Recent advances in photoacoustic contrast agents for in vivo imaging

Upputuri, Paul Kumar; Pramanik, Manojit

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Authors:

Paul Kumar Upputuri, [0000-0003-3091-7023](#)

School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459

Manojit Pramanik*, [0000-0003-2865-5714](#)

School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459. manojit@ntu.edu.sg

Abstract

Photoacoustic imaging (PAI) is a non-invasive hybrid imaging modality offering rich optical contrast and high depth-to-resolution ratio deep-tissue imaging. Endogenous chromophores present in the body such as haemoglobin, lipid, melanin, etc. provide strong photoacoustic contrast due to their strong light absorption in certain optical window. To enhance the performance of PAI further, researchers have developed several exogenous contrast agents such as metallic nanoparticles, carbon-based nanomaterials, quantum dots, organic small molecules, semiconducting polymer nanoparticles etc. These exogenous contrast agents not only help improving the imaging contrast, but also make targeted molecular imaging possible. In this review article, we first discuss the state-of-the-art PAI techniques with endogenous contrast mechanism. Later, we provide an overview of recent progress in the development of exogenous photoacoustic contrast agents for *in vivo* imaging applications. Finally, we present the pros/cons of the existing PA contrast agents along with future challenges of contrast agent-based PAI for biomedical applications.

1. INTRODUCTION

Optical microscopy techniques such as confocal fluorescence microscopy (CFM), two-photon microscopy (2PM), second harmonic generation (SHG) microscopy, coherent anti-Stokes Raman scattering (CARS) microscopy, play an important role in life sciences and biomedical imaging.(Ntziachristos, 2010; P. K. Upputuri et al., 2013; Lihong V. Wang & Wu, 2012; Wilson, Jermyn, & Leblond, 2018; Yun & Kwok, 2017) They can provide molecular contrast with high resolution, but the imaging depths are limited to ~1 mm (known as optical diffusion limit) in biological tissues due to strong optical scattering. Fortunately, with photoacoustic effect, it is possible to convert optical energy into ultrasound waves in biological tissues. Ultrasound waves are scattered much less than light in tissues, and hence they can reveal deeply seated biological structures and functions. Photoacoustic imaging (PAI) is a non-invasive hybrid imaging modality that is rapidly advancing towards preclinical and clinical applications.(Cox, Laufer, Arridge, & Beard, 2012; Manohar & Razansky, 2016; P. K. Upputuri & Pramanik, 2017b; J. Yao & Wang, 2018) In PAI, a pulsed (typically nanosecond) laser irradiates the tissue surface. Upon absorption of light by the tissue chromophores, it undergoes a time-varying thermal expansion-relaxation process, which leads to the generation of ultrasound waves [known as photoacoustic (PA) waves] in the tissue. The generated PA waves are collected at the tissue surface and the absorption maps within the tissue are obtained using various image

reconstruction techniques.(Gutta et al., 2017; C. Huang, Wang, Nie, Wang, & Anastasio, 2013; H. Huang, Bustamante, Peterson, & Ye, 2015; Prakash, Sanny, Kalva, Pramanik, & Yalavarthy, 2019; Schoeder, Olefir, Kronbichler, Ntziachristos, & Wall, 2018; Treeby, Zhang, & Cox, 2010) The key advantages of PAI over pure optical and acoustic imaging methods are: (i) PAI offers a non-invasive, speckle-free,(Z. Guo, Li, & Wang, 2009) and label-free imaging, (ii) imaging depth up to ~4 cm *in vivo*(Lin et al., 2018) and ~11.6 cm *in vitro*(Y. Zhou, Wang, et al., 2016) have been achieved in biological tissues, (iii) PAI can provide anatomical, functional, molecular, metabolic, genetic contrasts, biomarkers, oxygen metabolism, gene expression, etc.(Chuangsuwanich et al., 2018; D. Das & Pramanik, 2019; Deán-Ben, Gottschalk, Mc Larney, Shoham, & Razansky, 2017; Moothanchery, Seeni, Xu, & Pramanik, 2017; D. Pan et al., 2009; Sivasubramanian, Periyasamy, Dienzo, & Pramanik, 2018) (iv) PAI is a multi-scale imaging modality, i.e., it can image from cells to whole-organs in humans and small-animal whole-body with consistent contrast (mainly light absorption contrast).(Lihong V. Wang & Hu, 2012; L. V. Wang & Yao, 2016)

PAI is usually performed using a nanosecond pulsed laser with wavelength in the ultraviolet (UV), visible, or near-infrared (NIR) depending on the biological molecules to be imaged.(Guosong Hong, Antaris, & Dai, 2017) When light passes through biological tissues, it will be absorbed by the endogenous biological chromophores (Figure 1) such as water, lipids, oxy/deoxy-hemoglobin (HbO_2/Hb), melanin. Therefore, PAI can be used to image these endogenous contrast agents. Due to the strong absorption of light by hemoglobin in the visible region (400-780 nm)(Lu & Fei, 2014), 532 nm was widely used for PAI in the early days mainly for vasculature imaging.(Qu et al., 2018; X. D. Wang, Pang, Ku, Stoica, & Wang, 2003) Due to strong tissue scattering at shorter wavelengths (UV and Visible), the intensity of light, and hence the PA amplitude decreases exponentially along tissue depth with a decay constant of a few millimeters.(G. Ku & Wang, 2005) In the NIR region (780-2500 nm)(Lu & Fei, 2014) the tissue attenuation and scattering is low; hence these wavelengths are ideal for deep-tissue photoacoustic imaging.(He, Song, Qu, & Cheng, 2018; Smith, Mancini, & Nie, 2009) The NIR-I window (780-1100 nm) was extensively investigated for deep-tissue and small animal imaging.(Kalva, Upputuri, & Pramanik, 2019; Kalva, Upputuri, Rajendran, Dienzo, & Pramanik, 2019; K. H. Song, Kim, Cogley, Xia, & Wang, 2009; Paul Kumar Upputuri & Pramanik, 2015; Z. Wu et al., 2019; X. Yang, Skrabalak, Li, Xia, & Wang, 2007; Y. Zhou, Yao, & Wang, 2016) Due to the reduced blood absorption in the NIR region, the imaging depth is improved with a small sacrifice in contrast. However, for enhancing the PA contrast further and for applications like targeted molecular imaging, drug delivery, therapy, effective contrast agents are often necessary.(C. Kim, Favazza, & Wang, 2010; Geoffrey P. Luke, Yeager, & Emelianov, 2012; Sowers & Emelianov, 2018; Weber, Beard, & Bohndiek, 2016) Several types of nanoparticles based on metal,(Y. S. Chen et al., 2010; C. Kim, Cho, et al., 2010; W. Li & Chen, 2015; Dipanjan Pan et al., 2010; D. Pan, Pramanik, Wickline, Wang, & Lanza, 2011) carbon,(G. Hong, Diao, Antaris, & Dai, 2015; J.-W. Kim, Galanzha, Shashkov, Moon, & Zharov, 2009; Lalwani, Cai, Nie, Wang, & Sitharaman, 2013; Toumia et al., 2016) organic dye,(Dhiman Das, Sivasubramanian, Yang, & Pramanik, 2018; Gawale et al., 2017) semiconducting polymer,(S. Huang, Upputuri, Liu, Pramanik, & Wang, 2016; Jiang & Pu, 2017) were synthesized and demonstrated for PA imaging in the NIR-I window. Along with nanoparticles, reporter genes (RGs), fluorescent proteins, dyed-droplets were also used as PA contrast agents in the range 400-800 nm.(Brunker, Yao, Laufer, & Bohndiek, 2017) Melanoidin, commonly found in a variety of foods (coffee, bread, and soy sauce), has strong absorption around 420 nm.(H.-Y. Wang, Qian, & Yao, 2011) Glucose-glycine melanoidin (GG-melanoidin) was demonstrated as a PA agent for imaging as well.(Lee et al., 2016) In addition, there are several multimodal contrast agents that show great potential for PA imaging: MPR (magnetic resonance-photoacoustic-Raman) nanoparticles,(Kircher et al., 2012) carbon nanotubes as a contrast agent for photoacoustic and thermoacoustic imaging(Pramanik, Swierczewska, Green, Sitharaman, & Wang, 2009), etc.

FIGURE 1 Absorption coefficient of endogenous (intrinsic) tissue chromophores at their typical concentrations in the human body as a function of wavelength in the 400–1400 nm region, which covers the UV, visible, near infra-red I (NIR-I), and near infra-red II (NIR-II) windows respectively. Adapted from <http://omlc.org.edu>.

The performance of PAI can be further improved by moving the excitation window from NIR-I (700-1000 nm) to NIR-II (1000-1400 nm) window. (Antaris et al., 2016; Jiang & Pu, 2018; Kenry, Duan, & Liu, 2018; P. K. Upputuri & Pramanik, 2019) The NIR-II window offers: (i) more light energy on the tissues surface, this results in deeper light transmission with longer wavelength excitation. For example, the maximum permissible exposure (MPE) at 800 nm is $\sim 300 \text{ mW/cm}^2$ and MPE at 1064 nm is $\sim 1000 \text{ mW/cm}^2$ per pulse according to ANSI (American National Standards Institute) safety limit, ("American National Standard for Safe Use of Lasers ANSI Z136.1-2007 (American National Standards Institute, Inc., New York, NY, 2007),") (ii) reduced tissue scattering, makes the tissue more transparent at longer wavelengths, (iii) minimal auto-fluorescence, reduces the background signal in fluorescence imaging. Taking advantage of high MPE, whole body of mouse, (L. Li et al., 2017; D. Wang et al., 2017) rat, (L. Li et al., 2017) and human organs (Lin et al., 2018; Wray, Lin, Hu, & Wang, 2019) were successfully imaged at 1064 nm with blood as intrinsic contrast. However, the PA signal generated by NIR-II wavelengths is still weak and hence efforts were made to develop contrast agents with strong absorption in NIR-II window based on Inorganic materials (single walled carbon nanotubes, (Pramanik et al., 2009) CuS, (Geng Ku et al., 2012) Ag nanostructures, (Y. S. Chen, Homan, Xu, Frey, & Emelianov, 2012; Homan et al., 2010; G. P. Luke et al., 2013; Sivasubramanian et al., 2017; J. Zhou et al., 2018) metal-organic particles, (D. Zhang et al., 2018) Bi₂Se₃ nanoplates (Park et al., 2018)), and organic materials (phosphorous phthalocyanine, (Y. Zhou, Wang, et al., 2016) organic charge transfer complex, (Z. Wang, Upputuri, et al., 2019; Z. Wang, Zhen, et al., 2019) semiconducting nanoparticles (Bing Guo et al., 2017; Miao & Pu, 2018; J. Wu et al., 2017)). In this review, we first discuss PA imaging with intrinsic (endogenous) contrast and later, we will focus on the advances in exogenous PA contrast agents synthesized and demonstrated for *in vivo* photoacoustic imaging in the past few years.

2. PHOTOACOUSTIC IMAGING WITH INTRINSIC (ENDOGENOUS) CONTRAST

PAI provides structural and functional information of biological tissues with optical contrast and ultrasound spatial resolution. Due to available intrinsic contrast molecules in the body (e.g., hemoglobin), PAI has been widely used to visualize blood vessel structures, blood oxygenation

mapping, functional brain imaging, etc. In this section we will discuss the recent reports on structural and functional imaging of small animal whole body and human organs *in vivo*.

