A Micro-flow Phantom for Superficial Micro-vasculature Imaging

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Abstract. Visualization of cutaneous micro-vasculatures is a powerful approach assisting in the diagnosis of skin vascular disorders. These minute structures can be visualized by high-frequency ultrasound (HFUS) using ultrafast Doppler imaging. Ultrasound flow phantoms have been used as assessment tools to evaluate the performance of the ultrasound imaging system, however, to optimize the imaging system for visualization of micro-structures, flow phantom with micro-channels is required which are usually difficult to fabricate. Here, we design a simple approach for micro-flow phantom which is easy to fabricate and cast for detection of micro-circulation in superficial micro-structures. The proposed approach features (i) the micro-channels of 200-micron at the depth of 4 mm (ii) casted in the cryogel mixture of Poly-vinyl alcohol (PVA) and (iii) infused at flow speed of 30 mm/s using infusion pump. Visualization of micro-flow channel in power Doppler image obtained by HFUS ultrafast Doppler imaging reveals that the proposed micro-flow phantom could serve as a viable assessment tool for optimizing the system for in-vivo cutaneous micro-vasculature imaging.

1. Introduction

Micro-vasculatures are important constituents of blood circulatory system [1,2]. Examination of these micro-structures play a significant role in the diagnosis of vascular abnormalities. Cutaneous micro-vasculatures are superficial micro-vessels found in the dermis layer of the skin, at the depth of 1-4 mm [3]. In cutaneous imaging, high-frequency ultrasound (HFUS) has gained considerable importance to image the morphological structures and associated disorders. However, to image the micro-structures by HFUS has been quite challenging due to their minute dimensions [4]. To overcome this limitation, HFUS along with ultrafast ultrasound imaging can be used to detect the micro-circulation in microstructure because of its ultrafast signal acquisition which ultimately enhances the Doppler sensitivity to visualize the micro-structures [5]. Ultrafast ultrasound imaging is one of the most promising technique currently being used in the visualization of micro-structures in different applications of ultrasound imaging because of its higher spatio-temporal resolvability [6,7].

In Ultrasound imaging, phantoms are commonly known as assessment tools used to optimize and qualify the imaging systems; and to verify whether their imaging performance meets quality standards [8]. Doppler flow phantoms of various models have been developed for different applications to evaluate the imaging performance of the ultrasound system [9,10,11]. However, for microflow imaging, specific micro-flow phantoms are needed to perform both the experimental optimizations as well as performance evaluation of micro-vasculature flow imaging system. Several intensive approaches have been conducted to design and cast the micro vascularization phantoms in which micro tubes were
encapsulated in the tissue mimicking material (TMM) [12]. Albeit some studies used dialysis cartridge containing large number of cellulose capillaries as micro-phantoms [13], but the cartridge gets porous after certain period and can only visualize the top cellulose capillary structure.

New research studies demonstrated that the usage of microfluidic chips appears to be a propitious solution to create microvasculature phantom which mimics the complex microvascular networks [14,15]. As microfluidic chips are based on polydimethylsiloxane (PDMS) material, they encounter two major issues: (i) the speed of the sound in PDMS is 1076 m/s while speed of sound in human tissue is 1540 m/s (ii) the highly absorbent material nature which limits the depth penetration. On the other hand, PDMS based devices are difficult to fabricate due to complex procedures involving photolithography.

Recently, Grand-Perret et al., [8] developed a microflow phantom using strings of different diameters as straight channels and casting with soft silicon material. The developed micro phantom was interfaced with micro-fluidic flow control system to pass micro-flow.

Here, we report a simple approach for fabrication and casting of the micro-flow phantom, hereby overcome the technical drawbacks of previous solutions that involve complex procedures. We have used poly-vinyl (PVA) cryogel mixture as TMM instead of soft silicon material, by optimizing the protocol by Ho et al., [9]. The usage of PVA cryogel mixture as TMM makes micro-phantom more realistic to human tissue as acoustic properties of PVA are quite similar to human tissue. The geometrical design of the flow channel is straight, instead of using strings we have developed a different method to cast micro-flow phantom.

