The development and characterization of a novel yet simple 3D printed tool to facilitate phantom imaging of photoacoustic contrast agents

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**Abstract**

We report a new approach to preparing phantoms using 3D printing. This device supports plastic tubing containing the contrast agent and is immersed in a solution with absorption or scattering properties that mimic tissue. Up to 12 tubing samples could be placed in the device with sample-to-sample spacing as low as 0.3 mm and at a constant distance from the transducer (±0.16 mm), which is critical in validating photoacoustic contrast agents. We also studied different types of tubing and found that tubing with a larger outside diameter has more inherent signal. Both 40% India Ink and lipids in the immersion media modulated the signal. Finally, we created a depth phantom and found that signal decayed following a linear relationship ($R^2=0.997$) with respect to distance from the focal point. We include computer-assisted drafting code the community can use to print this phantom or customized versions of this phantom.

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1. Introduction

Photoacoustic imaging has attracted significant attention from the biomedical community because it combines good contrast of optical imaging with the temporal and spatial resolution of ultrasound [1]. A significant strength of photoacoustic imaging is its ability to collect images without exogenous contrast agents. That is, it can use the contrast of hemoglobin, deoxyhemoglobin, or melanin to produce images of hypoxia [2] and perfusion. However, for molecular imaging studies, contrast agents that produce signal only in the presence of a specific chemical cue are often needed. Photoacoustic contrast agents have been developed for a variety of targets including reactive oxygen species [3], drug levels [4], and cancer biomarkers [5,6].

There are many steps to validating molecular imaging agents including chemical synthesis, tissue culture studies, computational modeling, and small animal models [7]. One important step along this path is validation of the imaging agent with a phantom. These phantom studies are important because they characterize the signal intensity, signal stability, detection limits, and depth of penetration of the imaging agent/imaging hardware. Phantom studies can quickly evaluate the efficacy of imaging agents in vivo without using expensive and complicated animal models. However, phantom studies often must be repeated with each new iteration of the contrast agent or when changes are made to the photoacoustic equipment. Thus, it is important to have a fast, consistent, and facile approach to building phantoms for photoacoustic imaging.

There are a variety of phantoms available to the community each with advantages and limitations. One common approach is to simply use turkey [8], chicken [9], or pork [10] tissue. In this approach, plastic tubes containing the imaging agent are embedded into the meat prior to imaging. The advantage of this approach is that these tissues offer very good approximations of clinical samples. This tissue often contains proportions of muscle and fat tissue (but not blood) similar to that in humans. The limitation of this approach is that it is cumbersome and not reproducible—it is difficult to purchase or create tissue sections with the controlled diameters that are often needed for validation of imaging agents. This adds an extra variable to contrast agent development. This approach also has low temporal stability because meat quickly spoils.

Other phantoms described in the literature include mouse mimics [11] similar to the commercially available phantoms, finger cots [12] used in the histology lab, customized 3-D tubing arrays to simulate vasculature [13], or the use of a skin-equivalent-matrix with embedded vessel channels [14]. There have also been reports of carefully prepared photoacoustic calibration phantoms [15]. These calibration phantoms are particularly useful to model new photoacoustic hardware, validate reconstruction algorithms, and...
perform maintenance and quality control. However, these calibration phantoms have little utility in the development of new photoacoustic imaging agents because they are solid masses of polyvinyl chloride plastisol [16,17] that cannot be repurposed for new imaging agents.

Agarose is another common approach. People have created a variety of customized phantoms using agarose, e.g., in the shape of the state of Texas [18]. This agarose is often doped with lipids [19], inks [20], titania [21] and/or acoustic scatterers such that it more accurately recapitulates human, i.e., a tissue-mimicking phantom. In another iteration, the contrast agent can be placed inside of plastic tubing that is then sealed inside of the agar [22]. The advantages of agar include the wide flexibility in size, shape, and composition of the resulting phantom. The disadvantage is the relatively short temporal stability of the agar, the lengthy preparation time, difficulty in working with hot agarose (sample stability), challenges in making the dimensions consistent between replicate phantoms, and diffusion of contrast agent through the agar gel. Thus, having a more versatile phantom platform would be very advantageous to photoacoustic imaging researchers. An ideal system would be reproducible, easily tuned, affordable, and disposable.