2.1 *In vivo* whole-body imaging of small animals

FIGURE 2 (a) Schematic of the small animal whole-body photoacoustic computed tomography system with scanning spherical detection array. (b-d) Whole-body images acquired from a living intact mouse. (Legend): 1, left atrium; 2, cardiac ventricles; 3, liver; 4, spleen; 5, thoracic vessels; 6, kidney; 7, spine; 8, brown adipose tissue. Reproduced by permission from Ref.(Fehm, Deán-Ben, Ford, & Razansky, 2016)

Small animals such as rats, mice, etc. are ideal models for preclinical studies. They play a key role in studying human diseases and their effective treatments using modern medicine.(Xia & Wang, 2014) High-speed whole-body imaging system is always desired to visualize the biological events in real-time. A photoacoustic imaging system based on scanning of a spherical detection array was reported for 3D imaging of whole body of small animals.(Fehm et al., 2016) The schematic of the experimental **set-up** for whole body imaging is given in Figure 2a. A laser beam with 800 nm wavelength in the NIR-I window, 10 ns pulse duration, 100 Hz repetition rate from an optical parametric oscillator (OPO) laser was used to generate PA signals from the mouse body. The 800 nm beam was delivered to the animal through an opening in the center of the matrix array using a fiber bundle. The fluence on the surface of the mouse was about 19 mJ/cm². This is well below the ANSI (American National Standards Institute) limit 25 mJ/cm² at 800 nm.("American National Standard for Safe Use of Lasers ANSI Z136.1-2007 (American National Standards Institute, Inc., New York, NY, 2007),") The spherical matrix array, consists of 256 individual detection elements. Each detection element has an approximate area of 9 mm² and 100% bandwidth around a central frequency of 4 MHz. The time-resolved PA signals generated by each laser pulse were recorded at 256 individual detector locations of the spherical array, sampled by the data acquisition card (DAQ), and saved to computer for 3D image reconstruction. The system could provide high frame rate 100 frames per second within volumes of up to 1.5 cm³ and nearly isotropic spatial resolution in the range of 250-500 μm across fields of view covering the entire mouse body. For *in vivo* imaging, 50 frames were collected at each scanning position and a clustering approach was applied to minimize the effect of heartbeat and respiration on the image quality. Figures 2(b-d) display the reconstructed volumetric images of the living mouse from different view angles. The major anatomical structures such as left atrium, cardiac ventricles, liver, spleen, thoracic vessels, kidney, spine, brown adipose tissue are labelled with 1 to 8, respectively. A preclinical system, single-impulse panoramic photoacoustic computer tomography (SIP-PACT) was demonstrated for *in vivo* whole body imaging of small animals (mouse and rat) at 1064 nm.(L. Li et al., 2017) The anatomy of different organs (brain, heart, lungs, liver, spleen, kidney, caecum, intestine, etc.) of mouse was obtained with intrinsic hemoglobin contrast at 1064 nm. The whole-body of mouse

having trunk width ~ 28 mm, was imaged with high-contrast and high spatial resolution (~ 125 μm) at an imaging frame rate of 50 Hz. Another PACT system with a compact 1064 nm Nd:YAG laser was developed and demonstrated for imaging different organs of mouse with a resolution of 1.5 mm. (D. Wang et al., 2017) Brain, liver and kidney regions were imaged with 10 Hz pulse repetition frequency over 50 seconds, while the heart region was imaged with 50 Hz over 10 seconds.

2.2 *In vivo* imaging of human extremities

PACT was successfully applied for imaging human organs such as breast, (Lin et al., 2018) skin, (Schwarz, Aguirre, Omar, & Ntziachristos, 2016) thyroid, (Dima & Ntziachristos, 2016) hand fingers. (Deng & Li, 2016) Another interesting work, imaging angiographic structures in human extremities, including hands, arms, legs, and feet, was reported using 1064 nm PACT (Figure 3a). (Wray et al., 2019) The excitation light has NIR-II wavelength of 1064 nm, repetition rate of 10 Hz, and pulse duration between 8 to 12 ns. PA signals from hand were acquired within 100 μs using a full-ring ultrasonic transducer array with 512 elements, central frequency of 2.25 MHz, and one-way bandwidth of more than 95%. The in-plane resolution of the system is 255 μm . A volunteer's right hand was imaged using latitudinal mode illumination as shown in Figure 3a. Figures 3(b,c) show the 3D reconstructed maximum amplitude projection (MAP) and corresponding color-encoded depth-resolved image of the hand. Blood vessels can be visualized starting from the dorsal side of the wrist and ending at the palmar side of the fingers with high-contrast at 1064 nm. With an imaging depth of 1.8 cm on either side, hands, feet, wrist, upper forearm, leg, and bicep were imaged with 1064 nm while keeping the energy density within the ANSI limit (100 mJ/cm^2). This second window PACT system with excitation wavelength 1064 nm and blood contrast is a promising for clinical diagnosis of vascular diseases.

2.3 *In vivo* functional imaging on small animals

Along with structural information, PAI allows multi-scale functional imaging with the use of multiple-wavelengths excitation. (D. Pan, Kim, Wang, & Lanza, 2013; Rebling et al., 2018) A photoacoustic probe with AR (acoustic resolution) and OR (optical resolution) mode imaging facility was reported for both structural and functional imaging. (W. Liu et al., 2018) The probe includes two confocally arranged ultrasound transducers (Figure 4a) with central frequencies of 20 MHz (Low frequency-LF) and 40 MHz (High frequency-HF), respectively. The excitation wavelengths 532 nm and 640 nm were delivered through the aperture at the center of the ring-shaped focused UST. The axial resolution was 36 μm for OR-HF mode and AR-HF mode, and 72 μm for OR-LF mode and AR-LF mode, determined by the bandwidth of the transducer elements. For OR-HF mode and OR-LF mode, where the light focus was much tighter than the acoustic focus, the lateral resolution was optically determined by the light spot size of 6.9 μm .

The OR modes of the system were used to image the oxygen saturation of hemoglobin (sO_2) in the mouse head with the scalp removed and the skull intact. A traditional linear spectral unmixing method (Rebling et al., 2018) was used to quantify the relative concentrations of oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (HbR), based on the different optical absorption spectra of HbO_2 and HbR (Figure 4b). Figures 4c and 4d show the measured sO_2 of the mouse brain vasculature at two OR modes. The sO_2 levels are low in both the images, the only difference is that the 20-MHz transducer element provided stronger signals from the mouse cortex than the 40-MHz transducer element. This could be due to the skull's frequency-dependent acoustic attenuation.

FIGURE 3 (a) 1064 nm PACT system with latitudinal mode illumination for *in vivo* imaging of human hand. PACT of blood vasculature of the human hand (b) MAP image, (c) Color encoded depth image. Reproduced by permission from Ref.(Wray et al., 2019)

The molecular imaging capability of the AR/OR (Acoustic resolution/Optical resolution) quad system was demonstrated on the live mouse ear. The top and bottom surfaces of the ear were injected with bacteria expressing bacterial phytochrome monomeric near-infrared fluorescent protein (FP) mRFP670. This FP has strong optical absorption at 640 nm and has very low absorption at 532 nm whereas hemoglobin has the opposite trend, as shown in Figure 4b. Therefore, mRFP670 expressing bacteria can be identified by comparing the PA signals acquired at 640 nm and 532 nm. Figure 4e displays the AR-LF (AR-Low frequency) image of the mouse ear with mRFP670 signals in red and the blood signals in green. The proteins on both the top and the bottom surfaces of the ear are visible in the AR mode. The cross-sectional B-scan image of the AR-LF mode along the dashed yellow line (in Figure 4e) is shown in Figure 4f. Here, the top and bottom proteins are identified by the dashed yellow lines. The laser pulse energy used for imaging was 12 μ J. The quad AR/OR system was successfully demonstrated for multiscale morphological, functional, and molecular imaging in the mouse head, leg, and ear *in vivo*. The high scale flexibility of the system will enable broad applications in preclinical studies.

In the sections above, we discussed the recent applications of PAI using endogenous contrast haemoglobin (HbO₂ and HbR). There are several other biomolecules in the tissues that are used as contrast source for PAI, for example melanin,(Schwarz, Buehler, Aguirre, & Ntziachristos, 2016; Shu, Li, Dong, Sun, & Zhang, 2017) collagen,(P. Wang, Wang, Wang, & Cheng, 2012) lipid,(Sangha, Phillips, & Goergen, 2017; P. Wang et al., 2012) myoglobin,(Lin, Yao, Li, & Wang, 2016) bilirubin,(Y. Zhou, Zhang, Yao, & Wang, 2012) **Glucose**(Chatni et al., 2012; J. J. Yao et al., 2013), DNA and RNA.(D. K. Yao, Maslov, Shung, Zhou, & Wang, 2010) The advantage of using intrinsic contrast is that we don't need to inject any external contrast agent, which comes with issues such as biodegradability, bio safety, clearance from body, toxicity. However, sometimes intrinsic contrast is not enough for imaging. In such cases it is necessary to use exogenous contrast agent to enhance the imaging contrast. Exogenous contrast also helps in targeted molecular imaging. Another advantage of using exogenous contrast

agent is that their absorption peaks can be easily tuned to the wavelength of our need. In the next section we will discuss in detail the advancement of exogenous contrast agent in the recent past.

FIGURE 4 (a) Schematic of the dual-element ultrasound transducer with central frequencies at 20 MHz and 40 MHz. *In vivo* sO₂ mapping of a mouse brain with the scalp removed and the skull intact: (b) optical absorption spectra of the oxyhemoglobin (HbO₂), deoxyhemoglobin (HbR) and miRFP670. Measured sO₂ maps of the mouse brain vasculature at 532 nm and 640 nm by (c) OR-HF (optical resolution-high frequency) mode, and (d) OR-LF mode. Molecular imaging of bacteria expressing miRFP670 protein: (e) Top-view projection image of the mouse ear injected with bacteria, obtained by AR-LF with the dual-wavelength illumination at 532 nm and 640 nm. (f) Cross-sectional B-scan images obtained by AR-LF across the dashed line in (e). Reproduced with permission from Ref.(W. Liu et al., 2018)

3. PHOTOACOUSTIC IMAGING WITH EXOGENOUS CONTRAST ANGETS

Researchers have developed exogenous contrast agents to overcome the shortcomings of different endogenous molecules naturally present in the body. The absorption properties of exogenous agents

were usually adjusted to NIR region for PAI. Based on their chemical structure, the PA contrast agents are classified into (i) inorganic materials such as noble metal materials, metal chalcogenide materials, carbon-based nanomaterials, and (ii) organic materials such as small molecules, semiconducting polymer nanoparticles. Broadly, inorganic materials show higher photothermal conversion efficiency and good photothermal stability. On the other hand, organic materials show good biodegradability and good biocompatibility. In this section, we will discuss the recently reported inorganic and organic materials as contrast agent for PAI.

3.1 Inorganic materials

3.1.1 Metallic nanoparticles

In the field of nanomedicine, the role of metal nanoparticles (MNPs) has rapidly grown due to the characteristic properties at the nanoscale. Gold nanostructures were widely used for biomedical applications due to their unique optical and physicochemical properties. (Rivera, Ferri, & Marega, 2012) When the gold (Au) nanostructures are illuminated by a specific light, surface plasmons are generated in the metal nanosystem (Figure 5a, 5b). This LSPR (localized surface plasmon resonance) leads to the absorption of the light, whose energy is efficiently converted into heat, pressure, and then acoustic wave detectable by ultrasound transducers. PA spectra of gold nanoparticles of different shapes are shown in Figure 5c. Spherical AuNPs show absorption only in the visible region and hence they can hardly find applications in deep-tissue imaging. On the other hand, Au nanorods show a characteristic localized surface plasmon along the longitudinal direction of the nanorod, which generates a strong low-energy absorption band. The absorption band of the nanorods can be tuned to the NIR region by adjusting the aspect ratio of the nano-rod. Due to strong NIR absorption, Au nanorods are finding interesting applications in *in vivo* imaging.

