Fabrication and characterization of a multilayered optical tissue model with embedded scattering microspheres in polymeric materials

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Abstract: We report on a novel fabrication approach to build multilayered optical tissue phantoms that serve as independently validated test targets for axial resolution and contrast in scattering measurements by depth-resolving optical coherent tomography (OCT) with general applicability to a variety of three-dimensional optical sectioning platforms. We implement a combinatorial bottom-up approach to prepare monolayers of light-scattering microspheres with interspersed layers of transparent polymer. A dense monolayer assembly of monodispersed microspheres is achieved via a combined methodology of polyelectrolyte multilayers (PEMs) for particle-substrate binding and convective particle flux for two-dimensional crystal array formation on a glass substrate. Modifications of key parameters in the layer-by-layer polyelectrolyte deposition approach are applied to optimize particle monolayer transfer from a glass substrate into an elastomer while preserving the relative axial positioning in the particle monolayer. Varying the dimensions of the scattering microspheres and the thickness of the intervening transparent polymer layers enables different spatial frequencies to be realized in the transverse dimension of the solid phantoms. Step-wise determination of the phantom dimensions is performed independently of the optical system under test to enable precise spatial calibration, independent validation, and quantitative dimensional measurements.

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1. Introduction

Based on the principle of light scattering, optical coherence tomography (OCT) is a non-invasive, tomographic three-dimensional (3D) imaging modality capable of label-free detection of sub-surface biological inclusions or microstructures at a depth of several millimeters with micrometer scale sensitivity. OCT assesses tissue infrastructure by analyzing the temporal delay of back-scattered light using a low-coherence interferometer and is an emerging optical medical imaging platform with applications in evaluating disease states in an expanding range of clinical fields including ophthalmology, oncology, and cardiology, among others [1–3]. Another 3D imaging modality at higher resolutions for \textit{in vivo} applications is confocal microscopy which enables the visualization of superficial layers at a depth of up to a few hundred micrometers with submicrometer resolution. For these 3D imaging modalities, tissue models or phantoms with well-controlled optical properties (refractive index, scattering coefficient, anisotropy factor, and absorption coefficient) are routinely used for the evaluation of their key instrument characteristics such as point spread functions (PSFs) for the evaluation of lateral and axial resolutions, spectral responsivity for quantitative analyses of fluorescence and wavelength-dependent scattering, and detection sensitivity and dynamic range for tissue type-dependent optical densities and molecular concentration of target and image-contrast probes [4–6]. Phantoms have also been used for the calibration, inter-laboratory comparison, and standardization of imaging platforms for the same modality as well as for the validation of physical models and simulations to quantitatively interpret the image data [7,8]. Phantoms made from polymeric materials have been used routinely for their general biocompatibility, ability to form stable matrices that allow facile inclusion of various entities (e.g. polymer microspheres, cellular constituents, fluorescent dyes), and tunability of adsorption and scattering characteristics. A primary challenge, however, in optical standards work is the routine lack of consensus in phantom fabrication materials and methods, variable reproducibility of the ad hoc phantom approaches for one-of-a-kind devices, and no clear benchmarking to a “ground truth” tissue standard in the absence of a gold standard for precise optical device inter-comparison [9,10].

For lateral resolution benchmarks, the gold standard is the United States Air Force (USAF) resolution test chart, which consists of a fine chrome film pattern deposited on glass that conforms to the MIL-STD-150A standard and still widely accepted for system calibration as well as testing the quality and resolving power of optical imaging systems. The pattern
consists of groups of three bars or alternating dark/bright line pairs of differential periodicity with tailored dimensional ranges. For testing the quality of an optical system, the presence of simple periodic features enables the determination of the existence and magnitude of light diffraction from edge features and aberration due to the optics. The largest bar or line pairs the imager under test cannot discern defines the limitation of its resolving power. While the USAF standard target has been used to characterize and calibrate a wide variety of optical imaging systems for the determination of lateral resolution, a separate test target is needed to understand axial imaging performance in depth-resolving optical systems such as OCT and confocal microscopy. For axial resolution benchmarks, there is less of a consensus on a suitable test target. Such a length reference specimen is critically needed in the field of ophthalmology, wherein OCT is implemented to acquire optical biopsies of the retinal layers and to delineate the individual corneal layers which approach the resolution limit of OCT configurations in clinical use [11,12]. Furthermore, quantitative thickness measurements of the nerve fiber layer along with the other intra-retinal layers would aid in evaluation and clinical decision making for several ophthalmic conditions including age-related macular degeneration, diabetic retinopathy, and glaucoma [13].