Here, we report a new approach to preparing photoacoustic phantoms—a 3D printed tool that can quickly, easily, and reproducibly hold plastic tubes containing the liquid contrast agent. When placed inside of a beaker or evaporation dish, a variety of tissue mimicking materials can be added to module the optical and acoustic parameters from simple saline to liposyn or India Ink. This new approach can significantly reduce the time needed to prepare photoacoustic phantoms while maintaining the sample at exactly the same distance from the transducer, which is critical for reproducible photoacoustic measurements. We include CAD code that the community can use to prepare customized imaging phantoms.

2. Materials and methods

2.1. Raw materials

The raw material used for 3D printing was polyactic acid (PLA), which is a biodegradable thermoplastic. Polyethylene tubing was purchased from Harvard apparatus with an outer diameter (OD) of 1.27 mm and an inner diameter (ID) of 0.85 mm. Polytetrafluoroethylene (PTFE/Teflon) tubing was purchased from Newark Electronics with an OD of 1.01 mm and ID of 0.71. Polyethylene has a longitudinal speed of sound of 2100–2400 m/s\(^{-1}\), which depends on its density, whereas the speed of sound in Teflon is 1400 m/s\(^{-1}\) [23]. Teflon is used for protection against flammability and chemicals. Its tensile strength is 2000 psi and it holds its structure in a range from –75 to 260 °Celsius, according to the supplier. The melting point of polyethylene is between 115 and 135 °Celsius depending on density and polyethylene has a high plasticity. Methylene blue (98%) was purchased from Fisher.

To prepare the methylene blue samples, the reagent-grade powder was dissolved in phosphate buffered saline (PBS) and filtered through a 0.22 μm filter. The PBS was purchased in tablets from Fisher Scientific; one tablet was dissolved in 200 mL of deionized water to give 1X PBS. India Ink (0.2% in PBS buffer) was purchased from Alpha Aesar and used as a scattering solution in the media variations experiment. CD Lipid Concentrate (Thermo-Fisher, 11905031) was used to further simulate human tissue including scatter. The phantom was fixed to a beaker initially containing a 100 mL water solution. Then we gradually poured 1X India Ink to increase the concentration imaging at 1%, 10%, 25%, 40%, 50%, and 60%. A magnetic stir bar was used to ensure good miscibility. The gain was maintained at 12 dB throughout the whole experiment.

2.2. Computer assisted drafting

The phantom was designed in Autodesk Fusion 360 version 2016. The dimensions of the phantom are 3 × 3 × 2 cm for general purpose imaging, although we tuned the dimensions to perform a variety of quality control experiments. First, a 2D sketch was designed and then extruded to form the 3D structure. On this 3D structure, we drew the insertion holes and then performed a “sweep cut” to cut along the path chosen. All dimensions were carefully fixed to ensure height stability and alignment of the tubes. The final file was stored as a Stereolithography file (STL) and sent to the printer’s software, which interprets it as individual planar slices that determine how to deposit the plastic filament. The first design incorporated 16 equally spaced holes with diameters ranging from 1.7 to 2.5 mm. We tested the snugness of fit of the tubing in the hole and selected the best fit based on the force required to remove the tube from the hole. The CAD design was subsequently modified to create uniform hole diameters.

2.3. 3D printing

We used a MakerBot Replicator 2 Desktop 3D Printer. The printing resolution was at its maximum (0.1 mm). The infill, density or amount of material used for the internal structure of the phantom, was set at 15%. The number of shells determines the thickness of the external walls of the phantom and was left to the default 2. The extruder temperature was 230 °Celsius, which is the usual temperature used with PLA filament. The speed of the motor xy-stage was 150 mm/s.

A helper disk of thickness 0.1 mm was used to stabilize the base of the phantom during the printing process. The helper disk will also contribute to hold the phantom down during the imaging process; it is not part of the design in CAD but rather an add-on in the MakerBot’s software. The files used to prepare these 3D printed component can be found at https://github.com/yagoI994/phantom-designs-photoacoustics.

