PAM systems [133], it is still not commercially available due to the low pulsed energy and poor quality of the laser beam.

In the aspect of scanning protocol, the discussion focuses on PAM. For most conventional PACTs, the scanning process has been eliminated by using US arrays, in which linear/curved-array-based PACT can only provide two-dimensional image without additional scanning of UST array [65, 67, 71], and the spherical/plane-array-based PACT can realize volumetric imaging with a single pulse excitation [78, 81, 82]. Similar to conventional PACT, OAMes employs circular scanning around the samples [49]. To extend applications of OAMes, raster-scanning-based OAMes (RSOM) has been proposed and applied in animal studies [47, 95]. Furthermore, two semi-handheld RSOM systems have been applied in visualizing subcutaneous vasculature with a higher spatial resolution in deep tissue compared with PACT [20, 47, 49]. In PAM, galvanometer scanners and MEMS scanners have been widely used in handheld and semi-handheld systems to accelerate imaging speed and miniaturize volumetric size. Table 1 has presented a comparison of imaging speed between RSOM (70 s), AR-PAM (10 s, B-scan mode), and PAM (5 s) based on motorized translation stage and galvanometer [20, 47, 101–103]. To eliminate the relative translation between imaging interface and samples, portable OR-PAM employs a rotary scanning mechanism instead of conventional raster scanning mechanisms by introducing a motorized rotator [103, 104]. Although the SNR of portable OR-PAM is better than that of PAM based on a flat responsive UST, the volume size of imaging probe is difficult to reduce. With the development of MEMS techniques, three major MFM scanners have been introduced to replace galvanometer scanner for further miniaturization of OR-PAM [106–115]. Among them, electromagnetic MEMS scanner is water-immersible, and thus allows the scanning of both optical and acoustic beam simultaneously [114, 115]. However, the volume is too large compared with electrostatic and electrothermal MEMS scanners. In contrast, electrothermal MEMS scanners have the metrics of small size, low cost, large scanning view and safe driven voltage [106–112]. Latest study has demonstrated a water immersed electrothermal MEMS scanner which has great potential in the development of future handheld PAM [134].

Finally, in acoustic detection, various detectors including piezoelectric UST [20, 47, 101–103, 106, 113–115], UST arrays with different shapes [65, 67, 71, 78, 81, 82], and acoustic sensors based on optical effect [92] are proposed in handheld and semi-handheld PAI systems. In PACT, linear-, curved-, spherical-, and planar-arrays are suitable for different applications due to the advantages of compatibility with medical US, better imaging performance, high speed of volumetric imaging, and easy coupling in point-of-care testing, respectively [65, 67, 71, 78, 81, 82]. OAMes usually uses a single UST with an ultrawide bandwidth varying from 100–200 MHz. The development of ultrasound arrays with an ultrawide bandwidth is a potential solution to accelerate the imaging speed [20, 47, 49]. For PAM, single UST has been applied in handheld and semi-handheld systems [101–103, 106, 113–115]. Detecting with UST arrays in conjunction with micro-lens array is a potential solution to improve the frame rate from several Hz to real-time imaging [135, 136]. Besides, optical detectors such as Fabry–Pérot chamber [83–85], micro-ring resonator [86, 87], and non-interference remote sensing [137–139] are proposed in PAI, in which Fabry–Pérot chamber has been applied in semi-handheld PACT [90–92]. The optical methods can provide an ultrawide acoustic bandwidth of up to 280 MHz, resulting an isotropic spatial resolution [86, 87]. Furthermore, due to the optical transparency, optical detectors have specific merit of being compatible with pure optical imaging, such as OCT, OCT angiography, and fluorescence microscopy [83, 86, 90, 91].

