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Fabrication and application of heterogeneous printed mouse phantoms for whole animal optical imaging

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Abstract

This work demonstrates the usefulness of 3D printing for optical imaging applications. Progress in developing optical imaging for biomedical applications requires customizable and often complex objects for testing and evaluation. There is therefore high demand for what have become known as tissue-simulating “phantoms.” We present a new optical phantom fabricated using inexpensive 3D printing methods with multiple materials, allowing for the placement of complex inhomogeneities in complex or anatomically realistic geometries, as opposed to previous phantoms, which were limited to simple shapes formed by molds or machining. We use diffuse optical imaging to reconstruct optical parameters in 3D space within a printed mouse to show the applicability of the phantoms for developing whole animal optical imaging methods. This phantom fabrication approach is versatile, can be applied to optical imaging methods besides diffusive imaging, and can be used in the calibration of live animal imaging data.

1. INTRODUCTION

The interaction of light and matter at visible and near-infrared wavelengths has been used extensively in recent years for imaging biological processes both *in vitro* and *in vivo*. A rich spectrum of methods relying on the coherent or incoherent properties of light have been developed for applications ranging from focusing light through scatter [1–3] to protein folding and anticancer drug pharmacokinetics studies [4–6] to brain imaging [7–9]. The great challenge for these methods is overcoming the deleterious effects of scatter and absorption, which randomize the propagation direction of light and tend to decrease image resolution and quality. Evaluating, developing, and tuning these methods requires readily available and realistic tissue-like phantoms that provide known, controlled shapes and optical parameters. There is therefore a need for anatomically realistic tissue models.

3D printing is the ideal method for fabricating such phantoms. By adding material layer by layer, an object with virtually any desired shape can be built, allowing for the detailed design of the phantom geometry. By printing with multiple materials, it additionally becomes possible to place complex inhomogeneities at any desired location within the phantom, a feat which is difficult to accomplish (especially with high precision) using molds [10,11]. Such

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phantoms are useful for many applications, including tuning or testing measurement setups, characterizing spatial resolution and depth penetration, optimizing signal to noise, and comparing performance between systems. Here, for the first time, to the best of our knowledge, we describe a method to fabricate a heterogeneous whole animal phantom using 3D printing and show it is possible to image deeply embedded inhomogeneities.

Surprisingly, given its unique versatility and that it has been around since the 1980s, 3D printing has only recently been used to create phantoms for a few studies of near-surface (<1 mm depth) optical imaging methods, including spatial frequency domain imaging (SFDI) [12], hyperspectral reflectance imaging (HRI) [13], and speckle imaging [14]. In contrast, the phantoms fabricated here are for deep tissue (>2 cm depth, the location of major organs) imaging and the development of diffuse optical tomography (DOT) [15–18]. DOT is a whole animal optical imaging modality, allowing, for example, the determination of tumor sizes and locations [19], pharmacokinetic rates [6], and whole brain activation maps [20]. We note, however, that there is copious room to explore the uses of 3D printing for other imaging methods.

In optical diffusion imaging methods such as DOT, polystyrene beads [21], TiO_2 scatterers in acrylic [22], and Intralipid [23,24] have commonly been used as phantoms. However, phantoms created from these materials typically have simple shapes, such as those that can be formed by molds [10]. While such phantoms have proved useful, what is lacking is a method to create complicated geometries with inhomogeneities that better represent real objects of interest.

3D printing allows for the fabrication of geometrical replicas of animals or biological structures (such as blood vessels) with higher spatial resolution than has been demonstrated with DOT, making 3D printed phantoms ideal for testing and developing experimental setups and the various DOT algorithms. Additionally, the surface profile of a subject (such as a live mouse), which is captured during a DOT experiment, can be used to print a replica of the subject with known optical parameters that can be used for calibration, allowing for better image reconstructions of live mice than what have previously been possible.

In this paper we present reproducible heterogeneous solid phantoms fabricated using an inexpensive 3D printer. We used a popular publicly available whole body mouse atlas called Digimouse [25] to print realistic phantoms in the shape of mice from acrylonitrile butadiene styrene (ABS—used in Lego blocks), which has optical scatter and absorption characteristics similar to tissue [12,13]. Digimouse was constructed from coregistered x-ray, computed tomography (CT), and cryosection data of a normal nude male mouse and contains information on the geometrical structure of all major organs, fundamentally allowing a complete phantom to be printed from multiple materials which match the optical characteristics of the respective organs. For this work, we printed two inhomogeneities in the shape and at the location of the kidneys using two materials. Experimental data were captured and images were reconstructed with DOT using the open source TOAST++ package [26].