2.4. Scanner

The PA images were obtained using a Vevo LAZR Photoacoustic Imaging System from VisualSonics equipped with a 21 MHz-centered transducer (LZ250) as described previously [24]. The full field of view is 23 mm wide width and 30 mm deep with this transducer. The system uses a flashlamp-pumped Q-switched Nd: YAG laser with optical parametric oscillator and second harmonic generator. The operating frequency is 20 Hz and the wavelength of the laser can be tuned from 680 to 970 nm with a minimum step size of 2 nm. The pulse duration lies within 4 to 6 ns and the peak energy is 45 mJ ± 5 mJ at 20 Hz. The spot size associated with the LZ250 transducer is 1.25 mm x 25.4 mm, the fiber optic bundles are at an angle of 30° relative to the imaging plane, and the laser fluence is ~ 2–5 mJ/cm² [25]. The acquisition rate is 5 frames per second, and the transducer can be swept across a 5 cm region to create a three dimensional image.

Before scanning, the laser was optimized and calibrated using the built-in power meter and software. The gain of the images we used ranged from 5 to 39 dB depending on the sample being imaged; the ultrasound was set at a frequency of 21 MHz. The wavelength was set to 700 nm 3D scans were done over regions of 10 to 20 mm.
2.5. Image acquisition

The transducer was leveled prior to scanning. The outside of the tubes was cleaned and wiped with ethanol to remove any debris from the surface. Then they were placed in the phantom and loaded with the sample. We sealed one of the ends with a flame to prevent the sample from leaving the tube once immersed. The phantom was then placed in a beaker containing deionized water and held down using two weights on the helper disk. To reduce the ultrasound backscattering between the water and the platform, one of the clips could be placed across the base of the phantom.

The samples were aligned under the transducer at a depth of 11 mm—the focal point of the laser. The cross-section of the tubes was scanned longitudinally for the length of the phantom (typically 2 or 3 cm) to form the 3D images. The tubes were also scanned widthwise to ensure proper alignment.

In the lipid experiments, 20 mL of the agar/lipid solution (0%, 1%, 10%, 35%, and 50%) was allowed to gel in a petri dish. The sample holder was then added and 60 mL of agar/lipids solution was poured on top of the sample holder. The gain was maintained at 30 dB throughout the experiments with the lipids.

2.6. Image processing

We used ImageJ (v1.51a) [26] to quantitate the data. This includes photographs taken of the 3D printed phantom to analyze height, separation and diameters and compare them to the parameters set in SolidWorks. We photographed a micrometer together with the phantom to set the scale. Then, the distance from the top of the phantom to the top of each hole was measured to determine depth. To measure the relative roughness of the holes, a circle was drawn to delimit the perimeter and areas of material inside the perimeter were singled out and added together. We used this to measure the line profile in Fig. 2D. Images were analyzed as 8-bit images, and the data was analyzed with Excel (v15.23.2).

Furthermore, an image processing code was created using MATLAB (vR2015a). The code uses.tif files exported directly from the Vevo software and converts them to grayscale. Afterwards, it requests the user to input a threshold to scan the images. The output is the average pixel intensity from each tube, and this was exported to Excel for further analysis.

3. Results and discussion

The precision of the 3D printing enabled us to fabricate a phantom with customizable spacing values to measure the signal of different samples at a constant distance from the transducer. The phantom could be immersed into liquid solutions or sealed in gels to recreate different phantom conditions. The system could also be used to carefully hold samples at different depths.

3.1. Phantom construction

The phantom (Fig. 1A) is a 4.6-g 3 × 3 × 2 cm structure of PLA containing two 0.5 cm-thick walls facing each other and separated by 2 cm. In the face of these walls, there are 14 holes that are aligned from wall-to-wall at the same height with respect to the base. At the base of the printed phantom there is a 4-cm-diameter helper-disk (green arrow, Fig. 1B) used to help stick the phantom to the printing surface while printing. This secures it during photoacoustic imaging. On the rear face of the walls, there is a cavity for clips to be inserted to hold the tubes in place more tightly if necessary (blue arrows, Fig. 1A). Although up to 14 holes could be printed only 12 tubes could be imaged at one time.

