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3D Printed Calibration Micro-Phantoms for Super-Resolution Ultrasound Imaging Validation

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Abstract

This study evaluates the use of 3D printed phantoms for 3D super-resolution ultrasound imaging (SRI) algorithm calibration. The main benefit of the presented method is the ability to do absolute 3D micro-positioning of sub-wavelength sized ultrasound scatterers in a material having a speed of sound comparable to that of tissue. Stereolithography is used for 3D printing soft material calibration micro-phantoms containing eight randomly placed scatterers of nominal size 205 $\mu$m $\times$ 205 $\mu$m $\times$ 200 $\mu$m. The backscattered pressure spatial distribution is evaluated to show similar distributions from micro-bubbles as the 3D printed scatterers. The printed structures are found through optical validation to expand linearly in all three dimensions by 2.6% after printing. SRI algorithm calibration is demonstrated by imaging a phantom using a $\lambda$/2 pitch 3 MHz 62$\times$62 row-column addressed (RCA) ultrasound probe. The printed scatterers will act as point targets, as their dimensions are below the diffraction limit of the ultrasound system used. Two sets of 640 volumes containing the phantom features are imaged, with an intervolume uni-axial movement of the phantom of 12.5 $\mu$m, to emulate a flow velocity of 2 mm/s at a frame rate of 160 Hz. The ultrasound signal is passed to a super-resolution pipeline to localise the positions of the scatterers and track them across the 640 volumes. After compensating for the phantom expansion, a scaling of 0.989 is found between the distance between the eight scatterers calculated from the ultrasound data and the designed distances. The standard deviation of the variation in the scatterer positions along each track is used as an estimate of the precision of the super-resolution algorithm, and is expected to be between the two limiting estimates of $(\tilde{\sigma}_x, \tilde{\sigma}_y, \tilde{\sigma}_z) = (22.7$ $\mu$m, 27.6 $\mu$m, 9.7 $\mu$m) and $(\hat{\sigma}_x, \hat{\sigma}_y, \hat{\sigma}_z) = (18.7$ $\mu$m, 19.3 $\mu$m, 8.9 $\mu$m). In conclusion, this study demonstrates the use of 3D printed phantoms for determining the accuracy and precision of volumetric super-resolution algorithms.

Keywords: 3D printing, stereolithography, phantom, hydrogel, calibration, resolution, ultrasound

1. Introduction

Super-resolution ultrasound imaging (SRI) has recently emerged as a non-invasive technique, which enables imaging of the smallest vessels of the vasculature [1, 2, 3]. Micrometer sized gas filled bubbles provide high contrast in ultrasound imaging, and their path through the vasculature can be tracked over time to reveal the fine details of the vascular network. The conventional B-mode ultrasound images are replaced by cumulated maps of the super-localised centroids of the micro-bubbles, revealing vascular features which are much smaller than the diffraction limit of conventional ultrasound. However, a fundamental problem is to validate the spatial accuracy of these new techniques. Biological structures often have extremely complex geometries, with the added complications of liquid flow and tissue motion. Phantoms with well controlled dimensions are used for validation instead. As new imaging techniques are introduced, they are typically tested initially against numerically simulated data. This data could for instance be generated in Field II [4, 5, 6]. The next step would typically be to test the imaging techniques using phantoms, which have been adapted to suit the techniques. In the case of SRI, in which the end goal is to image vasculature on the scale of only a few tens of micrometers, the precision, the accuracy, and the repeatability of the phantom fabrication method all need to be improved. At the same time, it should ideally be possible to replicate the dimensionality of the vascular networks. Viessmann et al. [7] and Christensen-Jeffries et al. [8] employed tube phantoms of 3 mm and 200 $\mu$m diameters, respectively, to validate their SRI algorithms. Both of these are significantly larger than the vessels of interest, i.e. arterioles and venules with sub-100 $\mu$m dimensions and capillaries of 5-9 $\mu$m diameters [9]. Desailly et al. presented a phantom study in which the channel dimensions were reduced
to 40 × 80 µm² by utilizing the high resolution of silicon micro-fabrication UV lithography in polydimethylsiloxane (PDMS) [10]. While the latter dimensions of the channel were comparable to the sizes of capillaries, the ability to expand the phantom types to three dimensions are severely limited in all cases. A completely different approach uses the vasculature of chicken embryos, which is optically visible [11]. While the features are on the correct scale, and high resolution optical images can be taken, it is impossible to obtain a three-dimensional representation of the vascular network using commonly available optical microscopes. It should be noted that this is not a limitation of the chicken embryo model itself, since this will feature complex three-dimensional structures. But the characterisation of those networks is very complex, and not possible using regular optical microscopes. Optical mapping of the structures could be performed with other more complex methods such as optical coherence tomography [12]. All of the above mentioned methods are channel based, and thereby meant to provide an outer limit for the localization of the micro-bubbles which are tracked. But that leaves the inherent problem that it is not possible to control the position of the micro-bubbles within the tubes or vessels, and therefore, the source of the signal will not be precisely known. Apart from these presented examples of phantom studies, often no validation is performed, but inference of performance based on in vivo data needs to be made.

3D printing of phantoms is a promising new approach, which does not suffer from these limitations. It provides complete three-dimensional flexibility in fabrication and can replicate features in the sub-100 µm range [13]. Recently, 3D printed phantoms for ultrasound were demonstrated by Jacquet et al. [14], supposedly not with SRI in mind. The phantoms contained highly scattering solid features as small as 30 × 50 µm² in cross section, demonstrating the exciting potential for point spread function evaluation provided by the method, as well as other possibilities for phantom features and uses. Recently, we presented an alternative 3D printing method for phantom fabrication, namely stereolithography. The method is used to print hydrogels, a soft material with acoustic properties similar to tissue, and demonstrated that it is possible to obtain cavities of 100 × 100 × 100 µm³ and channels with a cross-section of 200 × 200 μm² in a hydrogel, which are suitable for SRI ultrasound [15]. These were used for 2D SRI experimentation. The same technique was used to print channel phantoms, which have been imaged using a row-column addressed (RCA) array to super-localize micro-bubbles in 3D [16]. The phantom consisted of a 200 µm diameter channel, and the super-resolved micro-bubble positions were determined in 3D using an RCA array. Through statistical analysis of the radial distribution of the micro-bubbles around the center line of the channel, the precision of the SRI system was determined to be less than 23 µm in all dimensions, which approaches the scale of the smallest vessels in tissue. In this case, the statistical analysis was used to mitigate the problem of not knowing the exact position of the micro-bubbles, since that of course is still a problem when using 3D printed channel based micro-phantoms.

