HHS Public Access

Author manuscript

IEEE Trans Ultrason Ferroelectr Freq Control. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

IEEE Trans Ultrason Ferroelectr Freq Control. 2017 August ; 64(8): 1205–1211. doi:10.1109/TUFFC.2017.2713599.

A Light Illumination Enhancement Device for Photoacoustic Imaging: *In Vivo* Animal Study

Jaesok Yu [Student Member, IEEE],

Center for Ultrasound Molecular Imaging and Therapeutics, Department of Medicine, Heart and Vascular Institute, University of Pittsburgh School of Medicine, with the University of Pittsburgh Medical Center, Pittsburgh, PA 15261 USA, and also with the Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261 USA

Joel S. Schuman,

Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261 USA, with the UPMC Eye Center, Department of Ophthalmology, Eye and Ear Institute, Pittsburgh, PA 15213 USA, and also with the Ophthalmology and Visual Science Research Center, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213 USA. He is now with the Department of Ophthalmology, NYU Langone Medical Center, New York, NY 10016 USA

Jung-Kun Lee,

Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA 15261 USA

Sang-Goo Lee,

IBULE PHOTONICS Inc., Incheon 21999, South Korea

Jin Ho Chang [Senior Member, IEEE], and

Department of Electronic Engineering, Sogang University, Seoul 04107, South Korea, and also with the Medical Solutions Institute, Sogang Institutes of Advanced Technology, Sogang University, Seoul 04107, South Korea

Kang Kim [Senior Member, IEEE]

Center for Ultrasound Molecular Imaging and Therapeutics, Department of Medicine, Heart and Vascular Institute, University of Pittsburgh School of Medicine, with the University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA 15261 USA, with the Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261 USA, and also with the McGowan Institute of Regenerative Medicine, University of Pittsburgh and UPMC, Pittsburgh, PA 15261 USA

Abstract

Photoacoustic (PA) imaging detects acoustic signals generated by thermal expansion of a light-excited tissue or contrast agents. PA signal amplitude and image quality directly depend on the light fluence at the target depth. With conventional PA imaging systems, approximately 30% energy of incident light at the near-infrared region would be lost due to reflection on the skin surface. Such light loss directly leads to a reduction of PA signal and image quality. A new light delivery scheme that collects and redistributes reflected light energy was recently suggested, which

is called the light catcher. In our previous study, proof of concept using a finite-element simulation model was shown and a laboratory-built prototype of the light catcher was applied on tissue-mimicking phantoms. In this paper, we present an elaborate prototype of a high-frequency PA probe with the light catcher fabricated using 3-D printing technology, which is conformal to a subcutaneous tumor in mice. The *in vivo* usefulness of the developed prototype was evaluated in a mouse tumor model. Equipped with the light catcher, PA signal amplitude from the clinical photosensitizer injected into the mouse tumor was enhanced by 33.7%, which is approximately equivalent to the percent light loss due to reflection on the skin.

Index Terms

Laser illumination scheme; light catcher; optical scattering; photoacoustic (PA) imaging; tumor imaging

I. Introduction

Photoacoustic (PA) technology is a promising state-of-the-art medical imaging modality to quantitatively provide a real-time optical contrast at depth by using energy conversion from absorbed light energy to acoustic wave in a tissue [1]. In PA imaging, the initial local PA pressure and PA image quality are directly affected by laser fluence. In the past few years, efforts have been made to further understand light propagation in the tissue and use it to improve light penetration in tissues, but with limited success. Kwon *et al.* [2] suggested a simulation model analyzing light propagation in the multiple layers of skin using mathematical models. They concluded that adjusting incident beam power and diameter, physically compressing the tissue, or using chemical agents can affect the light penetration depth. With such understanding, Yeo *et al.* [3] developed a compression-controlled low-level laser probe to increase photon density in soft tissues with the negative mechanical compression control technique. They successfully demonstrated a concentrated beam profile with enhanced laser photon density using their probe. Zhao *et al.* [4] suggested a new light illumination scheme for deep tissue PA imaging and the proof of concept was shown through a finite-element simulation. The proposed scheme was based on a fact that light energy is considerably lost by reflection at the skin surface depending on the incident angle. For example, experimental observation revealed that approximately 22%–29% of light at 800 nm reflects off human skin with an incident angle of 20° [5]. Moreover, it is known that the reflectance of 800-nm light increased by 13% (from 19% to 32%) as skin thickness increased from 0.43 to 1.60 mm [6]. Therefore, only approximately 70% of the near-infrared (NIR) light is expected to penetrate into the tissues to excite targets at depth. For the safety of human use, the maximum amount of allowed NIR light fluence exposure in PA imaging should comply with the laser exposure safety guideline of American National Safety Institute (ANSI, Z136.1—Safe Use of Lasers). To overcome this shortcoming, a device called the light catcher was developed to restore energy loss at the skin surface by collecting and redistributing reflected laser energy onto soft tissue and therefore enhancing the effective laser fluence. The proposed light catcher is a relatively simple concave-shaped light reflector integrated with an optical fiber bundle that is attached to an ultrasound probe. To collect and redistribute the light bouncing off the skin surface, the inner surface of the cavity

