Magneto-photo-acoustic imaging

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Abstract: Magneto-photo-acoustic imaging, a technique based on the synergy of magneto-motive ultrasound, photoacoustic and ultrasound imaging, is introduced. Hybrid nanoconstructs, liposomes encapsulating gold nanorods and iron oxide nanoparticles, were used as a dual-contrast agent for magneto-photo-acoustic imaging. Tissue-mimicking phantom and macrophage cells embedded in ex vivo porcine tissue were used to demonstrate that magneto-photo-acoustic imaging is capable of visualizing the location of cells or tissues labeled with dual-contrast nanoparticles with sufficient contrast, excellent contrast resolution and high spatial resolution in the context of the anatomical structure of the surrounding tissues. Therefore, magneto-photo-acoustic imaging is capable of identifying the nanoparticle-labeled pathological regions from the normal tissue, providing a promising platform to noninvasively diagnose and characterize pathologies.

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1. Introduction

Traditional imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US) have been developed to primarily provide morphological information. However, noninvasive, deep penetrating in vivo imaging of functional, molecular and cellular events is required to provide accurate localization and characterization of pathologies within the anatomical content of the surrounding tissues.

Among different imaging modalities, ultrasonography is widely used because it is a non-ionizing, cost-effective and portable imaging modality that could obtain an anatomical map of the tissue with excellent spatial and temporal resolution at reasonable penetration depth [1]. However, the sensitivity and specificity of ultrasound imaging are limited due to insufficient contrast between normal and pathologically transformed tissue [2]. To address the limitations of ultrasound imaging, various ultrasound-based techniques such as photoacoustic (PA) imaging [2–8] and magneto-motive ultrasound (MMUS) imaging [9–13] have been developed.

In PA imaging at large depth (beyond the quasi-ballistic photon regime [14]), the spatial resolution is determined by ultrasound while the imaging contrast is determined by optical absorption of tissue. To enable molecular PA imaging, plasmonic nanoparticles of various shapes and sizes, characterized by resonance absorption in a specific wavelength range, have been developed and used [7,8,15–17]. In molecular contrast-enhanced PA imaging, the distribution of nanoparticles could be measured with high sensitivity. However, significant background signals from endogenous chromophores in native tissue, such as hemoglobin, can interfere. Therefore, tissue regions labeled with plasmonic nanoparticles may have limited contrast compared to background tissue. To address this limitation, spectroscopic PA imaging...
has been developed to distinguish photoacoustic signals from gold nanoparticles and tissue based on the differences in optical absorption spectra [8]. However, spectroscopic PA imaging requires a tunable or multi-wavelength laser source that is significantly more expensive than a single wavelength laser. Furthermore, aggregation and random orientation of anisotropic nanoparticles, such as nanorods, may result in unpredictable plasmonic resonance shift as the nanoparticles interact with cells [18].

In MMUS imaging [9–13], magnetic excitation is applied to induce motion within the tissue labeled with magnetic nanoparticles. The ultrasound waves are used to track and image the magnetically induced motion within the tissue. Magneto-motive ultrasound imaging can identify the presence and location of magnetic nanoparticles inside tissue with excellent contrast because the typical magnetic susceptibility of superparamagnetic nanoparticles, such as iron oxide, is five orders higher than that of the native tissue. Furthermore, the biomechanical properties, such as stiffness, of the labeled tissue can be assessed using the magneto-motive displacements [9–12,19]. However, MMUS imaging exhibits limited contrast resolution. The magneto-motive motion is determined by the magnetic force acting on the nanoparticles and the mechanical properties of the labeled tissue. The magnetic nanoparticles inside the tissue, together with the adjacent regions of the tissue, are mechanically moved together under the magnetic excitation, resulting in a relatively uniform magneto-motive displacement inside the labeled regions. Therefore, MMUS imaging may not be able to differentiate the variations in distribution of nanoparticles within magnetically labeled tissue.

