Multimodal photoacoustic imaging as a tool for sentinel lymph node identification and biopsy guidance

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Abstract

As a minimally invasive method, sentinel lymph node biopsy (SLNB) in conjunction with guidance methods is the standard method to determine cancer metastasis in breast. The desired guidance methods for SLNB should be capable of precise SLN localization for accurate diagnosis of micro-metastases at an early stage of cancer progression and thus facilitate reducing the number of SLN biopsies for minimal surgical complications. For this, high sensitivity to the administered dyes, high spatial and contrast resolutions, deep imaging depth, and real-time imaging capability are pivotal requirements. Currently, various methods have been used for SLNB guidance, each with their own advantages and disadvantages, but no methods meet the requirements. In this review, we discuss the conventional SLNB guidance methods in this perspective. In addition, we focus on the role of the PA imaging modality on real-time SLN identification and biopsy guidance. In particular, PA-based hybrid imaging methods for precise SLN identification and efficient biopsy guidance are introduced, and their unique features, advantages, and disadvantages are discussed.

Keywords Sentinel lymph node · Sentinel lymph node biopsy · Photoacoustic imaging · Ultrasound imaging · Fluorescence imaging · Hybrid imaging · Contrast agents

1 Introduction

Photoacoustic (PA) imaging has been attracting much attention as an emerging imaging modality that has the ability to provide the advantages of an optical contrast and ultrasonic resolution. This imaging modality rests on the photoacoustic effect. Optical beam such as laser with a particular wavelength induces the vibrational and rotational oscillation of a specific molecule (e.g., hemoglobin in blood) that absorbs the incident laser pulses. This leads to converting the delivered energy into heat and subsequently transient thermoelastic expansion, thus generating acoustic waves (i.e., PA signals). Since the frequency range of PA signals is the ultrasonic range, ultrasound (US) transducers can detect the PA signals to construct PA images. The

intensity of PA signal is linearly proportional to the optical absorption coefficient of a molecule. PA imaging is categorized into PA microscopy (PAM), PA tomography (PAT) and cross-sectional PA imaging (CS-PAI), depending on signal detection scheme. Among them, CS-PAI is a viable method for clinical applications in real time, and it can be implemented by the minimal modification of a current ultrasound scanner [1–4]; PA and US images are inherently easy to be co-registered together, and thus this dual imaging modality can simultaneously provide anatomical and functional information. Figure 1 illustrates the process of PA signal generation and the detailed theoretical background of PA imaging can be found in [5].

The most popular application of PA imaging is blood vessel imaging and measurement of oxygen saturation [6–8], which is possible because hemoglobin is an excellent endogenous chromophore; it has the ability to highly absorb laser energy with wavelengths in a range of 400–600 nm, compared to other tissue components. In addition, oxygenated- and deoxygenated-hemoglobin in blood have different characteristic absorption spectra in response to incident laser. By using the optical absorption

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Fig. 1 Conceptual diagram describing the steps of photoacoustic signal generation

characteristics of lipid, the PA image of lipid core in atherosclerotic plaques was acquired [9]. Additionally, combined intravascular US and PA imaging transducers were developed for the real-time visualization of stent [10]. As a new clinical application, the possibility of the real-time imaging of breast microcalcifications, an early indicator of breast cancer, was demonstrated [11–13], and it was shown that multispectral PA imaging has the potential for differentiating cholesterol and neoplastic polyps (i.e., benign and malignant polyps) of the gallbladder [14]. Since a few endogenous chromophores exist in the body, various exogenous chromophores (i.e., PA contrast agents) based on non-biological materials such as gold nanoparticles, nanocages, and carbon nanotubes have been developed to expand the potential clinical applications of PA imaging [15–20]. In addition, biological material-based exogenous chromophores have been proposed as biocompatible PA contrast agents [21–23]. In conjunction with these contrast agents, it was demonstrated that PA imaging can be used for cancer detection, sentinel lymph node (SLN) identification, and image-guided therapy.