was coated with silver, which highly reflects (>90%) light in the NIR region [7]. In addition to an increase in light fluence at the skin surface, a more uniform distribution of the laser energy at depth caused by random light reflection between the silver coating surface and the skin surface was shown in the experiments using a tissue-mimicking phantom [4]. A conceptually similar technology was applied to a PA tomography system for brain imaging by Nie *et al.* [8]. This device, called the photon recycler, which was designed to recycle a scattered photon from the skin surface showed enhanced light transmittance *ex vivo* through a human skull. In [9], we manufactured an elaborate prototype light catcher for freehand scanning using 3-D printing technology and integrated it into a custom-made high-frequency ultrasound imaging probe. The developed system was tested *in vitro*. In this paper, we further investigated and quantitatively evaluated *in vivo* the effectiveness of the developed hand-held light catcher that was designed for small animal tumor study.

II. Materials and Methods

A. Photoacoustic Imaging

The initial local PA pressure rise p_0 by thermal expansion after a short-pulse laser excitation can be expressed by (1) with assumptions that the absorbed optical energy is completely converted into heat energy and nonthermal relaxation such as fluorescence is small enough to be negligible [10]

$$p_0 = \Gamma \mu_a F \quad (1)$$

where Γ is the Grüneisen parameter associated with the thermodynamic property of the tissue, μ_a is the absorption coefficient, and F denotes laser energy fluence [8]. The Grüneisen parameter is given by $\Gamma = \alpha / (\kappa \rho C_p)$, where α is the isobaric volume thermal expansion coefficient, κ is the isothermal compressibility, ρ is the density of the medium, and C_p is the specific heat capacity. Therefore, the initial local PA pressure is directly proportional to laser fluence. Given the input power of the laser, the light catcher is designed to maximize the laser delivery at depth for a higher PA signal amplitude and therefore increase imaging depth.

B. Light Catcher: A Prototype Manufacturing

A concave-shaped light catcher (hollow hemisphere, height: 12.7 mm, diameter: 25.4 mm) designed for a typical mouse subcutaneous tumor model was drawn by a 3-D computer-aided design (CAD) software (Solidworks 2012, Dassault Systèmes SOLIDWORKS Corp, MA, USA). Fig. 1 illustrates 3-D CAD drawing of the light catcher and the prototype of the light catcher in different views. All parts shown in Fig. 1(c)–(e) were produced by a stereolithography 3-D-layering printer (Viper SLA system, 3-D systems, Rock Hill, SC, USA) using water-resistant resin materials of high dimensional stability (Somos WaterShed XC11122, DSM, Heerlen, Netherlands). Silver was chosen as a coating material, which is highly reflective (>95%) in the NIR range. The silver coating process was performed at room temperature due to relatively low glass transition temperature (T_g , 39 °C–46 °C) of the

3-D printing material. A pair of custom-made optical fiber bundles (NA: 0.55, core diameter: 50 μm , fiber bundle width: 1.3 cm, Fiberoptic Systems Inc., Simi Valley, CA, USA) was integrated through two slits made in the concave reflecting mirror as shown in Fig. 1(c). The incident angle of each laser beam was designed to be 30° from the major axis of an ultrasound transducer to maximize the overlap area of two beams at the equator of the cavity. The approximate beam size at the equator of the light catcher (opening) is 2 cm $\times$ 1.3 cm. Multiple random reflections between the light catcher's inner surface and the skin surface are expected, which might result in more uniform light distribution in the tissue. To keep the geometrical setups of the optical fiber and the ultrasound transducer unchanged between experiments, a duplicate light catcher insertion without a concave reflecting mirror was fabricated as shown in Fig. 1(f).