From a phantom materials standpoint, the bottom-up assembly of microspheres into a two-dimensional ordered crystal can be accomplished by various techniques based on different physical mechanisms [14]. The goal of these techniques is to sequentially collect and organize microspheres, followed by binding of the structure formed onto a substrate material via concentration of monodispersed spherical particles. The collection can be achieved by simple sedimentation methods, but can also be directed and organized by convection and capillarity, or driven by external fields. The substrate particle of charged microspheres can be achieved by coating polyelectrolyte multilayers (PEMs) of opposing charge onto the substrate to facilitate particle adsorption. For our optical phantom construct, the primary challenge in establishing a scattering particle monolayer system was to maximize the yield of monodispersed microsphere monolayers with high coverage for subsequent particle transfer and inclusion in a viscoelastic host polymer. Direct deposition of microspheres onto an unmodified glass substrate typically resulted in both a low degree of coverage and particle aggregation in the dried state [15]. Therefore, the approach adopted herein for the phantom fabrication was to first chemically modify a glass substrate to serve as positively-charged anchor points for the negatively-charged polystyrene (PS) microspheres. The PEM-modified glass substrate was then heated for controlled water evaporation as the mechanism of convective particle flux to form an ordered monolayer array of particles.

An ideal candidate polymer for the multilayered tissue phantom fabrication approach would possess viscoelastic properties that both facilitate good molecular contact with the substrate and also exhibit strong resistance to stress thresholds during delamination or debonding. Striking the fine balance between the liquid and the solid character that determines the debonding process is integral to an adhesive’s performance. We chose as a model system a cross-linked polymer, polydimethylsiloxane (PDMS, Sylgard 182, Dow Corning, Midland, MI), which consists of a silicone oil base along with a curing agent that formed chemical cross-links between the polymer chains. This two component system thus represents a model system that provided a reproducible means to transition continuously from a viscous liquid to an elastic solid. Furthermore, cross-linked PDMS is an elastomer with a very low Young modulus of about 1 MPa, and very low surface energy of 22.7 mJ m \(^{-2}\) [16]. These two properties together allow a cured PDMS construct to reversibly stick to itself or other solids by means of van der Waals forces. This rather weak bonding (the adhesion work of PDMS and glass is 0.1 J m \(^{-2}\) to 0.2 J m \(^{-2}\)) is suitable for the phantom fabrication in which small pressures (≈35 kPa) are enough to delaminate the PDMS layer from the glass substrate without rendering significant defects or blistering [17].

In this work, we demonstrate a bottom-up method for the particle assembly of a monolayer of polystyrene (PS) microspheres and transfer them into the PDMS elastomer to
produce novel optical phantoms for the characterization of axial resolution and contrast in scattering-based measurements as would be made in clinical tissue thickness measurements. By varying the dimensions of the embedded microspheres and the thickness of intervening polymer layers, different spatial frequencies are replicated in the axial dimension of the phantom. Furthermore, these frequencies can provide a standardized approach to determine the axial contrast transfer function for the quantitative application in an OCT imaging system [6]. For precise spatial calibration, bulk phantom dimensions were independently measured using a surface interferometric technique. Such a test system enabled a more accurate and repeatable determination of sub-surface particle distributions within the polymeric material.

2. Materials and Methods

An ideal multilayered phantom for axial resolution and contrast characterization of an optical imaging system consists of periodic alternating reflective (or scattering) and transparent layers with known thickness. In scattering-based depth-resolving imaging modalities such as OCT, the reflective layers should also have homogeneous scattering characteristics to appear uniformly bright in the image, much like a metal film on glass would produce a laterally uniform brightness. We fabricated a set of multilayered phantoms to cover spatial frequencies on the order of the theoretical axial resolution of OCT, as estimated from the coherence length of the OCT source. We investigated a novel approach for fabricating the phantom based on layered fabrication of particle monolayers within a host elastomeric material. All phantoms incorporated polydimethylsiloxane (PDMS) with a refractive index of 1.41 as the host polymer to embed scattering polystyrene (PS) microspheres with nominal diameters of either 2 μm, 3 μm, 5 μm, or 10 μm, all with a refractive index of 1.57.