2. METHODS

A. Optical Experiment

Data were captured from the printed mice for reconstruction using an automated version of the measurement setup we have used previously for live mouse imaging [6]. The setup is shown in Fig. 1. A 633 nm pulsed laser (Horiba Jobin Yvon, pulse duration 230 ps) was used as the source and a cooled, gated, image-intensified CCD camera (Roper PIMAX, 512 × 512 pixels) was used for detection. A 3D topography laser line scanner was used to obtain the 3D profile of the printed mice, and these were used to determine the detector positions by projecting the CCD camera pixels to the Digimouse surface [27]. The position of the source was controlled using an X–Y motorized mirror mount from Zaber (T-MM2-KT04U), and the 3D topography scan was controlled using a 150 mm motorized linear stage, also from Zaber (T-LSM150A-KT04U). The microstep size of the X–Y mirror mount is 0.000086°, and the microstep size of the linear stage is 0.0476 μm. A script was written to control the stages and the camera for both the 3D scan and the gated measurement, fully automating the experiment. This automation not only allowed for much faster acquisition of the data but also better repeatability of the source positions for imaging the various printed mice. Gated transmission data were collected from the printed mice using a gate width of 1.56 ns and 80,000 gates with a sequential delay that varied from 17 to 35 ns for 41 images, where 17 ns is the delay required for the laser pulse triggered by the camera controller to reach the camera. The laser has an energy per pulse of 12 pJ and was triggered at 5000 Hz by the camera, giving an average power of 60 nW.

B. 3D Printing

3D Printing is an additive process by which material is added layer by layer to build an object. For this work we used the MakerBot Replicator 2X, as shown in Fig. 2(a), an inexpensive printer with dual extrusion, allowing us to print an object from two different materials. The printer operates by extruding ABS through two heated Teflon-coated nozzles (on the bottom of the extruders) onto a heated build plate. The nozzles' horizontal position is controlled by two stepper motors, and the plate is translated vertically to allow printing in three dimensions. For a typical print, a 3D profile is generated in a computer-aided design (CAD) program and converted to standard tessellation language (STL) format, which describes the raw unstructured triangulated surface by the unit normals and vertices of the surface triangles in Cartesian coordinates. This 3D surface profile is “sliced” by the MakerBot software and converted to ×3g format, which uses g-code to control the 3D printer. The ×3g file is loaded into the printer using a secure digital (SD) card.

The quality of the print is influenced by many setup parameters, including motor speed, nozzle temperature, layer thickness, and the temperature and leveling of the build plate. For our optical phantom, we printed at 100% fill with two materials, which required optimizing these parameters to achieve a successful print. Additionally, printing at 100% fill led to warping at the edges of the print due to uneven cooling. This problem was solved by printing on a raft with the object oriented so that the build surface area was maximized. To get the ABS to “stick” to the build plate, we built on sanded Kapton tape and cleaned the build area with acetone before each print.

C. 3D Printed Digimouse

The Digimouse mouse atlas [25] contains data on the geometrical structure of the major organs of a normal nude male mouse at a resolution of 0.1 mm. The data is organized in a Cartesian grid and consists of integers corresponding to different organs or types of tissue. We scanned through this data to select the kidneys and the skin only and created a surface profile of each, which was converted to an STL file in MATLAB. Figure 2(b) shows the resulting Digimouse isosurface with the kidneys only. It would be simple to include more organs, including the whole brain, heart, lungs, liver, skeleton, etc. in the print, but for this initial work we only printed the kidneys. Ideally, each organ would be printed from a different material with optical properties similar to the actual tissue.

The 3D printed Digimice are shown in Fig. 3. From left to right, the first mouse was printed without any inhomogeneities and was used for calibration. The second mouse was printed with highly absorbing inhomogeneities of black ABS in the shape of the kidneys. The third mouse, halted midprint, shows the location and quality of the absorbing inhomogeneities. The fourth mouse was printed with highly scattering inhomogeneities of white ABS in the shape of the kidneys. The fifth mouse was also halted midprint to examine the location and quality of the highly scattering inhomogeneities. The raft was carefully removed before imaging, and a sharp knife was used to remove excess support material on the bottom. A single mouse took approximately 3.5 h to print.