C. Experiment Setup of PA Imaging

Fig. 2 illustrates that the PA imaging system consists of a commercial clinical ultrasound scanner equipped with research package (SonixTouch, Analogic corporations, Peabody, MA, USA) to collect raw PA radio frequency (RF) data and an optical parametric oscillator tunable laser pumped by Nd:YAG Q-switched laser (Vibrant HE532I, OPOTek, Carlsbad, CA, USA) as a PA excitation source, with time synchronization between them. The laser wavelength can be tuned within 680–950-nm range. The short-laser pulse of 5 ns long was delivered through the custom-made bifurcated optical fiber bundles (Fiberoptic Systems Inc., Simi Valley, CA, USA) attached to both sides of an ultrasound transducer. The laser pulse fluence ($<10 \text{ mJ/cm}^2$ at 689 nm and $<15 \text{ mJ/cm}^2$ at 800 nm) was kept within ANSI safety limit. A custom built 128-element high-frequency linear array transducer centered at 15 MHz with the full-width-half-maximum bandwidth of 65% for small animal imaging with a footprint 15 mm long and 10 mm wide (iBule photonics, Incheon, South Korea) was connected to the ultrasound imaging system. The ultrasound gel was centrifuged to remove air bubbles before it was carefully applied on the inner surfaces of the light catcher and the subcutaneous tumor. The light catcher then was deliberately placed from one side of the tumor to avoid trapping bubbles. Any excessive gel was removed upon completion of positioning. For PA image reconstruction, the acquired raw PA RF data were processed off-line using delay-and-sum one-way dynamic receive beam forming. An envelope was extracted from the summed RF data applied with the Hilbert transform for PA image reconstruction. Ultrasound B-mode imaging was performed with 15 MHz transmit beam to focus at 10-mm depth. Acquired raw pulse-echo RF data were processed using a conventional B-mode processing method; delay-and-sum dynamic receive beam forming, a bandpass filter (Bandwidth: 10.5–19.5 MHz) and, envelope detection using the Hilbert transform. The PA signal amplitudes were normalized to the measured average laser fluence over 50 laser pulses measured near the laser source output via using a laser sensor (YAG EnergyMax, J-50MB-YAG, Coherent Inc., Santa Clara, CA, USA).

D. Material—Photoacoustic Contrast Agents

In this paper, a clinical photosensitizer that has an absorption peak at 689 nm in NIR was employed as a PA imaging agent. The NIR region is the most preferred wavelength range for *in vivo* PA imaging due to relatively low background absorption by both water and blood. Visudyne, also known as verteporfin (Liposomal verteporfin for injection, benzoporphyrin

derivative monoacid ring A, BPD-MA; Valeant Pharmaceuticals North America LLC, Bridgewater, NJ, USA) is FDA approved photosensitizer that has been used in photodynamic therapy (PDT) for an anatomical treatment for vaso-occlusion of the arteriolized neovessels. Even though the strongest absorption peak of the photosensitizer is nearby 430 nm (blue light), the absorption peak wavelength in the NIR, 689 nm, is mostly preferred for the PDT in the clinic as shown in Fig. 3(a). The blue light attenuates quickly because of hemoglobin that has strong absorption in the same wavelength range. Instead, the sharp secondary peak at 689 nm, albeit relatively small absorption magnitude, can be used for *in vivo* PA imaging, as background absorption by hemoglobin, fibrotic tissue, or a skin chromophore such as melanin is relatively low at this wavelength. A solution of the photosensitizer was prepared with dose for human administration guidance written in the manufacturer's instruction. A vial of the photosensitizer of 15 mg was reconstituted with 7 mL of sterile water for injection resulting in the dark green colored mother solution of 3.0 mM while the molar weight of the photosensitizer is 718.8 g/mol. The prepared mother solution was kept in dark storage to protect from ambient light. According to the clinical administration guideline, it would be diluted by using 5% dextrose to achieve a required dosage of the photosensitizer, 6 mg/m² of body surface area and the required total volume was 30 mL. Therefore, the clinical molar concentration of the photosensitizer should be kept under 0.7 mM. In addition, to test the light catcher efficiency at a different wavelength in NIR, indocyanine green (ICG) solution of 65 μ M with an absorption peak at 800 nm as shown in Fig. 3(b) was used in *ex vivo* experiment [11]–[13]. The ICG solution was prepared by mixing the ICG powder of 50.4 μ g and distilled water of 1 mL. Note the molar weight of the ICG is 774.96 g/mol.

E. Ex Vivo Experiment Using Chicken Breast Tissues

Fat and blood removed chicken breast tissues were used for an *ex vivo* evaluation of the developed light catcher. To support the light fluence enhancement at the target depth when using the light catcher, laser pulse energy was measured at the same depth, maintaining all experimental conditions same. Laser energy was measured over 50 times by using the laser sensor covered with thin clear plastic lab wrap (VWR, PA, USA), reducing variations from pulse-to-pulse laser energy fluctuation, at the bottom of the stacked chicken breast tissues with different thickness of 5, 10, and 15 mm, and compared with when the light catcher was not used as shown in Fig. 2(b). Each chicken breast tissues were prepared with the size of about 20 mm (width) $\times$ 20 mm (length) $\times$ 5 mm (thickness). To compare corresponding PA signal enhancement due to increased light energy, a polyethylene tube (PE-50, inner diameter: 580 μ m, outer diameter: 965 μ m, Becton Dickinson, Sparks, MD, USA) filled with PA contrast agents was placed below three stacked chicken breast tissues of the 'total thickness of 15 mm. A separate large piece of chicken tissue of 20-mm thickness was placed at the bottom of the tube. Top and bottom chicken breast tissues were fixed by using pin nails. Centrifuged ultrasound gel was carefully applied to fill up the cavity of the light catcher before gently placing it onto the prepared chicken breast tissues. A thin layer of ultrasound gel also was applied between the layers of the tissue. To test the consistency of the performance in different NIR wavelengths, two different PA contrast agents were introduced at a time into the PE-50 tube embedded in the chicken tissue; verteporfin solution with an absorption peak at 689 nm and ICG solution with an absorption peak at 800 nm