2.1. Microsphere assembly using polyelectrolyte multilayers (PEMs) and convective particle flux

Before assembling the PS microspheres into a scattering monolayer, we first successively dip-coated a plasma-treated glass substrate with several alternating charged bilayers of polyelectrolytes. Polyelectrolytes are polymers whose repeating units bear an electrolyte group containing free ions that make the substance electrically conductive. These groups will dissociate in aqueous solutions, making the polymers charged. The charged bilayers were formed using layer-by-layer assembly from aqueous solutions of the polycation poly(allylamine hydrochloride) (PAH) and the polyanion poly(sodium 4-styrene sulfonate) (PSS) (Sigma-Aldrich, St. Louis, MO) for 15 minutes each with successive 1 minute washings with DI water between as depicted in Fig. 1. Deionized (DI) water (Millipore, specific resistance = 18 MΩ·cm) was used for all experiments and washing steps.

In polymer research, the radius of gyration is the key parameter to describe not only the size of a polymeric molecule but also its shape. This describes the way in which the cross sectional areal density of the material is distributed around its central axis. Greater radius of gyration means that the mass is concentrated at the larger distance from the central axis, resulting in a greater resistance to deformation or buckling to affect the areal coverage upon attaching to the substrate surface. The radii of gyration for the two polyelectrolytes were approximately 31 nm (PSS in 0.5 mol/L NaCl) and 22 nm (PAH in 0.05 mol/L NaCl). Based on this parameter, the following polymer concentrations near the overlap concentration were prepared and initially used: (a) 4.1 mg/mL PSS and (b) 1.87 mg/mL PAH, each in 2.0 mol/L NaCl [18]. A modification in this procedure was also used at half (1.0 mol/L NaCl) the initial molar salt concentration for comparison. Due to the opposing charges of the polyelectrolyte solutions, alternating depositions up to 3.5 bilayers established a PEM with a specific positive charge at the surface. By flow coating the microspheres in aqueous solution, negatively surface charged PS microspheres (Polysciences, Warrington, PA) were adsorbed onto the PEM with a final positively-charged PAH layer at the top surface. In order to obtain a hexagonally packed monolayer of microspheres while avoiding nonplanar clustering, we first
pre-heated the PEM-modified glass substrate to 70 °C for 5 minutes. This step was immediately followed by flow coating with a PS microsphere suspension in order to induce convective particle flux as shown in Fig. 2.

By pre-heating the PEM-modified glass substrate upon which the particle suspension was flow coated, the two-dimensional monolayer array or crystal was assembled in the moving meniscus of the evaporating particle suspension. This capillary force-driven crystallization process occurred as the particles carried by the flux of liquid towards the drying front were concentrated and incorporated in the transition region between the meniscus and the drying crystal array of microspheres [19].

2.2. Multilayered phantom fabrication

After the PS particles were adsorbed onto the glass substrate, a PDMS elastomer formulation approximately 1 mm thick was cast onto the PS monolayer as shown in Fig. 3.

PS microspheres were embedded in the cured PDMS elastomeric construct and positioned at the glass-elastomer interface. When the construct was delaminated from the glass, the particles remained in the PDMS elastomer as a monolayer of PS particles axially positioned subjacent to the polymer surface. This construct was inverted and a transparent PDMS film with a thickness equivalent to the PS microsphere diameter could then be applied with a flow coater on the top surface of the initial construct [20]. Our in-house flow coating device was ideal for generating uniform polymer film thicknesses in the submicron regime. A secondary
Step 1: Adhesion of PS particles to PEM-modified glass substrate

Step 2: Polymer casting and delamination of PS particles

Step 3: Sandwich of particle layers with intervening flow coated transparent polymer layer

Fig. 3. Step-wise schematic for multilayered phantom fabrication approach. Step 1: flow coat PS microspheres onto glass substrates modified with 3.5 polyelectrolyte bilayers; Step 2: cast ~1 mm thickness PDMS base on glass substrate with PS microspheres and delaminate PS microspheres/polymer construct from glass substrate to transfer particles to polymer surface (top surface of PS microspheres exposed to illustrate monodispersed distribution); Step 3: flow coat intervening layer of polymer onto first PS/polymer construct; allow to cure and cap with other construct.