Printing a volume of some material completely enclosed by another volume of a different material is not typically done in 3D printing, although all 3D printers which can print with multiple materials have this capability. As such, little guidance was available and many iterations of printed mice were performed before a successful print was achieved. In fact, this may be the first time such an object has been printed on the MakerBot 2X, as there seem to be few reasons other than imaging for printing embedded objects. The process we used is described here. Two STL files were generated in MATLAB using the Digimouse atlas: one with the skin surface profile only and the other with both the skin and kidneys surface profiles. The first STL file only was used to generate the homogeneous printed mouse which was used for calibration. Both STL files together were used to generate the printed mice with inhomogeneities in the shape of the kidneys. This was done by placing both surface profiles at the same location on the build plate in the MakerBot software and designating a different extruder to each surface. When the STL files are sliced, the result is a single 3D volume with a different material placed within the volume of the kidneys. If the STL file containing the skin and kidneys surface profiles only is used, empty space instead of material would be present at the location of the kidneys. This type of print would be useful for fluorescence imaging experiments, for example, where a fluorophore in solution could be injected into the cavity.

The position and orientation of the mouse on the build plate is important for printing with multiple materials. The mouse was printed lying flat to maximize the surface area in contact with the heated build plate in order to minimize warping and maximize the likelihood of sticking. When printing with multiple materials, streaking, or the deposit of excess material at undesired locations, is a significant problem. Within the printing of a single layer, after the operating nozzle is switched to print with a different material, a small amount of material

remains on the tip of the now nonoperating nozzle. This excess material is deposited at unwanted locations on the mouse as both nozzles move across the object, causing streaking. To minimize this, purge walls were printed around the mouse, as can be seen in Fig. 2(a), and whenever the nozzles are switched, they are first dragged across the purge walls so that the excess material on the nonoperating nozzle is removed. Even with these purge walls, we saw significant streaking [as seen in the bottom mouse of Fig. 4(a)], until we changed the orientation of the mouse from that shown in Fig. 4(b) to that shown in Fig. 4(c). In the orientation of Fig. 4(c), only a single nozzle is positioned over the mouse at a time while printing head-to-tail, compared to Fig. 4(b), where both nozzles are positioned over the mouse and the chance of streaking is higher. Printing in Fig. 4(c) orientation effectively removed most of the streaking, although some can still be seen, for example, around the kidneys of the middle mouse in Fig. 3.

The quality of the first layer should be considered carefully as it must be laid almost flawlessly for a successful print. It is desirable to have as few gaps as possible in the path that the extruder takes in creating the first layer so that the entire layer will stick to the build plate with high probability. A simple way to accomplish this is to modify the first layer by changing the orientation of the entire printed object with respect to the extruders in order to change the path the extruders take during the print. We achieved this by rotating the printed mice in the clockwise direction (looking down on the mice) until there were almost no observed gaps in the first layer. This rotation is represented by θ in Fig. 4(c), where θ is the angle between the reference frame of the nozzles and the reference frame of the mouse. With a rotation of $\theta = 10^\circ$, few gaps were observed and this final position, as shown in Fig. 2(a), resulted in successful prints.

D. Calibration

The data set captured from the homogeneous printed mouse was used to calibrate the data captured from the printed mice with inhomogeneities [28] using

$$y_i = y_i^{\text{uncal}} \frac{y_i^{\text{comp}}}{y_i^{\text{base}}}, \quad (1)$$

where i represents the i th component of the data vector, y_i^{base} is raw data captured from the homogeneous mouse, y_i^{comp} is the forward solution data for the homogeneous mouse, and y_i^{uncal} is raw data captured from the mouse with inhomogeneities in the shape of the kidneys. For this work, the forward solution is the numerical solution to the diffusion equation at the detector positions due to excitation by the sources. Note that the calibration was done component by component, constituting unique calibration of every detector–source pair. Equation (1) is particularly convenient for calibrating data in the frequency domain (which was done here), as the camera response is contained within both y_i^{uncal} and y_i^{base} and is canceled out. The primary purpose of this calibration is to remove variations in the amplitude and phase of the measured data that are not described by the numerical model. The validity of this calibration deteriorates if the size and position of the *uncal* and *base*

objects vary; however, it is ideal for 3D printed objects of the same shape as long as they are placed carefully at the same position on the imaging platform. For a live mouse experiment this type of calibration becomes possible with 3D printing because a homogeneous phantom can be printed in the shape of the live mouse from the surface data acquired from the 3D line scan. This removes the need to calibrate through optimization-based inversion [18] and will allow better calibration of experimental data than what has been possible previously with live mice, likely resulting in higher quality images.