The 3D printed phantom was completely customizable and could be printed in 40 min. The height of the samples could be controlled from at least 2 mm below the transducer to 20 mm below the transducer; the spatial resolution of our printer is 100 µm. Up to 12 plastic tubes could be fit inside the phantom and imaged with our largest transducer (LZ250; 30 mm field of view; 21 MHz center frequency). When we attempted to add even more holes (blue arrow, Fig. 1A), the small separation distances (red arrow, Fig. 1C) caused printing defects that made the height inconsistent (yellow arrow, Fig. 1C).

Fig. 1. Physical construction of the phantom. a) CAD image of the 3D-printed phantom with tubes inserted and clips on the sides. b) Birds eye view of the phantom with 12 tubes inserted in it and loaded with dye solution. c) Side view of the phantom. d) Quantification of the disparities in depth from each hole with respect to the top of the phantom.
3.2. Phantom quality control

One initial goal was to characterize the consistency of hole placement. Fig. 1 shows that there is some variability in the depth of the holes. The variations are ±0.12 mm, which is roughly the spatial resolution of the printer. Fig. 1C illustrates the rough contour of the holes; the roughness constitutes 10% of the area of the circle. This roughness provided an extra grip for the tubes to be immobilized. We also measured the diameter of each hole and found the biggest difference to be around 0.1 mm. This was expected due to the resolution of the printer, but all the tubes were still easily placed in the phantom. Finally, we measured the hole-to-hole separation and found an interesting pattern in which the separation alternates from 0.3 to 0.5 mm from one tube to the next. This pattern can be appreciated in Fig. 2B. We hypothesize that this occurred due to inconsistencies associated with approaching the limit of resolution during the printing; this trend increased at smaller spacing values.

We studied two designs. The first design had more space between the tubes (0.87 mm; Table 1). It also incorporated a neck design in the insertion holes which gripped the tubes (Fig. 1A). However, this design wasted tubing because the scanning length was 30 mm. Furthermore, we could only fit 9 tubes at a time in the visual field of the LZ250 transducer (Table 2). The next iteration was smaller (faster printing & less material) and also reduced the tube-to-tube spacing to 0.4 mm, which allowed us to image up to 12 samples simultaneously. By removing the neck design, the diameter of the holes was reduced by 20% and the spaces were 54%, which allowed tighter packing. Importantly, the number of samples that can be scanned concurrently are highly dependent on the transducer used. Different transducers (Table 2) have different imaging areas and resolution and thus it is important to carefully match transducer and phantom to the end application.

The resolution of the printer limited the number of tubes that we could fit in the visual field. The manufacturer’s specifications [27] state that the printer offers 0.1 mm resolution for each layer of plastic. Fifteen 1.30-mm diameter tubes could theoretically be imaged at 0.1 mm separation between tubes. In practice, our best separation was ~0.4 mm, which limits the number of holes per unit length. Spacing values below 0.4 mm compromised the shape integrity of the holes; we noticed that some of the holes collapsed.

### Table 1
Parameters of phantoms. Dimensions, diameter of holes, tube-to-tube distance, and maximum number of tubes that fit on the visual field of the transducer for versions 1 and 2 of the phantom. Error margins represent the standard deviation of the average of all the diameters or tube-to-tube distances measured.

| Device version | Dimensions (x, y, z in mm) | Diameter (Ø in mm) | Tube-To-Tube Distance (mm) | Max # of tubes for imaging |
|----------------|---------------------------|-------------------|---------------------------|---------------------------|
| 1              | 71 x 50 x 20              | 1.62 ± 0.13       | 0.87 ± 0.13               | 9                         |
| 2              | 31 x 30 x 15              | 1.30 ± 0.08       | 0.40 ± 0.12               | 12                        |

### Table 2
Specifications of transducer. Frequency, resolution, and width and depth imaging dimensions for each transducer as specified by the manufacturer.

| Transducer | Frequency | Resolution | Width x Depth |
|------------|-----------|------------|---------------|
| LZ250      | 13–24 MHz | 75 µm      | 23 mm x 30 mm |
| LZ400      | 18–38 MHz | 50 µm      | 15 mm x 20 mm |
| LZ550      | 32–55 MHz | 40 µm      | 14 mm x 15 mm |
and there were large differences in depth ($\pm 0.16$ mm) and diameter ($1.28 \pm 0.30$ mm). To achieve the maximum resolution of the printer we might consider changing parameters such as the printing speed or the printing orientation. That will be the goal for future efforts.

