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Pulsed Laser Diode Based Optoacoustic Imaging of Biological Tissues

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Abstract

Optoacoustic tomography (OAT) is a promising hybrid imaging modality for many biomedical applications. However, the use of Nd:YAG laser as excitation source makes the OAT system expensive, and non-portable. Miniaturization of OAT system is an immediate task to make it a potential tool for both preclinical and clinical studies. In this work, we present an optoacoustic system that uses a miniaturized pulsed laser diode which can provide near infrared pulses at ~ 803 nm. With laser fluence ~ 0.3 mJ/cm² on the tissue surface, we achieved 3 cm-deep imaging in chicken tissue. The high pulse repetition rate of the diode laser allowed rapid acquisition of optoacoustic cross-sectional images with good image quality. The results promise that the proposed system has potential to be used as an alternative to Nd:YAG based OAT systems for biological imaging applications.

Keywords: Optoacoustic tomography, Pulsed laser diode, Deep tissue imaging, Biological imaging.

1. Introduction

Optoacoustic tomography (OAT) is an emerging hybrid imaging modality in which a nanosecond (ns) laser pulse irradiates the tissue and an ultrasonic transducer acquires the optoacoustic (OA) signals generated due to the absorption of light by the tissue chromophores [1-7]. The OA signal is generated due to the absorption of light by the chromophores present in the tissue (such as blood, melanin, water etc.) and subsequent ultrasound emission due to the thermoelastic expansion of the tissue. Optoacoustic imaging (OAI) has several unique advantages over pure optical and pure acoustic methods: (a) it has intrinsic optical absorption contrast, hence no labelling is required, (b) it has highly scalable spatial resolution (0.1-800 μ m) [8-11], (c) its penetration depth (0.1-80 mm) [8] is larger than other popular optical methods such as confocal microscopy, multi-photon microscopy, optical coherence tomography, etc., and (d) it can provide structural as well as functional information using multiple-wavelength. OAI has been successfully applied for imaging vasculature structures and its characterization [8], tumour angiogenesis [12, 13], molecular imaging [14], identifying venous valves [15], cancer screening [16, 17], breast imaging [3, 18], sentinel lymph node imaging [19-22], temperature monitoring [23], tissue engineering [24, 25] etc.

Usually, Nd:YAG / OPO laser, Ti: Sapphire laser, dye laser, etc., have been widely used as excitation sources in OAT systems [4, 26-28]. They could deliver ns-pulses with high energy (tens of milli Joules per pulse) for deep imaging. However, these lasers make the overall OAT system bulky,

expensive, and low image acquisition speed due to low-repetition rate (~ 10 Hz). So, there is a need to develop an OAT system that is compact, affordable, having quality imaging capabilities in biological tissues. Near Infrared pulse laser diode (PLD) could make the OAI system compact and relatively cheaper [29-32]. An OAT system with fiber based illumination and cylindrical scanning geometry was demonstrated on phantoms using PLD [33, 34]. OA imaging of superficial human blood vessels at a depth of ~ 1 mm below the skin was achieved [30]. Optoacoustic microscopy with PLD was reported and carbon fibers were imaged [35]. Recently, OA images were obtained using 805 nm laser diode with 0.5 mJ pulse energy. The imaging depth **up to 15 mm at 0.5 Hz frame rate was achieved using US transducer array** while operating at the maximum permissible exposure (MPE) of 1.3 mJ/cm^2 [32]. **However, the array based systems are very expensive and requires multi-channel parallel amplifications and data acquisition system. Thus to develop low-cost optoacoustic imaging system we need to use single-element ultrasound transducer, instead of array based transducer.** PLD source has not been used for deep tissue, real-time, **low-cost** photoacoustic imaging in biological tissues.

In this work, we present an Optoacoustic system that uses a cost-effective and compact pulsed laser diode (PLD) which can provide ~ 803 nm pulses with energy ~ 1.4 mJ per pulse at 7 kHz repetition rate. To study the performance of the state-of-art PLD source for OA imaging, first we acquired OA signals from animal blood (and black ink) samples embedded inside a chicken breast tissue. Then we performed OA cross-sectional imaging on blood and ICG embedded inside a chicken breast tissue.