PDMS construct with a subjacent PS monolayer was then inverted and placed on top of the semi-cured flow coated polymer film to eliminate discernible interfaces between the scattering and non-scattering polymer layers. Therefore, a bright-dark-bright layered structure was created, with these layers buried ≈1 mm beneath the top elastomeric surface. This thickness of the transparent layer between the top surface of the phantom and the initial scattering monolayer can be tuned by casting different amounts of polymer. An alternative approach we explored to control the intervening dark layer entailed flow coating exposed areas of the base layer with microsphere particle spacers resident at the edge. PDMS was then deposited between the spacers and the secondary PDMS construct positioned on top of the particle spacers.
2.3. Surface profilometry and confocal microscopy for high resolution imaging

We used a scanning white light interferometry (SWLI) (Zygo NewView 7300, Plainview, NY) with a 5X objective and 640x480 pixel camera, capable of measuring step height changes over a range of 50 nm to 1 mm, to measure surface profiles for the microsphere-embedded polymer construct and thicknesses of the successively layered fabricated using the multilayer buildup process. The surface profilometer was calibrated to a known NIST-traceable step height standard (Model #SHS-1.8QC, Serial #10783-08-16). The lateral and axial resolution of the surface profilometer is 0.1 nm and 360 nm, respectively. Relative axial positioning of the microspheres within the host elastomer were obtained by taking an XZ scan with a laser-scanning confocal Leica TCS SP5 microscope (Leica, Germany) using a 60 × 0.5 oil-immersion objective. The lateral and axial resolution of the confocal microscope is 170 nm and 600 nm, respectively.

2.4. OCT imaging and analysis

All phantom samples were imaged with a spectral domain OCT (SDOCT) system. The SDOCT system (Bioptigen, Raleigh, NC) operated at a center wavelength of 840 nm with 93 nm full-width at the half-maximum (FWHM) spectral bandwidth external source, yielding an approximate FWHM coherence length of approximately 3 μm. 6 mm x 6 mm wide rectangular OCT scans were captured from each phantom with 100 linear B-scans at 1000 A-scans per B-scan. The A-scans in each image were averaged together for quantitative assessment of resolution via the intensity peaks generated by two bright layers separated by a dark layer.

3. Results and Discussion

3.1. Key parameters in polyelectrolyte multilayer fabrication

As noted above, a process to produce a tightly packed PS microsphere coating on a glass substrate first involves the layer-by-layer deposition of electrostatic polyelectrolytes. A key parameter for polyelectrolyte suspensions involving dissociated electrolytes is the Debye or screening length scale beyond which the effect of the electrostatic charge no longer contributes to the surface attachment, as the counter-ions cluster near opposing charged surfaces to effectively screen the charge of a charged surface or polymer. This Debye length is given by

\[ \kappa^{-1} = \left( \frac{4\pi N_A L_b C_p}{\xi} \right)^{-0.5} \]  

for polyelectrolyte solutions without any added salt. \( N_A \) is Avagadro’s number, \( L_b \) is the Bjerrum length, \( C_p \) is the polyelectrolyte concentration, and \( \xi \) is the linear charge density of the polyelectrolyte. The Bjerrum length parameter describes the length scale at which the thermal energy equals the electrostatic potential energy between two elementary charges and is given by

\[ L_b = \frac{e^2}{4\pi \varepsilon kT} \]  

where \( e \) is the elementary charge, \( \varepsilon \) is the dielectric constant of the medium, \( k \) is the Boltzmann constant, and \( T \) is the absolute temperature. Significant charge separation can only occur when the distance between two opposite charges is greater than these length scales such that both electrostatic screening and thermal energy inhibit charge recombination. In the presence of salt in the polyelectrolyte solution, since the Debye length varies as follows:

\[ \kappa^2 = c + 2\xi^2 c_s \]
where \( z_e \) and \( c_e \) are the valency and concentration, respectively, of the salt counter-ions and \( c \) is the polymer concentration where the addition of salt decreases the Debye length due to enhanced screening from the additional charges.

Polyelectrolytes are generally more rigid than uncharged polymers due to the fact that the entropic driving force toward coiling and random chain conformations is inhibited by the intramolecular electrostatic repulsion of the charges along the chain backbone resulting in more correlated packing of the intermolecular polymer backbone. A measure of this rigidity is the persistence length \( L_p \), i.e. the length over which correlations in the direction of the backbone (at a given starting point) are lost or an essential measure of how long it takes for the chain to turn around. This length scale is given by the characteristic ratio, \( C_\infty \), and the bond length, \( L \) as follows:

\[
L_p = \frac{L}{2} (C_\infty + 1)
\]  