Among those applications, this review paper focuses on the role of the PA imaging modality on real-time SLN identification and SLN biopsy guidance. Especially, PA-based hybrid imaging methods for precise SLN identification and efficient SLN biopsy guidance are introduced and their unique features, advantages, and disadvantages are discussed. In the beginning, SLN identification and conventional SLN biopsy guidance methods are described to help the general readers understand the background of this field and the reason why multimodal photoacoustic imaging is attractive as a tool for SLN identification and biopsy guidance.

2 Sentinel lymph node identification and biopsy guidance methods

When tumor cells grow, new blood vessels and lymphatic vessels are created. At a certain stage, tumor cells have shed into the vasculature from the primary tumor and circulate in the bloodstream. It is known that the first target organ primarily reached by the circulating tumor cells is lymph nodes close to the tumor site. This lymph node is called sentinel lymph node. Therefore, it is important to identify SLN and detect the tumor cells inside the SLN to judge whether tumor metastasis occurs or not. For this, axillary lymph node dissection (ALND) has been conducted to diagnose tumor metastasis [24–27]. However, this conventional method may cause serious complications such as lymphedema, nerve injury, seroma formation, numbness or limited arm movement [28]. As a minimally-invasive method, SLN biopsy (SLNB) in conjunction with an imaging modality or a signal detector for SLN identification is widely used in clinics to alleviate the surgical complications by ALND [29].

SLNB is carried out through four steps as depicted in Fig. 2. As a first step, an exogenous dye or a radioactive tracer is injected around a primary tumor site. The injected material flows into SLN of which identification is conducted by means of visualization of the injected material or detecting signal from the material. After the identification, the suspicious SLN is surgically resected and subsequently examined by histopathology. Based on histopathological result, additional diagnosis including ALND are determined if the result is positive. For precise and effective SLNB, pre- and intra-operative SLNB guidance, which plays a role of locating SNL accurately and identify multiple basin drainages, is crucial [30]. Currently, several SLNB guidance methods are used in clinics, as

Fig. 2 Illustration of the general process of sentinel lymph node biopsy (SLNB): **a** injection of an exogenous contrast (EC) agent or a radioactive tracer into the primary tumor site and **b** identification of SLN by means of detecting signal from the injected material. **c** A

suspicious SLN reached by the injected material can be distinguished from lymph nodes (LNs) and is resected with the help of imaging guidance. The final step is to conduct histopathology of excised SLN to confirm whether tumor metastasis occurs or not

Table 1 Comparison of features of the conventional pre- and intra-operative SLNB guidance methods and photoacoustic imaging method

Method	Spatial resolution	Image/sensing depth	Exogenous contrast agent	Ionization Radiation	Performance
LS	20 mm	Very deep	Isosulfan blue, patent blue V, Evans blue	Yes	Preoperative
SPECT	500 μ s ~ 2 mm	Very deep	Radiometal (such as ^{99m}Tc and ^{111}In)	Yes	Preoperative
CT	20 μ s ~ 300 μ s	Very deep	Iodinated contrast agent	Yes	Preoperative
PET	1–2 mm	Very deep	Radioactive contrast agents (Gallium, ^{64}Cu -ATSM, Technetium-99 m)	Yes	Preoperative
MRI	10 μ s ~ 100 μ s	Very deep	Gadolinium, paramagnetic iron oxide	No	Preoperative
BD	Unaided visual resolution	~ 1 mm	Methylene blue	No	Intraoperative
RD	> 10 mm	> 50 mm	Radioactive tracer	Yes	Intraoperative
FL	~ 1 mm	~ 5 mm	ICG, MB	No	Intraoperative
US	400 μ s	~ 20 cm	Micro-bubble	No	Preoperative and intraoperative
PA	800 μ s	~ 7 cm	(Gold) NP, ICG, MB, Molecular dyes	No	Preoperative and intraoperative

summarized in Table 1, each with their own advantages and disadvantages.