2. Pulsed laser diode based optoacoustic imaging system

Figure 1. (a) Schematic of the pulsed laser diode based optoacoustic (OA) system. Photograph of (b) the OA system, (c) the front-view of the laser diode, (d) OAT system, (e) chicken breast tissue. UST - Ultrasonic transducer, DAQ - Data acquisition card, PR - Pulser/Receiver, PLD - Pulsed laser diode, M-Motor.

The schematic of the PLD optoacoustic system, which was used for signal study, is depicted in Figure 1(a). The PLD (Quantel, France) provides ~ 803 nm pulses with duration 136 ns and energy ~ 1.4 mJ at 7 kHz repetition rate. In Figure 1(a) LDU is laser driver unit which includes a 12 V, 4 A power supply (Votcraft, PPS-11810), a variable power supply (BASETech, BT-153) to control the laser power/pulse energy, and a temperature controller-TC (LaridTech, MTTC1410) to maintain the

temperature of the laser diodes. The pulse energy and repetition rate can be controlled independently. The dimensions of the laser head are 11.0 X 6.0 X 3.6 cm (weight ~250 grams), which is much smaller than the typical Nd:YAG laser head 78 X 18 X 19 cm (typical weight ~100 kgs). Light emitted by a stack of highly efficient diodes is in rectangular shape which diverges with an angle of $\sim 11.5^\circ$, and $\sim 0.65^\circ$ degree along the slow and fast axes, respectively. A function generator (FG250D, Funktionsgenerator) was used to provide TTL (Transistor-Transistor Logic) signals to synchronize the data acquisition (DAQ) and the laser pulses. The PA signals generated by the test sample (tissue) were received by an ultrasonic transducer (UST). In these experiments two different nonfocused USTs with central frequencies 2.25 MHz (Olympus-NDT, V306-SU) and 5 MHz (Olympus-NDT, V309-SU) having 12 mm diameter active area and $\sim 70\%$ nominal bandwidth were used. Since the pulse width of the excitation source is rather large (~ 136 ns), we can expect the maximum bandwidth of the generated PA signals will be in the order of 7.4 MHz. Hence, the USTs were chosen accordingly. The PA signals were subsequently amplified, and band pass filtered by a pulse/receiver (Olympus-NDT, 5072PR), finally digitized with a DAQ card (GaGe, compuscope 4227) and stored in a desktop computer. Figure 1(b) and 1(c) show the photographs of the system and the front-view of the laser-diode.

The OA signal study promoted us to do OA imaging. Figure 1(d) shows the schematic of the PLD-OAT system. The UST is driven by a stepper motor (M) (Lin Engineering, Silverpak 23C) to scan circularly around the sample. For imaging, we used continuous scanning instead of stop-and-go scan to increase the data acquisition speed. In continuous scan, stepper motor rotates the UST continuously at predefined speed, collects multiple OA signals as the transducer is moving; save the A-lines once the 360 degree rotation is completed. **In our experiments the maximum object size is ~ 10 mm (diameter), it takes ~ 6.7 μ s (speed of sound = 1.5 mm/ μ s) to record a single A-line. During this time, the maximal displacement of points within the object with respect to the UST surface can reach ~ 0.04 μ m (for a 10 second scan time).** The signals were averaged before the image reconstruction to reduce the computation load. For example, total number of A-lines were reduced to 500 by signal averaging 140 signals (in case of 10 s scanning, total A-lines = 10 x 7000 = 70000; after averaging A-lines = 70000/140 = 500). These A-lines data were used to reconstruct the cross-sectional OA image of the sample using a simple delay-and-sum back projection reconstruction algorithm. The transducer rotation, data collection, and the image reconstruction were done using MATLAB programs.

3. Experiments and results

3.1 Experiments on blood/ink sample

Figure 2. Optoacoustic signals received by (a) 2.25 MHz, and (b) 5 MHz USTs. The signal is averaged 700 times (0.1 sec). Inset (A, B) and (C, D) are PA signals received with no averaging (single pulse excitation) by the 2.25, and 5 MHz USTs, respectively.