To address the limitations of both PA and MMUS imaging, we introduce a hybrid imaging technique, magneto-photo-acoustic (MPA) imaging, to detect and characterize the tissue regions labeled with magneto-plasmonic nanoparticles with sufficient contrast, excellent contrast resolution and high spatial resolution. Magneto-photo-acoustic imaging requires dual-contrast (magneto-plasmonic) agent exhibiting enhanced optical absorption and magnetic susceptibility simultaneously. In MPA imaging, the high resolution photoacoustic signals from tissue regions labeled with magneto-plasmonic nanoparticles are identified using the magneto-motive motion, allowing the signals from background tissue regions to be suppressed. Therefore, the photoacoustic contrast between labeled and background tissue is significantly improved. Furthermore, photoacoustic signal amplitude filtered by the magnetically induced motion is indicative of the spatial distribution of nanoparticles. Thus, high contrast resolution is attainable in MPA imaging. Finally, MPA imaging visualizes the morphological and anatomical features of tissue with spatial resolution determined by ultrasound imaging system.

In this paper, MPA imaging was performed using liposomes encapsulating gold nanorods and iron oxide nanoparticles as a dual-contrast agent. Tissue-mimicking phantoms and macrophage cells embedded in ex vivo porcine tissue were used to demonstrate that MPA imaging can identify the location of cells or tissue labeled with dual-contrast nanoparticles in the context of anatomical structures of the surrounding tissues.

2. Materials and methods

2.1 Dual-contrast agent synthesis

The nanoconstructs, liposomal nanoparticles [20] used as dual-contrast agent for MPA imaging, were synthesized by incorporating superparamagnetic magnetite nanoparticles (Fe3O4 NPs) (Ocean NanoTech, LLC) and custom-made plasmonic gold (Au) nanorods (NRs) synthesized based on a published protocol into liposomes [21]. The fabrication of liposomal nanoparticles is shown schematically in Fig. 1. The synthesis of the dual-contrast agent began with the formation of a lipid cake. A solution of 1 mL egg phosphatidylcholine (Egg-PC) and 0.11 mL 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOPC) in chloroform (Avanti Polar Lipids, Inc.) was added to a 50 mL pear-shaped flask. Both Egg-PC and DOPC samples used in our experiments were 10 mg/mL. The solution was rotated at 100 rpm in room temperature water bath at a reduced pressure of 400 mbar in a rotary evaporator. After approximately 30 minutes, all of the chloroform evaporated from the lipid solution, resulting in an evenly
distributed film of Egg-PC and DOPC, called “lipid cake”, deposited on the inner wall of the flask. The flask was removed from the rotary evaporator and stored in a desiccator at reduced pressure overnight to ensure complete evaporation of chloroform from the lipid cake. Upon hydrating the lipid cake with an aqueous solution of nanoparticles, the nanoparticles would be trapped within the multi-lamellar liposomes (MLLs) as they spontaneously assembled. To ensure the liposomal nanoparticles exhibited both plasmonic and magnetic properties, the lipid cake was hydrated with a 1X phosphate buffered saline (PBS) solution containing 15 nm Fe₃O₄ NPs and ~10 nm by ~50 nm Au NRs. The lipid cake was hydrated with the nanoparticles solution for 30 minutes at atmospheric pressure at 75 rpm and 45°C. The MLLs containing Fe₃O₄ NPs and Au NRs underwent five freeze-thaw cycles (FTCs) to produce single-lamellar liposomes (SLLs). In the freeze-thaw cycles, the liposomal solution was frozen in dry ice for approximately 15 minutes and immediately thawed in a 45°C water bath. The FTCs fracture the outer lipid bilayers of the MLLs, leaving the liposomal nanoparticles with a single lipid bilayer. The diameters of the SLLs obtained after the FTCs were substantially varied due to the spontaneous formation of the MLLs upon hydration of the lipid cake. The SLLs were extruded through a 200 nm filter to obtain nanoparticles with mean diameter of 200 nm. In the prepared solution of dual-contrast liposomal nanoparticles, the concentration of Au NRs was approximately 28 nM (1.18 mg/mL), while the concentration of Fe₃O₄ NPs was approximately 250 nM (1 mg/mL). In each liposomal nanoparticle, there were 2 ± 1Au NRs and 35 ± 18 Fe₃O₄ NPs (mean/standard deviation). Dynamic light scattering (DLS) analysis indicated that the empty liposomes prepared using the same protocol had an average diameter of 213.0 nm and a polydispersity index of 0.205.