[Fig. 3]. PA images on short axis of the tube were acquired for both contrast agents using the laser pulse tuned at each absorption peak with and without the light catcher at the same depth. Mean and standard deviation of PA signal amplitude were calculated over tube area of each PA image. For each dye, PA images when using and without using the light catcher were shown in same dynamic range after being normalized to the peak PA signal amplitude when using the light catcher. The statistical significance was measured between mean values of paired PA images (with using the light catcher versus without using the light catcher) by using t-test.

F. In Vivo Experiment Using Mouse Tumor Model

EMT-6 breast cancer cells acquired from the American type culture collection (ATCC, Manassas, VA, USA) were cultured in Waymouth's MB 752/1 with L-glutamine culture medium (Gibco, Life Technologies, Grand Island, NY, USA) added with 15% fetal bovine serum albumin (FBS, Atlanta biological, GA, USA) and 2% penicillin-streptomycin (Lonza, Basel, Switzerland). Cells were cultured in the cell incubation t-flask chamber at 37 °C with 5% CO₂ condition until reaching a number of cells $>8 \times 10^6$. Cultured cells were withdrawn from incubated t-flask to a vial of 1×10^6 cells for each mouse. Each sample was washed 3 times and suspended in 0.2 mL hyclone hank's balanced salt solution (HBSS, Thermo Scientific, South Logan, UT, USA). Tumors were developed on 6-weeks old female BALB/c-albino mouse by subcutaneously injecting cells on her lower back. Tumor-bearing mice were taken care of until each tumor size reached up to 10 mm in diameter [Fig. 2(d)]. The prepared verteporfin solution of 50 μ L (3.0 mM) was locally administrated into the center of tumor of each anesthetized mouse using an injection needle of gauge 26. PA signal amplitudes from injected verteporfin were measured when using and without using the light catcher. For each animal, the paired PA images were normalized to the maximum PA signal intensity in the PA image when using the light catcher. All images are shown in the same normalized dynamic range. The total number of mice used in this paper was eight and significance of the measurements was determined by calculation of p-value using t-test statistical analysis. The *in vivo* animal study was conducted under an approved protocol of Institutional Animal Care and Use Committee of the University of Pittsburgh.

III. Results and Discussion

A. Ex Vivo Study: Light Delivery Enhancement With the Light Catcher in Chicken Breast Tissues

Fig. 4 compares laser energy at different depths in the chicken breast tissues between the cases when using and without using the light catcher. The white and black bars represent the mean value of measured laser energy with and without applying the light catcher, respectively, at three different depths of 5, 10, and 15 mm, with an error bar of the standard deviation over 50 measurements. Using the light catcher increases the laser energy by 27.9% (17.3 ± 1.1 mJ versus 12.3 ± 0.8 mJ, $p < 0.05$, when using the light catcher versus without using the light catcher for the rest of this paper) and 33.3% (26.4 ± 1.5 mJ versus 17.8 ± 1.0 mJ, $p < 0.05$) at 5 mm, 31.0% (11.7 ± 0.7 mJ versus 8.1 ± 0.5 mJ, $p < 0.05$) and 34.9% (17.4 ± 1.0 mJ versus 11.2 ± 0.7 mJ, $p < 0.05$) at 10 mm, and 30.4% (9.4 ± 0.6 mJ versus 6.3 ± 0.4 mJ, $p < 0.05$) and 34.4% (13.0 ± 1.0 mJ versus 8.5 ± 0.6 mJ, $p < 0.05$) at 15 mm for 689 nm

as shown in Fig. 4(a) and 800 nm as shown in Fig. 4(b), respectively. Note that the measured laser energy without chicken breast (at depth 0 mm) are considered as same for both cases when using or without using the light catcher at each wavelength (36.3 ± 1.1 mJ versus 35.6 ± 0.9 mJ, $p < 0.05$ for 689 nm, 57.7 ± 1.5 mJ versus 57.3 ± 1.3 mJ, $p < 0.05$ for 800 nm). One of the limitations of this experiment is that laser pulse energy was measured instead of local laser fluence that directly affects initial local PA pressure because measuring local laser fluence could be erroneous without being able to know exact light propagation paths and beam profile. However, we believe that the increment ratio of total through-transmission of laser energy with the device would be very close to if not exactly same as the increment ratio of local laser fluence, while all experimental conditions were maintained the same.