A low density polyethylene (LDPE) tube (inner diameter: 0.59 mm) filled with black ink and UST were mounted inside water as shown in Figure 1(b). The tube was placed at ~ 4 cm distance from the laser window. The OA signal received by the UST was band pass filtered (1-10 MHz) and amplified with 50 dB gain. Finally, the signal was digitized by a DAQ card at 50 Ms/s and stored in computer. A total of 7000 A-lines (1 sec) were collected. Similarly, OA signal from blood was also acquired. To confirm that the LDPE tube has no contribution to the OA signals, we repeated the experiment with unfilled LDPE tube as well. Figure 2 shows the OA signals averaged 700 times (0.1 sec). The OA signals obtained with single pulse excitation (no averaging) are also shown as profiles A, B, C, and D in Figure 2 (inset). The OA signal generated by black ink is as strong as that generated by blood which indicates that they have similar optical absorption coefficients at ~ 803 nm. At 800 nm, the absorption coefficient for whole blood is $\sim 5 \text{ cm}^{-1}$.

3.2 Experiments on blood/ink embedded inside chicken breast tissue

The LDPE tube filled with black ink or blood was embedded in the chicken breast tissue (CBT). The tube was still kept the same distance 4 cm from the laser window. The photograph in Figure 1(e) shows the cut pieces of CBT having 2, 4, 6 cm thickness. The LDPE tube was embedded in the middle of the tissue sample. OA signals were collected when the tube was placed at 1, 2, or 3 cm deep from the laser illuminated tissue surface. The generated PA signal also needs to travel 1, 2, or 3 cm inside the attenuating chicken breast tissue before it is received by the transducer. The incident laser energy density on the tissue surface area was $\sim 0.3 \text{ mJ/cm}^2$, which is much less than the “maximum permissible exposure (MPE)” of 32 mJ/cm^2 at 803 nm [36-38]. Figures 3(a) and (b) show the OA signals collected at these depths by the 2.25 and 5 MHz UST, respectively. Similarly, the above experiment was repeated with LDPE tube filled with blood embedded inside the tissue. Figures 3(c) and (d) show the PA signals from blood collected by the 2.25 and 5 MHz UST, respectively. In Figure 3, each signal was averaged 700 times. In our current experiments, both black ink and blood were successfully detected in chicken breast tissue at depth of ~ 3.0 cm.

Figure 3. Optoacoustic signals collected at different depths of the chicken breast tissue. The OA signals generated from black ink (a,b) and blood (c,d) in a LDPE tube, embedded inside the tissue at different depths. Signal is averaged 700 times (0.1 sec). Depth (D) values are indicated in the plots.

3.3 Signal-to-noise ratio (SNR) measurements

The SNR was calculated using the relation, $SNR = V/n$, where V is the peak-to-peak PA signal voltage and n is the standard deviation of the background noise. The SNR in terms of dB can be expressed as $SNR(dB) = 20 \times \log_{10} \left(\frac{V}{n} \right)$. The SNR as a function of square root of number of signal averaging ($\sqrt{N}$) for 5 MHz UST is shown in Figure 4(a). The graph clearly shows the improvement in SNR with increase of N . Figure 4(b) shows the SNR versus penetration depth (D) inside the chicken breast tissue. Due to the overwhelming light scattering in the chicken tissue, the intensity of light, and hence the SNR decreased with increasing depth. The maximum penetration depth measured with black ink or blood in chicken breast tissue was ~ 3.0 cm. The SNR at this depth reached ~ 15 for black ink and ~ 10 for blood, after averaging 700 times.

Figure 4. (a) Signal-to-noise ratio (SNR) of OA signals generated from black ink (A) and blood (B) in a 2 cm-thick chicken breast tissue as a function of square root of number of averages ($\sqrt{N}$) for the 5 MHz transducer. (b) SNR versus penetration depth (D) in the chicken breast tissue for 5 MHz UST.