From this, we can infer that the polyelectrolyte chain conformation becomes more rod-like or stiffer with a concomitant increase in the charge density along the chain. When the concentration of counter-ion in a polyelectrolyte solution is increased, counter-ion condensation effectively reduces the charge density along the backbone, resulting in the increase of the mean spacing, \( b \), between charges on the chain, then so does the Debye length according to the following equation and Eq. (1):

\[
\xi = \frac{L_p}{b}
\]  

Since the Debye length is linearly correlated with the persistence length, \( L_p \), it increases as well and results in a stretched chain at higher charge densities. When \( \xi \) is unity, the charge spacing equals the Bjerrum length and the entropic driving force towards coiling equals the electrostatic driving force toward chain stretching. When charge density increases further, polyelectrolyte chain stretching predominates over coiling which may result in an uniformly spaced charge distribution and lesser extent of interdigitation with an oppositely charged polyelectrolyte chain. For our polymer-microsphere system, the NaCl salt concentration was varied to identify the optimal regime for maximal particle-substrate binding yet mitigate the extent by which screening salt counter-ions interfere with the subsequent electrostatic adsorption of PS microspheres to the PEM and the charge-density dependent stretching of the polyelectrolyte chain conformation.

3.2. PS particle binding and recovery in delaminated polymer construct

Prior to particle transfer and inclusion into the host polymer, we modified the glass substrate with PEMs to confer charged attachment sites for PS microsphere immobilization. Compared to a sedimentation control group where microspheres were physiosorbed onto the unmodified glass substrate yielding predominately disorganized three-dimensional particle aggregates, microspheres adsorbed onto a PEM-modified glass substrate yielded qualitatively better surface attachment, more uniform spacing, and more coplanar two-dimensional array of microspheres. As previously stated, NaCl salt concentration in our PEM solutions were modulated to further augment the electrostatic adsorption of PS microspheres by the mechanism of polyelectrolyte chain stretching and mitigation of the screening counter-ions to expose higher charge densities for particle adsorption. For illustration, a comparison of the 5 \( \mu \text{m} \) PS microsphere binding for the two polyelectrolyte solution salt concentrations (2.0 mol/L NaCl versus 1.0 mol/L NaCl) is shown in Figs. 4A and B.

Compared to its higher salt concentration counterpart, the 1.0 mol/L NaCl polyelectrolyte salt concentration samples exhibited a larger number of substrate-bound PS microspheres with the shorter interparticle spacing. On the other hand, stacked or nonplanar clustering of
microspheres was evident in the 2.0 mol/L NaCl polyelectrolyte salt concentration samples. In Fig. 4A, non-uniform absorption and sporadic clustering (indicated by bright and black halos off the focal plane) of microspheres may have been attributed to the coiling of the PEM chains in the presence of high salt counter-ions, thereby exposing non-uniform and relatively low charge density regimes available for inter-particle electrostatic binding.

After the viscous PDMS liquid was cast on the PS microsphere anchored PEM glass substrate and allowed to cure into an elastic solid, the cured elastomeric layer was delaminated to transfer the microspheres onto its surface. The transfer efficiency of microspheres to the elastomer for the three experimental conditions was quantified from images of different regions (n = 5) for particle counts on the PS-bound glass substrate followed by the transferred particle counts in the elastomeric construct. Particle counts were done from these images displayed by the ImageJ software. The fractional recovery of particles in the PDMS elastomeric construct after polymer casting, curing, and delamination for the two molar salt concentrations in Figs. 4A and B were 0.93 ± 0.08 and 0.74 ± 0.18, respectively. The particle transfer yield was higher for the high polyelectrolyte salt concentration due to a decrement in the initial bonding of the microspheres to the PEM-modified glass substrate, thereby facilitating ease of transfer to the elastomer. The particle transfer yield was lower for the low polyelectrolyte salt concentration case where higher charge density on the substrate enhanced microsphere-glass substrate bonding, requiring higher delaminating threshold forces to increase the particle transfer. For the fabrication of an ideal phantom, both the number density of microspheres and the transfer efficiency must be maximized. The increased density of microspheres would be achieved by using 1.0 mol/L NaCl solution as shown in Fig. 4B. However, at this lower salt concentration, the transfer rate was lowest due to higher initial binding affinity of microspheres to the substrate. To mitigate the electrostatic interaction between spheres and a PEM-modified glass substrate prepared at 1.0 mol/L NaCl solution, the substrate was preheated prior to binding of spheres. To further decrease the interparticle spacing and to increase the yield of bound microspheres as well, the PEM-modified glass substrates were pre-heated to initiate convective particle flux into a densely packed monolayer. There was concern that the heating would affect the thermal energy dependent Bjerrum length (Eq. (2)), and thereby reduce the affinity of the PS microspheres to the PEM-modified glass substrate. However, this did not prevent the relative monodispersity of packed microspheres. On the other hand, the initial binding of PS spheres onto the substrate was further increased as shown in Fig. 4C. Furthermore, the transfer rate
was increased to 0.83 ± 0.11, compared with the case without pre-heating, indicating a higher transfer rate than both cases without pre-heating. This implies that the electrostatic bonds were relaxed with the introduction of thermal energy.