This research study aims to design an easy to fabricate and cast micro-phantom circumventing the challenges of complex procedures of photolithography and other protocols. The proposed design approach of micro-structure encapsulated in TMM is at the depth of 1–5 mm, thereby enabling the probability to visualize cutaneous micro-vessels in in-vivo at the depth of 1–4 mm in the dermis layer of skin. To infuse the micro-flow in micro-channel here, we present an efficient and effective workflow circuit assembly infusing the micro-flow with the help of infusion pump. Finally, the straight tilted micro channel in micro-phantom is visualized by performing ultrafast power Doppler ultrasound imaging. Taken together, the proposed approach is expected to assist in optimizing and developing the imaging systems for visualization of superficial microvascular structures. We anticipate that this method will find broader applications in vascular ultrasound microflow imaging.

2. Material and Methods
A Doppler micro-flow phantom with flow channel of 200-micron in diameter was designed and fabricated to visualize the superficial micro-flow tract, by HFUS ultrafast Doppler imaging at the depth of 1-5 mm. Detailed description about the designing and casting of micro-phantom is described in the subsequent sections.

2.1. Micro-flow phantom
A flow tract of micro-dimension was created by using a simple approach which includes a wire and IV catheters. The wire was encapsulated in the PVA cryogel mixture which mimic the elasticity of the human tissue.

2.1.1. Micro-phantom Design Approach.
The design approach for fabrication and casting of micro-flow phantom involves the following steps, First the phantom casting case was designed in a CAD software (SolidWorks, Dassault System SolidWorks Corp.) and printed by a 3D printer (Tiertime UP 300) by using polylactic acid (PLA) filament (PolyLite™). In the 3D printed phantom case, appropriate holes of 2 mm diameter were printed on both sides at the depth of 3.5 mm from the surface of the case.

After printing holes in the casting case, an intravenous ethylene tetrafluoroethylene (ETFE) catheters of 26 G dimension were placed through the holes and tightly fixed at both sides of the phantom case. A copper wire of 200-micron diameter used as the straight channel which is passed from the catheters on both sides and suspended in the phantom case by tightly fixing the wire with adhesive tape as shown in
Figure 1(a). Figure 1(b) is an advanced version of the design approach in which instead of straight channel, a slightly tilted channel was devised for better processing of micro-flow Doppler signal.

2.1.2. Fabrication and casting of tissue mimicking material (TMM).

Once the phantom casting case was prepared along with suspended micro-wire, the tissue mimicking material was fabricated and cast by following the published protocol from [9]. The materials for the cryogel mixture of Poly-vinyl alcohol (PVA) were comprised of 10 wt% of PVA powder (341584; Sigma-Aldrich) and 3 wt% of graphite particles (282863; Sigma-Aldrich).

![Diagram](image1)

**Figure 1.** Design approach of micro-phantom. IV catheters (Pink) were placed through the holes and tightly fixed on both sides of casting case. Copper wire (Blue) passed from catheters and suspended in the casting case. (a). Straight channel of 200-micron diameter. (b). Slightly tilted straight channel of 200-micron diameter.

Before following the casting procedure, the IV catheter on respective sides of phantom casting case filled with PVA cryogel mixture with the help of syringe to fill the gap between the IV catheter and the wire to eliminate the chances of any blockage in the micro-flow channel after casting procedure. Figure 2 shows the stepwise procedure of micro-phantom fabrication. After two respective freeze and thaw cycles, 200 micron-wire from the obtain micro-phantom PVA slab was eject out with respective attached catheter and later the PVA slab was removed from the phantom casting box. In this manner the micro-scale flow channel in PVA phantom was designed and prepared for micro-scale micro-flow imaging.

For perfusion of micro-flow, we used blood mimicking fluid (BMF) consist of small particles which mimics the blood scatterers. The BMF used [11] was composed of 10 wt% of glycerol and 5 wt% of silica spheres (Average diameter: 8-13 micrometer, Godd ball B-25C, Suzuki Yushi Corp.)

2.2. Experimental Setup

2.2.1. Flow setup.

Following by casting of micro-phantom of flow channel of 200 micron in diameter, the PVA micro-phantom slab was connected with the flow circuit by inserting the IV catheters again on respective side of the micro-flow phantom. The KD scientific legato 200-series infusion pump was used to infuse the BMF in the micro-flow channel. 20 ml syringe was used as infusion volume to set the flow rate of 56 μl/min at flow speed of 30 mm/s in 200-micron diameter of micro flow channel. To connect the infusion pump with the micro-phantom, we used an appropriate and effective flow circuit assembly which connects all the parts of the flow setup tightly to eliminate the chances of leakages in the micro-flow
setup as shown in Figure 3. It is comprised of lure connectors of 1.5 mm inner diameter and plastic tubes of 1.5 mm inner diameter whose arrangement are also shown in Figure 3.