3.3. Replicates study

In the next experiment we imaged a set of methylene blue replicates to quantify the variations in their signal. The PA images below were taken at a gain of 6 dB. Maintaining a constant distance between the transducer and the phantom is critical in photoacoustic imaging because variations in this distance can markedly increase of decrease signal. Fig. 2A confirmed the height consistency ($\pm 0.12$ mm) of our phantom. Next, we studied the photoacoustic intensity of the samples. We measured the signal of each sample and found out that it is 17% lower on the sides than in the center (Fig. 2C). We hypothesize that this is because the laser is weaker on the sides of the transducer. Fig. 2B also shows that the spacing between tubes is not complexly uniform. However, the line profile in Fig. 2D confirmed that the height was consistent throughout the length of the tube with no bending of the tubes. The peaks in the graph correspond to regions in the PA image with contamination such as dust or dye aggregates.

3.4. Background study

Photoacoustic contrast agents are often validated by placing the sample in plastic tubing, but the background signal of this tubing can compromise the testing. Thus, we next modified the phantom design to test the background signal of the different types of tubing (Fig. 3). The phantom contained 10 holes tuned to match the diameter of the tubes. We placed all the holes on a tangent line that intersects the upper region of the circles to ensure that all the tubes were illuminated by the laser at the same depth. Thus, the laser illumination was consistent regardless of tubing size.

The table in Fig. 3A shows the specifications of the tubes used. We increased the gain to 39 dB to image the background signal. Both Teflon and the Harvard Apparatus polyethylene have the lowest signal; interestingly, they also have the most similar properties in terms of ID and OD. However, Teflon's higher melting point makes it difficult to handle because its walls are not easily malleable, which is problematic when sealing the ends; thus polyethylene is preferred. Contrary to what would be expected, the signal from tube #4 is only 17% stronger than tube #5, although its wall is 2x thicker. Instead, the tube with the biggest outside diameter (2) had the highest PA signal by two-fold.

We also performed additional spectral imaging of Teflon, polyethylene, and polyactic acid from 680 to 970 nm. The polyethylene had 3- to 4-fold higher signal than PLA and Teflon from 750 to 970. This relative increase was even larger from 680 to 750 (up to 10-fold). We emphasize that these data were collected at very high gain ($\sim 39$ dB), and thus this signal has a minor impact on contrast agent validation. The PLA material was used to construct the phantom and had low photoacoustic signal except for versions.

3.5. Phantom media variations

The signal in photoacoustic imaging is highly dependent on absorption and scatter from the surrounding tissue. Thus, it is critical that this phantom be compatible with tissue-mimicking materials. With our system, this is done by immersing the phantom in a dish containing the media of interest. Fig. 4 quantifies the intensity change as a function of India Ink concentration. This ink was poured around the phantom to simulate background absorption in tissue[28]. The image quality and intensity decreased

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**Fig. 3.** Analysis of plastic tubing used with the 3D printed phantom. a) Table containing specifications of the different tubes. The inner and outer diameters were measured with a 0–150 mm Neiko Digital Caliper. Error margins represent the standard deviation of 3 measurements per parameter. b) Quantification of imaging data showing that sample 2 has the highest signal. Scans were done in duplicate in four different positions for a total of 12 scans. The labels 1–5 in panel b correspond to the row numbers in panel a. Error bars in panel b represent the standard deviation of the mean of two different positions. c) Spectral imaging of the different types of plastics used. Polyethylene has 3- to 4-fold higher signal from 750 to 970 nm and up to 10-fold higher signal at 680 nm but with 39 dB gain.
as a function of dye concentration. The relationship between dye concentration and signal intensity was linear with $R^2 = 0.9686$. The steepest decrease occurred at 10% India Ink where the signal decreased by 7% from the 1% India Ink. This experiment demonstrates the capability of using the phantom under different media conditions and the ability to add new mixtures into the solution without constructing an entirely new phantom.