The liposomal nanoparticles were characterized with transmission electron microscopy (TEM) and UV-vis spectroscopy. The TEM image of liposomal nanoparticle, presented in Fig. 2(a), shows that Fe₃O₄ NPs and Au NRs were successfully packaged within the liposomes. The UV-vis spectra of 1X PBS solution containing Fe₃O₄ NPs and Au NRs for hydrating the lipid cake (dashed line) and the final liposomal nanoparticles (solid line) are shown in Fig. 2(b). The PBS solution containing both Fe₃O₄ NPs and Au NRs exhibits a strong plasmonic resonance at 798 nm. After hydrating lipid cake with the PBS solution, the dual-contrast liposomal nanoparticles exhibit a broader plasmonic resonance at 815 nm. The red-shift and broader range of resonance absorption of the liposomal nanoparticles compared to the PBS solution reflects a change in the local dielectric field resulting from the interaction of Fe₃O₄ NPs and Au NRs when they were encapsulated in the liposomes [22].

2.2 Phantom construction

To initially demonstrate the feasibility of MPA imaging, the experiments were performed using the tissue-mimicking phantom with six inclusions. The background of the tissue-mimicking phantom was prepared by mixing 2 wt% gelatins with 0.002 wt% graphite to represent the endogenous chromophores in native tissue. The inclusions were prepared by mixing 10 wt% gelatins with different types of nanoparticles at different concentrations. Specifically, the first inclusion contained gelatin only and was used as control (inclusion I).
The other inclusions contained 0.70 mg/mL Au NRs (inclusion II), 0.60 mg/mL Fe$_3$O$_4$ NPs (inclusion III), high concentration (0.70 mg/mL Au and 0.60 mg/mL Fe$_3$O$_4$) of dual-contrast liposomal nanoparticles (inclusion IV), and low concentration (0.47 mg/mL Au and 0.40 mg/mL Fe$_3$O$_4$) of dual-contrast liposomal nanoparticles (inclusion V). Finally, a composite inclusion (inclusion VI) was made consisting of adjacent regions with high and low concentrations of liposomal nanoparticles. The concentrations of liposomal nanoparticles in these regions of inclusion VI were the same as that in inclusions IV and V, respectively. In other words, inclusion VI was equivalent to inclusions IV and V placed together and interconnected. In addition, 0.1 wt% and 0.2 wt% of 30 μm silica particles, acting as ultrasound scatters, were added to the background material and inclusions, respectively.

To further demonstrate the MPA imaging, excised porcine *longissimus dorsi* muscle tissue sample injected with macrophages which were labeled with Fe$_3$O$_4$ NPs and Au NRs and suspended in 12% gelatin solution, was used. To prepare the sample, the J774A.1 macrophages were cultured in Dulbecco's Modified Eagle Media (DMEM), and supplemented with 5% FBS at 37°C in 5% CO$_2$. To load cells with the dual-contrast agent (Fe$_3$O$_4$ NPs and Au NRs), macrophages were incubated with a 3 mL suspension of sterilized 0.1 mg/mL Au NRs and 0.3 mg/mL Fe$_3$O$_4$ NPs for 24 hours. The cells and particles were centrifuged at 110 g for 3 min, and unbound particles in the supernatant were discarded. The darkfield images of native cells and the labeled cells are shown in Fig. 3(a) and Fig. 3(b), respectively. The bright color of the labeled cells comes from the internalized nanoparticles. The extinction spectra of native macrophages and macrophages labeled with both Fe$_3$O$_4$ NPs and Au NRs are shown in Fig. 3(c).