Fig. 5 shows short-axis PA images from a tube filled with two different dyes embedded in chicken breast tissue at depth of 15 mm; verteporfin as shown in Fig. 5(a) and ICG as shown in Fig. 5(b). A dotted white circle represents the tube with inner diameter of $580 \mu\text{m}$ as a region of interest (ROI). PA signals were only detected on the top and bottom of the tube due to the linear array transducers geometrical limited view [13], [14]. When using the light catcher, PA signal amplitudes over the tube area for both agents were significantly enhanced as shown in Fig. 5(c); verteporfin at 689 nm: 37.6% enhancement, PA amplitudes of 39.9 ± 11.7 versus 24.9 ± 4.4 , and ICG at 800 nm: 32.5% enhancement, PA amplitudes of 70.2 ± 26.7 versus 47.4 ± 16.1 . A significant difference with $p < 0.05$ using t-test was shown for both cases. PA signal enhancement in two different wavelengths of 689 and 800 nm are in good agreement with laser fluence increase shown above and our previous *in silico* and *in vitro* results [4], [9]. This experiment result implies that PA signal would be amplified by increased laser fluence at depth when using the light catcher.

B. In Vivo Study: PA Signal Enhancement With the Light Catcher in Mouse Tumor Model

A representative PA image overlaid on ultrasound B-mode image is shown in the Fig. 6(a). Ultrasound B-mode image was log-compressed with 40-dB dynamic range. The dashed yellow contour draws the tumor of the mouse, and the top layer of the tumor is hair-removed skin. The dashed white box (height: 1.2 mm, width: 2 mm) in the image indicates the injection site within the tumor volume. The bright color at the center of the ROI indicates PA signal from the injected photosensitizer. The PA signals with much lower amplitudes found in the periphery of ROI are believed to be from the vascular network. In Fig. 6(b), the reconstructed PA images in ROI of animal tumor models with and without the light catcher are compared next to each other. Overall, PA images acquired when using the light catcher (bottom panels in each row) clearly exhibit with higher signal levels than without using light catcher (top panels in each row).

Table I summarizes PA signal amplitudes averaged over ROI from each mouse and PA signal enhancement ratio of with and without the light catcher. With the light catcher, the percentage of PA signal enhancement ranges from 26.1% (mouse no. 4) to 37% (mouse no. 8) with an average of 33.7%.

The statistical power analysis is shown in Fig. 6(c). Circles represent the averaged PA signal amplitudes within ROI of each mouse with the light catcher, and triangles are averaged PA signal amplitudes without the light catcher. Central line in the boxplot represents the median,

and both edges of the box indicate 25th and 75th percentiles. Two whiskers above and bottom of the boxplot display the range of measurements for the maximum and the minimum value, respectively. A p-value lower than 0.05 determines that PA signal level is significantly enhanced by an average of 33.7% when using the light catcher.

In summary, enhancement of the laser fluence in tissues using the developed light catcher is evidenced by the increased PA signal amplitudes measured *ex vivo* in the chicken breast tissues. In an *in vivo* study using the mouse tumor model, significantly improved PA amplitudes are observed when using the light catcher. This suggests the potential of an increased PA imaging depth when using the light catcher. In addition, the light catcher may protect the operator and the subject from unwanted exposure of laser excitation that may allow free hand PA imaging without needing eye protection goggles. For further translation of this technology and device, a few technical challenges have to be overcome. The current prototype of the reflecting mirror cone is fabricated using rigid, opaque material that makes difficult to use it on nonflat skin surfaces and prevents the operator from seeing the scanning area. Materials that are mechanically compliant and optically transparent to visible light, yet still reflective to the NIR, will be ideal for the reflecting mirror cone. In addition, the cone will have to be reasonably flat as well for easier free hand scanning, without potentially trapping air bubbles inside. As long as the cone fully covers the skin surface, a similar efficiency can be expected for different geometry. Moreover, the light catcher can be applicable to other optic-based technology such as PDT that is expected to increase light delivery efficiency thus for potentially improved treatment.