A 3D printing solution will in principle allow for arbitrarily complex 3D structures or channel networks to be made. However, the presented work demonstrates an alternative to localisation of micro-bubbles in channel systems for SRI algorithm calibration, which allows for elimination of the issue of the uncertainty in the micro-bubble positions within micro-channels. It is based on [15], which also demonstrated that small 3D printed cavities will scatter sound. By keeping them smaller than the ultrasound wavelength, they can be used as point targets to evaluate imaging performance for regular B-mode imaging. Thereby, it becomes possible to fixate small scatterers at very precise locations within the hydrogel. These structures will be stable in time, enabling repeated imaging, in direct contrast to small channels and micro-bubbles.

The scatterers do not provide the exact same ultrasound response as conventional micro-bubbles. It should be noted that many different contrast sequences are utilised in SRI today, and each of them will result in different micro-bubble responses. Yet, all of them provide isolated point-like structures in the ultrasound images, of which the centroids can be determined for creation of the SRI images. While the scatterers can not be utilized for optimisation of the micro-bubble response, they provide a scattering structure which is stable in time and space, and can therefore be used to validate the rest of the SRI processing chain.

In this paper, we demonstrate how these stable, fixated scatterers can be used as an alternative to conventional tube phantoms to determine the accuracy and precision of SRI hardware and algorithms independently in all three dimensions. The 3D printing method allows for absolute positioning accuracy and precision unparalleled by other types of phantom fabrication methods. It is also shown how the high degree of control of phantom features using this method can illuminate additional problems in a SRI pipeline, such as distortion. The phantoms can potentially be used to demonstrate local variations in the SRI properties based on the scatterer position within the localization field of view.

2. Materials and Methods

This section describes the phantom fabrication method, and the details of the phantoms which have been designed for these experiments. Additionally, the experimental procedures are described, as well as the SRI pipeline structure.

2.1. Fabrication of the phantoms

Calibration phantoms were fabricated by stereolithographic 3D printing of an aqueous solution of poly(ethylene glycol) diacrylate (PEGDA, Mn 700 g/mol, 455008, Sigma-Aldrich) to form a hydrogel solid. Stereolithography is
based on printing a stack of individual thin layers of materials, calling for prior digital slicing of the targeted 3D design into separate layer designs matching the printing system. The method and components have previously been presented in more detail [15]. Each layer is printed by spatially confined illumination of the targeted solid areas, which leads to localized photochemically induced solidification of the printing solution. A custom-designed stereolithographic printer that projects a full image of the current layer was used. Each layer image is a one-to-one projection of a digital image generated on a Digital Mirror Device (DMD, DLP9500UV, Texas Instruments, TX) with a center-to-center pixel spacing of 10.8 µm in both lateral dimensions. Thus, there will inherently be a physical mapping of the targeted phantom design layers onto a square grid of 10.8 µm spacing. Phantom shapes were generated directly as a series of layer images matching the DMD pixel pitch using a MATLAB (MathWorks, MA) script and with a layer thickness of 20 µm. The aqueous printing solution contained 20% (weight by volume) PEGDA as pre-polymer, 5 mg/mL LAP (lithium phenyl-2,4,6-trimethylbenzoylphosphinate, 900889, Sigma-Aldrich) as photoinitiator, and 12 mg/mL Quinoline Yellow (309052, Sigma-Aldrich) as absorber. Each patterned layer motif was illuminated with 365 nm light at an intensity of 20 mW/cm² for 3 seconds (in the bulk of the phantom) to 23 seconds (locally on the scatterer perimeter), depending on the features being printed. The phantoms were printed on 22 × 22 × 0.40 mm³ cover glasses (MENZ-ZDA022022AHE0, Menzel Gläser, DE) pretreated with (3-glycidyloxypropyl)trimethoxysilane (440167, Sigma-Aldrich) to enhance the adhesion to the printed PEGDA. The resulting printed structures are not in equilibrium with water directly after printing, but will swell slightly when subsequently transferred to water. Previous work showed that after four hours, the printed structure reaches its equilibrium swelling [17]. However, the time to equilibrium is likely dependent on the dimensions of the printed structure and the dimensions of the phantoms used in this work are larger than those used to conduct the swelling experiment. The test structures measured 10 × 10 × 3.5 mm³, and the phantoms used in this work measured 21.1 × 11.9 × 11.9 mm³. However, the phantoms were in all cases left in water at least overnight, and often more than a full day, before being used for experimentation, which should be sufficient to reach equilibrium.

Acoustic parameters of a hydrogel solid with a layer thickness of 20 µm, the rear of the hydrogel sample. 17 different samples were measured, with the analysed signals having been averaged from 32 emissions. The average speed of sound was found to be 1577 m/s, with a standard deviation of 14 m/s. The speed of sound correlates well to the speed of sound found in selected typical human tissues [18], such as vessels. The 3D printed acoustic setup used for determining speed of sound was also used to determine attenuation, by mounting two transducers with the hydrogel sample placed in between. The attenuation was fitted across four samples of different thicknesses to eliminate surface reflection losses. It was fitted as a power law model given by μ = a · f^b, where f is the ultrasound frequency in MHz, a is the attenuation coefficient at 1 MHz and b describes the degree of nonlinearity of the dependence on frequency [19]. This fit was made between 2 and 9 MHz, limited by the transducer bandwidth. The parameters were determined as a = 0.15 dB/[MHz cm] and b = 1.5. As the phantom is submerged in water during experimentation, the speed of sound in the entire imaged volume does not match perfectly. Beamforming compensation for different speeds of sound is a complex matter as illustrated in [20]. For this experiment, we have chosen to approximate the entire volume as having a speed of sound equal to that of water at room temperature (1480 m/s). An optimal compromise in the choice of speed of sound might exist, minimising any potential errors due to the mismatch between water and hydrogel. It is important to note that while this could potentially be employed to reduce errors in receive, it is not possible to adjust the speed of sound assumptions in the transmit signals after the experiment.