To demonstrate the ability of this system to characterize absorption and scatter from lipids, we added lipids at 0%, 1%, 10%, 35%, and 50% into an agar gel and measured photoacoustic signal (Fig. 5). The addition of lipids has been shown to increase ultrasound attenuation by absorption and scattering [28]. The PA signal increased as the turbidity of the sample increased from 1%-35%, the PA signal increased. However, at a lipid concentration of 50%, the PA signal decreased due to scattering in the agar media. These experiments demonstrate how this phantom can systematically perturb individual conditions without constructing an entirely new phantom.

3.6. Depth study

Measuring signal intensity as a function of depth is an important goal during the validation of photoacoustic contrast agents and photoacoustic imaging equipment. Previous approaches have used layers of agarose or fishing line [29], but these are time-consuming options, and it is difficult to accurately control the height. Our goal was to design a phantom that could easily control the depth of the samples. Hence, we updated the design of our phantom to have a height gradient on the holes for the tubes; the height gradient was $1 \pm 0.1$ mm from 7.4 to 15.5 mm below the transducer (Fig. 6).

The signal at 15.5 mm is more than 2 fold lower than at the focal point (~11 mm), but this was at a gain of 16 dB, which is far from the maximum (40 dB). However, having a higher gain caused excessively high signal from the more proximal tubes. The signal can “bleed” into neighboring voxels and inaccurately contribute signal. While we could image deeper with higher gains, the goal here was to demonstrate the depth-specific imaging capabilities of our phantom. The reduction in intensity from 10.3 to 15.5 mm followed a linear relationship with an $R^2 = 0.997$. Additional experiments showed methylene blue could be imaged at least 20 mm deep with higher gain.

4. Conclusions

We designed and fabricated phantoms for PA imaging using 3D printing. While 3D printed phantoms have been described for magnetic resonance imaging [30], biophotonics [14], and
computed tomography [31], to the best of our knowledge, this is
the first report of such a system tailor made exclusively for pho
toacoustic imaging. These tools improve upon the current technique
used in making phantoms for PA imaging. First, it markedly
reduces the time spent in preparing agarose phantoms because
our method is rapid and reusable.

The speed of sound of PLA is 2260 m·s⁻¹ [32], which when
immersed in water (1482 m·s⁻¹) causes a large contrast in the US
signal. This contrast is strong enough to clearly determine the
boundaries of the phantom. Future work will study the background
correlation coming from the phantom and test other 3D printing
materials such as acrylonitrile butadiene styrene (ABS), resins, etc.
We also plan to increase the number of tubes that can fit in a given
area so more samples can be imaged at once without compromis-
ing the height stability. We have shown that 3D printing provides a
fast, inexpensive and stable method for creating phantoms suitable
for testing contrast agents in photoacoustic imaging. The files can be
found at https://github.com/yago1994/phantom-designs-pho
toacoustics. These are free to use or customize for other
applications.

Conflict of interests

The authors declare that there is no conflict of interests
regarding the publication of this paper.

Acknowledgements

Jesse V. Jokerst acknowledges funding from NIH HL117048 and
HL137187 and infrastructure from S10 OD021821. The authors also
thank the American Cancer Society Institutional Research [grant
number 14-250-42] provided through the Moores Cancer Center,
University of California, San Diego. We also knowledge UC San
Diego Library’s Digital Media Lab for providing us with 3D printers
and Chris Barback for assistance in managing and operating the
photoacoustic equipment.