E. Parameter Estimation

In the time domain and for homogeneous scatter, the photon flux density, $\phi(\mathbf{r}, t)$ (W/mm²), satisfies the diffusion equation [28–30]

$$\frac{1}{c} \frac{\partial}{\partial t} \phi(\mathbf{r}, t) - D \nabla^2 \phi(\mathbf{r}, t) + \mu_a \phi(\mathbf{r}, t) = S(\mathbf{r}, t), \quad (2)$$

where $\mathbf{r}$ denotes the position, μ_a (mm⁻¹) is the linear absorption coefficient, $D = 1/[3(\mu_a + (1 - g)\mu_s)]$ (mm) is the diffusion coefficient, μ_s is the linear scattering coefficient, g is the mean cosine of the scattering angle accounting for anisotropy, c is the speed of light in the medium, and $S(\mathbf{r}, t)$ is the photon source. This equation can be solved analytically for the transmitted photon flux density in an infinite slab geometry by first assuming that all incident photons are initially scattered at a depth $z_0 = 1/[(1 - g)\mu_s]$, or $z_0 = 1/\mu'_s$, and placing point image sources such that $\phi(\mathbf{r}, t) = 0$ on an extrapolated slab boundary [29]. We will define one surface of the slab as being at $z = 0$. We then define the transmittance at the $z = d$ boundary, where d is the thickness of the slab, as the magnitude of the current density from Fick's law in cylindrical coordinates, where the current density $\mathbf{J}(\rho, d, t)$ is given by

$$\mathbf{J}(\rho, d, t) = -D \nabla \phi(\rho, z, t)|_{z=d} \quad (3)$$

with $\phi(\rho, z, t)$ the Green's function solution to Eq. (2). Using the discrete dipole approximation to enforce a zero flux boundary in a slab geometry, and retaining four dipoles, the transmittance $T(\rho, d, t)$ through a detector aperture of radius ρ_o is

$$T(\rho, d, t) = 4\pi D c t \left[1 - \exp \left(\frac{-\rho_o^2}{4D c t} \right) \right] T_o(d, t), \quad (4)$$

where $T_o(d, t)$ is a function containing the dipole information and is given by

$$\begin{aligned}
T_o(d, t) = & (4\pi Dc)^{-3/2} t^{-5/2} \exp(-\mu_a ct) \\
& \times \left\{ (d - z_o) \exp \left[\frac{-(d - z_o)^2}{4Dct} \right] \right. \\
& - (d + z_o) \exp \left[\frac{-(d + z_o)^2}{4Dct} \right] \\
& + (3d - z_o) \exp \left[\frac{-(3d - z_o)^2}{4Dct} \right] \\
& \left. - (3d + z_o) \exp \left[\frac{-(3d + z_o)^2}{4Dct} \right] \right\}. \quad (5)
\end{aligned}$$

A simplified solution for Eq. (4) can be found by taking the two-term Taylor expansion with respect to ρ before integrating across the detector aperture.

A gated measurement was performed on an 8 cm by 8 cm 3D printed slab of ABS with $d = 0.5$ cm using the setup described in Section 2.A. The data were fit to the convolution of the camera response and Eq. (4) by finding the minimum mean square error (MMSE) for various μ'_s and μ_a . The estimated scatter and absorption parameters at 633 nm were $\mu'_s = 1.43$ and $\mu_a = 0.0018 \text{ mm}^{-1}$, respectively, which are close to the values found in other works [12,13]. These parameter estimates were used for calibration and as the initial values in the reconstruction. The optical scatter and absorption characteristics of ABS are close to what is found in tissue [13], showing that these phantoms are ideal for evaluating and developing whole animal optical imaging methods.