The foundation for all experiments in this work is a phantom containing eight randomly placed scatterers. The outer dimensions of the phantom is 21.1 × 11.9 × 11.9 mm³ with each scatterer being 205 × 205 × 200 µm³. While the printing setup allows for printing significantly smaller scatterers, it was necessary with an increased size to obtain reflections with intensities larger than background scattering due to unavoidable small random print errors in the phantom. The scatterers will function as point targets in regular B-mode volumes, when the imaging wavelength is larger than the scatterer size, in this case for any frequency below 6 MHz. They were positioned with a minimum separation distance of 3 mm, which will eliminate overlapping signals for any frequency above 0.5 MHz. The designed layout is shown in Fig. 1, in which the blue points represents the randomly positioned scatterers. Separate droplines lead from the points out along the y-axis and along the z-axis respectively. The droplines end up 1 mm from the respective surfaces in the collapsed x-y-plane version (red) and the collapsed x-z-plane version (turquoise) of the scatterers.

Hollow structures will initially be filled with unpolymerized printing solution during printing. When submerged in water, this will over time partly be replaced by water since the hydrogel is diffusion open to water. The cavities referred to in this work are only cavities in terms of not containing solid PEGDA hydrogel.

The printed phantoms should be stored in water. When
Figure 1: The designed layout of the scatterers within the $\sim 21.1 \times 11.9 \times 11.9$ mm$^3$ phantom. The blue points are the randomly positioned scatterers. The droplines are included to aid the 3D perception of the scatterer placement. For the optical correlation experiment, one set of phantoms had the scatterers collapsed into the $x$-$y$-plane near the top surface (red), and the other set had the scatterers collapsed into the $x$-$z$-plane near the side (turquoise).
exposed to air, the absorbed water starts evaporating resulting in shrinkage of the phantom. After a few hours, the shrinkage will result in breaking of the phantom. However, when stored in water, there has been no indication of phantom destruction. The phantom lifespan has been observed to be more than a month.

2.2. Back-scatter analysis

The scatterers in this phantom differ from standard micro-bubbles used in vivo in terms of materials and geometry, which could produce differing backscatter characteristics. While the phantom scatterer has similar acoustic impedance inside the feature (water) as the surrounding medium (PEGDA), micro-bubbles have considerably lower acoustic impedance inside (gas) than outside (blood plasma). The more extreme acoustic impedance ratio of the micro-bubbles would tend to produce a stronger backscatter reflection than the phantom scatterers. On the other hand, micro-bubbles are typically much smaller than these phantom scatterers, which would tend to make the scattered pressure from the micro-bubbles much lower. In addition, the smaller diameter would typically produce a more spatially-uniform scattering pattern across a wider frequency range.

The Anderson model [21] was employed to understand the competing relationships between material and geometry differences in these two scenarios. This model has been experimentally validated for micron-sized fluid objects [22]. Both the scattered pressure spatial distribution and the axial (0-degree) backscatter magnitude were evaluated across the frequency range relevant to this study. The micro-bubbles were approximated as air-filled spheres suspended in water with radii of 1.5, 2.5, and 4.5 µm, representing the range of bubble sizes, which have been found to provide the majority of the scattered signal from SonoVue [23]. The phantom scatterer was approximated as a water-filled sphere with 100µm radius suspended in a fluid with the density and bulk sound speed of the PEGDA phantom. For comparison, an additional phantom scatterer with smaller radius (50 µm) was also included. Although commercial micro-bubbles are typically encapsulated with a shell, the influence of the shell was excluded from this initial model. The shell effect depends on the particular shell material, and some shells even produce characteristics similar to the “free bubble” modelled by the Anderson model [24, 25].

The results are plotted in Fig. 2. Fig. 2(a)-(d) are polar plots of the scattered pressure spatial distribution. These illustrate how the scatterers produce similar reflected pressure distributions as the micro-bubbles across ±60 degrees, which is an angular range relevant for the imaging algorithm under development. Only the backscattered part of the signal is included in the figures since the signal needs to be received by the transducer again. All micro-bubbles had uniform spatial distribution because they were much smaller than a wavelength, while the phantom scatterers exhibited some spatial non-uniformity. The greatest deviation at 4 MHz for 60 degrees was less than 2.5 dB for the 100 µm radius scatterer and less than 0.5 dB for the 50 µm radius scatterer. Above 4 MHz, however, the 100 µm radius scatterer produced an increasingly non-uniform spatial distribution. For instance, the difference at 5 MHz for 60 degrees was nearly 8 dB, which could cause a significant off-axis enhancement artifact. Accordingly, 4 MHz was considered the upper frequency limit to retain reasonably uniform backscattered pressure from this 100 µm scatterer.

The 0-degree backscatter magnitude spectrum also showed similarity between the micro-bubble and the 100 µm radius scatterer. Unlike the micro-bubbles, this scatterer did not show strong resonance characteristics, but its magnitude was similar to the off-resonance reflection magnitude of the micro-bubbles. Between 2 and 4 MHz, the backscattered pressure differed from the nominal 2.5 µm micro-bubble by less than 6 dB. While these differences must be considered when analysing the SNR limitations of the algorithms (for instance, when estimating penetration depth), they are small enough to consider the scattered signal as a good approximation of the micro-bubble for the purposes of algorithm development. The 50 µm radius scatterer showed similar backscatter magnitude to the micro-bubbles at the higher frequencies but was 18 dB weaker than the 2.5 µm micro-bubble at 2 MHz. The similarity between the 100 µm radius scatterer and the micro-bubble models between 2 and 4 MHz supports the use of the phantom scatterers as they were designed and fabricated for use in this study. Studies at higher frequencies may require smaller phantom scatterers to maintain acceptable spatial distribution and reflection magnitude similarity to micro-bubbles.