Lymphoscintigraphy (LS) is a common method used in clinics for SLN mapping. This method uses gamma emitting radionuclides such as Tc-99 m that is detected for lymph drainage imaging [31–33]. Other dyes used for LS are isosulfan blue, patent blue V, Evans blue, and fluorescent dyes that stain live tissues and cells, which is the reason why they are called the vital dye [34]. The vital dyes are used for visualizing overall lymphatic vessels and drainages [35, 36]. The disadvantages of LS are a high risk

of radioactive exposure and a poor spatial resolution [37–40]. Computed tomography (CT) is a good tool for identification of lymph nodes due to its high spatial resolution [41–44], but high radiation dose is a burden on patients and it is difficult to obtain the information about lymphatic flow due to its low temporal resolution [45]. Contrast-enhanced magnetic resonance imaging (CE-MRI) with various nanoparticles is capable of visualizing tissue structures with high spatial and contrast resolutions [46–49]. However, although CE-MRI can provide the structural and functional information about SLN due to its

high spatial resolution (i.e., 10–100 μm) and it is known as a safe method, small SLNs with early-stage micrometastasis is possibly failed for identification [50]. Additionally, CE-MRI is an expensive tool and cannot be used intra-operatively because it is not a real-time imaging modality. Positron emission tomography (PET) is the most sensitive to physiological change due to cancer occurrence, but its low spatial resolution (i.e., 1–2 mm) hampers detection of small SLN [51] and the potential interference from infection and lymph-node inflammation is problematic [52]. Additionally, PET suffers from a high risk of radioactive exposure. In contrast, contrast-enhance ultrasound imaging (CE-US) with microbubble contrast agents has shown promise for SLN identification pre-operatively due to its cost-effectiveness, real-time imaging capability, and good spatial and contrast resolution [53, 54]. In intra-operative assessment, its low contrast resolution without contrast agents and high user dependence make it difficult to be used for accurate SLN identification although its real-time imaging capability is an attractive aspect of biopsy guidance [55]. For intra-operative SLNB guidance, visual identification of injected blue dye (BD) is widely used, but its shallow visibility makes it impossible to identify deep-lying SLNs, especially in obese patients [56, 57]. In contrast, radioactivity detection with a gamma-ray probe (RD) has good depth coverage in SLN identification as well as an excellent SLN identification ratio of up to 93% [56]. Radiation risk and no visual information are the main drawback of this method. Recently, fluorescence (FL) imaging has attracted attention as a SLN identification method because of its high sensitivity to the administered dyes and its wide-field *en face* imaging capability [58]. In Table 2, the sensitivity and specificity of each method for SLN identification, which are measured in human subjects, are presented.

Table 2 Sensitivity and specificity of the conventional methods for SLN identification, which were measured in human subjects

Method	Sensitivity (%)	Specificity (%)	References
Lymphoscintigraphy	31	100	[34]
PET/CT	85.1	22.7	[59]
MRI	78.6	68.9	[60]
BD	96.8	86.36	[61]
CT	20	22.7	[62]
FL	71	84.6	[63]
US	13.6	96.9	[64]

3 PA imaging for SLN identification and biopsy guidance

Compared to ALND, SLNB in conjunction with those guidance methods facilitates the reduction of the surgical complications and operation time [65–68]. However, the surgical complications still remain as a problem to be solved [69, 70]. Fine needle aspiration biopsy (FNAB) under US imaging guidance has the potential for minimal surgical complications, but it still suffers from a high false-negative rate of 11–20% and highly variable sample rate (0–53%) [71]. The desired SLNB guidance should be capable of precise SLN localization for accurate diagnosis of micro-metastases at an early stage of cancer progression and reducing the number of SLN biopsies for minimal surgical complications. For this, high sensitivity to the administered dyes, high spatial and contrast resolutions, deep imaging depth, and real-time imaging capability are pivotal requirements. The PA imaging modality with various contrast agents is a possible method to meet the requirements.