References

[1] L.V. Wang, S. Hu, Photoacoustic tomography: in vivo imaging from organelles
to organs, Science 335 (6075) (2012) 1458–1462.
[2] S. Mallick, K. Watanabe, D. Timerman, D. Schoenfeld, T. Hasan, Prediction of
tumor recurrence and therapy monitoring using ultrasound-guided
photoacoustic imaging, Theranostics 5 (3) (2015) 289–301.
[3] K. Pu, J. Mei, J.V. Jokerst, G. Hong, A.L. Antaris, N. Chattopadhyay, A.J.
Shuhendler, T. Kurosawa, Y. Zhou, S.S. Gambhir, Diketopyrrolopyrrole-Based
semiconducting polymer nanoparticles for In vivo photoacoustic imaging, Adv.
Mater. 27 (5) (2015) 1984–1990.
[4] K.J. Cash, C. Li, J. Xia, L.V. Wang, H.A. Clark, Optical drug monitoring:
photoacoustic imaging of nanosensors to monitor therapeutic lithium in vivo,
ACS Nano 9 (2) (2015) 1692–1698.
[5] S. Mallick, T. Larson, J. Tam, P.P. Joshi, A. Karpiousk, K. Sokolov, S. Emelianov,
Multiwavelength photoacoustic imaging and plasmon resonance coupling of
gold nanoparticles for selective detection of cancer, Nano Lett. 9 (8) (2009)
2825–2831.
[6] A. De La Zerda, C. Zavaleta, S. Keren, S. Vaithilingam, S. Bodapati, Z. Liu, L. Levi,
B. R. Smith, T.-J. Ma, O. Oralkan, Carbon nanotubes as photoacoustic molecular
imaging agents in living mice. Nat. Nanotechnol. 3 (9) (2008) 557–562.
[7] M.L. James, S.S. Gambhir, A molecular imaging primer: modalities, imaging
agents, and applications, Physiol. Rev. 92 (2) (2012) 897–965.
[8] J. Riefel, F. Chen, J. Kim, G. Chen, W. Shao, S. Shao, U. Chitgupi, R. Hernandez,
S. A. Graves, R.J. Nickles, Hexamodal imaging with porphyrinophospholipid-
crated upconversion nanoparticles, Adv. Mater. 27 (10) (2015) 1785–1790.
[9] S.-R. Kothapalli, T.-J. Ma, S. Vaithilingam, O. Oralkan, B.T. Khuri-Yakub, S.S.
Gambhir, Deep tissue photoacoustic imaging using a miniaturized 2-D
capacitive micromachined ultrasonic transducer array, IEEE Trans. Biomed.
Eng. 59 (5) (2012) 1199–1204.
[10] M. Pramanik, M. Swierczewska, D. Green, B. Sitharaman, L.V. Wang, Single-
walled carbon nanotubes as a multimodal-thermooptical and photoacoustic-
contrast agent, J. Biomed. Opt. 14 (3) (2009) 034018-034018-8.
[11] M.S. Mohamed, A.C. Pouloue, S. Veeraranarayanan, R.R. Abuerto, T. Mitcham, Y.
Suzuki, Y. Sakamoto, P.M. Ajayan, R.R. Bouchard, Y. Yoshida, Plasmonic fluorescent
CdSe/Cu 2S hybrid nanocrystals for multichannel imaging and
cancer directed photo-thermal therapy, Nanoscale 8 (15) (2016) 7876–7888.
[12] R.J. Papkoski, A. Forbrich, E. Huyoh, J. Chen, J.D. Lewis, G. Zheng, R.J. Zemp,
Porphyrin nanostructures: sub-micrometer ultrasound and photoacoustic
contrast imaging agents, Small 12 (3) (2016) 371–380.
[13] E. Zhang, J. Lauder, P. Beard, Backward-mode multil wavelength photoacoustic
scanner using a planar Fabry-Perot polymer film ultrasound sensor for high-
resolution three-dimensional imaging of biological tissues, Appl. Opt. 47 (4)
(2008) 561–577.
[14] C. Chen, F. Klaempfl, C. Knipfer, M. Riemann, R. Kanawade, F. Stelzle, M. Schmidt,
Preparation of a skin equivalent phantom with interior micron-scale vessel
structures for optical imaging experiments, Biomed. Opt. Express. 5 (9) (2014)
3140–3149.
[15] S.E. Bohndiek, S. Bodapati, D. Van De Sompel, S.-R. Kothapalli, S.S. Gambhir,
Development and application of stable phantoms for the evaluation of
photoacoustic imaging instruments, PLoS One 8 (9) (2013) e75533.
[16] W.C. Vogt, C. Jia, K.A. Bear, B.S. Garra, T.J. Pfefer, Biologically relevant photoacoustic imaging phantoms with tunable optical and acoustic properties, J. Biomed. Opt. 21 (10) (2016) 101405–101405.