3.4 PLD-OAT imaging

The good signal strength and SNR of the OA signals generated using PLD promoted us to do imaging. For OAT imaging we have used continuous scan [39] in which the stepper motor rotates the UST continuously at predefined speed, collects signals while moving, saves the A-lines once the rotation is complete. Imaging experiments were performed on two different samples. Sample-1 is horse hair phantom prepared in a triangular shape as shown in Figure 5(a). Here the horse-hair was glued on plastic tubes. The hair has side-length of ~ 8 mm and diameter of ~ 150 μm . The images of hair phantom obtained by collecting OA signals in 20, and 10 sec scan time using 2.25 MHz UST (Figures 5b, 5c) and 5 MHz UST (Figures 5d, 5e). The line profiles along A, B [indicated on Figures 5(b) and 5(d)] are compared in Figure 5(f). The full width at half-maximum (FWHM) values are ~ 435 μm and ~ 285 μm for 2.25 MHz and 5 MHz UST, respectively. To study the effect of scan speed on the quality of image, we calculated the SNR of images acquired at different scan speeds. The SNR values are 175 and 130 (with 2.25 MHz), 160 and 115 (with 5 MHz) for images in Figures 5b, 5c and 5d, 5e, respectively. The images from 2.25 MHz UST have better SNR because there is more OA energy in low-frequency range, hence it can receive stronger signal than other transducer. But the images from 5 MHz UST are sharper (higher spatial resolution). Reducing the scan time reduces the number of recorded signals (A-lines) and hence, the SNR of the reconstructed images reduces with scan time for both the transducers. For 2.25 MHz UST, reducing the scan time from 20 to 10 s, reduce the SNR from 175 to 130. However, the PLD-OAT system promises to provide good image quality even at high scan speed of 10 s. The Nd:YAG laser based PAT systems will take several minutes for A-line collection, due to low-repetition rate, to generate a good quality image.

Figure 5. Reconstructed images of horse-hair phantom: (a) Photograph of the triangular shaped horse-hair phantom glued on transparent plastic tubes, OAT images acquired using 2.25 MHz transducer (b,c), 5 MHz transducer (d,e). All images have the same scale bar as shown in b. Scan time for the image acquisition was (b), (d) 20 seconds, (c), (e) 10 seconds. (f) Line profiles along A, and B indicated on images in Figures 2(b) and 2(d), respectively.

Deep-tissue imaging experiments were carried out on sample-2, which is made of two LDPE tubes (~12 mm long and ~0.59 mm inner diameter), one filled with mice blood and other filled with indocyanine green (ICG). The two LDPE tubes were placed on a chicken breast tissue as shown in Figure 6(a). For imaging they were covered by tissues of various thicknesses as shown in Figure 6(b). The ICG solution was prepared with 323 μM concentration to have an absorption peak ~800 nm. The tissue cross-section containing the LDPE tubes was imaged when tissue slices were sequentially placed to make the tubes 1 cm, and 2 cm deep from laser-illuminated tissue surface. OAT images were acquired on sample-2 using 2.25 MHz at 20 sec, 10 sec scan time. Figures 6(c,d) and 6(e,f) show the OAT images acquired at 1 cm, and 2 cm depth, respectively. **The SNR values of blood, ICG measured at 1 cm are ~25, ~32 and that measured at 2 cm are ~ 8, ~14, respectively. Both the tubes were clearly visible even at 2 cm under the chicken breast tissues.** Both the tubes were clearly visible even at 2 cm under the chicken breast tissues.

Figure 6. Deep-tissue imaging of blood and ICG tubes hidden inside chicken breast tissue: Photograph of the (a) LDPE tubes filled with blood and ICG on chicken tissue, (b) stack of chicken tissue layers inside which the blood and the ICG tubes were hidden. OAT images acquired using 2.25 MHz transducer at 1 cm (c,d), and 2 cm (e,f) depth from the chicken breast tissue surface.