Like the conventional procedure for SLN identification, PA contrast agents are injected around the primary tumor site before PA imaging. Song et al. [72] reported that methylene blue (MB) can serve as a PA contrast agent for SLN identification and the image of SLN located at a depth of up to 30 mm was obtained. Additionally, indocyanine green (ICG) was used for PA imaging of SLN at a depth of 22 mm [73]. These results imply that PA imaging has high possibility of rapid clinical translation for SLN identification with minimal side effects because both MB and ICG are Food and Drug Administration-approved dyes. However, these materials have the drawback of concentration-dependent optical absorbance and high flowing speed [74], which hampers tracking the injected dye over time at an optimal optical absorption. Due to the ability to adjust for high optical absorbance at a desired laser wavelength, gold-based particles have been intensively investigated as PA contrast agents for SLN identification [17, 75–77]. To improve biocompatibility, Lee et al. [78] synthesized organic nano-formulated naphthalocyanines for dual-color PA imaging and demonstrated the possibility of dual-color SLN identification. Silica-coated gold nanoplates (Si-AuNPs) were developed to maintain thermodynamic stability that is associated with optical absorbance of particles and typically degraded during continuous PA imaging for SLN identification [79]. Koo et al. [80] developed single-walled carbon nanotubes conjugated with ICG (SWNTs-ICG) to enhance the ability of PA signal induction (i.e., PA signal amplitude four times higher than the conventional SWNTs) for the high contrast image of deep-lying SLNs.

Regardless of whether PA contrast agents are made of non-biological or biological materials, the injection of contrast agents is a burden on both clinicians and patients. In this light, there have been a few attempts to identify SLN and diagnose its metastasis without administering any PA contrast agents. The first study was to diagnose melanoma metastasis in SLNs [81]. The proposed method is based on the fact that melanoma is an endogenous chromophore. Therefore, it is possible to acquire PA signals from metastatic SLNs when the melanoma cells exist in the SLNs, and preliminary experimental results showed the feasibility although further studies are necessary to confirm the usefulness of the proposed method. Another interesting study is that metastatic SLNs can be identified by measuring oxygen saturation in lymph nodes by using spectroscopic PA imaging [82]. In the study, it was found that the metastatic lymph nodes have a considerably low oxygen saturation, compared to the healthy lymph nodes; the *P* value was 0.018. The experimental results are impressive, but it is unknown whether micro-metastases at an early stage of cancer progression can be diagnosed; a very small number of cancer cells may not significantly affect change in blood oxygen saturation. If this is true, SLNB is still necessary to confirm lymph node metastasis.

4 PA-based hybrid imaging methods for SLN identification and biopsy guidance

Except blood vessels, PA imaging is not suitable for obtaining the structural information of the organs. Therefore, combining PA imaging with other imaging modalities responsible for anatomic information is beneficial to precise SLN identification. For this purpose, US imaging modality is the best because an ultrasound transducer for PA signal reception is also used for acquiring US images and the same imaging system can be used for both imaging modalities. To this end, PA images can be exploited to visualize the accumulation of injected contrast agent in SLN over time because US images keep providing anatomical information about SLN and surrounding tissues [83–85]. Additionally, the combined PA and US imaging system may facilitate precise percutaneous FNAB to extract SLNs [86, 87]; US imaging is responsible for showing anatomic information surrounding the suspicious SLNs and PA imaging is used to locate the SLNs and track the position of a fine needle. Recently, carbon nanoparticles-incorporated liquid–gas phase-transition nanodroplets have been developed for PA and US dual imaging of SLNs and photothermal therapy [88]; carbon nanoparticles act as a PA contrast agent and triggers liquid perfluorohexane to gaseous phase after converting light energy into heat. The

gaseous perfluorohexane becomes a US contrast agent. In addition, this contrast agent can be used for photothermal therapy because carbon nanoparticles have photothermal-conversion effect. To enhance efficacy of photothermal therapy using this nanodroplets, the dual thermal therapeutic method may be viable [89].