### 2.3 Magneto-photo-acoustic (MPA) imaging

A block diagram of the MPA imaging system is shown in Fig. 4. Either tissue-mimicking phantom or tissue sample was placed in a water cuvette attached to a 3-D positioning stage. For PA imaging, a tunable optical parametric oscillator (OPO) pulsed laser system operating at an 800 nm wavelength (peak absorption of Au NRs) and pulse duration of 5 ns was used to irradiate the sample and to generate photoacoustic signals. The laser light was delivered from the top of the sample using a 1.5 mm diameter optical fiber. The photoacoustic signals were detected using a single element ultrasound transducer (25 MHz center frequency, 25.4 mm focal depth) connected to ultrasound pulser/receiver. For ultrasound imaging, the ultrasound pulse was transmitted 30 μs after the laser pulse to ensure that the photoacoustic transients...
were completely captured, and the backscattered ultrasound echo signals were detected using the same ultrasound transducer. Finally, the pulsed magnetic field (8 ms pulse duration) was produced using a magnetic pulser connected to a solenoid with cone-shaped-tip core to focus the field. The magnetic field generated the magnetically-induced motion inside the tissue while the ultrasound pulse-echo signals were acquired at 1 kHz pulse repetition rate to track the magneto-motive displacement. Therefore, at each lateral position of the ultrasound transducer, photoacoustic signal and a series of ultrasound pulse-echo signals before and after the application of magnetic field were captured. A custom-built LabVIEW application controlled the time sequences of pulsed laser system, ultrasound pulser/receiver, pulsed magnetic field generator, data acquisition unit, and motion axes for mechanical scanning [7,8]. The 2-D cross-sectional and spatially co-registered images were obtained by mechanically moving the sample in horizontal (lateral) direction.

During the offline processing, a digital band-pass (5-45 MHz) filter was applied to photoacoustic and ultrasound radiofrequency signals to filter the out-of-band noise and to improve signal-to-noise ratio. The analytic signals were obtained by applying the Hilbert transform on the filtered signals. The absolute values of the photoacoustic and ultrasound analytic signals were spatially resolved to form PA and US images, respectively. The magneto-motive displacement was detected using a block-matching motion tracking algorithm based on 2D cross-correlation [23]. At each pixel, the displacement between reference signal and ultrasound pulse-echo signals collected at 1 kHz pulse repetition rate during and after the magnetic excitation was estimated from the position of the maximum of correlation coefficient calculated using a correlation kernel measuring 750 μm axially and 100 μm laterally. Once the temporal profile of the displacement was calculated, the maximum displacement at each pixel was mapped in the MMUS image. It should be noted that the background displacement of MMUS imaging was defined as the displacement from a stationary reference (e.g., bottom of the water cuvette) and was subtracted from the estimated displacement of the sample. To form the MPA image, the ultrasound gray-scale B-scan image was used as a background map. Then, using co-registered PA and MMUS images, the magnitude of photoacoustic signal at each pixel was multiplied by the normalized magneto-motive ultrasound signal at the same pixel, and the resulting values were color-coded using logarithmic scale and displayed over the ultrasound image.
3. Results and discussion