By using different strategies of laser delivery, scanning protocol, and acoustic detection in PACT, OAMes, and PAM, PAI reach multiscale, multiparametric, and multi-parametric imaging. In general, handheld PACTs can resolve vascular in deep tissues around 1–10 cm with a spatial resolution of hundreds of microns as shown in Table 1 [65, 71, 78, 82, 92]. In addition to hemoglobin, by inducing PA signals with multiple wavelengths, PAI can resolve other chromophores such as lipid, protein, and ICG [71, 78, 81]. Due to the deep penetration, handheld PACT focuses on studies of imaging guided biopsy [65, 67, 81, 82], visualization of vasculature in deep tissues [67, 71, 78], and imaging of vasculature in human extremities [67, 78, 82]. Compare to PACT, handheld and semi-handheld PAMs have a better spatial resolution around several microns in lateral. However, the penetration depth is limited to ~ 1 mm and the FOV is restricted in ~ 2 × 2 mm². Due to the high resolution, poor penetration, and small imaging domain of PAM, most handheld and semi-handheld systems are applied in visualization of superficial microvasculature in human skin and oral mucosa, which are related to melanoma and oral cancer, respectively [102, 103, 106, 113–115]. Apart from PACT and PAM, OAMes has compromise resolution (tens of microns) and penetration depth (below 1 cm). Hence semi-handheld RSOMs focus on vascular and microvasculature distributing from epidermis to dermis with biomarkers related to systemic sclerosis and psoriasis [20, 47].

Briefly, to address the challenges of handheld and semi-handheld PAI, new optical delivery/excitation strategies, novel optical scanning protocols, and advanced acoustic detection methods have been proposed to promote clinical translation and extend the fundamental applications of PAI. As a promising technique, there are still several aspects requiring further focus in the future: 1) A true handheld/portable PAIs without any cable and optical fiber, which are normally connected to the imaging platform for light delivery and signal transmission, is preferred. On-chip LEDs and wireless transmission of signals have the potential to achieve this goal; 2) In most PAIs, scanning is time-consuming. For PACT, scanning can be eliminated by using a 2D/3D transducer array. However, in PAM, scanning is inevitably required to achieve the high spatial resolution. The use of micro-lens array may provide an opportunity to achieve parallel scanning and reduce the scanning time; 3) In detection, comparing with piezoelectric transducer, optical method is a more attractive technique. With the improvement of the robustness of existing optical detections, PAI can be further miniaturized by using optical detections instead of conventional USTs.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A

Appendix Fig. A1(A) presents the schematic of PACT to clarify several definitions in PAI. For a conventional PACT, a single element transducer scans along the circle around the sample. The tangential and axial resolutions refer to the resolution along the tangential direction of the scanning track and the axial direction of the transducer, respectively [6, 7]. In general, the axial resolution is determined by the frequency of
ultrasound, in which a transducer with a higher center frequency and wider bandwidth brings a better axial resolution. The temporal resolution depends on the aperture of transducer, which refers to the active detection area of the detecting element. There is a tradeoff between high temporal resolution and detecting sensitivity. A small aperture means a high temporal resolution but a poor sensitivity. Another important feature of the imaging performance is the directivity of the transducer, which refers to the acoustic radiation angle when the radiation power decreases to −6 dB of the peak power in the center of the transducer. Thus, a larger directivity also means a better acceptance angle of a transducer. For a transducer array (as shown in appendix Fig. A1(A)), the angular aperture refers to the angle coverage as shown in appendix Fig. A1(A). Since the PA pressure signal is a spherical wave, a limited view array (as shown in appendix Fig. A1(A)) will lose signals out of the view, while a full-view (360° angular aperture) array can receive all the signals in the imaging plane.

For raster scanning PAM or RSOM, two parameters, lateral and axial resolution, characterize the spatial resolution of imaging systems. Similar to PACT, the axial resolution in PAM and RSOM means the resolution in axial direction of the transducer. The axial resolution is primary determined by the acoustic diffraction limit, which is same as that in PACT. A transducer with a higher center frequency and wider bandwidth can bring a better axial resolution. Lateral resolution refers to the resolution in scanning directions (x, y) of light or transducer as shown in appendix Fig. A1(B). In AR-PAM and RSOM, the lateral resolution usually depends on the size of the acoustic focus. In OR-PAM, the lateral resolution depends on the size of the optical focus. In PAM, the type of transducer will affect the SNR/detection sensitivity. As shown in appendix Fig. A1(B), PAMs with a focused UST can achieve a high SNR because the response is greater at focus. On the contrary, a flat transducer has a uniform response in entire acoustic detection filled but the sensitivity is smaller.

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Fig. A1. Several definitions in PAL. (A) Schematic of the conventional PACT and transducer-array-based PACT. (B) Schematic of raster-scanning-based PAM.