FIGURE 5 Dipolar surface plasmon resonances of (a) gold nanospheres, (b) gold nanorods, and (c) PA spectra of gold nanospheres and nanorods. Au nanospheres show absorption peak only in the visible region. Au nanorods show weak absorption in the visible region corresponding to transverse mode (TM) and strong absorption in the NIR region corresponding to longitudinal mode (LM). Reproduced with permission from Ref. (El-Brolosy et al., 2008)

Recently, gold/silver hybrid nanoparticles were demonstrated for PA imaging of bacterial infection. Here PA imaging was used to effectively trigger and monitor the release of Ag⁺ ions from Au nanorods. (T. Kim, Zhang, Li, Zhang, & Jokerst, 2018) Ag⁺ ions are a well-known antibacterial agent,

and Ag nanoparticles act as a reservoir of these Ag⁺ ions for targeted therapy of bacterial infections. First, the Au-nanorods with strong absorption in NIR were coated with silver (Ag), which decreased their NIR absorption and hence PA signal. Then, the addition of ferricyanide solution (1 mM) resulted in oxidative etching of the silver shell. Thus, the PA signal from Au-nanorods is recovered as the silver is released, and this PA signal offers noninvasive monitoring of localized release of Ag⁺ ions. The synthesis procedure is illustrated in Figure 6a with corresponding TEM images. Photoacoustic ON and OFF behavior of AuNRs, Au/AgNRs, and etched Au/AgNRs was first confirmed with the PA spectra (Figure 6b) and *in vitro* PA imaging (Figure 6c). Here, AuNRs produced good PA signal (ON), Au/AgNRs produced no PA signal (OFF), and etched Au/AgNRs produced strong PA signal (ON).

To monitor the release of silver ions *in vivo*, Au/Ag particles in 200 μ L PBS solution was subcutaneously injected into wild-type male mouse. PA images were collected 4 h after intraperitoneal injection of 1 mM silver etchant [hexacyanoferrate (III) in PBS (500 μ L)]. The anatomical B-mode ultrasound images confirm the injection and imaging area. No PA signal was detected on Au/AgNRs (Figure 6d) and later strong PA signal appeared upon the *in vivo* silver etching (Figure 6e). The recovered PA contrast indicates the localized release of silver from the Au/Ag nanoparticles. These results prove that the hybrid Au/Ag nanoparticles are a useful theranostic agent for the PA imaging and treatment of bacterial infections.

FIGURE 6 (a) Schematic and TEM images of AuNRs, Au/AgNRs, and etched Au/AgNRs. Photoacoustic spectra (b) and *in vitro* photoacoustic image (c) of AuNRs, Au/AgNRs, and etched Au/AgNRs. The addition of the Ag shell effectively quenches the PA signal from AuNRs. However, the PA contrast is regenerated upon the etching of the Ag shell. B-mode ultrasound (gray scale) and photoacoustic (red) images of subcutaneously injected Au/AgNRs (d) and Au/AgNRs with successive addition of silver etchant (e). PA imaging was done at 800 nm (NIR-I). Reproduced with permission from Ref.(T. Kim et al., 2018)

Another interesting photoacoustic nano-agent, hyper-branched Au-plasmonic blackbodies (AuPBs) of compact sizes (<50 nm), was reported for photothermal therapy and second window PA imaging.(J. Zhou et al., 2018) The schematic of the one-pot synthesis of AuPB, which contained multiple Au nanoparticle assemblies in polydopamine, is shown in Figure 7a. These AuPBs showed very strong and uniform absorption in the entire wavelength range from 400 to 1350 nm (Figure 7b). Such strong optical absorption is due to the intraparticle plasmonic coupling effect among branches. Simulation of the electric field reveals that the hyperbranched nanostructure carries optical hot-spots due to strong coupling of the closely spaced branches (Figure 7c). Electric fields at the joints and gaps inside the superstructure were enhanced by more than 2 orders of magnitude, resulting in strong localization of heat generation. Here, the heat power density generated by 808 nm (NIR-I) and 1064 nm (NIR-II) lasers is similar, indicating the comparable photothermal transduction at these two wavelengths. Here, same

power density 1.0 Wcm^{-2} was used at 808 nm and 1064 nm. These AuPBs are suitable for PA imaging and photothermal therapy at any wavelength in the range 400-1350 nm. This specific plasmonic Au-nanostructure was successfully demonstrated for tumor imaging on live mouse at 1064 nm (Figure 7d). The compact plasmonic broadband nano-agents with tailored surface properties hold potential for a wide spectrum of light-mediated applications.

For clinical translation of AuNPs, their size should be below 10 nm to undergo renal clearance. Due to the direct relationship between their absorption and size, synthesizing small AuNPs for PAI is challenging. Miniature gold nanorods (8 nm x 49 nm) with strong absorption in NIR-II window were synthesized and showed 4.5 times greater photoacoustic contrast on living tumor-bearing mouse at 1064 nm. (Y.-S. Chen, Zhao, Yoon, Gambhir, & Emelianov, 2019) An antibody-targeted 5 nm AuNPs were demonstrated as molecularly specific contrast agents for PAI. (Han, Bouchard, & Sokolov, 2019) Copper-based NPs are promising candidates for biological applications due to their good biocompatibility, low cost, and strong absorbance in both NIR-I and NIR-II windows (650 to 1300 nm). They can enhance the quantitative accuracy for multispectral PA imaging when the illumination wavelength is over 1000 nm. Recently, Copper Sulphide (CuS) nanoparticles were demonstrated for deep-tissue and *in vivo* PA imaging on 4T1 subcutaneous tumor bearing nude mouse. The CuS NPs showed similar PA amplitudes at 700 nm and 1050 nm and images obtained at 1050 nm have 1.5 times higher SNR (Signal-to-Noise Ratio) compared to images at 700 nm. (Y. Liu, Gao, Xu, & Yuan, 2019)

FIGURE 7 Gold plasmonic blackbody (AuPB) for high-contrast photoacoustic imaging: (a) one-pot synthesis of AuPBs, (b) optical absorbance spectra of dopamine and AuPBs at different stages of synthesis; inset: photographs of dopamine solution (left) and AuPB dispersion (right), (c) simulation results of the heat power density inside the AuPBs illuminated by light at 808 and 1064 nm with same power density 1.0 Wcm^{-2} , (d) *In vivo* PA imaging of mouse tumor (highlighted by yellow circles) at 1064 nm excitation at different times after intravenous injection of 100 μL of AuPBs (μg/mL). Reproduced with permission from Ref. (J. Zhou et al., 2018)

3.1.2 Quantum dots

Optical absorption properties of semiconducting nanoparticles (or quantum dots, QDs) can be easily tuned to the NIR region by choosing the appropriate particle size. They possess high absorption cross-sections, good photostability, and high quantum yield comparable with most small molecule dyes.

Recently, MoO_{3-x} quantum dots (QDs) were demonstrated for PA imaging. MoO_{3-x} QDs were fabricated via a hydrothermal method in which metallic Mo (molybdenum) and chitosan were used. The chitosan acts as a capping and reducing agent at 80°C for 24 h. Figure 8a shows the TEM image of the homogeneously dispersed QDs with size around 2.5 nm. HR-TEM images of couple of QDs are shown in Figure 8b, the lattice fringes are clearly visible indicating a definite crystalline nature of QDs. The MoO_{3-x} QDs show strong optical absorption in the optical window of 700-1300 nm (Figure 8c), such broad absorption comes from the free electron induced LSPR effect. PA images of *in vitro* phantoms at 880 nm are shown in Figure 8d, here the PA signal from MoO_{3-x} QD dispersion linearly increased with its concentration showing its potential for quantitative measurements.

FIGURE 8 (a) TEM image, (b) HR-TEM image of MoO_{3-x} Quantum Dots. Inset in (a) is the schematic of the quantum dot. (c) Optical absorption spectra of MoO_{3-x} QDs at different concentrations. (d) PA images of MoO_{3-x} QD at different concentrations. (e,f) *In vivo* photoacoustic images of mouse bearing HeLa tumor before and after injection of MoO_{3-x} QDs at different time points. Reproduced by permission of The Royal Society of Chemistry. (Ding et al., 2017)

These MoO_{3-x} QDs were tested for tumor imaging on live mouse. The HeLa tumor-bearing mice were injected with 100 μL of MoO_{3-x} QDs (2 mg mL^{-1}) via intratumoral or tail intravenous injection. For the intratumoral injection group shown in Figure 8e, the PA signal from tumor site was increased and the tumor was clearly visible till 24 h, indicating a long-time accumulation of QDs in the tumor. On the other hand, for the tail intravenous injection group shown in Figure 8f, the strongest PA signal was observed at 4 h and thereafter the PA signal from tumor decreased slowly and almost vanished after

12 h. The passive tumor accumulation and clearance after intravenous injection may be due to the small size of quantum dots (about 2.5 nm). All these results suggest that MoO_{3-x} QDs is a promising PA contrast agent for tumor imaging via either intratumoral or intravenous injection. New carbon nanodots, porphyrin-implanted nanodots (PNDs) were developed by selective pyrolysis of porphyrin macrocycles and citric acid.(F. Wu et al., 2018) These PNDs show good aqueous dispersibility, and favourable biocompatibility. The PNDs displayed strong PA signals at 686 nm in a neutral or slightly acidic environment and weaker signals with a pH in a range from 7 to 8. Such carbon NDs can find applications in *in vivo* photodynamic cancer treatment.

3.2 Organic materials

3.2.1 Small organic molecules

Small molecule dyes (SMDs) are polyconjugated organic chromophores. For PA contrast agent applications, the dye should have absorption in the NIR and moreover, the fluorescence quantum yield of the dye should be as low as possible to maximize photoacoustic efficiency. Indocyanine green (ICG) is already largely used in medical diagnostics. ICG can serve as a contrast agent for both fluorescence and photoacoustic imaging.(C. Kim, Song, Gao, & Wang, 2010; Koo et al., 2012; Rajian, Fabiilli, Fowlkes, Carson, & Wang, 2011; Sivasubramanian, Periyasamy, & Pramanik, 2018) It has a concentration-dependent absorption band tunable in the range 700-900 nm; it is water soluble and biocompatible as well. However, ICG suffers from some important drawbacks: poor aqueous stability, concentration-induced aggregation and fast clearance from the body. The fast up-take and clearance of the ICG dye with absorption around 800 nm was monitored in living rat by a pulsed laser diode based PAT (PLD-PAT) system.(P. K. Upputuri & Pramanik, 2017a)

FIGURE 9 (a) Schematic diagram of the adopted procedure for ICG-labeling and *in vivo* detection of MSCs. (b) Raw average PA intensity over time after spectral unmixing. (c) Representative PA monitoring of the cell engraftment over days (excitation wavelength: 810 nm) after spectral unmixing of signal in the right hindlimb. Reproduced with permission from Ref.(Filippi et al., 2019)

Recently, ICG dye was used to label murine bone marrow-derived mesenchymal stem cells, also known as medicinal signaling cells (MSCs).(Filippi et al., 2019) The transplantation of MSCs holds great promise for the treatment of a plethora of human diseases. PAI was used to monitor stem cells labeled

with ICG. The labeling procedure was performed by detaching and incubating MSCs with ICG-containing medium at 37°C as shown in Figure 9a. About 3.0×10^5 ICG-labeled MSCs were locally transplanted into the gastrocnemius muscle of the right hindlimb of healthy C57BL/6J live mouse. In this study, to distinguish between the signals arising from the intracellular ICG and the free molecule, spectral unmixing was performed on the PA images acquired from right hindlimb as shown in Figure 9b. The PA signal from the intracellular ICG first increased to about 2.5 times with respect to the pre-injection signal, and later became stable for more than 50 hours as shown in Figure 9b. On the other side, the PA signal from free ICG increased after 40 hours, thus suggesting the occurrence of a dye release process. In the PA representative images shown in Figure 9c, the PA signals from intracellular ICG, free ICG, oxy-hemoglobin, and deoxy-hemoglobin are shown in green, purple, red, and blue colors respectively. This approach may find applications in transplantation-related studies by providing the *in vivo* cell fate surveillance with safety and good endogenous contrast among soft tissues.