![Figure 2](image)

**Figure 2.** Fabrication and casting of micro-flow phantom (a). Phantom casting case with slightly tilted micro-channel (b). Casting of micro-flow phantom by using Poly-vinyl cryogel mixture (c). micro-flow phantom PVA slab after process of casting (d). Obtained micro-flow phantom with appropriate inlet to connect with micro-flow setup.

![Figure 3](image)

**Figure 3.** Micro-flow setup with appropriate flow circuit connections including the infusion pump to infuse the micro-flow in micro-flow channel.
2.2.2. Imaging System.
For imaging experiments, to visualize the micro-flow channel in the micro-phantom, HFUS ultrafast compound Doppler imaging was performed by using a research ultrasound platform (Vantage 256 high-frequency configuration; Verasonics Inc., Kirkland, WA, USA) which is equipped with a L38-22v CMUT linear array transducer (KOLO silicon, USA). For transmission and reception of ultrasound pulses 128 channels of array transducer were used at centre frequency of 30 MHz. In ultrafast compound imaging seven plane waves were transmitted at compounding angles (-1 degree to 1 degree) for acquiring IQ signal from the micro-phantom. At determined flow rate setting, the acquired beamformed images at various steering angles were summed up to create the compounded images. Note that the Vantage system has limited sampling rate of 62.5 MHz that is insufficient for acquiring high-frequency signals. Instead, we used the approach of bandwidth sampling [16] with a controlled sampling rate of 41.6 MHz. Obtained compounded images at pulse repetition frequency of 7000 Hz were processed to extract the micro-flow signal by implementing a singular value decomposition (SVD) as a clutter filter to extract the slow-time signal from the micro-channel. In the extraction of the signal, time threshold value was set to detect the slow-time signal efficiently. Finally, the power Doppler image of micro-flow channel was obtained after power Doppler computation.

3. Results and Discussion
The HFUS ultrafast B-mode image of micro-phantom shown in Figure 4 clearly visualize the IV catheters in Figure 4 (a) and Figure 4 (c) as brighter object which indicates the inlet and outlet of micro-phantom. However, the micro-flow channel in Figure 4 (b) is not clear due to the micro-dimension of the micro channel. On the other hand, the power Doppler image shown in Figure 5 clearly illustrate the visualization of micro-flow tract by mapping the micro-flow in the channel. The high intensity signal of flow acquired with ultrafast Doppler technique assists in the visualization of flow tract at depth of 4 mm in micro-phantom.

![Figure 4](image_url).

**Figure 4.** Ultrafast Compound B-mode image of devised micro-flow phantom with appropriate IV catheters as inlet and outlet in the phantom.

The obtained micro-phantom images by HFUS ultrafast Doppler imaging shown that the devised approach of micro-phantom fabrication and casting can be used to cast the micro-channel of any diameter in a simple and easy manner. Also, the PVA cryogel mixture as TMM used in this study has acoustic compatibility within physiological range as well as stable enough to preserve for long duration of time. The devised approach could be the feasible procedure in the fabrication of micro-flow phantoms for superficial ultrasound imaging.

4. Conclusion
In this research study, we report the development of a simple approach for micro-flow phantom which is easy to fabricate and cast without following any complex procedures. The designed approach is based on micro-channel of 200-micron at the depth of 1-5 mm embedded in PVA cryogel mixture with proper
inlet and outlet of IV catheter to precisely connect with the flow circuit. Obtained B-mode and power Doppler images from the devised micro-phantom revealed that it can be used as an assessment tool for optimizing the ultrasound systems for visualization of superficial micro-structures in much better manner. The devised approach can be further improved to make it more realistic to complex vascular network by designing the micro-channels of complex geometries.

![Micro-flow channel](image)

**Figure 5.** Ultrafast Compound Doppler image of micro-flow phantom with micro-channel of 200-micron.

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