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*Design and Performance Validation of Phantoms Used in Conjunction with Optical
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Deformable and durable optical phantoms with controlled density of scatterers

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ABSTRACT

We developed optical tissue phantoms with a novel combination of matrix and scatterers. These phantoms have a well known scattering microstructure of monodisperse silica microspheres, embedded in elastic silicone. We characterize their mechanical properties and, some of their optical properties. We also validate the control over the density of scatterers achieved with our proposed fabrication technique. The properties obtained are a practical combination of deformability, durability and simplicity of the microstructure. These are illustrated by results on speckle statistics in optical coherence tomography.

Keywords: Optical tissue phantoms, optical coherence tomography, silicone, microspheres

1. INTRODUCTION

In biomedical optics, tissues phantoms are most often produced to replicate the optical coefficients of specific tissues. However, in some cases, other tissue-like or non tissue-like properties are sought for specific needs. In studies on speckle statistics and elastography using optical coherence tomography (OCT), the following characteristics are important.

The first one is the simplicity of the microstructure. It is obviously easier to first understand how a system interacts with a simple sample, before moving on to complex materials such as biological tissues. A simple optical model for biological tissues is a random distribution of discrete scatterers. This can be obtained by using very small particles mixed in a matrix. The second property is a good control of the density of scatterers contained in the phantoms. This is essential because our study of speckle consists in looking at the variation of statistical parameters in OCT images with the density of scatterers. The third essential property is deformability. For elastography, we primarily need our phantoms to be elastic enough to easily apply deformations and track changes in the speckle pattern of our OCT images. Ideally, the phantoms should also mimic the mechanical behavior of some tissues or organs.

In section 2, a few solutions found in literature are discussed. In section 3, our phantoms of silica microspheres in silicone together with their fabrication process are presented. Then, results of characterization experiments including scanning electron microscopy (SEM), tensile tests, and OCT amplitude profiles are presented in section 4. Finally, in section 5, results obtained on speckle analysis of OCT images illustrate the usefulness of the phantoms.

2. EXISTING SOLUTIONS

A number of combinations of matrices and scattering materials previously reported in the literature appeared to have the required characteristics. They can be divided in two classes: inorganic powders in silicone, and microspheres in water, gelatin or other matrices.

2.1 Inorganic powders in silicone

Room temperature vulcanizing (RTV) silicones have previously been mixed with non-organic powders and absorbents to replicate specific optical properties and coefficients of various tissues [1, 2]. Because of their elasticity, RTV silicone matrices have also been used to simulate mechanical properties of tissues or organs [3].

Non-organic powders are often used as scatterers because they are widely available with average sizes in the range of one or two microns, therefore small compared to the resolution of standard OCT systems (10 – 15 μm). However, even with very high quality samples, inorganic powders are always provided with a wide range of particle sizes (a variation of few microns) and shapes. With such a distribution of scatterer sizes, it is almost impossible to obtain a precise evaluation of the number density of scatterers.

2.2 Microspheres in various matrices

With the use of microspheres, precise information about the size, the nature and the shape of the scatterers is obtained. They provide discrete scatterers that can easily be modeled. Furthermore, since the microspheres can be obtained with low dispersion in size, the number of particles in a sample can be evaluated by weighing the microspheres in the fabrication process. A popular choice for microsphere phantoms is polystyrene microspheres in water [4, 5]. However, in water, the microspheres are constantly subjected to Brownian motion, which is not suitable for our needs. Another solution is the use of gelatin and agarose to solidify aqueous suspensions of microspheres but those have the major inconvenience of degrading quickly [6]. Finally, microspheres were also previously mixed in matrices of polyester [7] and epoxy resin [8] but those are too stiff for elastography.

3. FABRICATION

In order to obtain deformable phantoms with a simple microstructure and a good control over the number density of scatterers, our approach is to mix dried silica microspheres in silicone. According to the manufacturer (Bangs Laboratories), the refractive index of the silica microspheres is between 1.43 and 1.45.

The silicone we use is Sylgard 184 sold as a resin – catalyst kit mixed in a 10:1 ratio and curing in 48 hours at room temperature. The ratio of the optical to the geometrical paths in OCT yields a group refractive index of $n_g = 1.41$ at the 1.3 μm wavelength of our OCT system. At that wavelength, the silicone is transparent.