FIGURE 10 (a) Schematic illustration of the preparation of Polipo-IR NPs. (b) Optical spectra of Polipo-IR NPs in water and the free IR-1061 dye in CCL₂. The inset image shows the appearance of free IR-1061 dye in CCL₂ (left) and Polipo-IR NPs in water (right). (c) Photograph of the chicken phantom and embedded NPs for deep-tissue imaging. (d) Comparison of PA images at 808 nm and 1064 nm. (e) SNR as a function of tissue depth at 1064 nm and 808 nm. PA imaging of nude mouse bearing orthotopic HCC: photograph (f), ultrasound image (g), and PA images of pre-injection (h), 0.5 h (i), 2 h (j), 3 h (k), and 4 h (l), respectively. Reproduced by permission of The Royal Society of Chemistry.(Q. Chen et al., 2019)

In parallel, other classes of dyes such as methylene blue, Evans blue, IRdye800, etc.,(Laramie, Smith, Marmarchi, McNally, & Henary, 2018) have been explored as contrast agents for molecular PA

imaging. Recently, a new type of small molecular dye-loaded lipid nanoparticles with intense NIR-II absorption was reported for photoacoustic imaging.(Q. Chen et al., 2019) Figure 10a illustrates the synthesis procedure in which IR-1061 dyes, CH, and DSPC were first dissolved in dichloromethane for rotary evaporation, and then modified with DSPE-PEG₂₀₀₀. More details on these chemicals and reagents are given in Ref.(Q. Chen et al., 2019) To overcome the inherent hydrophobicity of the IR-1061 dye and expand its use for the biomedical application, Polipo-IR NPs were synthesized by the rotary evaporation method. The optical absorbance of Polipo-IR NPs in water and the free IR-1061 dye in CCL₂ are show in Figure 10b. It can be observed that the absorption peak of the Polipo-IR NPs is wider i.e., ranging from 970 nm to 1350 nm (NIR-II). The use of Polipo-IR NPs for deep-tissue imaging was demonstrated on chicken tissue phantom (Figure 10c). This phantom was imaged at two different wavelengths 808 nm (NIR-I) and 1064 nm (NIR-II). The maximum depth of the phantom was 12.05 mm, and the PA signals of the four phantoms were all obtained using both wavelengths (Figure 10d). In all four imaged phantoms, the SNRs of 1064 nm images were superior compared to those of 808 nm (Figure 10e). All these results confirmed that 1064 nm is more suitable for deep-tissue imaging than 808 nm.

The NIR-II dye was tested on mice bearing orthotopic HCC (Hepatocellular carcinoma). The ultrasound image of the tumor before the administration of Polipo-IR NPs is shown in Figure 10g. Before injection, there was no obvious PA signal in the whole body of mice as shown in Figure 10h. The PA signal of the skin increased at 0.5 h post-injection, while no strong PA signal appeared in the region of the liver. With the continued circulation of the Polipo-IR NPs in mouse body, two distinct enhanced PA signals appeared in the liver after 2 h of injection, which was consistent with the corresponding pre-operative ultrasound images. The PA signal reached the maximum value at 3 h post-injection with a maximum SNR of 5.98 ± 0.23 (Figure 10k), mainly due to the EPR (enhanced permeability and retention) effect and strong PA signal from the Polipo-IR NPs. These results exhibit the great potential of Polipo-IR NPs for *in vivo* diagnosis of orthotopic HCC and deep-tissue imaging in photoacoustic systems.

3.2.2 Organic dyed droplets

The spatial resolution of PAT is fundamentally limited by acoustic diffraction. Single dyed droplets that can flow in blood vessels were used to break the acoustic diffraction limit.(P. Zhang, Li, Lin, Shi, & Wang, 2019) The droplets were prepared by dissolving hydrophobic IR-780 iodide dye in oil (67% clove oil+33% peanut oil), followed by mixing with water. The sizes of the droplets range from 4 to 30 μm , with a mean value of approximately 10 μm . These droplets can flow easily in the blood vessels and can generate PA signal that is 2 times higher in amplitude compared to the blood. The PACT system was equipped with a full-ring ultrasonic transducer array and a 780 nm excitation laser. The fluence on the mouse head was maintained at 30 mJ/cm^2 . First, the mouse brain was imaged using conventional PACT, where the contrast is due to blood absorption at 780 nm (Figure 11a). The brain images were continuously acquired, while droplets were injected into the heart through the catheter. The droplet suspension was prepared with 20 μL of the dye solution (2 mM) in 1 mL of water to achieve a droplet concentration of about $4 \times 10^7 \text{ mL}^{-1}$. Figure 11b shows the super-resolution PACT of the brain vasculature due to droplets in the blood stream. The amplitude profiles of conventional and super-resolution images are compared in Figure 11c. In the super-resolution image, the blood vessels appear sharper, and the closely neighbouring vessels can be resolved. This dyed droplet technique improved the resolution from 150 μm to 25 μm , this approach is promising for imaging thin blood vessels and monitoring targeted drug delivery in deep tissues.

FIGURE 11 *In vivo* image of the cortical layer of a mouse brain with conventional PACT using hemoglobin as the contrast (a), with super-resolution PACT image with localizing single droplets. (c) PA amplitude profiles of conventional (blue) and super-resolution (red) images along the lines that are shown in (a) and (b). (d) and (e) are magnified images of the selected regions in (a) and (b). (f) PA amplitude profiles along the dotted lines in (d) and (e). Reproduced with permission from Ref.(P. Zhang et al., 2019)

3.2.3 Biliverdin nanoparticles

Biodegradable nanoparticles for photoacoustic imaging are rare and limited to a few polymeric nano-agents. The development of biodegradable NPs is important for clinical applications. Metabolically digestible and inherently photoacoustic imaging probes were developed from nanoprecipitation of biliverdin, a naturally occurring heme-based pigment.(Fathi et al., 2019) The synthesis of biliverdin nanoparticles (BVNPs) is shown in Figure 12a. The properties of BVNPs were found to be highly dependent on the nature of the reaction media. Reactions were conducted in water, in 0.1 M 2-(N-morpholino) ethanesulfonic acid (MES) buffer, and in 0.15 M aqueous NaCl solution. The resultant BVNPs are water-BVNPs, MES-BVNPs, and NaCl-BVNPs, respectively. Figure 12b shows the absorption and emission wavelengths of the three different BVNPs.

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FIGURE 12 (a) Schematic of BVNP synthesis: The use of water, MES, and NaCl results in water-BVNP (iii), MES-BVNP (i), and NaCl-BVNP (ii), respectively. Dialysis of MES-BVNP with water (iv) results in water-BVNP. Nanoparticles degrade in the presence of biliverdin reductase (v). (b) MES-BVNP has a red-shifted fluorescence compared to the water-BVNP and NaCl-BVNP. All compositions exhibit high absorbance at 365 and 680 nm, resulting in fluorescent and photoacoustic properties. (c) Photoacoustic imaging of sentinel lymph nodes using BVNP at 680 nm wavelength. PA images of mouse before nanoparticle injection, 10 min and 30 min after injection. Scale bar-5 mm (white), 1 mm (yellow). LN = lymph node; LV= lymphatic vessel. Reproduced with permission from Ref.(Fathi et al., 2019)

To evaluate the feasibility of using these BVNPs as a diagnostic imaging probe, *in vivo* experiments were performed on the lymphatic system of mice. Water-BVNP, MES-BVNP, NaCl-BVNP, or a vehicle control was administered in the hind limb of live mouse via hock injection. No abnormalities were observed in the mouse body due to the injection of the nanoparticles. *In vivo* PA images of the lymph node and lymphatic vessels at 680 nm are shown in Figure 12c. All three nanoparticle types showed passive accumulation in the lymph nodes. The PA signal intensity was also found to vary with time, indicating a time-dependent nanoparticle uptake and clearance from the lymphatic system. Thus, the BVNPs are promising nano-agents for biocompatible biological imaging due to their inherent photoacoustic and fluorescent properties as well as their complete metabolic digestion.

3.2.4 Semiconducting polymer nanoparticles

Semiconducting polymer nanoparticles (SPNs) are the advanced organic nano-agents which show several advantages for both fluorescence and photoacoustic imaging compared to the biomaterials discussed above.(Jiang, Cui, et al., 2017; Xie, Upputuri, Zhen, Pramanik, & Pu, 2017) *In vivo* brain imaging was demonstrated in NIR-I and NIR-II window using SPNs. The readers can refer to the Ref.(Jiang, Upputuri, et al., 2017) for more details on SPNs having absorption in NIR-I, and SPN-II having broad absorption in NIR-I and NIR-II region. The schematic of SPN-II nanoparticle and optical absorption spectra of SPN-I and SPN-II are shown in Figure 13(a) and 13(b), respectively. The SPN-II type nanoparticles showed a spectrum with a maximum peak at 1253 nm. The SPN-II (6 mg/mL, 300 μ L per rat) was injected in rat through intravenous injection. After injection, brain images were acquired using a dual-wavelength PAT scanning system. The system was equipped with a OPO pumped by a Nd:YAG laser. The fundamental of Nd:YAG laser i.e., 1064 nm (NIR-II) and 750 nm (NIR-I) from

OPO were used for imaging. After injection, brain images were acquired at 750 nm up to 70 min. It was measured that PA signals from the blood vessels were increased by 66% and SNR was enhanced by about 1.5-fold after injection. The SNR was stabilized for 70 min, indicating the long-circulation of SPN-II in blood owing to its small diameter (~ 54 nm) and PEG-passivated surface. For comparison, brain vasculature images at 750 nm and 1064 nm are shown in Figure 13(c) and 13(d). The SNR at 1064 nm (27.7 ± 4.1 dB) was 1.5-fold of that at 750 nm (18.2 ± 4.9 dB). These results confirmed the advantage of NIR-II over NIR-I PA imaging.