Experiments using tissue-mimicking phantom were performed to demonstrate that MPA imaging is capable of detecting and identifying the regions labeled with dual-contrast nanoparticles with sufficient contrast and excellent contrast resolution. Figure 5(a) shows the B-scan ultrasound image of the tissue-mimicking phantom where six inclusions were embedded in the phantom are marked. One of the inclusions (inclusion I) did not contain any contrast agent and was used as control, while other inclusions contained different types of contrast agents at different concentrations. The structure of the tissue-mimicking phantom and the locations of the inclusions were depicted in the B-scan ultrasound image. However, the US image cannot differentiate the inclusions due to the insignificant ultrasound contrast from the nanoparticles. In contrast, strong photoacoustic signals were detected only from the inclusions containing photoacoustic contrast agent: Au NRs (inclusion II) or dual-contrast liposomal nanoparticles (inclusions IV, V and VI). To quantitatively investigate the contrast enhancement in the PA image by nanoparticles, the magnitude of the averaged photoacoustic signal from each marked region was calculated and displayed in Fig. 5(b). The height of each column represents the magnitude of the averaged photoacoustic signal from the corresponding region, and the error bar shows the standard deviation of the photoacoustic signal. The PA image of the tissue-mimicking phantom is also shown in Fig. 5(b).

As evident from Fig. 5(b), the inclusions that contain either Au NRs or liposomal nanoparticles have elevated optical absorption compared to the background. In addition, PA imaging is sensitive to the spatial variations of optical absorption; the differences in photoacoustic signals from the inclusions were consistent with the concentrations of the plasmonic nanoparticles. As shown in Fig. 5(b), the control inclusion (inclusion I) and the inclusion containing Fe₃O₄ NPs (inclusion II) did not generate significant photoacoustic signals. This was expected since light absorption at a wavelength 800 nm was insignificant. Inclusions II, IV and the left region of inclusion VI containing high concentration of Au NRs,
Fig. 5. (a) The ultrasound (US) image of the tissue-mimicking phantom with six inclusions. The background of the phantom was prepared by mixing gelatin with optical contrast agent to represent the endogenous chromophores in native tissue. The inclusions were prepared by mixing gelatin with different types of contrast agents at different concentrations. The first inclusion contained gelatin only and was used as a control (inclusion I). Other inclusions contained 0.70 mg/mL Au NRs (inclusion II), 0.60 mg/mL Fe₃O₄ NPs (inclusion III), high concentration (0.70 mg/mL Au and 0.60 mg/mL Fe₃O₄) of dual-contrast liposomal nanoparticles (inclusion IV), and low concentration (0.47 mg/mL Au and 0.40 mg/mL Fe₃O₄) of dual-contrast liposomal nanoparticles (inclusion V). Inclusion VI consists of two regions, which were equivalent to inclusions IV (left region) and V (right region) placed together and interconnected. (b) The magnitude of the averaged photoacoustic (PA) signal from each marked region in Fig. 5(a), and the PA image of the tissue-mimicking phantom. (c) The magneto-motive displacement of each marked region and the magneto-motive ultrasound (MMUS) image of the phantom. (f) Magneto-photo-acoustic (MPA) image of the tissue-mimicking phantom. Each image covers area measuring 7.7 mm axially and 56 mm laterally.

either isolated or encapsulated in liposomal nanoparticles, generated intense photoacoustic signals. Inclusion IV and the right region of inclusion VI contained low concentrations of liposomal nanoparticles, which encapsulated fewer Au NRs, therefore produced weaker photoacoustic signals. Overall, the magnitude of photoacoustic signal is representative of concentration of plasmonic nanoparticles. However, there are also noticeable photoacoustic signals generated from the background material (mimicking endogenous chromophores in the native tissue). These background signals reduce the contrast of PA imaging and ability to identify the regions labeled by nanoparticles.