In order to confirm 1.0 mol/L NaCl solution as the optimal molar salt concentration for particle transfer yield in the polymer, a parametric study was carried out for the following salt concentrations: 0.5 mol/L, 1.0 mol/L, 1.5 mol/L, 2.0 mol/L, 2.5 mol/L, and 3.0 mol/L. In Fig. 5, for 2 μm particle monolayers, XZ-scanned confocal reflectance images of subjacent PS microspheres demonstrated highest density particle packing subjacent to the elastomeric surface for the 1.0 mol/L NaCl solution. As the molar salt concentration increases periodically from 1.0 mol/L to 3.0 mol/L, a concomitant decrease in particles adsorb onto the glass substrate as non-uniform and relatively low charge density regimes predominate due to polyelectrolyte chain coiling. An interesting inflection point occurs between 1.0 mol/L and 0.5 mol/L as further decrease in the salt concentration leads to failure of the constituent polyelectrolyte chains to interdigitate with the opposing charged chains. This results in discontinuities in the buildup of the multilayer polyelectrolytes at the 0.5 mol/L molar salt concentration and decrease in adsorption particles and subsequent transfer of particles into the polymer as shown in Fig. 5A.

In all cases, a predominance of coplanar subjacent microspheres was observed.

3.3. Surface profile of PS microspheres within monolayer

For the fabrication of the ideal axial resolution target with particle embedded polymer layers, it is important to control the axial position of the microspheres relative to the layer interfaces. Since axial resolution is critical to quantifying OCT resolution, the PS particle layer should have minimal deviation in the axial direction. This would allow for the stacked layers of particles and PDMS to have well-defined placement regardless of measurement location. Therefore, to determine axial locations the microspheres, the transfer pattern of the microspheres to the surface of delaminated elastomer was investigated with high resolution surface profilometry.

In the ideal case (Case I), the particle layer should be completely buried within the PDMS matrix, with the top of the particle contacting the exposed surface. When imaged by surface

Fig. 6 Variants of microsphere axial distributions within polymer
High resolution surface profilometry allows for the identification of four different particle transfer patterns with regard to relative axial positions of particles as these unique patterns are illustrated in Fig. 6: (1) particles completely submerged in the polymer and subjacent to surface; (2) particles protruding from the surface with polymer coverage of microsphere; (3) particles protruding from surface with no polymer coverage of microsphere; and (4) sunken particles submerged in the polymer and protruding from surface.

Firstly, for Case I, particles were completely submerged in the elastomer with an axial position directly subjacent to the polymer surface as a result of the delamination force required to detach the elastomer from glass being tantamount to that required to exfoliate the relatively weak elastrostatically stabilized particles from the PEM layer. Next, in Case II, the slight peaks above the polymer surface represented particles protruding from the surface capped with a slight amount of PDMS on top. This resulted when the polymer was cast over the PS-attached PEM glass substrate and the uncured PDMS intercalated between the particle and glass substrate. This intercalation of PDMS was attributed to the presence of negligible electrostatic interactions between particle and PEM where the microspheres were effectively physisorbed onto the glass substrate. In Case III, particle protrusions above the polymer surface were observed due to the smaller delamination force required to detach the elastomer from the glass substrate compared to that required to mechanically exfoliate the strong elastrostatically stabilized particles from the PEM layer. The resultant partially embedded microspheres assumed a higher axial position relative to the polymer surface after trailing behind the polymer during the delamination step. These particles were represented in Fig. 6B primarily as red areas with ring-like bases corresponding to particles projecting through the surface and the ring representing a contact edge for the PDMS and particle. Finally, in Case IV, due to surface tension effects, some particles were not completely submerged in PDMS, and we observed divots typified by a depressed halo surrounding a black hole as shown in Fig. 7A. For SWLI profilometry, regions of high curvature cannot be measured, so null data points are positions unresolvable by the measurement technique. Since particles on a PEM substrate were unresolvable on using SWLI profilometry, these voids were indicative of exposed particles. This is corroborated by Fig. 4A, where particle aggregates were randomly dispersed on the transfer substrate. For aggregates, intercalation of PDMS prior to curing would be difficult, leading to incomplete wetting and defects as described in Case IV.