FIGURE 13 (a) Schematic of SPN-II nanoparticles, (b) optical absorption spectra of SPN-I and SPN-II (40 $\mu\text{g/mL}$), *in vivo* PA images of rat brain at 750 nm (c) and 1064 nm (d) acquired 70 min after injecting SPN-II (6 mg/mL, 0.3 mL per rat). Energy density used was 5.5 mJ/cm². Reproduced with permission from Ref.(Jiang, Upputuri, et al., 2017)

Another SNPs, ultra-narrow bandgap semiconducting polymer poly (thienoisindigo-alt-diketopyrrolopyrrole) (denoted as PIGD), were designed and demonstrated for PA imaging at 1064 nm.(P. K. Upputuri et al., 2018) The synthesis of PIGD is shown in Figure 14a. First, the cerebral cortex of a rat brain was imaged at 1064 nm using blood as contrast agent. In this case, the vascular contrast at 0 min (before injection) is poor as shown on Figure 14b. After a single injection of PIGD NPs (0.25 mL per rat, 2 mg/mL), brain images were acquired at different time points. The cross-sectional images of the rat brain at 40 min and 70 min after injection are shown in Figure 14c and 14d, respectively. The SNR of the image at 0 min, and 70 min were 18 dB and 37 dB, i.e., a ~ 2 -fold enhancement in PA signal due to the accumulation of PIGD NPs in the brain vasculature. The long-circulation of PIGD NPs could be due to their suitable size of ~ 25 nm and the PEGylated surfaces. This *in vivo* brain imaging proved that even though blood absorption is low at 1064 nm (NIR-II window), by using suitable long-circulating nano-agents it is possible to achieve high contrast PA imaging. Several nano-agents demonstrated for small animal brain imaging at 1064 nm can be found in the Ref.(B. Guo et al., 2018; P. Zhang et al., 2017) Moreover, using high energy density of 50 mJ/cm² at 1064 nm, the cross-section of relatively large animal (Monkey) brain cortex with skull thickness ~ 2 mm was imaged using blood contrast.(Xinmai Yang & Wang, 2008) With strong 1064 nm agent, PAT can potentially image through even thicker skull bones. Biomaterial researchers are working on synthesizing biodegradable NIR-II contrast agents based on SPNs for PA imaging.(Jiang et al., 2019)

FIGURE 14 *In vivo* PA imaging of rat brain vasculature using PIGD NPs: (a) schematic of the synthesis of PIGD nanoparticles, brain vascular images at (b) 0 min (before injection), (c) 40 min, (d) 70 min post-injection of PIGD NPs (2 mg/mL, 0.25 mL per rat). Energy density used was 5 mJ/cm². Reproduced with permission from Ref.(P. K. Upputuri et al., 2018)

Along with the novel nanomaterial discussed above, there are voltage-sensitive dyes(VSD)(Kang et al., 2019; Rao, Zhang, & Wang, 2016) used for photoacoustically detecting direct neural activities *in vivo*. Recently another VSD named PAVSD800-2 was demonstrated for monitoring membrane potential for brain imaging.(H. K. Zhang et al., 2017) *Drosophila* brain neurons stably express GCaMP5G, a calcium-sensitive fluorescent protein whose optical absorption coefficient changes with calcium influx during action potentials. PA imaging was used to monitor the odor-evoked neuronal activities in a *drosophila* model. About ~20% odor-evoked fractional PA signal increase was recorded at all depths of the *drosophila* brain *in vivo*, with and without removal of the brain cuticle.(R. Zhang, Rao, Rong, Raman, & Wang, 2016)

4. Conclusions and Outlook

In this review, we briefly discussed the recent developments in the photoacoustic imaging systems, new contrast agents, and their preclinical and clinical applications. Using blood as the endogenous contrast, PAT could image small animal whole-body including internal organs such as heart, liver, spleen, kidney, spine at high-resolution.(Fehm et al., 2016; L. Li et al., 2017; D. Wang et al., 2017) PAT was also successfully applied for imaging human organs such as whole-breast,(Lin et al., 2018) skin,(Schwarz, Aguirre, et al., 2016) thyroid gland,(Dima & Ntziachristos, 2016) finger joints,(Deng & Li, 2016) hands, feet, wrist, forearm, leg, bicep(Wray et al., 2019) using blood as the main contrast. In all these applications, blood absorption at 1064 nm was used to image the anatomy of blood vessels deep inside animal body and human organs. The NIR-II wavelength (1064 nm) was widely used due to the availability of high MPE of 1000 mW/cm² (about 4-time higher than the MPE of 250 mW/cm² at 750 nm NIR-I wavelength), and due to reduced blood absorption and tissue scattering in the NIR-II region.(P. K. Upputuri & Pramanik, 2019) The high energy 1064 nm wavelength helped the researchers to quickly realise the potential of PAT for both animal and human imaging applications.

To broaden the applications of PAI modality and to improve the sensitivity, specificity, and contrast of PAI further, several exogenous contrast agents with strong NIR absorption and high photo-thermal conversion efficiency were developed. We discussed the recently reported PA exogenous contrast

agents, such as metallic nanomaterials (Au/Ag,(T. Kim et al., 2018) AuPB(J. Zhou et al., 2018)), quantum dots (MoO_{3-x}),(Ding et al., 2017) small molecule ICG dye,(P. K. Upputuri & Pramanik, 2017a) IR-1061 dye loaded lipid NPs,(Q. Chen et al., 2019) biodegradable Biliverdin NPs,(Fathi et al., 2019) organic dyed-droplets,(P. Zhang et al., 2019) semiconducting polymer nanoparticles.(Bing Guo et al., 2017; Jiang, Upputuri, et al., 2017; Miao & Pu, 2018; P. K. Upputuri et al., 2018; L. Zhou, Zhou, & Wu, 2018) We classified these PA contrast agents into inorganic and organic materials based on their chemical structure, and summarised their advantages and disadvantages in Table 1. Semiconducting polymer nanoparticles are the advanced biomaterials which show good optical properties for *in vivo* PA imaging in recent years.

Ideally, the contrast agents should be nontoxic while in the body, and then completely cleared. Toxicity factor of NPs is a very important concern and toxic effect should be eliminated. The toxicity of inorganic NPs is a critical issue that might hinder their application in biomedical imaging. In general, it is thought that organic nanoparticles are less toxic than inorganic ones, because they are often fully digested within the human GIT (gastrointestinal tract) and are not bio-persistent. Nevertheless, there may be certain circumstances where they could cause toxicity. The toxicity of inorganic and organic NPs are broadly discussed in the Ref.(Cho, Glaus, Chen, & Xia, 2010; McClements & Xiao, 2017)

Table 1 List of exogenous contrast agents for photoacoustic imaging.

Type of material	Type of nano-agents		Pros(+) / Cons(-) of nano-agents	Ref.
Inorganic nanomaterials	Metal-based	Nobel metal nanomaterials Ex. Au / Ag nanocrystals, Small Au NPs, Pd nanosheets, copper NPs, etc	(+) Good biocompatibility (+) Tunable chemical properties (-) Poor biodegradability (-) size/morphology dependent optical properties	(M. Chen et al., 2014; W. Li & Chen, 2015; Maturi, Locatelli, Monaco, & Comes Franchini, 2019)
		TMC (transition metal chalcogenides)-based nanomaterials Ex.: CuS, WS_2 , MoS_2 , Ag_2S , Co_9Se_8 , ZnS, Nb_2C , Bi_2S_3 , etc	(+) Good Photo-thermal stability (+) High photo-thermal conversion efficiency (+) Drug loading (-) Poor biodegradability	(Cheng et al., 2014; Fu, Zhu, Song, Yang, & Chen, 2019; Park et al., 2018; X. R. Song et al., 2015; S. Wang, Lin, Wang, Chen, & Huang, 2016)
	Carbon-based	Carbon-based nanomaterials Ex: CNTs, Graphene NPs, Carbon dots, etc	(+) Good Photo-thermal stability (-) Heterogeneity (-) Poor biodegradability (-) Poor clearance from the body	(Gao, Hu, Liu, Sheng, & Zheng, 2019; Ge et al., 2015; G. Hong et al., 2015; Pramanik et al., 2009)
Organic nanomaterials	Small organic molecules Ex: ICG, Methylene blue, IR800-dye, Evans Blue, etc		(+) Good biocompatibility (+) Low toxicity (-) Fast clearance (-) short circulation time (-) Poor photostability	(Laramie et al., 2018; Y. Liu, Bhattarai, Dai, & Chen, 2019; D. Wu, Huang, Jiang, & Jiang, 2014; Yoo, Jung, Min, Kim, & Lee, 2018)
	Semiconducting nanoparticles Ex.: semiconducting polymers, oligomers, etc		(+) Tunable absorption (+) Large absorption coefficient (+) High photo-stability (+) Low toxicity (+) Size independent optical properties	(K. Huang, Zhang, Lin, & Huang, 2019; Miao & Pu, 2018; Pu et al.,

		(-) more studies are needed to understand the biodegradation of SPNs	2015; L. Zhou et al., 2018)
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All these exciting studies show the potential of PAI for animal and human imaging. However, conventional PAI (with endogenous agents) has few fundamental and technological challenges: (i) the blood absorption and hence the PA signal is weak in the NIR region, which can be somewhat compensated by using high energy light illumination. But the energy of some of the commercial NIR lasers are still not enough to fully utilise the MPE limit permitted by ANSI safety standards. (ii) The depth-dependent light attenuation and intermixing of unwanted background signals impose great challenges in the accurate quantification of biological parameters by PAI *in vivo*, such as pH value in tumors. (iii) Thick bone penetration for imaging of brain-tumors and imaging of air-filled cavities such as imaging of lung tumors are still challenging. (iv) Due to high blood volume in the liver, imaging tumor inside liver is not straight forward. (v) The large variations in the tissue transmission across different organs at different wavelengths, makes it difficult to carry out quantitative imaging. Numerous exogenous contrast agents have been reported to enhance the performance of conventional PAI. However, there are several challenges and improvements needed before PA agents can be translated into clinical applications: (i) biomaterial studies mostly focus on developing nanomaterials with low quantum yield to enhance their PA efficiency as a contrast agent. But the major focus should be on the elucidation of the biocompatibility, and long-term toxicological profile which are crucial for medical applications. (ii) The ratiometric studies show that there is a trade-off between the drug dose and PA signal enhancement. So, the emerging PA contrast agents should be able to provide contrast enhancement even at low concentrations for effective clinical application. (iii) All the developed nanomaterials were tested only in proof-of-concept applications such as lymph node imaging, cancer imaging, brain tumor imaging, etc., in small animals. (iv) PA contrast agents with excellent biocompatibility, favourable clearance profiles, and low-toxicity similar to those of the clinically approved small molecule dyes (ICG, methylene blue, Evans blue), are still needed. The small molecule dyes show low-toxicity, but they are not considered ideal candidates for imaging applications due to their poor photostability and fast clearance. (v) Semiconducting polymer nanoparticles show high photo-stability, photo-thermal conversion efficiency, low-toxicity, but more studies are needed to understand the biodegradability of SPNs in the body. Recently, metabolizable semiconducting polymer nanoparticles were synthesised and were tested on small animals for brain and tumor imaging at 1064 nm.(Jiang et al., 2019) More *in vivo* studies in realistic animal models are needed in this direction.

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References

- American National Standard for Safe Use of Lasers ANSI Z136.1-2007 (American National Standards Institute, Inc., New York, NY, 2007). In.
- Antaris, A. L., Chen, H., Cheng, K., Sun, Y., Hong, G., Qu, C., . . . Dai, H. (2016). A small-molecule dye for NIR-II imaging. *Nat Mater*, 15(2), 235-242.
- Brunker, J., Yao, J., Laufer, J., & Bohndiek, S. E. (2017). Photoacoustic imaging using genetically encoded reporters: a review. *Journal of Biomedical Optics*, 22(7), 070901.