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The MMUS image in Fig. 5(c), on the other hand, could identify the regions labeled with magnetic nanoparticles with high contrast because the tissues have fairly low magnetic susceptibility compared to magnetic nanoparticles. The graph shown in Fig. 5(c) indicates the mean value and standard deviation of magneto-motive displacement measured within a rectangular window measuring 1.5 mm axially and laterally located at the center of each inclusion. The detected displacement in the background of the tissue-mimicking phantom was around 5 μm. The control inclusion (inclusion I) and the inclusion with Au NRs (inclusion II) exhibit almost no contrast in the MMUS image. Other inclusions containing Fe₃O₄ NPs or liposomal nanoparticles could be easily identified in the MMUS image. The largest magneto-motive displacement (around 35 μm) was measured in the inclusion containing Fe₃O₄ NPs (inclusion III). Although inclusion IV contained the same concentration of Fe₃O₄ NPs encapsulated in liposomes, the detected displacement from inclusion IV was around 30 μm. The slight difference in the displacements between inclusion III and IV is likely due to additional weight of Au NRs in liposomal nanoparticles. Comparing the displacements detected from inclusions IV and V, which contained different concentrations of liposomal nanoparticles, it is clear that as the concentration of the magnetic nanoparticles decreases, the induced displacement decreases accordingly. However, the map of magneto-motive displacement in inclusion VI was approximately uniform although the inclusion contained two regions (left and right) with different concentrations of liposomal nanoparticles. In fact the difference of concentrations of nanoparticles between left and right regions in inclusion VI was exactly the same as the difference between inclusion IV and inclusion V. Therefore, the MMUS imaging has limited ability to distinguish the regions containing spatially varying concentrations of magnetic nanoparticles if the regions are close to each other and mechanically connected. Thus, MMUS imaging exhibits low contrast resolution within the tissue with sub-regions containing different concentration of nanoparticles. However, the MPA image of the tissue-mimicking phantom shown in Fig. 5(d) retains the best properties of each subsequent imaging technique. Specifically, the MPA image identifies the inclusions containing dual-contrast agent, liposomes encapsulating Fe₃O₄ NPs and Au NRs. Indeed, only the inclusions containing dual-contrast liposomal nanoparticles (inclusions IV, V and VI) were color-coded in the MPA image, and the signals from other inclusions and background were highly reduced. Furthermore, the regions with different concentrations of dual-contrast nanoparticles could be clearly distinguished using MPA imaging. Therefore, MPA imaging was capable of accurate representation of the tissue labeled with dual-contrast agents in the phantom.

The ex-vivo experiment with macrophages was designed to test the ability of MPA imaging to detect cells labeled with nanoparticles within the tissue. The US, PA, MMUS and MPA images of a tissue sample injected with nanoparticle-labeled macrophages are shown in Fig. 6. The B-scan ultrasound image shown in Fig. 6(a) visualizes the cross-sectional view of the tissue sample with injected macrophages. However, the macrophages cannot be easily identified because ultrasound backscattering from macrophages is similar to that of the background tissue, and the nanoparticles internalized by the cells do not provide significant contrast in ultrasound images. The PA image shown in Fig. 6(b) could visualize the labeled cells in the tissue based on the optical absorption from the nanoparticles. But noticeable photoacoustic signals were also detected from background tissue, especially in the upper boundary of the tissue, because of the strong light absorption by endogenous chromophores within the tissue. Therefore, the contrast in the PA image is reduced. The MMUS image shown in Fig. 6(c) identifies the presence of nanoparticles inside the tissue and suggests the location of nanoparticle-labeled macrophages with sufficiently high contrast. Indeed, the magneto-motive displacement in the region containing macrophages was around 100 μm, while the displacement from the background tissue was around 8 μm. Finally, the MPA image, obtained from the co-registered PA, MMUS, and US images and displayed in Fig. 6(d), identified the nanoparticle-labeled macrophages with sufficient contrast, excellent contrast resolution and high spatial resolution with the anatomic features of the imaged tissue.
Our results indicate the feasibility of MPA imaging to visualize the presence and location of nanoparticles inside tissue/cells with the spatial resolution of ultrasound imaging and enhanced contrast based on both the optical absorption and the magnetic susceptibility of the dual-contrast agent. Magneto-photo-acoustic images of the tissue-mimicking phantom and tissue sample were obtained by combining the co-registered US, PA and MMUS images. In the MPA image, only the signals from cells or tissue labeled with dual-contrast agent were selected and displayed over the anatomical content of the tissue, while the background or nonspecific signals were significantly suppressed. Therefore, MPA imaging enhances the contrast between the nanoparticle-labeled cells or tissues and the surrounding tissue. In the desired region labeled with dual-contrast nanoparticles, the MPA image provides high contrast resolution utilizing the sensitivity of photoacoustic signals to the variation of optical absorption caused by different concentration of nanoparticles. In addition, MPA imaging retains high spatial resolution determined by the ultrasound imaging system. Finally, the alignment of the data sets from different modalities in MPA imaging is simple and accurate because of the shared detection system. Therefore, the MPA image allows the improved spatial localization of the desired cells or tissue regions labeled with magneto-plasmonic nanoparticles with high sensitivity.