4. Discussions and conclusions

NIR wavelength window is known for its higher optical penetration depth in biological tissue due to lower blood and water absorption [40]. This laser diode emitting at wavelength 803 nm is very suitable for deep tissue imaging. As seen from Figure 2, **the single shot OA signals have relatively strong amplitude ~100-200 mV and SNR ~ 3-7**. Moreover, 7 kHz repetition rate of the laser will enable faster data acquisition compared to the standard high energy pulsed lasers used in traditional OAT imaging systems (typical repetition rate is 10-20 Hz) [3, 4, 18, 21]. Even with 700 signal averaging (with improved *SNR*) we will be able to achieve the same data acquisition speed as that of the traditional lasers. But since, the OA signal received is quite strong signal averaging may not be even needed. Of course with deeper tissue imaging the signal strength as well as *SNR* will eventually decrease (Figures 3 and 4). In such cases having higher repetition rate is particularly useful as more signals averaging can be done to improve the *SNR*. Moreover, the signal strength and hence the imaging depth can be further enhanced using contrast agents. There are a plethora of contrast agent reported for OAI in the NIR wavelength range, such as, ICG, gold nanocages, gold nanorods, gold nanobeacons, carbon nanotubes, etc. [41-47]. In our current experiments, a maximum penetration depth of ~3.0 cm was achieved in the chicken breast tissue. The depth can be improved to some extent by simply focusing the diverging laser beam on the tissue surface.

In summary, we developed an ultra-portable (laser head weighs ~250 grams compared to ~50-100 kg traditional pulsed OPO lasers used in OAT) and less-expensive (nearly five times less expensive than traditional lasers used in OAT) pulsed laser diode based optoacoustic system for biomedical imaging applications. It was successfully demonstrated on ex vivo tissue sample with embedded blood vessel like tube filled with animal blood/black ink. At a depth up to ~3 cm the OA signals were clearly visible. At this depth the *SNR* reached ~15 for black ink and ~10 for blood with 700 signal averaging. 2D OAT images were acquired in 10 s to form a good quality image. It was demonstrated that blood and ICG embedded at 2 cm depth in chicken breast tissue can be clearly imaged with good contrast. The signal strength, image contrast, deep imaging capabilities the presented PLD-OAT system promises that it can potentially image deep vasculature in the breast, brain, and other organs as well. We are currently working to use PLD-OAT system for small animal brain imaging to demonstrate its potential for preclinical applications.

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References

- [1] Ermilov SA, Khamapirad T, Conjunteau A, Leonard MH, Lacewell R, Mehta K, et al. Laser optoacoustic imaging system for detection of breast cancer. *J Biomed Opt.* 2009;14(2):024007.
- [2] Wang LV, Hu S. Photoacoustic Tomography: In Vivo Imaging from Organelles to Organs. *Science.* 2012;335(6075):1458-62.
- [3] Xia W, Piras D, Singh MKA, van Hespén JCG, van Leeuwen TG, Steenbergen W, et al. Design and evaluation of a laboratory prototype system for 3D photoacoustic full breast tomography. *Biomed Opt Express.* 2013;4(11):2555-69.
- [4] Cai X, Kim C, Pramanik M, Wang LV. Photoacoustic tomography of foreign bodies in soft biological tissue. *J Biomed Opt.* 2011;16(4):046017.
- [5] Su YX, Zhang F, Xu KX, Yao JQ, Wang RKK. A photoacoustic tomography system for imaging of biological tissues. *J Phys D Appl Phys.* 2005;38(15):2640-4.