The fabrication process begins by weighing the microspheres and by adding the needed volume of the resin part of the silicone. The reactive is not added at that point. To insure good incorporation of the microspheres, the same volume of hexane is added. Hexane is a very good solvent for Sylgard 184 silicone and greatly decreases the mixture viscosity [9]. This mixture is then placed in an ultrasonic bath for about two hours, breaking down most aggregates while efficiently and uniformly dispersing the microspheres in the silicone matrix. Afterwards, the hexane is evaporated under vacuum for a few hours, leaving the silicone still capable of polymerization. The reactive is finally added to the batch in proper proportion and mixed manually. This mixture can be cast in molds of any shape and left alone for polymerization. Fig. 1 shows examples of different shapes obtained. We use the disk shape phantom for bench-top experiments and the cylindrical phantom for testing our catheterized OCT system.

4. CHARACTERIZATION

To assess some of the properties of the phantoms, characterization experiments like scanning electron microscopy (SEM), tensile testing, and OCT imaging are performed. We use SEM to qualitatively observe the incorporation of the microspheres into the matrix. As can be seen in Fig. 2, the particles are very well inserted, well wetted, and their distribution appears to be uniform and random. To further assess the randomness of this distribution, the autocorrelation of the SEM images is calculated. One is presented in Fig. 3, where the single peak at center without secondary lobes and surrounded by an almost constant gray level reveals an absence of regularity in the positioning of the microspheres. The weak vertical pattern is caused by vertical cutting artifacts (see Fig. 2).

Fig. 1 : On the left, a disk shaped silica in silicone phantom, on the right, a cylindrical silica in silicone phantom

Fig. 2 : SEM image of a cross section cut into a phantom containing $7,0 \times 10^9$ part/ml of $2.34 \mu\text{m}$ silica microspheres

Fig. 3 : 2D autocorrelation of the previous SEM image

Image processing of the SEM images is also used to count the particles and verify that a good control over the density of scatterers is obtained. Two series of seven and eight phantoms of various densities of scatterers were produced, one with 2.34 μm diameter silica microspheres, and the other with 1.86 μm diameter silica microspheres. For each phantom, six images of a cut cross section are sampled, filtered and thresholded to count the particles. As seen in Fig. 4, there is a good linear relation between the nominal concentration and the average number of particles in the images for both series. The difference in slopes between both series can be attributed to the fact that the bigger particles have a higher chance of appearing at the surface when cutting the cross-section.

Fig. 4 : Average number of scatterers in SEM image compared to the nominal concentration for 2 series of phantoms

The second type of characterization we performed was to measure some mechanical properties of our phantoms. It was previously reported that the ratio of resin to catalyst used to cure silicones has an influence on their elasticity [3]. We produced blank silicone samples with different concentration of catalyst and also phantoms with microspheres to perform uniaxial tensile tests. In a first experiment, rectangular strips of these samples are stretched at low speed (approx. 10 mm/min) until they ruptured while the true stress is measured. In Fig. 5, the results of these experiments are presented together with a curve from a tensile test performed on the circumferential axis of a porcine aorta. Many observations can be drawn from this figure. First, as expected, the stress to strain ratio is significantly decreased when the catalyst concentration is lowered from the standard 10 : 1 resin to catalyst ratio to a 15 : 1 ratio. However, a further decrease to a 20 : 1 ratio does not lower the slope of the curves (namely the stiffness) and also yields sticky samples. Second, we can also observe that the incorporation of microspheres does not significantly affect the stiffness but lowers the rupture stress. This is not surprising since the microspheres are good fracture initiation sites. Third, the phantoms exhibit strain hardening, a non-linear behavior also observed in soft biological tissues (see the curve of the porcine aorta). Finally, at low stretch ratios, the porcine aorta and the 15 : 1 phantoms have similar stiffness but the phantoms are still a bit stiffer.