3. OPTICAL IMAGING OF PRINTED MOUSE PHANTOMS

In DOT, the forward model describes the propagation of modulated incoherent light through tissue using the diffusion equation, and the absorption (μ_a) and scatter (μ'_s) parameters are reconstructed as a function of position from boundary measurements using an inverse solver. The inverse problem is ill-posed, and the challenge resided in finding the global minimum of an objective function [31]. TOAST++, an open-source collection of libraries for sparse matrix algebra and finite-element analysis, can be used to solve nonlinear inverse problems such as DOT [26].

Here, boundary data from the printed mouse with highly scattering inhomogeneities in the shape of the kidneys was captured using the experimental setup described in Section 2.A. The data were calibrated according to Eq. (1) and imported into the TOAST++ environment in MATLAB, along with the source and detector positions obtained using the 3D line scan. An unstructured finite element mesh was generated using TetGen [32] using 22,489 nodes (4881 surface nodes) from a subset of the Digimouse data. Alternatively, the 3D line scan could have been used to generate the mesh, as we have done previously for imaging mice where the 3D surface profile is not known [6,27], however, the mesh generated from the Digimouse data is more accurate as it was used to print the mouse. For reconstruction, images were formed on a Cartesian grid with voxel size $(1 \text{ mm})^3$ and a nonlinear conjugate

gradient iterative solver was used with first-order Tikhonov regularization. The hyperparameter (α) which weights the penalization imposed by the regularization was set to 10^{-5} , a relatively small number. Using modulation frequency data at 36 MHz, μ_a and μ'_s were simultaneously reconstructed. Data from seven source positions on the bottom and 346 detector positions on the top of the printed mice were used (see Fig. 5), giving 2422 measurements. The data, which was captured in the time domain, was low-pass filtered and then transformed to the frequency domain with a fast Fourier transform. Two outlier data points were removed. A relatively low modulation frequency was used because the calibrated detected phase data were found to most closely match simulated forward data at this frequency. Using a 3.47 GHz Intel X5690 with 96 GB RAM, the reconstruction converged in 20 iterations lasting 1.3 h, where convergence is five consecutive iterations in which the change in the objective function is less than 1% of the mean of the five largest objective function reductions.

The reconstruction of the printed mouse with highly scattering inhomogeneities in the shape of the kidneys is shown in Fig. 6. Figure 6(a) shows the μ_a mesh cross section at the $z = 15$ mm plane, Fig. 6(b) shows the μ_a isosurface, Fig. 6(c) shows the μ'_s mesh cross section at the $z = 16$ mm plane, and Fig. 6(d) shows the μ'_s isosurface. As expected for the highly scattering inhomogeneities, μ_a is small, and μ'_s is large compared to the background values. Comparing to Fig. 2(b), the reconstructed μ_a shows the size and location of the kidneys more accurately than the reconstructed μ'_s , likely because the large contrast between the scattering inhomogeneities and the background made convergence to the exact solution of μ'_s more difficult. These results show that it is possible to image inhomogeneities in 3D printed phantoms using DOT and that these phantoms are a useful tool for evaluating and developing DOT experimental setups and algorithms.

4. DISCUSSION

We have used a mouse atlas to print geometrical replicas of mice with inhomogeneities in the shape of the kidneys, but other atlases can be used to print other objects of interest, such as a human breast or brain with inhomogeneities in the shape of tumors. Objects with more complicated inhomogeneities can be used for general image evaluation, such as a bar pattern for determining spatial resolution. Cavities can be placed within the object that can be filled with a fluorescent solution using a syringe, useful for evaluations of fluorescence diffuse optical tomography [28]. Progress in bioprinting, the printing of complex 3D functional living tissues from biocompatible materials, cells, and supporting components [33], could enable the fabrication of extremely realistic phantoms. Shells can be printed using clear resins to contain conformable objects (such as a mouse brain) so that the 3D surface profile is known, avoiding the need for the 3D topography laser line scan. For mouse or animal imaging, the surface profile that is captured during a DOT experiment can be used to print a replica of the subject with known optical parameters that can be used for calibration, allowing for better image reconstructions of mice than what have previously been possible.

Although there are broad applications of 3D printed optical phantoms due to the versatile nature of 3D printing, there are restrictions due to the limited materials available and their static optical properties. One solution to this problem was proposed by Avtzi *et al.* [34], where solid 3D printed objects were used to create silicone molds. These molds were then filled with epoxy resin with controlled optical properties to create phantoms. Although useful for creating molds, placing complex inhomogeneities becomes difficult with this method compared to 3D printing phantoms and inhomogeneities with multiple materials. This problem of static optical properties may be addressed by adding varying amounts of India ink, Intralipid, or polystyrene beads to ABS or an equivalent printing material to control the optical properties, similar to what was done by Sheng *et al.* [14] to simulate skin tissue, although this must be done carefully to not disrupt 3D printing. Additionally, fluorescent chemicals could be added to the printing material in order to print fluorescent inhomogeneities.