There are a few studies to combine PA imaging and nuclear medicine. For this, the research focus was to develop dual-mode contrast agents. Akers et al. [90] radiolabeled MB with ^{125}I for both PA imaging and single-photon emission computed tomography (SPECT) and Liu et al. [91] developed ^{64}Cu -labeled nanoparticles for both PA imaging and positron emission tomography (PET). Clinical translational strategy of this dual-modal contrast agents is that PET-CT (or SPECT-CT) imaging is used in pre-operative assessment of SLNs (i.e., surgical planning) and intra-operative SLN mapping is conducted by PA imaging. However, the risk of radiation exposure still remains as a crucial problem.

In contrast, FL imaging is a safe method because there is no problem associated with ionized radiation. Additionally, both ICG and MB generally used for PA imaging have played a role of contrast agents for FL imaging; several studies demonstrated usefulness of ICG and MB as a contrast agent of combined PA and FL imaging [73, 92–94]. Liu et al. [95] developed fluorescent dye-loaded mesoporous silica nanoparticles for combined near-infrared (NIR) FL and PA imaging for the purpose of SLN mapping. Akers et al. [96] found that FL quenching leads to increased contrast for PA imaging and this finding was used to develop NIR dye-loaded perfluorocarbon-nanoparticles for combined FL and PA imaging of SLNs. Furthermore, Kang et al. [3] have recently reported the implementation of a real-time tri-modal imaging system for combined US, PA, FL imaging and demonstrated that the tri-modal imaging system enables surgeons to take full advantages of the complementary information obtained from the combined images, i.e., precise pre-operative localization of SLNs and intra-operative biopsy guidance [97].

5 Implications for future research

Most techniques for SLN identification and biopsy guidance inevitably use various exogenous contrast agents. The performance of the agents is one of key factors in determining the sensitivity and specificity of each method for SLN identification (see Table 2). In this respect, there have been many attempts to develop high-performance exogenous contrast agents. For the multimodal PA imaging techniques, especially, a few multimodal contrast agents have been proposed [98–100]. However, most of new

contrast agents are made from non-biological materials, which has sparked controversy as to whether the non-biological materials are safe for clinical use [101, 102]. It is necessary to focus on the development of exogenous contrast agents based on biological materials for efficient translation of the multimodal PA imaging techniques into clinical practice for SLN identification and biopsy guidance. To increase the clinical usefulness, additionally, developing real-time methods for increasing light penetration is an important research topic [103, 104], although PA imaging allows for a relatively deep imaging depth. This also make it possible to exploit (multimodal) PA imaging in various clinical applications. On the other hand, the translation of a new technique into clinical practice requires changing the conventional workflow, and thus an efficient clinical protocol for SLN identification and biopsy guidance using the multimodal PA imaging should be developed.

6 Conclusion

PA imaging modality has the advantages of real-time imaging capability, high spatial and contrast resolutions, and non-ionizing signal generation, and it has received much attention in the last decade due to those attractive features. However, PA imaging has less capability of providing anatomical information except blood vessels. Therefore, combining PA and other imaging modalities for complementing the limitation of PA imaging is the best option to take full advantage of PA imaging; we believe that its key application is SLNB guidance requiring precise SLN localization for accurate diagnosis of micro-metastases at an early stage of cancer progression. In the near future, we will see the multimodal photoacoustic imaging that plays an important role in SLNB guidance in clinics.

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Compliance with ethical standards

Conflict of interest All authors declare no conflicts of interest.

Ethical approval This article does not contain any studies conducted by the authors on humans or animals.

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