- Chatni, M. R., Xia, J., Sohn, R., Maslov, K., Guo, Z., Zhang, Y., . . . Wang, L. V. (2012). Tumor glucose metabolism imaged in vivo in small animals with whole-body photoacoustic computed tomography. *Journal of Biomedical Optics*, 17(7), 076012.
- Chen, M., Tang, S., Guo, Z., Wang, X., Mo, S., Huang, X., . . . Zheng, N. (2014). Core-shell Pd@Au nanoplates as theranostic agents for in-vivo photoacoustic imaging, CT imaging, and photothermal therapy. *Advanced Materials*, 26(48), 8210-8216.
- Chen, Q., Chen, J., He, M., Bai, Y., Yan, H., Zeng, N., . . . Fang, C. (2019). Novel small molecular dye-loaded lipid nanoparticles with efficient near-infrared-II absorption for photoacoustic imaging and photothermal therapy of hepatocellular carcinoma. *Biomaterials Science*, 7, 3165–3177.
- Chen, Y.-S., Zhao, Y., Yoon, S. J., Gambhir, S. S., & Emelianov, S. (2019). Miniature gold nanorods for photoacoustic molecular imaging in the second near-infrared optical window. *Nature Nanotechnology*, 14(5), 465-472.
- Chen, Y. S., Frey, W., Kim, S., Homan, K., Kruizinga, P., Sokolov, K., & Emelianov, S. (2010). Enhanced thermal stability of silica-coated gold nanorods for photoacoustic imaging and image-guided therapy. *Optics Express*, 18(9), 8867-8878.
- Chen, Y. S., Homan, K., Xu, D., Frey, W., & Emelianov, S. (2012). *Feasibility of contrast-enhanced photoacoustic liver imaging at a wavelength of 1064 nm*. Paper presented at the Biomedical Optics and 3D Imaging OSA, Miami, FL.
- Cheng, L., Liu, J., Gu, X., Gong, H., Shi, X., Liu, T., . . . Liu, Z. (2014). PEGylated WS(2) nanosheets as a multifunctional theranostic agent for in vivo dual-modal CT/photoacoustic imaging guided photothermal therapy. *Advanced Materials*, 26(12), 1886-1893.
- Cho, E. C., Glaus, C., Chen, J., & Xia, Y. (2010). Inorganic nanoparticle-based contrast agents for molecular imaging. *Trends in Molecular Medicine*, 16(12), 561-573.
- Chuangsuwanich, T., Moothanchery, M., Yan, A. T. C., Schmetterer, L., Girard, M. J. A., & Pramanik, M. (2018). Photoacoustic imaging of lamina cribrosa microcapillary in porcine eyes. *Applied Optics*, 57(17), 4865-4871.
- Cox, B., Laufer, J. G., Arridge, S. R., & Beard, P. C. (2012). Quantitative spectroscopic photoacoustic imaging: a review. *Journal of Biomedical Optics*, 17(6), 061202.
- Das, D., & Pramanik, M. (2019). Combined ultrasound and photoacoustic imaging of blood clot during microbubbles-assisted sonothrombolysis. *Journal of Biomedical Optics*, 24(12), 121902.
- Das, D., Sivasubramanian, K., Yang, C., & Pramanik, M. (2018). On-chip generation of microbubbles in photoacoustic contrast agents for dual modal ultrasound/photoacoustic in vivo animal imaging. *Scientific Reports*, 8(1), 6401.
- Deán-Ben, X., Gottschalk, S., Mc Larney, B., Shoham, S., & Razansky, D. (2017). Advanced optoacoustic methods for multiscale imaging of in vivo dynamics. *Chemical Society Reviews*, 46(8), 2158-2198.
- Deng, Z., & Li, C. (2016). Noninvasively measuring oxygen saturation of human finger-joint vessels by multi-transducer functional photoacoustic tomography. *Journal of Biomedical Optics*, 21(6), 061009.
- Dima, A., & Ntziachristos, V. (2016). In-vivo handheld optoacoustic tomography of the human thyroid. *Photoacoustics*, 4(2), 65-69.
- Ding, D., Guo, W., Guo, C., Sun, J., Zheng, N., Wang, F., . . . Liu, S. (2017). MoO₃-x quantum dots for photoacoustic imaging guided photothermal/photodynamic cancer treatment. *Nanoscale*, 9(5), 2020-2029.
- El-Brolossy, T. A., Abdallah, T., Mohamed, M. B., Abdallah, S., Easawi, K., Negm, S., & Talaat, H. (2008). Shape and size dependence of the surface plasmon resonance of gold nanoparticles studied by Photoacoustic technique. *The European Physical Journal Special Topics*, 153(1), 361-364.
- Fathi, P., Knox, H. J., Sar, D., Tripathi, I., Ostadhossein, F., Misra, S. K., . . . Pan, D. (2019). Biodegradable Biliverdin Nanoparticles for Efficient Photoacoustic Imaging. *Acs Nano*, 13(7), 7690-7704.

- Fehm, T. F., Deán-Ben, X. L., Ford, S. J., & Razansky, D. (2016). In vivo whole-body optoacoustic scanner with real-time volumetric imaging capacity. *Optica*, 3(11), 1153-1159.
- Filippi, M., Garello, F., Pasquino, C., Arena, F., Giustetto, P., Antico, F., & Terreno, E. (2019). Indocyanine green labeling for optical and photoacoustic imaging of mesenchymal stem cells after in vivo transplantation. *J Biophotonics*, 12(5), e201800035.
- Fu, Q., Zhu, R., Song, J., Yang, H., & Chen, X. (2019). Photoacoustic Imaging: Contrast Agents and Their Biomedical Applications. *Advanced Materials*, 31(6), e1805875.
- Gao, D., Hu, D., Liu, X., Sheng, Z., & Zheng, H. (2019). Recent advances in functional nanomaterials for photoacoustic imaging of glioma. *Nanoscale Horizons*.
- Gawale, Y., Adarsh, N., Kalva, S. K., Joseph, J., Pramanik, M., Ramaiah, D., & Sekar, N. (2017). Carbazole Linked NIR Aza-BODIPY Dyes as Triplet Sensitizers and Photoacoustic Contrast Agents for Deep Tissue Imaging. *Chemistry - A European Journal*, 23(27), 6570-6578.
- Ge, J., Jia, Q., Liu, W., Guo, L., Liu, Q., Lan, M., . . . Wang, P. (2015). Red-Emissive Carbon Dots for Fluorescent, Photoacoustic, and Thermal Theranostics in Living Mice. *Advanced Materials*, 27(28), 4169-4177.
- Guo, B., Sheng, Z., Hu, D., Lin, X., Xu, S., Liu, C., . . . Liu, B. (2017). Biocompatible conjugated polymer nanoparticles for highly efficient photoacoustic imaging of orthotopic brain tumors in the second near-infrared window. *Materials Horizons*, 4(5), 1151-1156.
- Guo, B., Sheng, Z., Hu, D., Liu, C., Zheng, H., & Liu, B. (2018). Through Scalp and Skull NIR-II Photothermal Therapy of Deep Orthotopic Brain Tumors with Precise Photoacoustic Imaging Guidance. *Advanced Materials*, 30(35), 1802591.
- Guo, Z., Li, L., & Wang, L. V. (2009). On the speckle-free nature of photoacoustic tomography. *Medical Physics*, 36(9), 4084-4088.
- Gutta, S., Kadimesetty, V. S., Kalva, S. K., Pramanik, M., Ganapathy, S., & Yalavarthy, P. K. (2017). Deep neural network-based bandwidth enhancement of photoacoustic data. *Journal of Biomedical Optics*, 22(11), 116001.
- Han, S., Bouchard, R., & Sokolov, K. V. (2019). Molecular photoacoustic imaging with ultra-small gold nanoparticles. *Biomedical Optics Express*, 10(7), 3472-3483.
- He, S., Song, J., Qu, J., & Cheng, Z. (2018). Crucial breakthrough of second near-infrared biological window fluorophores: design and synthesis toward multimodal imaging and theranostics. *Chemical Society Reviews*, 47(12), 4258-4278.
- Homan, K., Kim, S., Chen, Y. S., Wang, B., Mallidi, S., & Emelianov, S. (2010). Prospects of molecular photoacoustic imaging at 1064 nm wavelength. *Optics Letters*, 35(15), 2663-2669.
- Hong, G., Antaris, A. L., & Dai, H. (2017). Near-infrared fluorophores for biomedical imaging. *Nature Biomedical Engineering*, 1(1), 0010.
- Hong, G., Diao, S., Antaris, A. L., & Dai, H. (2015). Carbon Nanomaterials for Biological Imaging and Nanomedicinal Therapy. *Chemical Reviews*, 115(19), 10816-10906.
- Huang, C., Wang, K., Nie, L., Wang, L. V., & Anastasio, M. A. (2013). Full-Wave Iterative Image Reconstruction in Photoacoustic Tomography with Acoustically Inhomogeneous Media. *IEEE Transactions on Medical Imaging*, 32(6), 1097-1110.
- Huang, H., Bustamante, G., Peterson, R., & Ye, J. Y. (2015). An adaptive filtered back-projection for photoacoustic image reconstruction. *Medical Physics*, 42(5), 2169-2178.
- Huang, K., Zhang, Y., Lin, J., & Huang, P. (2019). Nanomaterials for photoacoustic imaging in the second near-infrared window. *Biomaterials Science*, 7(2), 472-479.
- Huang, S., Upputuri, P. K., Liu, H., Pramanik, M., & Wang, M. (2016). A dual-functional benzobisthiadiazole derivative as an effective theranostic agent for near-infrared photoacoustic imaging and photothermal therapy. *Journal of Materials Chemistry B*, 4(9), 1696-1703.
- Jiang, Y., Cui, D., Fang, Y., Upputuri, P. K., Pramanik, M., Ding, D., & Pu, K. (2017). Amphiphilic semiconducting polymer as multifunctional nanocarrier for fluorescence/photoacoustic imaging guided chemo-photothermal therapy. *Biomaterials*, 145, 168-177.