To mitigate these effects, a slight modification in the PEM method was implemented with a two-fold decrement in the PEM salt concentration to 1.0 mol/L NaCl to mitigate the polyelectrolyte chain charge screening effect of the counter-ions. PS particles under these modified conditions where the PEM charges were exposed resulted in a greater extent of electrostatic interaction. Under these modified conditions, the PEM were arranged in more linear chains containing higher charge density, resulting in greater number of particles in the same axial plane available (i.e. fewer stacked aggregates) for transfer into the polymer. A comparison of the different salt concentrations no divots in the polymer for the lower concentration 1.0 mol/L NaCl case in Figs. 7A–7C. The particle packing density was increased by heating the PEM-modified glass substrate prior to coating with the PS microspheres. This induced convection particle flux maintained the relative coplanar arrangement of the PS microspheres on the PEM-modified glass substrate while reducing the interparticle spacing. However, this application of heat likely had the adverse effect of altering the polyelectrolyte chain conformation to a more coiled state and thereby blunting the electrostatic bond between the PS and PEM-modified glass substrate. The result was the re-emergence of particles resembling that in Case II with slightly surface exposed particles with polymer coverage in Figs. 7C and 7D. These samples contain both ideal properties for an optical phantom, with the highest packing density (Fig. 4C) and minimal axial drift (≈0.1 µm) in comparison to other processing conditions.
Fig. 7. Surface profilometry of PS-embedded elastomer constructs. Five constructs were fabricated for each experimental group with surface profile sampling at four randomly selected regions. Contour plots shown were 1.50 mm x 1.10 mm in size. Traces for the height measurements or XZ profiles representing samples with maximum total deviation from flat surface are provided in the Supplemental Information. (A) A contour plot and corresponding height measurement for embedded 5 μm diameter PS microspheres in 2.0 mol/L NaCl polyelectrolyte solvent showed presence of buried aggregates leading to localized bowing of the elastomeric material. (B) Lower polyelectrolyte solvent concentration increasing charged PS-PEM interactions resulting in greater number of surface particle protrusions. (C) Modified experimental condition of pre-heating substrate to initiate convective particle flux flattened profile. (D) 10 μm embedded PS microspheres for particle size comparison showing similar profile flattening.

3.4. Phantom bulk layer thickness validation

The layered phantom fabrication method as described in Fig. 3 yielded four types of sample specifications: 10 μm nominal thick PS monolayers separated by a 10 μm thickness PDMS film, and, similarly, 5 μm PS nominal thick monolayers separated by a 5 μm thickness PDMS film, 3 μm PS monolayers separated by a 3 μm nominal PDMS film, and, similarly, 2 μm PS monolayers separated by a 2 μm nominal PDMS film. The intervening dark layer in the phantoms was measured using surface profilometry and the PS monolayers thickness was calculated using the PS microsphere manufacturer’s diameter specifications provided in tandem with axial deviations from the polymer surface measured using confocal microscopy. The mean ± standard deviation of the bright and dark phantom layer thicknesses for the 10 μm, 5 μm, 3 μm, and 2 μm PS microsphere embedded constructs were 10.0 μm ± 0.4 μm and 11.3 μm ± 0.3 μm, 4.8 μm ± 0.4 μm and 4.9 μm ± 0.3 μm, 3.2 μm ± 0.3 μm and 4.2 μm ± 0.3 μm, and 2.1 μm ± 0.2 μm and 3.1 μm ± 0.2 μm, respectively.