2.3. Experimental setup and procedure

2.3.1. Optical validation of phantoms

The phantom fabrication method accuracy should be verified by another characterisation method. Although the printer specifications have been stated, they only specify the lower limit of the attainable feature sizes and accuracies. Furthermore, the phantom expansion due to post-printing swelling needs to be determined to compensate the designed feature sizes before using the phantom as a calibration tool. Optical characterisation using an optical microscope can be used to locate phantom features with high precision. Unfortunately, the printed hydrogel scatters light, rendering it impossible to use the base phantom with the eight randomly placed scatterers, since these are placed too far inside the phantom. Instead, the same coordinates were used to make two new phantoms, in which the coordinates were collapsed either into the x-y-plane and placed near the top of the phantom (red in Fig. 1), or into the x-z-plane and placed near the side of the phantom (turquoise in Fig. 1). By placing them near the surfaces, the light scattering is minimised and the scatterers become
Figure 2: (a)-(d) show a comparison of the scattered pressure spatial distribution for micro-bubbles and hydrogel scatterers for 2 to 5 MHz. The micro-bubbles were modelled as air-filled scatterers with radii of 1.5, 2.5, and 4.5 µm (black), and the hydrogel scatterer was modelled as a water-filled 100 µm radius scatterer as in the presented experiments (red) as well as 50 µm radius scatterer (magenta). Only the backscattered part of the signal is included in the polar plots since the signal needs to be received by the transducer again. Each curve within each plot has been normalised to its 0-degree intensity. Note that the three micro-bubbles are indistinguishable in (a)-(d). (e) shows the 0-degree backscattered magnitude pressure where each magnitude spectrum has been normalised to the pressure from Microbubble R2.5 μm at 3 MHz.
clearly visible in the optical microscope. Each scatterer was physically moved into a defined centre point in the optical viewfield using an X-Y microscope stage with integrated linear encoders for accurate readout of the actual position. This procedure circumvents possible measurement errors due to distortions in the optical components. The measurements were performed using a Zeiss LSM 700 upright microscope with a Zeiss 130x85 PIEZO stage having a positioning reproducibility of ±0.6 μm.

The positioning accuracy of the procedure was assessed by repeatedly locating the same scatterer. The position was found with a standard deviation of 1.3 μm along both the x-axis and the y-axis (n = 50). The analysis procedure is sketched in Fig. 3. The distance between all scatterers can be determined from the scatterer positions. The distances can then be correlated to the corresponding design distances from the 3D model, and the correlation should be linear. The slope of the correlation is the factor by which the printed structure has expanded relative to the design. If the printed structures are a perfect replication of the design, the correlation will be a linear relationship with a slope of 1.

2.3.2. Super-resolution ultrasound imaging calibration

With the correlation determined, the true distances between the scatterers in the 3D version of the scatterer phantom will be known, and can be used to compare against those found by ultrasound. When aiming to measure position changes on the order of a few micrometers, vibrations of the measurement setup will be detrimental. A 3D printed holder was fitted to the phantom dimensions, enabling mounting of the phantoms on top of an absorbing polyurethane rubber sheet (Sorbothane, Inc., Kent, Ohio, USA). The holder was mounted to the bottom of a water tank which in turn was mounted on a 8MR190-2-28 rotation stage (0.01° resolution) combined with a 8MTF-75LS05 x-y translation stage (0.31 μm resolution) (Standa, Vilnius, Lithuania). To minimize the effect of vibrations, the water tank on top of the rotation and translations stages were mounted on a Newport PG Series floating optical table (Irvine, California). An aluminium bridge was also mounted to the table, to hold the ultrasound transducer above the water tank, thereby also minimizing vibrations of the transducer. A sketch of the setup can be seen in Fig. 4.

The phantom was translated relative to the ultrasound probe using the translation stage along a single axis; in the first experiment along the x-axis, and in the second experiment along the y-axis. The inter-volume stage movement in both experiments was 12.5 μm, corresponding to a 2 mm/s velocity acquired at a volume rate of 160 Hz. This speed corresponds to common flow velocities in small vessels. By moving the phantom in between volume acquisitions, any differences depending on the phantom placement within the field of view of the transducer will be included in the analysis, instead of simply testing the SRI pipeline parameters locally within the transducer field of view.

The imaging probe was a prototype 62 + 62 elements 3 MHz piezo-electric, row-column addressed (RCA) array [26]. The probe was connected to the experimental scanner SARUS [27], which is capable of storing channel data for offline processing. A single frame is a summation of 32 defocused emissions using a synthetic aperture (SA) imaging approach [16]. Rows were transmitting and columns were receiving, thereby resulting in 62 channels in receive per emission. The phantom was stationary while a frame was being measured to avoid intra-frame motion artefacts. In total 2 × 640 volumetric frames were acquired over the 2 × 640 positions. The volumetric frames were then passed to the SRI pipeline.

2.4. Super-resolution pipeline

The SRI pipeline has been described in detail in [16]. It is briefly summarised in the following. The super-resolution pipeline consists of three steps. The first is SA beamforming. Each imaged volume spans a volume of 14.86 × 14.86 × 7.43 mm³, corresponding to 61 × 61 × 243 voxels. Each high resolution volume was a summation of 32 volumes beamformed from 32 emissions, using a specialised beamformer [28] implemented on a GPU [29]. The volume was dynamically focused in receive (F-number of 1.5) and synthetically in transmit (F-number of 1), with an optimized sequence for SA B-mode. This was done for all 2 × 640 frames. In the next step, a stationary echo filter was applied to remove stationary tissue. In a micro-bubble experiment, this would remove the signal stemming from the tissue as it is stationary, leaving only the micro-bubble signal. However, since the entire phantom was translated between each frame in this experiment, the stationary echo filter would have no effect on the results. The final step is to determine the points scatterer positions based on local maxima. Sub-pixel positioning is obtained by interpolating the peak location using a second order polynomial in all three dimensions. The 3D coordinates \( \{x_p, y_p, z_p\} \) of the detected points is then provided as the output from the third stage. Tracks of the individual scatterers can then be formed by collecting spatially similar coordinates across all imaged frames. The pipeline was implemented in MATLAB, and was processed offline [16].

Acquisition of data took approximately 20 minutes, primarily due to slow movement of the translations stage. The processing time depends on multiple factors such as storage type (SSD, HDD), processing unit (CPU) and GPU. The computation/processing time was tested on a PC running Ubuntu 16.04.1, with an Intel® core™ i7-4770 CPU @3.40 GHz CPU, and a GeForce GTX 1050 Ti GPU, with MATLAB (R2018a). The pipeline itself is divided into two overall sections, namely 1) beamforming and 2) tracking and localisation, which were not optimised for fast run time for these experiments. Beamforming a single 61 × 61 × 243 voxel volume took approximately 12.5 seconds, corresponding to a run time of 1 hour 46 minutes.
for the 640 volumes for each direction. Localization and tracking across all 640 volumes took less than 2 minutes, translating to a combined runtime of 1 hour and 48 minutes for each direction. It is of course important to improve the run time for the clinical application, but it was not an essential component for this study of calibration phantoms.