- Jiang, Y., & Pu, K. (2017). Advanced Photoacoustic Imaging Applications of Near-Infrared Absorbing Organic Nanoparticles. *Small*, 13(30), 1700710.
- Jiang, Y., & Pu, K. (2018). Molecular Fluorescence and Photoacoustic Imaging in the Second Near-Infrared Optical Window Using Organic Contrast Agents. *Advanced Biosystems*, 2(5), 1700262.
- Jiang, Y., Upputuri, P. K., Xie, C., Lyu, Y., Zhang, L., Xiong, Q., . . . Pu, K. (2017). Broadband Absorbing Semiconducting Polymer Nanoparticles for Photoacoustic Imaging in Second Near-Infrared Window. *Nano Letters*, 17(8), 4964-4969.
- Jiang, Y., Upputuri, P. K., Xie, C., Zeng, Z., Sharma, A., Zhen, X., . . . Pu, K. (2019). Metabolizable Semiconducting Polymer Nanoparticles for Second Near-Infrared Photoacoustic Imaging. *Advanced Materials*, 31(11), 1808166.
- Kalva, S. K., Upputuri, P. K., & Pramanik, M. (2019). High-speed, low-cost, pulsed-laser-diode-based second-generation desktop photoacoustic tomography system. *Optics Letters*, 44(1), 81-84.
- Kalva, S. K., Upputuri, P. K., Rajendran, P., Dienzo, R. A., & Pramanik, M. (2019). Pulsed laser diode-based desktop photoacoustic tomography for monitoring wash-in and wash-out of dye in rat cortical vasculature. *Journal of Visualized Experiments*, 147, e59764.
- Kang, J., Zhang, H. K., Kadam, S. D., Fedorko, J., Valentine, H., Malla, A. P., . . . Gjedde, A. (2019). Transcranial recording of electrophysiological neural activity in the rodent brain in vivo using functional photoacoustic imaging of near-infrared voltage-sensitive dye. *Frontiers in Neuroscience*, 13, 579.
- Kenry, Duan, Y., & Liu, B. (2018). Recent advances of optical imaging in the second near-infrared window. *Advanced Materials*, 30(47), e1802394.
- Kim, C., Cho, E. C., Chen, J., Song, K. H., Au, L., Favazza, C., . . . Wang, L. V. (2010). In vivo molecular photoacoustic tomography of melanomas targeted by bioconjugated gold nanocages. *Acs Nano*, 4(8), 4559-4564.
- Kim, C., Favazza, C., & Wang, L. V. (2010). In vivo photoacoustic tomography of chemicals: high-resolution functional and molecular optical imaging at new depths. *Chemical Reviews*, 110(5), 2756-2782.
- Kim, C., Song, K. H., Gao, F., & Wang, L. V. (2010). Sentinel Lymph Nodes and Lymphatic Vessels: Noninvasive Dual-Modality in Vivo Mapping by Using Indocyanine Green in Rats—Volumetric Spectroscopic Photoacoustic Imaging and Planar Fluorescence Imaging. *Radiology*, 255(2), 442-450.
- Kim, J.-W., Galanzha, E. I., Shashkov, E. V., Moon, H.-M., & Zharov, V. P. (2009). Golden carbon nanotubes as multimodal photoacoustic and photothermal high-contrast molecular agents. *Nature Nanotechnology*, 4(10), 688-694.
- Kim, T., Zhang, Q., Li, J., Zhang, L., & Jokerst, J. V. (2018). A Gold/Silver Hybrid Nanoparticle for Treatment and Photoacoustic Imaging of Bacterial Infection. *Acs Nano*, 12(6), 5615-5625.
- Kircher, M. F., de la Zerda, A., Jokerst, J. V., Zavaleta, C. L., Kempen, P. J., Mittra, E., . . . Gambhir, S. S. (2012). A brain tumor molecular imaging strategy using a new triple-modality MRI-photoacoustic-Raman nanoparticle. *Nature Medicine*, 18(5), 829-U235.
- Koo, J., Jeon, M., Oh, Y., Kang, H. W., Kim, J., Kim, C., & Oh, J. (2012). In vivo non-ionizing photoacoustic mapping of sentinel lymph nodes and bladders with ICG-enhanced carbon nanotubes. *Physics in Medicine and Biology*, 57(23), 7853.
- Ku, G., & Wang, L. V. (2005). Deeply penetrating photoacoustic tomography in biological tissues enhanced with an optical contrast agent. *Optics Letters*, 30(5), 507-509.
- Ku, G., Zhou, M., Song, S., Huang, Q., Hazle, J., & Li, C. (2012). Copper sulfide nanoparticles as a new class of photoacoustic contrast agent for deep tissue imaging at 1064 nm. *Acs Nano*, 6(8), 7489-7496.
- Lalwani, G., Cai, X., Nie, L., Wang, L. V., & Sitharaman, B. (2013). Graphene-based contrast agents for photoacoustic and thermoacoustic tomography. *Photoacoustics*, 1(3-4), 62-67.

- Laramie, M. D., Smith, M. K., Marmarchi, F., McNally, L. R., & Henary, M. (2018). Small Molecule Optoacoustic Contrast Agents: An Unexplored Avenue for Enhancing In Vivo Imaging. *Molecules*, 23(11), 2766.
- Lee, M. Y., Lee, C., Jung, H. S., Jeon, M., Kim, K. S., Yun, S. H., . . . Hahn, S. K. (2016). Biodegradable Photonic Melanoidin for Theranostic Applications. *Acs Nano*, 10(1), 822-831.
- Li, L., Zhu, L., Ma, C., Lin, L., Yao, J., Wang, L., . . . Wang, L. V. (2017). Single-impulse Panoramic Photoacoustic Computed Tomography of Small-animal Whole-body Dynamics at High Spatiotemporal Resolution. *Nature Biomedical Engineering*, 1(5), 0071.
- Li, W., & Chen, X. (2015). Gold nanoparticles for photoacoustic imaging. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 10(2), 299-320.
- Lin, L., Hu, P., Shi, J., Appleton, C. M., Maslov, K., Li, L., . . . Wang, L. V. (2018). Single-breath-hold photoacoustic computed tomography of the breast. *Nat Commun*, 9(1), 2352.
- Lin, L., Yao, J., Li, L., & Wang, L. V. (2016). In vivo photoacoustic tomography of myoglobin oxygen saturation. *Journal of Biomedical Optics*, 21(6), 61002.
- Liu, W., Shcherbakova, D. M., Kurupassery, N., Li, Y., Zhou, Q., Verkhusha, V. V., & Yao, J. (2018). Quad-mode functional and molecular photoacoustic microscopy. *Scientific Reports*, 8(1), 11123.
- Liu, Y., Bhattarai, P., Dai, Z., & Chen, X. (2019). Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer. *Chemical Society Reviews*, 48(7), 2053-2108.
- Liu, Y., Gao, D., Xu, M., & Yuan, Z. (2019). Multispectral photoacoustic imaging of cancer with broadband CuS nanoparticles covering both near-infrared I and II biological windows. *J Biophotonics*, 12(3), e201800237.
- Lu, G., & Fei, B. (2014). Medical hyperspectral imaging: a review. *Journal of Biomedical Optics*, 19(1), 010901.
- Luke, G. P., Bashyam, A., Homan, K. A., Makhija, S., Chen, Y. S., & Emelianov, S. Y. (2013). Silica-coated gold nanoplates as stable photoacoustic contrast agents for sentinel lymph node imaging. *Nanotechnology*, 24(45), 455101.
- Luke, G. P., Yeager, D., & Emelianov, S. Y. (2012). Biomedical applications of photoacoustic imaging with exogenous contrast agents. *Annals of Biomedical Engineering*, 40(2), 422-437.
- Manohar, S., & Razansky, D. (2016). Photoacoustics: a historical review. *Advances in Optics and Photonics*, 8(4), 586-617.
- Maturi, M., Locatelli, E., Monaco, I., & Comes Franchini, M. (2019). Current concepts in nanostructured contrast media development for in vivo photoacoustic imaging. *Biomaterials Science*, 7(5), 1746-1775.
- McClements, D. J., & Xiao, H. (2017). Is nano safe in foods? Establishing the factors impacting the gastrointestinal fate and toxicity of organic and inorganic food-grade nanoparticles. *NPJ Science of Food*, 1(1), 1-13.
- Miao, Q., & Pu, K. (2018). Organic semiconducting agents for deep-tissue molecular imaging: second near-infrared fluorescence, self-luminescence, and photoacoustics. *Advanced Materials*, 30(49), 1801778.
- Moothanchery, M., Seeni, R. Z., Xu, C., & Pramanik, M. (2017). In vivo studies of transdermal nanoparticle delivery with microneedles using photoacoustic microscopy. *Biomedical Optics Express*, 8(12), 5483-5492.
- Ntziachristos, V. (2010). Going deeper than microscopy: the optical imaging frontier in biology. *Nature Methods*, 7(8), 603-614.
- Pan, D., Kim, B., Wang, L. V., & Lanza, G. M. (2013). A brief account of nanoparticle contrast agents for photoacoustic imaging. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 5(6), 517-543.
- Pan, D., Pramanik, M., Senpan, A., Wickline, S. A., Wang, L. V., & Lanza, G. M. (2010). A facile synthesis of novel self-assembled gold nanorods designed for near-infrared imaging. *Journal of Nanoscience and Nanotechnology*, 10(12), 8118-8123.

- Pan, D., Pramanik, M., Senpan, A., Yang, X., Song, K. H., Scott, M. J., . . . Lanza, G. M. (2009). Molecular photoacoustic tomography with colloidal nanobeacons. *Angewandte Chemie International Edition*, 48(23), 4170-4173.
- Pan, D., Pramanik, M., Wickline, S. A., Wang, L. V., & Lanza, G. M. (2011). Recent advances in colloidal gold nanobeacons for molecular photoacoustic imaging. *Contrast Media and Molecular Imaging*, 6(5), 378-388.
- Park, S., Park, G., Kim, J., Choi, W., Jeong, U., & Kim, C. (2018). Bi₂Se₃ Nanoplates for contrast-enhanced photoacoustic imaging at 1064 nm. *Nanoscale*, 10(44), 20548-20558.
- Prakash, J., Sanny, D., Kalva, S. K., Pramanik, M., & Yalavarthy, P. K. (2019). Fractional Regularization to Improve Photoacoustic Tomographic Image Reconstruction. *IEEE Transactions on Medical Imaging*, 38(8), 1935-1947.
- Pramanik, M., Swierczewska, M., Green, D., Sitharaman, B., & Wang, L. V. (2009). Single-walled carbon nanotubes as a multimodal-thermoacoustic and photoacoustic-contrast agent. *Journal of Biomedical Optics*, 14(3), 034018.
- Pu, K., Mei, J., Jokerst, J. V., Hong, G., Antaris, A. L., Chattopadhyay, N., . . . Rao, J. (2015). Diketopyrrolopyrrole-Based Semiconducting Polymer Nanoparticles for In Vivo Photoacoustic Imaging. *Advanced Materials*, 27(35), 5184-5190.
- Qu, Y., Li, L., Shen, Y., Wei, X., Wong, T. T., Hu, P., . . . Wang, L. V. (2018). Dichroism-sensitive photoacoustic computed tomography. *Optica*, 5(4), 495-501.
- Rajian, J. R., Fabiilli, M. L., Fowlkes, J. B., Carson, P. L., & Wang, X. (2011). Drug delivery monitoring by photoacoustic tomography with an ICG encapsulated double emulsion. *Optics Express*, 19(15), 14335-14347.
- Rao, B., Zhang, R., & Wang, L. V. (2016). *Photoacoustic imaging of voltage signals (Conference Presentation)*. Paper presented at the Photons Plus Ultrasound: Imaging and Sensing.
- Rebling, J., Estrada, H., Gottschalk, S., Sela, G., Zwack, M., Wissmeyer, G., . . . Razansky, D. (2018). Dual-wavelength hybrid optoacoustic-ultrasound biomicroscopy for functional imaging of large-scale cerebral vascular networks. *J Biophotonics*, 11(9), e201800057.
- Rivera, V. A. G., Ferri, F. A., & Marega, E. (2012). Localized Surface Plasmon Resonances: Noble Metal Nanoparticle Interaction with Rare-Earth Ions. In *Plasmonics - Principles and Applications*.
- Sangha, G. S., Phillips, E. H., & Goergen, C. J. (2017). In vivo photoacoustic lipid imaging in mice using the second near-infrared window. *Biomedical Optics Express*, 8(2), 736-742.
- Schoeder, S., Olefir, I., Kronbichler, M., Ntziachristos, V., & Wall, W. A. (2018). Optoacoustic image reconstruction: the full inverse problem with variable bases. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 474(2219), 20180369.
- Schwarz, M., Aguirre, J., Omar, M., & Ntziachristos, V. (2016). Optoacoustic Imaging of Skin. In *Imaging in Dermatology* (pp. 375-385).
- Schwarz, M., Buehler, A., Aguirre, J., & Ntziachristos, V. (2016). Three-dimensional multispectral optoacoustic mesoscopy reveals melanin and blood oxygenation in human skin in vivo. *J Biophotonics*, 9(1-2), 55-60.
- Shu, X., Li, H., Dong, B., Sun, C., & Zhang, H. F. (2017). Quantifying melanin concentration in retinal pigment epithelium using broadband photoacoustic microscopy. *Biomedical Optics Express*, 8(6), 2851-2865.
- Sivasubramanian, K., Mathiyazhakan, M., Wiraja, C., Upputuri, P. K., Xu, C., & Pramanik, M. (2017). Near Infrared light-responsive liposomal contrast agent for photoacoustic imaging and drug release applications. *Journal of Biomedical Optics*, 22(4), 041007.
- Sivasubramanian, K., Periyasamy, V., Dienzo, R. A., & Pramanik, M. (2018). Hand-held, clinical dual mode ultrasound-photoacoustic imaging of rat urinary bladder and its applications. *J Biophotonics*, 11(5), e201700317.
- Sivasubramanian, K., Periyasamy, V., & Pramanik, M. (2018). Non-invasive sentinel lymph node mapping and needle guidance using clinical handheld photoacoustic imaging system in small animal. *J Biophotonics*, 11(1), e201700061.