3.5. OCT imaging

OCT images of the phantom samples as shown in Fig. 8 were acquired with the OCT system configuration operating in the spectral domain (FDOCT). The 3 μm and 2 μm embedded particle monolayer phantoms are shown since the theoretical axial resolution limit could be addressed at these phantom dimensions. In Fig. 8C, the axial distance in microns was calibrated from the 3 μm particle monolayer sample by taking the peak-to-peak axial pixel separation. This separation is defined by the center-to-center separation of the bead monolayers. This is determined by summing the radii of the beads in the monolayers and the intervening particle spacing. The monolayer thickness was determined by confocal microscopy to be 3.2 μm ± 0.3 μm. The interparticle spacing was independently verified by
Fig. 8. 6 mm x 6 mm wide rectangular OCT scan of multilayered phantom constructs with 100 linear B-scans at 1000 A-scans per B-scan for 3 μm and 2 μm scattering PS particles. The scattering and intervening transparent layer thicknesses were validated with confocal microscopy and surface profilometry, respectively. (A, C) Representative OCT cross-sectional B-scans with accompanying axial profiles in (B, D) respectively are plotted as an average sampling of 50 A-scans along lateral extent of phantom to minimize effect of bending of phantom sample on the mean axial intensity measurement. The axial distance between two layers was calibrated from the sample in (B) by equating the peak-to-peak axial pixel separation between the particle monolayers to the transparent intervening layer thickness of 4.2 μm measured by surface profilometry. The OCT measured axial distance of 3.3 μm between two scattering monolayers in (D) was in statistical agreement with the result, 3.1 μm ± 0.2 μm, validated independently by surface profilometry. (E-F) 3D OCT image reconstructions highlight the separation between particle monolayers extending to the entire field of view of 500 μm x 500 μm. The theoretical axial resolution $R_{OCT}$ for these OCT imaging systems is given by the following relation:

$$R_{OCT} = \frac{l}{2} \approx 0.44 \frac{\lambda^2}{\Delta\lambda}$$

surface profilometry to be 4.2 μm ± 0.3 μm. The peak-to-peak separation of 7.4 μm ± 0.6 μm led to a calibration of the OCT B-scan image with 2.0 pixels/μm in the axial dimension. This calibration scale was then used to measure the interparticle spacing shown in Fig. 8C. The OCT measured axial distance of 3.3 μm between two scattering monolayers in Fig. 8D was in statistical agreement with the result, 3.1 μm ± 0.2 μm, validated independently by surface profilometry. Furthermore, the 3D OCT reconstruction illustrates uniform coverage of the particle monolayer in the field of view.

The theoretical axial resolution $R_{OCT}$ for these OCT imaging systems is given by the following relation:
where \( l_c \) represents the coherence length, \( \lambda_0 \) is the source center wavelength and \( \Delta \lambda \) is the source bandwidth. Therefore, the FDOCT system operated at a center wavelength of 840 nm with 93 nm FWHM spectral bandwidth has a theoretical axial resolution of \( \approx 3 \mu m \).

The layered phantoms did not demonstrate any surface specular reflections, because the layers of interest were buried \( \approx 1 \) mm beneath the top PDMS layer. The bright and dark layers of both layered phantoms with embedded 4.2 \( \mu m \) and 3.1 \( \mu m \) particles were resolvable by SDOCT. However, the layers of the 3.1 \( \mu m \) spaced sample were less resolvable, where the intervening dark layer between the particle monolayers approached the resolution limit. For both specifications, both the primary and secondary peaks were observable, but the intensity trough between the peaks in the 3.1 \( \mu m \) case was shallower as the spacing between the bright particle monolayers approached the theoretical axial resolution for the optical imaging system. Furthermore, significant index mismatch between the PS and PDMS polymers may present a challenging imaging condition, particularly at larger spatial frequencies as the stronger reflections at the boundaries of the microspheres may obscure the intervening transparent layer.

4. Conclusions

We have fabricated and characterized initial phantoms for assessment of the OCT depth of field measured parallel to the optical axis, thus contributing to the determination of the axial resolution, a fundamental figure-of-merit of imaging systems. The assembly of concentrated monodispersed microspheres in a two-dimensional crystal array was achieved via a combined methodology of PEM for particle binding and heat-induced convective particle flux towards a densely packed particle organization. Modulation of the PEM counter-ions was also recognized to optimize particle transfer from a glass substrate into an elastomer while preserving the relative axial positioning in a monolayer. This enabled a multilayered phantom approach to fabricate accurate length reference specimens as a suitable test target for quantitative dimensional measurements with depth-resolved imagers such as OCT. Future work will involve fabrication of more phantoms to cover a larger range of spatial frequencies, exploration of other polymer-microsphere material combinations, and further investigation of the optical property characteristics to encode in such phantoms. As the resolving power and detection sensitivity of OCT continue to increase, these multilayered axial resolution targets could offer both researchers and end-users of depth-resolving optical modalities both an effective means to critically assess and validate a key metric of device performance as well as provide greater confidence in a clinical measurement.

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