- [6] Wang L, Xia J, Yao J, Maslov KI, Wang LV. Ultrasonically Encoded Photoacoustic Flowgraphy in Biological Tissue. *Physical Review Letters*. 2013;111(20).
- [7] Xia J, Chatni MR, Maslov K, Guo Z, Wang K, Anastasio M, et al. Whole-body ring-shaped confocal photoacoustic computed tomography of small animals in vivo. *J Biomed Opt*. 2012;17(5):050506.
- [8] Hu S, Wang LV. Photoacoustic imaging and characterization of the microvasculature. *J Biomed Opt*. 2010;15(1):011101.
- [9] Upputuri PK, Wen Z-B, Wu Z, Pramanik M. Super-resolution photoacoustic microscopy using photonic nanojets: a simulation study. *J Biomed Opt*. 2014;19(11):116003.
- [10] Danielli A, Maslov K, Garcia-Uribe A, Winkler AM, Li C, Wang L, et al. Label-free photoacoustic nanoscopy. *J Biomed Opt*. 2014;19(8):086006.
- [11] Wang L, Zhang C, Wang LV. Grueneisen Relaxation Photoacoustic Microscopy. *Phys Rev Lett*. 2014;113(17):174301.
- [12] Wang X, Pang Y, Ku G, Xie X, Stoica G, Wang LV. Non-invasive laser-induced photoacoustic tomography for structural and functional imaging.pdf. *Nature Biotechnology*. 2003;21:803-6.
- [13] Kolkman RGM, Thumma KK, ten Brinke GA, Siphanto RI, van Neck H, Steenbergen W, et al. Photoacoustic imaging of tumor angiogenesis: Spie; 2008.
- [14] Pan D, Pramanik M, Senpan A, Allen JS, Zhang H, Wickline SA, et al. Molecular photoacoustic imaging of angiogenesis with integrin-targeted gold nanobeacons. *FASEB J*. 2011;25(3):875-82.
- [15] Bosschaart N, Kolkman R, van Leeuwen T, Steenbergen W, editors. Imaging of venous valves with photoacoustics. *Proceedings of SPIE*; 2006.
- [16] Xie Z, Roberts W, Carson P, Liu X, Tao C, Wang X. Evaluation of bladder microvasculature with high-resolution photoacoustic imaging. *Optics Letters*. 2011;36:4815-7.
- [17] Wang T, Yang Y, Alqasemi U, Kumavor PD, Wang X, Sanders M, et al. Characterization of ovarian tissue based on quantitative analysis of photoacoustic microscopy images. *Biomed Opt Express*. 2013;4(12):2763-8.
- [18] Pramanik M, Ku G, Li C, Wang LV. Design and evaluation of a novel breast cancer detection system combining both thermoacoustic (TA) and photoacoustic (PA) tomography. *Med Phys*. 2008;35(6):2218.
- [19] Pan D, Pramanik M, Senpan A, Ghosh S, Wickline SA, Wang LV, et al. Near infrared photoacoustic detection of sentinel lymph nodes with gold nanobeacons. *Biomaterials*. 2010;31(14):4088-93.
- [20] Pramanik M, Song KH, Swierczewska M, Green D, Sitharaman B, Wang LV. In vivo carbon nanotube-enhanced non-invasive photoacoustic mapping of the sentinel lymph node. *Phys Med Biol*. 2009;54(11):3291-301.
- [21] Erpelding TN, Kim C, Pramanik M, Jankovic L, Maslov K, Guo Z, et al. Sentinel Lymph Nodes in the Rat : Noninvasive Photoacoustic and US imaging with a clinical US system. *Radiology*. 2010;256(1):102-10.
- [22] Grootendorst DJ, Jose J, Wouters MW, van Boven H, Van der Hage J, Van Leeuwen TG, et al. First experiences of photoacoustic imaging for detection of melanoma metastases in resected human lymph nodes. *Lasers Surg Med*. 2012;44(7):541-9.
- [23] Pramanik M, Wang LV. Thermoacoustic and photoacoustic sensing of temperature. *J Biomed Opt*. 2009;14(5):054024.
- [24] Cai X, Zhang YS, Xia Y, Wang LV. Photoacoustic Microscopy in Tissue Engineering. *Materials today*. 2013;16(3):67-77.
- [25] Talukdar Y, Avti P, Sun J, Sitharaman B. Multimodal ultrasound-photoacoustic imaging of tissue engineering scaffolds and blood oxygen saturation in and around the scaffolds. *Tissue engineering Part C, Methods*. 2014;20(5):440-9.
- [26] Beard P. Biomedical photoacoustic imaging. *Interface Focus*. 2011;1:602-31.
- [27] Xu M, Wang LV. Photoacoustic imaging in biomedicine. *Rev Sci Instrum*. 2006;77(4):041101.
- [28] Ke H, Erpelding TN, Jankovic L, Liu C, Wang LV. Performance characterization of an integrated ultrasound, photoacoustic, and thermoacoustic imaging system. *J Biomed Opt*. 2012;17(5):056010.
- [29] Robb RA. 3-D visualization in biomedical applications. *Annual Review of Biomedical Engineering*. 1999;1(1):377-99.