The liposomes containing Au NRs and Fe₃O₄ NPs were used as the dual-contrast agent in our experiments. The nanoconstructs were designed to exhibit both plasmonic resonance in near–infrared (NIR) spectral region and high magnetic susceptibility. The liposomal nanoparticles allowed flexibility in the loading ratio of Au NRs to Fe₃O₄ NPs. In addition to liposomal nanoparticles, various other hybrid nanoparticles could also be used as the contrast agent for MPA imaging [22,24,25]. Furthermore, new particles optimized for MPA imaging can be designed [20].

Magneto-photo-acoustic imaging is a promising tool for various biomedical applications. For instance, MPA imaging can assess mechanical and optical properties of soft tissue. Photoacoustic imaging can map optical absorption property of the tissue [3–8], while the displacements measured in MMUS imaging can indicate the elasticity and viscosity of the soft tissue [12,13,19]. Since there is a significant correlation between diseases and local changes of soft tissue properties detected using MPA imaging, the MPA imaging technique has potential...
to detect the pathologies at early stages [6,8,12,13]. Furthermore, the MPA imaging system can be used to guide and assess photothermal therapy by using the dual-contrast agent [13]. Because of the presence of the magnetic component, the dual-contrast nanoparticles can be actively accumulated into the desired region using an external magnetic field. Based on the optical absorption property, the nanoparticles can lead to localized thermal damage by absorbing the radiant energy from the laser. The efficient targeting of nanoparticles through the magnetic field increases the effectiveness of the treatment and reduces the required dosage of photoabsorbers, thereby reducing the side effects associated with general systematic administration of nanoparticles. In addition, the dynamic MPA imaging of the targeted tissue can indicate the presence of the photo absorbers and assess the therapeutic outcome. The temperature maps measured with PA imaging [26] and the tissue elasticity measured with MMUS imaging [12,13,19] are important parameters expected to change significantly during the photothermal therapy.

Finally, molecular MPA imaging could be realized by functionalizing the surfaces of the dual-contrast nanoconstructs, allowing for improved spatial localization of the targeted cells in the context of the anatomic map of the tissue. Since the molecular localization of nanoparticles could be indicative of specific physiology, MPA imaging might provide a promising platform to noninvasively diagnose and characterize pathologies.

4. Conclusion

In conclusion, MPA imaging using the dual-contrast agent characterized by elevated optical absorption and magnetic susceptibility was developed. The liposomes containing Au NRs and Fe₃O₄ NPs were designed and used as the dual-contrast agent for MPA imaging. We have investigated multi-modal MPA imaging to image a tissue-mimicking phantom and tissue sample injected with cells labeled by a dual-contrast agent, and obtained the MPA images by co-registering B-mode US, PA and MMUS images from the same cross-section of the samples. The MPA image could clearly define the localization of the nanoparticle-labeled cells or tissue with enhanced contrast, excellent contrast resolution and high spatial resolution in the context of anatomical landmarks of the surrounding tissues. The precise morphological information from MPA imaging provides an important basis for diagnosis, characterization and treatment of pathologies.

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