- Smith, A. M., Mancini, M. C., & Nie, S. (2009). Second window for in vivo imaging. *Nature Nanotechnology*, 4(11), 710-711.
- Song, K. H., Kim, C., Cobley, C. M., Xia, Y., & Wang, L. V. (2009). Near-infrared gold nanocages as a new class of tracers for photoacoustic sentinel lymph node mapping on a rat model. *Nano Letters*, 9(1), 183-188.
- Song, X. R., Wang, X., Yu, S. X., Cao, J., Li, S. H., Li, J., . . . Chen, X. (2015). Co(9) Se(8) nanoplates as a new theranostic platform for photoacoustic/magnetic resonance dual-modal-imaging-guided chemo-photothermal combination therapy. *Advanced Materials*, 27(21), 3285-3291.
- Sowers, T., & Emelianov, S. (2018). Exogenous imaging contrast and therapeutic agents for intravascular photoacoustic imaging and image-guided therapy. *Physics in Medicine and Biology*, 63(22), 22TR01.
- Toumia, Y., Domenici, F., Orlanducci, S., Mura, F., Grishenkov, D., Trochet, P., . . . Paradossi, G. (2016). Graphene Meets Microbubbles: A Superior Contrast Agent for Photoacoustic Imaging. *ACS applied materials & interfaces*, 8(25), 16465-16475.
- Treeby, B. E., Zhang, E. Z., & Cox, B. T. (2010). Photoacoustic tomography in absorbing acoustic media using time reversal. *Inverse Problems*, 26(11), 115003.
- Upputuri, P. K., Lin, J., Gong, L., Liu, X., Wang, H., & Huang, Z. (2013). Circularly polarized coherent anti-Stokes Raman scattering microscopy. *Optics Letters*, 38(8), 1262-1264.
- Upputuri, P. K., & Pramanik, M. (2015). Pulsed laser diode based optoacoustic imaging of biological tissues. *Biomedical Physics & Engineering Express*, 1(4), 045010-045017.
- Upputuri, P. K., & Pramanik, M. (2017a). Dynamic in vivo imaging of small animal brain using pulsed laser diode-based photoacoustic tomography system. *Journal of Biomedical Optics*, 22(9), 090501.
- Upputuri, P. K., & Pramanik, M. (2017b). Recent advances toward preclinical and clinical translation of photoacoustic tomography: a review. *Journal of Biomedical Optics*, 22(4), 041006.
- Upputuri, P. K., & Pramanik, M. (2019). Photoacoustic imaging in the second near-infrared window: a review. *Journal of Biomedical Optics*, 24(4), 040901.
- Upputuri, P. K., Yang, C., Huang, S., Wang, K., Wang, M., & Pramanik, M. (2018). Contrast-enhanced photoacoustic imaging in the second near-infrared window using semiconducting polymer nanoparticles. *Journal of Biomedical Optics*, 24(3), 031002.
- Wang, D., Wang, Y., Wang, W., Luo, D., Chitgupi, U., Geng, J., . . . Xia, J. (2017). Deep tissue photoacoustic computed tomography with a fast and compact laser system. *Biomedical Optics Express*, 8(1), 112-123.
- Wang, H.-Y., Qian, H., & Yao, W.-R. (2011). Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry*, 128(3), 573-584.
- Wang, L. V., & Hu, S. (2012). Photoacoustic Tomography: In Vivo Imaging from Organelles to Organs. *Science*, 335(6075), 1458-1462.
- Wang, L. V., & Wu, H.-I. (2012). *Biomedical Optics: Principles and Imaging*. New Jersey: John Wiley & Sons, Inc.
- Wang, L. V., & Yao, J. (2016). A practical guide to photoacoustic tomography in the life sciences. *Nature Methods*, 13(8), 627-638.
- Wang, P., Wang, P., Wang, H. W., & Cheng, J. X. (2012). Mapping lipid and collagen by multispectral photoacoustic imaging of chemical bond vibration. *Journal of Biomedical Optics*, 17(9), 96010-96011.
- Wang, S., Lin, J., Wang, T., Chen, X., & Huang, P. (2016). Recent Advances in Photoacoustic Imaging for Deep-Tissue Biomedical Applications. *Theranostics*, 6(13), 2395.
- Wang, X. D., Pang, Y. J., Ku, G., Stoica, G., & Wang, L. V. (2003). Three-dimensional laser-induced photoacoustic tomography of mouse brain with the skin and skull intact. *Optics Letters*, 28(19), 1739-1741.

- Wang, Z., Upputuri, P. K., Zhen, X., Zhang, R., Jiang, Y., Ai, X., . . . Xing, B. (2019). pH-sensitive and biodegradable charge-transfer nanocomplex for second near-infrared photoacoustic tumor imaging. *Nano Research*, 12(1), 49-55.
- Wang, Z., Zhen, X., Upputuri, P. K., Jiang, Y., Lau, J., Pramanik, M., . . . Xing, B. (2019). Redox-activatable and acid-enhanced nanotheranostics for second near-infrared photoacoustic tomography and combined photothermal tumor therapy. *Acs Nano*, 13(5), 5816-5825.
- Weber, J., Beard, P. C., & Bohndiek, S. E. (2016). Contrast agents for molecular photoacoustic imaging. *Nature Methods*, 13(8), 639-650.
- Wilson, B. C., Jermyn, M., & Leblond, F. (2018). Challenges and opportunities in clinical translation of biomedical optical spectroscopy and imaging. *Journal of Biomedical Optics*, 23(3), 030901.
- Wray, P., Lin, L., Hu, P., & Wang, L. V. (2019). Photoacoustic computed tomography of human extremities. *Journal of Biomedical Optics*, 24(2), 1-8.
- Wu, D., Huang, L., Jiang, M. S., & Jiang, H. (2014). Contrast agents for photoacoustic and thermoacoustic imaging: a review. *Int J Mol Sci*, 15(12), 23616-23639.
- Wu, F., Su, H., Cai, Y., Wong, W.-K., Jiang, W., & Zhu, X. (2018). Porphyrin-Implanted Carbon Nanodots for Photoacoustic Imaging and in Vivo Breast Cancer Ablation. *ACS Applied Bio Materials*, 1(1), 110-117.
- Wu, J., You, L., Lan, L., Lee, H. J., Chaudhry, S. T., Li, R., . . . Mei, J. (2017). Semiconducting polymer nanoparticles for centimeters-deep photoacoustic imaging in the second near-infrared window. *Advanced Materials*, 29(41), 1703403.
- Wu, Z., Li, L., Yang, Y., Hu, P., Li, Y., Yang, S. Y., . . . Gao, W. (2019). A microrobotic system guided by photoacoustic computed tomography for targeted navigation in intestines in vivo. *Science Robotics*, 4, eaax0613.
- Xia, J., & Wang, L. V. (2014). Small-animal whole-body photoacoustic tomography: a review. *IEEE Transactions on Biomedical Engineering*, 61(5), 1380-1389.
- Xie, C., Upputuri, P. K., Zhen, X., Pramanik, M., & Pu, K. (2017). Self-Quenched semiconducting polymer nanoparticles for amplified in vivo photoacoustic imaging. *Biomaterials*, 119, 1-8.
- Yang, X., Skrabalak, S. E., Li, Z. Y., Xia, Y., & Wang, L. V. (2007). Photoacoustic Tomography of a Rat Cerebral Cortex in vivo with Au Nanocages as an Optical Contrast Agent. *Nano Letters*, 7(12), 3798-3802.
- Yang, X., & Wang, L. V. (2008). Monkey brain cortex imaging by photoacoustic tomography. *Journal of Biomedical Optics*, 13(4), 044009.
- Yao, D. K., Maslov, K., Shung, K. K., Zhou, Q., & Wang, L. V. (2010). In vivo label-free photoacoustic microscopy of cell nuclei by excitation of DNA and RNA. *Optics Letters*, 35(24), 4139-4141.
- Yao, J., & Wang, L. V. (2018). Recent progress in photoacoustic molecular imaging. *Current Opinion in Chemical Biology*, 45, 104-112.
- Yao, J. J., Xia, J., Maslov, K. I., Nasirivanaki, M., Tsytsarev, V., Demchenko, A. V., & Wang, L. V. (2013). Noninvasive photoacoustic computed tomography of mouse brain metabolism in vivo. *Neuroimage*, 64(1), 257-266.
- Yoo, S., Jung, D., Min, J.-J., Kim, H., & Lee, C. (2018). Biodegradable Contrast Agents for Photoacoustic Imaging. *Applied Sciences*, 8(9), 1567.
- Yun, S. H., & Kwok, S. J. J. (2017). Light in diagnosis, therapy and surgery. *Nature Biomedical Engineering*, 1(1), 0008.
- Zhang, D., Xu, H., Zhang, X., Liu, Y., Wu, M., Li, J., . . . Yuan, Z. (2018). Self-quenched metal-organic particles as dual-mode therapeutic agents for photoacoustic imaging-guided second near-infrared window photochemotherapy. *ACS applied materials & interfaces*, 10(30), 25203-25212.
- Zhang, H. K., Yan, P., Kang, J., Abou, D. S., Le, H. N., Jha, A. K., . . . Boctor, E. M. (2017). Listening to membrane potential: photoacoustic voltage-sensitive dye recording. *Journal of Biomedical Optics*, 22(4), 045006.

- Zhang, P., Li, L., Lin, L., Hu, P., Shi, J., He, Y., . . . Wang, L. V. (2017). High-resolution deep functional imaging of the whole mouse brain by photoacoustic computed tomography in vivo. *J Biophotonics*, 11(1), e201700024.
- Zhang, P., Li, L., Lin, L., Shi, J., & Wang, L. V. (2019). In vivo superresolution photoacoustic computed tomography by localization of single dyed droplets. *Light: Science & Applications*, 8(1), 36.
- Zhang, R., Rao, B., Rong, H., Raman, B., & Wang, L. V. (2016). *In vivo photoacoustic neuronal imaging of odor-evoked calcium signals in the drosophila brain (Conference Presentation)*. Paper presented at the Photons Plus Ultrasound: Imaging and Sensing.
- Zhou, J., Jiang, Y., Hou, S., Upputuri, P. K., Wu, D., Li, J., . . . Duan, H. (2018). Compact Plasmonic Blackbody for Cancer Theranosis in Near-Infrared II Window. *Acs Nano*, 12(3), 2643-2651.
- Zhou, L., Zhou, H., & Wu, C. (2018). Semiconducting polymer nanoparticles for amplified photoacoustic imaging. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 10(5), e1510.
- Zhou, Y., Wang, D., Zhang, Y., Chitgupi, U., Geng, J., Wang, Y., . . . Lovell, J. F. (2016). A Phosphorus Phthalocyanine Formulation with Intense Absorbance at 1000 nm for Deep Optical Imaging. *Theranostics*, 6(5), 688-697.
- Zhou, Y., Yao, J., & Wang, L. V. (2016). Tutorial on photoacoustic tomography. *Journal of Biomedical Optics*, 21(6), 061007.
- Zhou, Y., Zhang, C., Yao, D. K., & Wang, L. V. (2012). Photoacoustic microscopy of bilirubin in tissue phantoms. *Journal of Biomedical Optics*, 17(12), 126019.