3. Results

This section presents the results of the optical validation of the printed structures, as well as the accuracy and precision of the SRI pipeline.

3.1. Optical validation of scatterer positions

Two replicates of each of the two projected-scatterer phantoms for optical validation were made. Each scatterer was located using the optical microscope and the translation stage coordinates of each scatterer was determined. Subsequently, the scatterer coordinates were used to determine the distance between the scatterers. The correlation between the optically measured distances and the designed distances can be seen in Fig. 5. In addition to analysing the direct correlation between measured distances and design distances, it was also investigated whether there was any difference between the two sets of cross-planes (\(x-y\) and \(x-z\)), which could potentially be explained by the anisotropic voxels. The different phantoms were also modelled as a random factor, to test and compensate for print-to-print variability. The combination of fixed and random factors makes the fitted model a linear mixed effects model. Such a model can be analysed using the lmerTest package [30] in R [31]. A summary of the data types and the factors included in the analysis can be seen in Table 1.

The initial mixed effects model is given as

\[
Y_i = \mu + \alpha(\text{Plane}_{i}) + (\beta_1 + \beta_2(\text{Plane}_{i})) x_{\text{design}, i} + c(\text{Phantom}_{i}) + \epsilon_i,
\]

(1)
Figure 5: Correlation between the distance between the designed scatterer positions and the distances measured using an optical microscope. The black line is the final reduced model seen in Eq. (2).

where $Y_i$ is the optically measured distances, $\mu$ is the overall intercept, $\alpha(\text{Plane}_i)$ is an intercept addition due to the Plane factor, $\beta_1$ is the average slope of the model, $\beta_2(\text{Plane}_i)$ is a plane dependent correction to the slope, $c(\text{Phantom}_i) \sim N(0, \sigma^2_{\text{Phantom}})$ is a random offset from phantom to phantom, and $\epsilon_i \sim N(0, \sigma^2)$ is the residual error, with $N(\mu, \sigma^2)$ being a normal distribution with mean $\mu$ and standard deviation $\sigma$, all for the $i$th response. All $c(\text{Phantom}_i)$’s and $\epsilon_i$’s are independent.

The model reduction was conducted by removing only a single term at a time, based on a 5% level of significance. Neither the random effect of the individual phantoms ($c(\text{Phantom}_i)$), nor the Plane dependent intercept addition ($\alpha(\text{Plane}_i)$), nor the Plane dependent slope ($\beta_2(\text{Plane}_i)$) were significant at 5%. Thereby the model reduction converged at the final model

$$Y_i = \mu + \beta_1 \cdot x_{\text{design},i} + \epsilon_i.$$  

(2)

The model coefficients and confidence intervals of the reduced model can be seen in Table 2. The analysis showed that the phantom swelling is isotropic, since there was no effect of the Plane factor. There was no significant difference between the four test phantoms, indicating good print repeatability. The parameter estimate of $\beta_1$ indicates that the phantom expands by approximately 2.6% along all dimensions. The residual standard error of the model is 36.6 µm. Model diagnostics showed that the residuals appeared to be normally distributed. Thereby, the model is a good describer for the phantom expansion. The overall good correlation of all points to the straight line indicate that the expansion is uniform and isotropic in the investigated region of the print area. The analysis showed a significant intercept of 23 µm, which was unexpected. Given that the intercept lies outside of the data range of interest, it will not be analysed any further. It should be noted that the confidence interval for the intercept varies from less than a single voxel width, to four voxel widths.

3.2. Ultrasound super-resolution pipeline calibration

3.2.1. Scatterer localisation

Fig. 6 shows three selected cross planes of a B-mode volume. The coloured dots mark the localised positions of the scatterers detected in one of the 640 volumes. The example cross planes have been chosen such that they all contain the scatterer marked by a blue dot. The $x - z$ cross plane (Fig. 6c) also contains an additional scatterer (red). Five scatterers were correctly detected within the presented volume. The choice of cross-planes results in three of them not being directly visible in the figure. The large reflection at $x \approx 3.5$ mm and $z \approx 4$ mm does not correlate with any of the designed scatterer positions, and likely stems from a print artefact.

The localised positions of the 3D printed scatterers, accumulated over the 640 volumes, can be seen in Fig. 7. The colours group the tracked points of the individual scatterers, while the black tracks illustrate the expected tracks based on the design coordinates. Droplines are included to aid the 3D perception. The black tracks are included for visual confirmation that the localisations are indeed

<table>
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<td>Plane</td>
<td>XY, XZ</td>
<td>Random factor</td>
</tr>
<tr>
<td>Phantom</td>
<td>1, 2, 3, 4</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Summary of the variables and their data types used in the optical correlation analysis.

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Standard Error</th>
<th>2.5%</th>
<th>97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept [mm]</td>
<td>0.023</td>
<td>0.009</td>
<td>0.005</td>
</tr>
<tr>
<td>$\beta_1$ (slope)</td>
<td>1.026</td>
<td>0.002</td>
<td>1.022</td>
</tr>
</tbody>
</table>

Table 2: Model parameter estimates of the final reduced model including confidence intervals of correlation between optical measurements and design distances.
Figure 6: (a) B-mode volume containing scatterers. Three cross planes of the B-mode volume are shown, (b) x-y, (c) x-z, and (d) y-z. The super-localised positions of the scatterers are marked by coloured dots. The selected cross-planes were aligned to the position of the blue scatterer. The incidental alignment to the red scatterer is not perfect. Slight adjustments of the plane position would increase the apparent intensity of the red scatterer, at the expense of that of the blue scatterer.
the designed scatterers. It is recommended to always include such a comparison to confirm that the localisations indeed correspond to the features of the designed phantom. Thereby, it is a simple process to dismiss localisations from printing artefacts, and limit the analysis to localisations stemming from the designed scatterers. The black expected tracks are included for all positions, even when the scatterer is no longer detected, and the plot field of view is limited to the scatterer localisations. Thus, the mismatches between black and coloured tracks, seen for instance for the dark red track in Fig. 7(a) indicates a loss of detection midway through the translation of the phantom.

The two scatterers in Fig. 6 were coloured equally as in Fig. 7, with the positions in Fig. 6 corresponding to the starting positions of the blue and red point in Fig. 7 marked by the droplines. The horizontal field of view in the figures have been limited to the measured data tracks, removing parts of the black tracks. The actual cross-sectional field of view of the probe is 14.86 x 14.86 mm².