IV. Conclusion

A significant PA signal enhancement by about 33.7% is shown when using the developed light catcher in an *in vivo* mouse tumor model injected with a clinical photosensitizer, verteporfin, that has an absorption peak at 689 nm in the NIR region. Such enhancement of PA signal amplitude is mainly attributed to improved effective laser fluence at the target depth when using the light catcher that collects and redistributes the reflected light. This is further supported by both increased laser fluence and PA signal intensity in *ex vivo* chicken breast tissue experiments when using the light catcher. All these *ex vivo* and *in vivo* experiment results are in good agreement with our earlier simulation model [4], [9].

Acknowledgments

This work was supported by the International Collaborative R&D Program (M0000053, Development of a Novel Multi-modal In vivo Imaging System for Animal-to-Human Use) funded by the Ministry of Trade, Industry and Energy (MOTIE), Korea.

References

1. Song KH, Wang LV. Deep reflection-mode photoacoustic imaging of biological tissue. *J Biomed Opt.* 2007; 12(6):060503-1–060503-3. [PubMed: 18163798]
2. Kwon K, Son T, Lee KJ, Jung B. Enhancement of light propagation depth in skin: Cross-validation of mathematical modeling methods. *Lasers Med Sci.* Jul; 2009 24(4):605–615. [PubMed: 19030946]
3. Yeo C, et al. Development of compression-controlled low-level laser probe system: Towards clinical application. *Lasers Med Sci.* Sep; 2010 25(5):699–704. [PubMed: 20393768]

4. Wang Z, Ha S, Kim K. A new design of light illumination scheme for deep tissue photoacoustic imaging. *Opt Exp*. Sep; 2012 20(20):22649–22659.
5. Hardy JD, Muschenheim C. Radiation of heat from the human body. V. The transmission of infra-red radiation through skin. *J Clin Invest*. Jan; 1936 15(1):1–9. [PubMed: 16694368]
6. Hardy JD, Hammel HT, Murgatroyd D. Spectral transmittance and reflectance of excised human skin. *J Appl Physiol*. Sep; 1956 9(2):257–264. [PubMed: 13376438]
7. Han P, Tseng YC. Spectral shift amplification and polarization-controlled spectral shift with silver metal. *Metals*. Dec.2015 6(1):7.
8. Nie L, Cai X, Maslov K, Garcia-Urbe A, Anastasio MA, Wang LV. Photoacoustic tomography through a whole adult human skull with a photon recycler. *J Biomed Opt*. 2012; 17(11):110506. [PubMed: 23123972]
9. Yu, J., et al. Enhancement of photoacoustic signal using a novel light illumination improvement device: *In vivo* feasibility animal study. *Proc. IEEE Int. Ultrason. Symp. (IUS)*; Sep. 2014; p. 349-352.
10. Wang LV. Tutorial on photoacoustic microscopy and computed tomography. *IEEE J Sel Topics Quantum Electron*. Jan-Feb;2008 14(1):171–179.
11. Landsman ML, Kwant G, Mook GA, Zijlstra WG. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. *J Appl Physiol*. Apr; 1976 40(4):575–583. [PubMed: 776922]
12. Alander JT, et al. A review of indocyanine green fluorescent imaging in surgery. *Int J Biomed Imag*. Feb.2012 2012 Art. no. 940585. doi: 10.1155/2012/940585
13. Beard P. Biomedical photoacoustic imaging. *Interface Focus*. Jun.2011 1(4):602. [Online]. Available: <http://rsfs.royalsocietypublishing.org/content/1/4/602.abstract>. [PubMed: 22866233]
14. Xu M, Wang LV. Photoacoustic imaging in biomedicine. *Rev Sci Instrum*. Apr.2006 77(4):041101.

Biographies

Jaesok Yu (S'14) received the B.S. and M.S. degrees in electronic engineering from Sogang University, Seoul, South Korea, in 2009 and 2011, respectively. He is currently pursuing the Ph.D. degree in bioengineering with the University of Pittsburgh, Pittsburgh, PA, USA.

He was a Researcher with the Medical Solution Institute, Sogang University, where he was involved in developing a novel ultrasound imaging platform, and Doppler and nonlinear imaging technologies. He is currently with the Multimodality Biomedical Ultrasound Imaging Lab, Center for Ultrasound Molecular Imaging and Therapeutics, University of Pittsburgh Medical Center, Pittsburgh, under the supervision of Dr. K. Kim, where he was involved in ultrasound technologies and its clinical applications. His current research interests include molecular photoacoustic imaging and its clinical translations, phase-transition droplet for ultrasound and photoacoustic imaging, novel ultrasound imaging technology such as super-resolution imaging, and biomedical signal processing.

Joel S. Schuman received the M.D. degree from the Mount Sinai School of Medicine, New York, NY, USA, in 1984.