- [30] Kolkman RGM, Steenbergen W, van Leeuwen TG. In vivo photoacoustic imaging of blood vessels with a pulsed laser diode. *Lasers Med Sci.* 2006;21(3):134-9.
- [31] Wang T, Nandy S, Salehi HS, Kumavor PD, Zhu Q. A low-cost photoacoustic microscopy system with a laser diode excitation. *Biomed Opt Express.* 2014;5(9):3053-8.
- [32] Daoudi K, van den Berg PJ, Rabot O, Kohl A, Tisserand S, Brands P, et al. Handheld probe integrating laser diode and ultrasound transducer array for ultrasound/photoacoustic dual modality imaging. *Opt Express.* 2014;22(21):26365.
- [33] Allen JS, Beard P. Pulsed near-infrared laser diode excitation system for biomedical photoacoustic imaging. *Opt Lett.* 2006;31(23):3462-4.
- [34] Allen TJ, Cox BT, Beard PC, editors. Generating photoacoustic signals using high-peak power pulsed laser diodes. *Proceedings of SPIE*; 2005.
- [35] Zeng L, Liu G, Yang D, Ji X. 3D-visual laser-diode-based photoacoustic imaging. *Opt Express.* 2012;20(2):1237-46.
- [36] Zhang HF, Maslov K, Wang LHV. In vivo imaging of subcutaneous structures using functional photoacoustic microscopy. *Nat Protoc.* 2007;2(4):797-804.
- [37] Wang LV. Tutorial on Photoacoustic Microscopy and Computed Tomography. *IEEE Journal of selected topics in Quantum electronics* 2008;14 171-9.
- [38] American National Standard for Safe Use of Lasers. ANSI Standard Z1361-2000, NY. 2000.
- [39] Ma R, Taruttis A, Ntziachristos V, Razansky D. Multispectral optoacoustic tomography (MSOT) scanner for whole-body small animal imaging. *Opt Express.* 2009;17(24):21414-26.
- [40] Wang LV, Wu H-I. *Biomedical Optics: Principles and Imaging.* New Jersey: John Wiley & Sons, Inc.; 2009.
- [41] Ku G, Wang LV. Deeply penetrating photoacoustic tomography in biological tissues enhanced with an optical contrast agent. *Opt Lett.* 2005;30(5):507-9.
- [42] Kim C, Cho EC, Chen J, Song KH, Au L, Favazza C, et al. In vivo molecular photoacoustic tomography of melanomas targeted by bioconjugated gold nanocages. *Acs Nano.* 2010;4(8):4559-64.
- [43] Pan D, Pramanik M, Senpan A, Yang X, Song KH, Scott MJ, et al. Molecular photoacoustic tomography with colloidal nanobeacons. *Angew Chem Int Ed Engl.* 2009;48(23):4170-3.
- [44] Pan D, Pramanik M, Wickline SA, Wang LV, Lanza GM. Recent advances in colloidal gold nanobeacons for molecular photoacoustic imaging. *Contrast Media Mol Imaging.* 2011;6(5):378-88.
- [45] Pan D, Pramanik M, Senpan A, Wickline SA, Wang LV, Lanza GM. A facile synthesis of novel self-assembled gold nanorods designed for near-infrared imaging. *Journal of Nanoscience and Nanotechnology.* 2010;10(12):8118-23.
- [46] Pramanik M, Swierczewska M, Green D, Sitharaman B, Wang LV. Single-walled carbon nanotubes as a multimodal-thermoacoustic and photoacoustic-contrast agent. *J Biomed Opt.* 2009;14(3):034018.
- [47] Kim C, Qin R, Xu JS, Wang LV, Xu R. Multifunctional microbubbles and nanobubbles for photoacoustic and ultrasound imaging. *J Biomed Opt.* 2010;15(1):010510.