[17] M. Fonseca, R. Zegers, P. Beard, R. Cox, Characterization of a PVC-based tissue-mimicking phantom for quantitative photoacoustic imaging, European Conferences on Biomedical Optics, International Society for Optics and Photonics (2015) pp. 953911–953911-9.

[18] Y.-S. Chen, W. Frey, S. Kim, P. Kruijzinga, K. Homan, S. Emelianov, Silica-coated gold nanorods as photoacoustic signal nanoamplifiers, Nano Lett. 11 (2) (2011) 348–354.

[19] A.B. Kargioui, S.R. Aghayamov, S. Mallidi, J. Shah, W.G. Scott, J.M. Rubin, S.Y. Emelianov, Combined ultrasound and photoacoustic imaging to detect and stage deep vein thrombosis: phantom and ex vivo studies, J. Biomed. Opt. 13 (5) (2008) 054061-054061-8.

[20] L. Ding, X.L. Deln-Ben, C. Lutzweiler, D. Razansky, V. Ntziachristos, Efficient non-negative constrained model-based inversion in optoacoustic tomography, Phys. Med. Biol. 60 (17) (2015) 6733.

[21] C. Avigo, N. Di Lascio, P. Armanetti, C. Rusmich, L. Cavigl, F. Ratto, S. Meucci, C. Masciullo, M. Cecchini, R. Pini, Organosilicon phantom for photoacoustic imaging, J. Biomed. Opt. 20 (4) (2015) 046008–046008.

[22] J. Levi, S.-R. Kothapalli, S. Bohndiek, J.-K. Yoon, A. Dragulescu-Andrasi, C. Nielsen, A. Tisma, S. Bodapati, G. Gowrishankar, X. Yan, Molecular photoacoustic imaging of follicular thyroid carcinoma, Clin. Cancer Res. 19 (6) (2013) 1494–1502.

[23] The Speed and Attenuation of Sound, (2015) (Accessed 11 January 2017).

[24] J.V. Jekjohn, A.J. Cole, D. Van de Sompele, S.S. Gamblir, Gold nanorods for ovarian cancer detection with photoacoustic imaging and resection guidance via Raman imaging in living mice, ACS Nano 6 (11) (2012) 10366–10377.

[25] A. Needles, A. Heinmiller, P. Ephrat, C. Bilan-Tracey, A. Trujillo, C. Theodoropoulos, D. Hixon, F. Foster, Development of a combined photoacoustic micro-ultrasound system for estimating blood oxygenation, IEEE Int. Ultrason. Symp. IEEE 2010 (2010) 390–393.

[26] M.D. Abrámoff, P.J. Magalhães, S.J. Ram, Image processing with ImageJ, Biophotonics Int. 11 (7) (2004) 36–42.

[27] MakerBot Replicator 2.

[28] J.R. Cook, R.R. Bouchard, S.Y. Emelianov, Tissue-mimicking phantoms for photoacoustic and ultrasonic imaging, Biomed. Opt. Exp. 2 (11) (2011) 3193–3205.

[29] A. Nikoozadeh, L.W. Choe, S.-R. Kothapalli, A. Moini, S.S. Sanjani, A. Kamaya, Oralkan O, S.S. Gamblir, P.T. Khuri-Yakub, Photoacoustic imaging using a 9F microLinear CMUT ICE catheter, IEEE Int. Ultrason. Symp. IEEE 2012 (2012) 24–27.

[30] M.J. Burfeindt, T.J. Colgan, R.O. Mays, J.D. Shean, N. Behdad, B.D. Van Veen, S.C. Hagnes, MRI-derived 3-D-printed breast phantom for microwave breast imaging validation, IEEE Antennas Wireless Propagation Lett. 11 (2012) 1610–1613.

[31] J. Wang, J. Coburn, C.-P. Liang, N. Woolsey, J.C. Ramella-Roman, Y. Chen, T.J. Pfefer, Three-dimensional printing of tissue phantoms for biophotonic imaging, Opt. Lett. 39 (10) (2014) 3010–3013.

[32] N. Parker, M. Marhef, S. Morgan, P. Povey, Longitudinal acoustic properties of poly(lactic acid) and poly(lactic-co-glycolic acid), Biomed. Mater. 5 (5) (2010) 055004.

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