He is Director of the NYU Langone Eye Center, Professor and Chairman of Ophthalmology, and Professor of Neuroscience and Physiology at NYU Langone Medical Center, NYU School of Medicine, and Professor of Electrical and Computer Engineering at NYU Tandon School of Engineering. Prior to arriving at NYU in 2016, he was Distinguished Professor and Chairman of Ophthalmology, Eye and Ear Foundation Endowed Chair in Ophthalmology, Director of UPMC Eye Center (2003–2016) and before that was with Tufts University from 1991 to 2003, where he was Residency Director (1991–1999) and Glaucoma and Cataract Service Chief (1991–2003). In 1998, he became Professor of Ophthalmology, and Vice Chair in 2001. He has published more than 350 peer-reviewed scientific journal articles.

Dr. Schuman and his colleagues were first to identify a molecular marker for human glaucoma, published in *Nature Medicine* in 2001. Continuously funded by the National Eye Institute as a principal investigator since 1995, he is an inventor of optical coherence tomography (OCT), used world-wide for ocular diagnostics. He is a Fellow of the American College of Surgeons.

Jung-Kun Lee received the B.S. and M.S. degrees in inorganic materials, and the Ph.D. degree in materials science and engineering acoustics from Seoul National University, Seoul, South Korea, in 1994, 1996, and 2000, respectively.

After receiving his Ph.D. degree, he moved to Los Alamos National Laboratory, NM, USA, for almost 6 years as Technical Staff Member and Director's Funded Postdoctoral Fellow. In 2007, he joined the University of Pittsburgh (Pitt), Pittsburgh, PA, USA. He is currently Associate Professor and William Kepler Whiteford Faculty Fellow with the Department of Mechanical Engineering and Materials Science, Pitt. His current research interests include electronic and optical properties of semiconductor materials and their energy application

with emphasis on solar energy harvesting, and also include sophisticated processing of materials and processing-functional property relation.

Sang-Goo Lee received the Ph.D. degree from Seoul National University, Seoul, South Korea, in 1995.

From 1995 to 1997, he worked on the control of crystal morphology and size in various reaction systems as a Manager of Process Development Team, HANWHA Corp. From 1997 to 1999, he handled the growth of relaxor ferroelectric (lead magnesium niobate) single crystal which is a very important material for electrostrictive, electro-optic devices at the Center for Advanced Material Research, Stanford University, Stanford, CA, USA. The research result of this process was presented in *Applied Physics Letters* in 1999. In 1999, he founded iBULe Photonics, Incheon, South Korea, a new material development company for commercializing this material. It is in a leading position to develop and produce piezoelectric PMN-PT/PIN-PMN-PT single crystal materials, wafers, and applications with patented technology. iBULe Photonics now produces PMN-PT/PIN-PMN-PT and adopts it to its new products, medical ultrasound probe, SONAR sensor, NDT, accelerometer, flame detector, and energy harvest.

Jin Ho Chang (S'00–M'08–SM'15) received the B.S. and M.S. degrees in electronic engineering from Sogang University, Seoul, South Korea, in 2000 and 2002, respectively, and the Ph.D. degree in biomedical engineering from the University of Southern California, Los Angeles, CA, USA, in 2007.

From 2002 to 2003, he was a Research Engineer with the Digital Media Research Laboratory, LG Electronics Inc., Seoul. He was a Postdoctoral Research Associate with the NIH Resource Center for Medical Ultrasonic Transducer Technology, University of Southern California. In 2010, he joined the Department of Biomedical Engineering, Sogang Institute of Advanced Technology, and the Department of Electronic Engineering, Sogang University, Seoul, as Assistant Professor and was promoted to Associate Professor in 2014. His current research interests include photoacoustic imaging and its clinical applications,

high-frequency ultrasound imaging systems, therapeutic ultrasound, and biomedical signal processing.

Kang Kim (M'06–SM'17) received the B.S. degree in educational physics from Seoul National University, Seoul, South Korea, in 1986, the M.S. degree in physics from the University of Paris VI (Universite de Pierre et Marie Curie), Paris, France, in 1989, and the Ph.D. degree in acoustics from the Pennsylvania State University, State College, PA, USA, in 2002.

He was a Research Associate with the Agency for Defense Development (ADD), Chinhae, South Korea. He then became a Senior Research Associate leading a SONAR Development Team starting in 1995. He was a Postdoctoral Research Fellow with the Biomedical Engineering Department, University of Michigan, Ann Arbor, MI, USA, where he was involved in ultrasound tissue elasticity imaging in medical applications and then became Assistant Research Scientist. He is currently Associate Professor of Medicine with tenure, School of Medicine, Department of Medicine and Heart and Vascular Institute, and Associate Professor of Bioengineering, Swanson School of Engineering, Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA. His current research interests include noninvasive ultrasound-based multimodality imaging techniques such as nonlinear tissue elasticity imaging, 3-D elasticity imaging, thermal strain imaging, photoacoustic molecular imaging, and engineered tissue characterization.