5. CONCLUSION

We have demonstrated that 3D printing is useful for creating realistic tissue-like phantoms with complicated geometries and inhomogeneities for evaluating and developing DOT. 3D printing is an effective tool (and perhaps in some cases the only tool) for creating complicated phantoms with inhomogeneities for imaging. The phantoms fabricated here were designed for imaging with DOT, but likely all optical imaging methods can benefit from customizable 3D printed phantoms. In addition to fabricating phantoms, 3D printing can be used to create optics equipment [35], lenses, and optical fibers [36], and is useful for customizing experimental setups, making 3D printers a useful addition to any imaging or optics lab.

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Fig. 1.

Experimental setup. Pulsed time-domain data transmitted through the mouse is captured by the gated, intensified CCD camera. The motorized X-Y scan mirror changes the source position before each gated measurement. The computer is used to control the camera and the motorized X-Y stage, automating the data capture.

(a)

(b)

Fig. 2.

(a) MakerBot Replicator 2X used for 3D printing. The dual extruders are visible at the top right. The purge walls printed around the mouse allow excess material to be swept off the extruders when changing print color, helping to minimize spotting caused by the dual extrusion. Printing on a raft reduced warping at the edges. Kapton tape on the heated build plate helps to increase sticking. (b) Digimouse mesh with kidneys only. This mesh was used to generate the STL file for 3D printing.

Fig. 3.

3D printed Digimice with highly absorbing and scattering inhomogeneities of black and white ABS in the shape of the kidneys. All mice were printed from ABS at 100% fill with a layer height of 0.1 mm. Starting from the left, the first mouse was printed without any inhomogeneities and was used for calibration. The second mouse was printed with highly absorbing inhomogeneities of black ABS in the shape of the kidneys. The third mouse was halted midprint due to the misprinted front right limb and shows the location and size of the absorbing inhomogeneities. The fourth mouse was printed with highly scattering inhomogeneities of white ABS in the shape of the kidneys. The fifth mouse was also halted midprint to show the position and shape of the highly scattering inhomogeneities. The raft was carefully removed before imaging, and a sharp knife was used to remove excess support material on the bottom.

(a)

(b)

(c)

Fig. 4.

Changing the orientation of the printed object can drastically improve quality. The top mouse in (a) was printed in the orientation shown in (c), and the bottom mouse in (a) was printed in the orientation shown in (b). Parts (b) and (c) show a top view, where the black box represents the build plate, the red squares represent the extruders, and the black circles represent the heated nozzles. The orientation in (c) is the same as in Fig. 2(a), where θ is an angle signifying a rotated reference frame with respect to the nozzles. In the (b) orientation, excess material was smeared across the head-to-tail length of the mouse as both nozzles were dragged to the purge walls, resulting in substantial streaking, or deposit of unwanted material, as seen in the bottom mouse in (a). For the mouse in the (c) orientation, only a

single nozzle is positioned over the mouse at a time while printing head-to-tail, reducing the chance of streaking.

Fig. 5.

Source (red) and detector (blue) positions on the Digimouse surface nodes (green). Data from the seven source positions on the bottom were captured at the 346 detector positions on the top of the printed mice, giving 2422 measurements. The surface profile obtained from the 3D line scan was used to project the CCD camera pixels (detectors) and the incident light from the pulsed laser diode (sources) to the Digimouse surface.

Fig. 6.

Simultaneous reconstruction of absorption and scatter parameters from calibrated data collected from the printed mouse with highly scattering inhomogeneities in the shape of the kidneys using TOAST++. Note that the reconstruction for the mouse with highly absorbing inhomogeneities is not shown. First-order Tikhonov regularization was used with $\tau = 10^{-5}$: (a) shows the μ_a mesh cross section at the $z = 15$ mm plane, (b) shows the μ_a isosurface, (c) shows the μ_s' mesh cross section at the $z = 16$ mm plane, and (d) shows the μ_s' isosurface.