Although eight scatterers were printed, not all were found in the two experiments: seven scatterers were correctly localised for the movement along the x-axis (Fig. 7(a)) and five scatterers were correctly localised for the movement along the y-axis (Fig. 7(b)). In addition, the track length varies from 81 localisations to 633 localisations, across the 640 volumes. Two additional tracks, which did not align with the design coordinates, have been omitted from the images and the analyses. It is expected that these tracks stem from print artefacts, resulting in unintended cavities in the phantom, which therefore reflect the ultrasound similarly as the designed scatterers. They aligned well with the reflection seen in Fig. 6c at x ≈ 3.5 mm and z ≈ 4 mm. While these print artefacts would also be fixed in position, and be moved along the same trajectory as the designed scatterers, the print artefact geometry is not known. If a print artefact is significantly larger than the imaging wavelength, localisation of the centroid might be ambiguous, and therefore, these tracks were omitted from the analysis.

3.2.2. Super-resolution accuracy

The SRI pipeline accuracy was investigated in a similar manner to the optical validation, by comparing the known distances between the designed points to the measured distances between points from the ultrasound experiments. There are two main differences to the optical experiment: The scatterers are now positioned not in collapsed planes but in 3D, visualised as the blue points in Fig. 1, and the design distances are compensated for the expansions according to the results in Table 2 before analysing the correlation between the designed distances and those calculated from the ultrasound data. After the compensation, the correlation should be a straight line with a slope of 1, in the case of perfect correlation. Since there are two sets of experiments, one for each direction of motion of the translation stage, the variables of the analysis are the compensated design distances, the measured ultrasound distances, and a factor separating the data into the x- and y-motion, all summarised in Table 3. In this experiment, the entire beamformed volume has been assumed to have a speed of sound equal to that in pure water, 1480 m/s.

As was mentioned in Section 3.2.1 and shown in Fig. 7, an unequal number of scatterers were localised by the SRI pipeline in the two experiments, and the tracks were of unequal length. This means there will be more data for the x-direction of motion, resulting in an unbalanced dataset from a statistical point of view. In addition, our analysis of the variation in the data showed that the data was heteroscedastic, meaning the variation in the data was not constant across the entire dataset. A uniform variation is a fundamental assumption of most common statistical analysis methods. Furthermore, modelling the correlation of the raw distances between points might be heavily biased toward certain parts of the data simply due to the large and varying number of samples in different parts of the dataset. To mitigate these issues, a weighted least squares analysis of the distance distributions was conducted. This was performed by modelling the mean distance between each point across all measurements, with each mean value being weighted by the variance of the measurements contributing to that mean.

The correlation between the compensated design distances and the mean of the distances calculated by the SRI pipeline is shown in Fig. 8.

The initial linear model is given as

\[ Y_i = \mu + \alpha(\text{Motion}_i) + (\beta_1 + \beta_2(\text{Motion}_i))x_{\text{design},i} + \epsilon_i, \]

(3)

where \( Y_i \) is the mean of the distance between points calculated from the SRI pipeline output, \( \mu \) is the overall intercept, \( \alpha(\text{Motion}_i) \) is an intercept addition due to the motion factor, \( \beta_1 \) is the average slope of the model, \( \beta_2(\text{Motion}_i) \) is a Motion dependent correction to the slope, and \( \epsilon_i \sim N(0, \sigma^2) \) is the residual error, with \( N(\mu, \sigma^2) \) being a normal distribution with mean \( \mu \) and standard deviation \( \sigma \), all for the \( i \)th response. All \( \epsilon_i \)'s are independent.

The model reduction was conducted by removing only a single term at a time, based on a 5% level of significance. Neither the overall intercept (\( \mu \)), nor the direction of motion dependent addition to the intercept (\( \alpha(\text{Motion}_i) \)), nor the direction of motion dependent correction to the slope (\( \beta_2(\text{Motion}_i) \)) were significant at 5%, and were therefore removed. Thereby the model reduction converged at the final model

\[ Y_i = \beta_1 \cdot x_{\text{design},i} + \epsilon_i. \]

(4)

The model coefficient and confidence interval of the reduced model are presented in Table 4. The analysis showed no dependence of the direction of motion, nor any intercept of the correlation. The modelled average behaviour of the fitted line has a slope of 0.989, close, yet not equal, to a perfect correlation with a slope of 1. Based on the
Figure 7: Cumulated localized scatterers acquired over 640 volumes. The phantom was translated in two separate experiments, (a) along the transducer x-axis and (b) along the transducer y-axis. The black tracks illustrate the expected tracks based on the design coordinates. Droplines end on the z=8 mm plane, and are included to aid the 3D perception.

<table>
<thead>
<tr>
<th>Sample values</th>
<th>Variable type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound distance [mm]</td>
<td>8.717, 3.730,..., 6.279</td>
<td>Numerical values</td>
</tr>
<tr>
<td>Compensated design distance [mm]</td>
<td>8.719, 3.811,..., 6.384</td>
<td>Numerical values</td>
</tr>
<tr>
<td>Motion</td>
<td>X, Y</td>
<td>Fixed factor</td>
</tr>
</tbody>
</table>

Table 3: Summary of the variables and their data types used in the ultrasound correlation analysis.
Calculated distance (ultrasound) [mm]

Figure 8: Correlation between the compensated design distances and the mean of the distances calculated by the SRI pipeline. The line represent the final reduced model seen in Eq. (4).