Fig. 1.

Developed high-frequency ultrasound transducer integrated with the light catcher for PA imaging. (a) and (b) 3-D drawing of the light catcher prototype. All parts were fabricated using 3-D printing technology. (a) and (d) Handheld style housing was made for freehand scanning. (b), (c), and (e) Detailed configurations of the ultrasound transducer and bifurcated optical fiber bundle integrated with the light reflecting mirrors. The angle between the transducer and the optical fiber is 30° . The concave shape light catcher is 12.7 mm in height and 25.4 mm in diameter. The light catcher inner surface was coated with silver. (f) For experimental evaluation of the device under the same geometrical setups of the optical fiber and the ultrasound transducer, two types of insertion with and without the light reflecting mirror were built. UT: Ultrasound transducer; w/LC: with the light catcher; w/o LC: without the light catcher.

Fig. 2.

(a) Experimental setups for *ex vivo* tissue and *in vivo* animal study. A commercial research ultrasound scanner was synchronized with a tunable laser system. A short laser pulse of 5 ns long was delivered through a bifurcated optical fiber attached to both sides of the US transducer. The concave reflecting mirror of the light catcher was placed on the target covered with ultrasound gel. (b) Laser fluence was measured at different depths (5, 10, and 15 mm) in chicken breast tissues (thickness of each tissue slab: 5 mm) at 689 and 800 nm. (c) PA signal was measured from a polyethylene tube (inner diameter: 580 μm , outer diameter: 965 μm) filled with a contrast agent that was placed between chicken breast tissues of varying thickness. (d) For *in vivo* animal study, a contrast agent was locally injected into a subcutaneous tumor (diameter: 1 cm) on the back of a mouse.

Fig. 3.

(a) Normalized absorption spectra of the verteporfin solution and (b) the ICG solution at $65\mu\text{M}$. In addition to its major absorption peak at the visible light at nearby 430 nm (blue light), a distinct secondary absorption peak of verteporfin in the NIR at 800 nm.

Fig. 4.

Ex vivo experiment results: Measured laser energy at (a) 689 nm and (b) 800 nm at different depths in chicken breast tissues. Laser energy is measured at 5-, 10-, and 15-mm depth of chicken breast tissues with (white) and without (black) the light catcher. Each bar represents the mean of laser energy over 50 measurements with an error bar of the standard deviation. w/o LC: without the light catcher; w/LC: with the light catcher.

Fig. 5.

Ex vivo experiment results: Short-axis PA images from a tube filled with (a) verteporfin taken at 689 nm. (b) ICG taken at 800 nm that was embedded in chicken breast tissue at depth of 15 mm. The left panels are for the case without using the light catcher and the right panels are for the case when using the light catcher. Dotted white circles represent a polyethylene tube. (c) PA signal was enhanced by 37.6% for verteporfin at 689 nm and by 32.5% for ICG at 800 nm.

Fig. 6.

In vivo experiment results: Normalized PA images with and without the light catcher. (a) PA image overlaid on ultrasound B-mode image with 40-dB log-compression. The dashed yellow contour represents the tumor location of the mouse and the top layer of the tumor is skin with hair removed. The dashed box (height: 1.2 mm, width: 2 mm) indicates the ROI where the photosensitizer is administered. (b) PA images of ROI when using (bottom row) and without using the light catcher (top row) for each mouse ($n = 8$) within a depth of 14.6–15.8 mm. All PA images are normalized by measured laser fluence and shown in the same dynamic range. (c) Statistical analysis of *in vivo* study results (Table I). Solid circles and triangles represent averaged PA signal amplitudes in ROI with and without the light catcher, respectively. The central line of the boxplot is median, the edges of the box are 25th and 75th percentiles, and two whiskers above and below of the box are the maximum and the minimum of measurements.

TABLE I

In Vivo Experiment Results: The Normalized and Averaged PA Signal Amplitudes (A.U.) in the ROI and Enhancement Ratio When Using the Light Catcher

Mouse No.	1	2	3	4	5	6	7	8
PA amplitude w/o LC (a.u.) *	0.46	0.45	0.42	0.49	0.44	0.44	0.44	0.45
PA amplitude w/LC(a.u.) *	0.68	0.67	0.66	0.66	0.70	0.66	0.65	0.71
Enhancement (%)	32.8	32.9	34.9	26.1	36.9	32.9	31.6	37.0
Averaged enhancement (%)								
33.7								

* PA signal amplitude of each mouse was mean value in ROI of the injected photosensitizer ($n = 8$). PA signal amplitudes were normalized by the laser fluence. All values are in arbitrary unit (a.u.). w/o LC: without the light catcher; w/LC: with the light catcher.