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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.

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

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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 agarose 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 polylactic 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 ms^{-1}, which depends on its density, whereas the speed of sound in Teflon is 1400 ms^{-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 um 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 (ThermoFisher, 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 x 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/yago1994/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/cm^2 [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.