Table 4: Model parameter estimates of the final reduced model including confidence intervals of correlation between ultrasound distances and compensated design distances.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>2.5%</th>
<th>97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_1$ (slope)</td>
<td>0.989</td>
<td>0.003</td>
<td>0.982</td>
<td>0.996</td>
</tr>
</tbody>
</table>

3.2.3. Super-resolution precision

The same ultrasound data was used to estimate the SRI pipeline precision. The precision was estimated by investigating the variation of the individual localisations relative to the trajectories of the translated scatterers. The tracks with motion along the $x$-direction were used to estimate the precision in $y$. The tracks with motion along the $y$-direction were used to estimate the precision in $x$. Both datasets were used to estimate the precision in $z$. To visualise the variation, the mean $x$-, $y$- and $z$-coordinate were subtracted from each individual track, to centre the tracks around the transducer coordinate-system origin. This is illustrated in Fig. 9, where two cross-planes ($x$-$y$ and $x$-$z$) are shown for the tracks with motion along the $x$-axis, which corresponds to the tracks in Fig. 7 a). The colour of the points represent the tracks of the different design points, and are matched to those of the tracks in Fig. 7 a). The movement was uni-axial along the translation stage $x$-axis. However, slight misalignment between the ultrasound transducer and the translation stage have resulted in the localisation tracks not being perfectly aligned to the transducer axes. This can be observed in Fig. 9, in which the black line is the average trajectory of all tracks in the dataset. It should be noted however, that the axes are not equally scaled in the main plots, but only in the small inserts. The misalignment angle is 0.49° in the $x$-$y$ plane, and 0.79° in the $x$-$z$ plane. This misalignment should be compensated for when determining the variation of the tracks. The scatterers are fixed in the phantom and have been moved collectively by the translation stage. Then all tracks should have moved in the same direction, and the average trajectory of the tracks would be a good estimate of that. An estimate of the precision was determined as the variation relative to the average trajectory, i.e. by determining the deviation from each localisation to the average trajectory, and calculating the standard deviation of these deviations. The precision along all three dimensions based on the variability relative to the average trajectory is displayed in Table 5 (“Average trajectory”). However, the coloured lines indicate that the tracks are in fact not parallel, but at small angles to each other. It is fairly small angles relative to the average trajectory, with the largest angle in any plane being 3.1°. This indicates that there is an error somewhere in the SRI pipeline, and that determining the precision relative to the average trajectory might be misleading. As an alternative, the estimate of the precision could be determined relative to the individual trajectories of the tracks. The precision along all three dimensions based on the variability relative to the individual trajectories is displayed in Table 5 (“Individual trajectories”). However, given that the tracks should have been parallel, this latter estimate of the precision might also be misleading. It is expected that the two presented estimates of the precision are limiting cases, and that the true precision of the SRI pipeline will lie somewhere in between.

4. Discussion

The 3D printed phantoms have successfully been used for SRI pipeline characterisation. The presented results illustrate that it is possible to obtain estimates for precision and accuracy, using these specialised phantoms. The obtained precision indicates an improvement in resolution of at least a factor of $\approx 18$ when comparing the worst precision estimate ($27.6 \, \mu m$) to the utilized ultrasound wavelength, $\approx 500 \, \mu m$. It is particularly worth noting that even the worst obtained estimates for precision are comparable to the size of the smallest vessels in tissue. Thereby it is clear that the used method is suitable for resolving features at the size of the smallest vessels in tissue in three dimensions, and the stability of the phantom features allows for documentation of this.
Figure 9: Cross-planes of the tracks with motion along the $x$-axis ((a)-(b)) and motion along the $y$-axis ((c)-(d)), offset to be centred around the coordinate system origin. The black lines show the average trajectory within each plot, while the coloured lines are linear fits to the individual trajectories of the different scatterers. The scaling is equal across the main plots for ease of comparison, but the main plots do not have equally scaled axes. The narrow graphs on top in all figures show the same linear fits, with equally scaled axes.
The scatterers were $205 \times 205 \times 200 \mu m^3$. Thereby they are significantly larger than the micro-bubbles typically used in SRI. Even so, the theoretical analysis of the backscatter distribution showed that the scattered pressure for the utilised frequency, observed in Fig. 2(b), is similar in terms of the spatial distribution, deviating by less than 1 dB for the 100 $\mu m$ radius scatterer across $\pm 60$ degrees, which is the angular range relevant for the presented experiments.

It should be noted that there is good agreement between the SRI pipeline property estimates determined using these calibration phantoms, and the estimates for precision obtained using a flow phantom, as has been presented in [16]. In the article, the 3D localisation precision based on a 3D printed flow phantom containing a single 200 $\mu m$ diameter channel, imaged using the same ultrasound equipment and SRI pipeline as utilized in this study. The experimental procedure for such an experiment consists of creating an adequate micro-bubble concentration, using a micro-flow controller to control the volume flow rate to obtain an optimised flow velocity within the channel system, and optimising the imaging sequences to avoid destroying the micro-bubbles during imaging. From these localisations, it was possible to determine the localisation precision based on the micro-bubble localisation distribution in the flow channel. The precision was found to be 16.5 $\mu m$ in the $y-z$ plane and 23 $\mu m$ in the $x-z$ plane. Note that the precision was determined through analysis of the radial distribution of micro-bubbles within the channel. By doing that, all precision estimates are a mixture of the axial precision and lateral precision, which are not expected to be equal. By instead measuring the distribution of fixed points as presented here, the analysis is not limited to a radial distribution, but independent estimates of the precision along $x$, $y$, and $z$ have been obtained. The scatterer phantoms presented in this article avoids all of the experimental setup complexities of a flow phantom, and resulted in localisation precision estimates perfectly in line with the estimates based on a micro-bubbles in a flow phantom. It is, thus, a much simpler validation method.

For the initial optical characterisation of the phantoms, a 36.6 $\mu m$ residual error was found for the correlation between the designed distances and those measured using an optical microscope. This is significantly larger than the position repeatability claimed by the microscope stage manufacturer and the experimentally validated position repeatability which was tested. A possible explanation might be that the experiment to determine the position repeatability was made by locating the same scatterer multiple times. On the other hand, the correlation in Fig. 5 was made localising many different scatterers. Local distortion of the printed structures might make the scatterer shapes slightly unequal, resulting in localisation of comparative features (for instance a specific corner) more difficult between scatterers, than when locating the same feature on the same scatterer. It should be noted that the model diagnostics showed that the residuals appeared to be normally distributed, indicating that the model is a good describer for the phantom expansion.

The two translations in the ultrasound experiment were not conducted from the same starting point. The second translation was started from the end point of the first translation. It is expected that the discrepancy between the number of detected scatterers in the two experiments could have been avoided by starting the two translations from the same position. However, this was of course a post-analysis discovery.