Fig. 5 : Uniaxial tensile tests until rupture showing variation of the elasticity with resin : catalyst ratio for silicone blanks, phantoms and a porcine aorta

In other uniaxial tests, phantoms were stretched periodically up to a 1.5 ratio while the true stress was also measured. It can be seen in Fig. 6 that the stress while stretching slightly differs from the stress when releasing. This hysteresis is also a common property of soft tissues like the porcine aorta shows in Fig. 5. Because they have strain hardening, hysteresis and approximately similar elasticity, silicones like Sylgard 184 have previously been used to mimic the mechanical properties of organs like coronary arteries. To fabricate mechanical phantoms, the difference in stiffness can be compensated by adjusting the ratio of the wall thickness to inner diameter of silicone tubes in order to match the compliance of the coronary vessels [10].

Fig. 6 : Tensile tests with repetitive stretches showing hysteresis

Our next characterization experiment was to compare a model of the dependency of the OCT signal amplitude along the optical axis with the concentration of scatterers (C) in a sample with measurements on phantoms with various concentrations. These comparisons are depicted in Fig. 7 for three phantoms made of $2.34 \mu\text{m}$ silica microspheres. A single factor is used for all cases to adjust the amplitude between theory and experiment. The model based on a discrete sum of random phasors suggests that the amplitude should be proportional to $C^{1/2}$. The relative amplitudes of the measured OCT signal agree with this prediction, again confirming that there is good control on the density of scatterers in the fabrication process. Also, the agreement between the modeling and the experimental results is very good across the depth of the sample, a further confirmation of the homogeneity of the samples.

5. APPLICATIONS

To illustrate the usefulness of these phantoms, we present some results of an investigation of the speckle size in OCT. The homogeneity and control over the density of scatterers are the two essential properties for this work on speckle statistics. It is common knowledge that the speckle size is affected by the OCT system resolution and by the optics used in the sample arm. But in fact, even if these two parameters are of major influence, the speckle size is also related to the microstructure of the sample. An interpretation of a model based on single scattering led to the identification of two limiting regimes: low and high densities of scatterers. At low densities, the speckle size is the envelope of the point spread function (PSF) of the system which can be separated into an axial component equal to the OCT resolution and a transverse component related to the incident beam spot size by a factor of $1/\sqrt{2}$. At high densities, the light scattered by many particles interferes, which causes a decrease in the speckle size after the extraction of the envelope of the OCT signal. This problem was studied by Wagner et al. for ultrasound B-scans [11] and their results applied to OCT measurements predict that the low density speckle sizes in the focal region should be reduced by about 30% in the high density regime. The speckle size is expected to decrease monotonically between these two regimes. This interpretation is investigated by measuring the speckle size on images of the silica in silicone phantoms as well as on simulated images. As an example of the results, Fig. 8 shows the evolution of the axial speckle size in the focal region with respect to an index called the effective number of scatterers (ENS). The ENS is the average number of scatterers in a volume delimited by the optical spot size and the OCT resolution [5].

Fig. 7 : Average OCT signal profiles (dotted lines) compared to modeling (continuous lines).

Fig. 8 : Axial speckle size at focus for simulations and phantom experiments with theoretical upper and lower limits

In the figure, we see a good agreement between the results from simulations and phantom images. The values of the two boundaries match pretty well and an evolution is observed in between. The discrepancies that are observed are mainly caused by the difficulty of evaluating the speckle size precisely but the tendency is clear. In this study, we also look at the speckle size in the transverse direction, as well as the evolution of the speckle size along a focusing optical axis. More details about the modeling and other results can be found elsewhere [12]. Agreement between modeling and imaging would have been very difficult without phantoms containing discrete scatterers, where their density can be precisely evaluated and are homogeneously distributed in a solid matrix.

There is also more to the results on speckle analysis presented here. The measurements were performed more than a year after the phantoms were fabricated. The durability of the phantoms is therefore a great asset of this technique. Since both silica and silicone are inorganic and very stable materials, these phantoms will last a very long time.

6. CONCLUSIONS

The incorporation of silica microspheres in silicone combines many advantages. It provides a very good control over the density of scatterers. It produces phantoms that are deformable while some adjustment of the mechanical properties is possible. Molding into various shapes is easy, and it is quite practical since the samples have a very long life. As an example of their usefulness, they were used with success in a study of the speckle size in OCT. With future work on characterization of the optical properties and on the shaping of the phantoms in specific geometries, this type of phantoms should find many uses in various fields of biomedical optics.

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