The high positioning control has allowed for the detection of distortion in the SRI pipeline, through the non-parallel tracks, which would not have been possible using conventional phantoms. The tracks should have been parallel given that the scatterers are fixated in the phantom, and that they have only been moved collectively using the translation stage. The distortion is the reason for the discrepancy between the precision estimates. However, it was quite small with an angular distortion of at most 3.1°. A possible explanation could be that the experiment has been conducted assuming a speed of sound of 1480 m/s in the entire beamformed volume. This was chosen, since the phantom was submerged in water, and the phantom itself consists of $\approx 75\%$ water. However, the speed of sound of the phantom has been measured to be $\approx 1577 \text{ m/s}$, which will lead to distortion. A way to decrease the distortion would be to match the speed of sound of the immersion medium to that of the phantom. However, as the phantom is permeable to water, a glycerol- or salt water solution will also diffuse into the phantom itself. When the solution enters the cavities, the contrast would likely go down. This should be investigated in future experiments.

An alternative or additional explanation could be that the ultrasound system has both a spatially dependent sensitivity and a spatially dependent point spread function, which changes in shape and intensity. This would not only explain the non-parallel tracks, but could also explain the difference in the number of tracks detected in the two ultrasound experiments, and that the eighth scatterer was not localised in either experiment. A consequence of a spatially dependent point spread function could be that full calibration of a SRI pipeline should perhaps be performed with local parameter estimates throughout the field of view of the probe instead of globally, as presented here. Thus the properties of a SRI pipeline would then be given by accuracy and precision estimates, both as functions of the $x$, $y$, and $z$ coordinates. This might even be necessary, illustrated by the results in this paper, as proper thresholding can become difficult to implement globally in the field of view. Phantoms containing many scatterers distributed across the entire field of view of the imaging probe could be implemented to characterise the spatially varying point spread functions.

The presented phantom illustrates an alternative solution for SRI pipeline calibration to regular tube phantoms. However, this does not mean that it is irrelevant to create phantoms, which allow flow of micro-bubbles to be tracked.
Given that it is a 3D printing method, these could easily be made, as was demonstrated in previous work [15, 16]. The 3D printing method allows for creating any arbitrary complex structure, making it possible to mimic complex vascular systems. The complexity is in principle only limited by the printer specifications and the printing material governing the achievable minimum voxel size.

The presented phantom concept could be expanded to investigate other aspects of super-resolution algorithms and systems, such as resolvability and separability. The separation of 3 mm was chosen to ensure no overlap between the reflected signals from the individual scatterers, thereby mimicking how many SRI pipelines work today. Thereby, the resolution that can be expected from a SRI pipeline will be given by the variability of the positions, presented here as the σ values in Table 5. Phantoms could be developed with scatterers placed much closer, to tune algorithms to be able to separate signals from partially overlapping reflections. This would be highly relevant for instance for some of the new types of SRI schemes which seek to be able to separate reflections much closer than the wavelength [32].

The 205 × 205 × 200 μm³ scatterer size limits the phantom to be used with imaging frequencies equal to or less than 6 MHz. However, imaging probes capable of using larger frequencies are widely used. The phantom feature sizes are by no means the limit of the printing system. In [32] we presented another 3D printed phantom containing 45 × 45 × 1000 μm³ scatterers for 2D imaging. Integration of the signal across the elevation plane allows for an increased intensity even though the scatterers were significantly smaller in cross-section than those used in this work. That is not possible for 3D imaging. However, no optimization of the scatterer size has been done for this work. Additional optimization through local dose changes resulting in local acoustic parameter changes might allow for obtaining even larger intensities from the same sized scatterer. A different approach would be to not only consider increasing the intensity from the scatterers, but also decreasing the background noise from the bulk of the phantom. Some of the unintended structures observed in ultrasound might originate from issues in the printer system, which could potentially be optimised.

The 3D printed scatterer phantom provides feature positioning accuracy for phantom fabrication techniques unparalleled by any other phantom fabrication technique. However, it does not provide a perfect replication of the reflection signals from micro-bubbles. The contrast from the scatterers is significantly lower than what is obtainable using micro-bubbles through contrast imaging sequences. The observed drop in localisations in certain parts of the imaging field of view is likely not an issue when imaging micro-bubbles. The presented printing method can still be improved to increase the contrast. The 3D printing method inherently provides design freedom to create complex structures, potentially replicating real vascular structures. However, for controlled validation, simplified structures are often desirable, to provide a better overview and predictability of the outcomes. This work has presented a fundamental study, demonstrating some of the simpler possibilities, but have clearly not exhausted the potential.

Hopefully, the demonstration of this new technique can inspire other researchers to either improve on the technique, or develop even better techniques for validation.

The precision, accuracy and repeatability of the 3D printed phantoms would be incredibly difficult to achieve, if not impossible, using the traditional types of tube phantoms or chicken embryos. Yet, it still provides the opportunity of creating complex three-dimensional phantom features, allowing for full volumetric characterisation of an ultrasound system, which is not offered by any other phantom fabrication method available today.

5. Conclusion

We have presented 3D printed micro-phantoms containing absolute 3D micro-positioned sub-wavelength sized ultrasound scatterers for calibration of super-resolution ultrasound imaging (SRI) pipelines. The presented phantoms contain fixed scatterers and can therefore be used for long acquisitions, producing repeatable results, unlike traditional tube phantoms. The resulting printed structures have been characterised using an optical microscope, and it has been shown that the printed structures systematically expand isotropically by 2.6% relative to the design. The phantom was used to calibrate a super-resolution ultrasound imaging (SRI) pipeline by correlating the distances between the cavities calculated through the SRI pipeline to the phantom design distances. The analysis showed a correlation slope of 0.989, close to a perfect correlation of 1. The variability of the super-localised positions of the individual scatterers across 640 volumes were used as an estimate of the precision of the SRI pipeline. Based on the analysis, it is expected that the precision of the SRI pipeline lies between the two limiting estimates of (˜σx, ˜σy, ˜σz) = (22.7 μm, 27.6 μm, 9.7 μm), when estimated relative to the average trajectory off all tracks, and (σx, σy, σz) = (18.7 μm, 19.3 μm, 8.9 μm), when estimated relative to the individual track trajectories. Both of these precision estimates are on the same scale as the features intended to be resolved in vivo, namely vessels of only a few tens of micrometers.

The presented phantom has proven to be a useful tool in validating accuracy and precision for a SRI pipeline, as well as unveiling distortion in the SRI pipeline, the latter of which would have been impossible using a traditional tube phantom. The study demonstrates the use of 3D printed phantoms for determining the accuracy and precision of volumetric super-resolution algorithms.


