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Fast Optical Transillumination Tomography with Large-Size Projection Acquisition

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Abstract

Techniques such as optical coherence tomography and diffuse optical tomography have been shown to effectively image highly scattering samples such as tissue. An additional modality has received much less attention: Optical transillumination tomography (OT), a modality that promises very high acquisition speed for volumetric scans. With the motivation to image tissue-engineered blood vessels for possible biomechanical testing, we have developed a fast OT device using a collimated, non-coherent beam with a large diameter together with a large-size CMOS camera that has the ability to acquire 3D projections in a single revolution of the sample. In addition, we used accelerated iterative reconstruction techniques to improve image reconstruction speed, while at the same time obtaining better image quality than through filtered back projection. The device was tested using ink-filled PTFE tubes to determine geometric reconstruction accuracy and recovery of absorbance. Even in the presence of minor refractive index mismatch, the weighted error of the measured radius was less than 5% in all cases, and a high linear correlation of ink absorbance determined with a photospectrometer of $R^2 = 0.99$ was found, although the OT device systematically underestimated absorbance. Reconstruction time was improved from several hours (standard arithmetic reconstruction) to 90 seconds per slice with our optimized algorithm. Composed of only a light source, two spatial filters, a sample bath, and a CMOS camera, this device was extremely simple and cost-efficient to build.

Keywords

Optical Tomography; Algebraic Reconstruction Technique; Tissue-Engineering

INTRODUCTION

In the industrial world, cardiovascular diseases such as arteriosclerosis are the major cause of morbidity and mortality³. Typical treatments for a arterial occlusions include angioplasty and stenting, but these methods are limited if the diameter of vessels is smaller than 6mm^{5,23,27}. Although the supply from animal or cadaver vessels is possible, these approaches suffer from tissue degradation, dilatation and aneurysm formation^{1,7,11,14,19}. Recently, a completely new approach to grow a blood vessel consisting of only the patient's own cells and without any synthetic materials was introduced^{17,18}. This new technique carries the promise that a vast supply of vascular grafts, particularly of small diameter, can now become available. The production of this type of tissue-engineered blood vessel (TEBV) is relatively expensive

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and needs to be performed on a per-patient basis. This implies that non-destructive and minimally invasive testing methods to monitor growth, perform quality control and indirectly predict biomechanical properties are of key importance in the production process. Specific optical imaging methods, such as optical coherence tomography, with high spatial resolution and good fluid-tissue contrast would have the desired properties.

One possible modality is optical transillumination (OT) tomography. We have recently presented a first-generation pencil-beam OT scanner for the purpose of acquiring cross-sectional images of TEBV¹⁰. While contrast and resolution were clearly sufficient to identify tissue geometry, inhomogeneities and defects, the presented scanner suffered from long acquisition times. First-generation devices are characterized by a combination of translational and rotational motion to acquire projections. In its most advanced state, the device required 3 minutes per cross-sectional slice, leading to a total scan time of several hours for an exhaustive scan¹⁰. The main goal of this study was to investigate the feasibility to use a full three-dimensional (3D) projection by means of a large-diameter excitation beam and a large-size CMOS detector for acquisition projection. In other words, one revolution of the sample provides enough projections to reconstruct a 3D volume of the CMOS detector's height. Since this process requires the simultaneous reconstruction of many cross-sectional slices, particular attention was given to the optimization of reconstruction time. We present an optimized variation of the well-known algorithm to reconstruct images from projections using the iterative arithmetic reconstruction technique (ART²) with ordered subsets (OS-SART) that reduced reconstruction time from 500 seconds per slice (ART) to 90 seconds per slice.

MATERIALS AND METHODS

A. System design

Figure 1 shows a sketch of the setup of the OT system. A red light source was chosen because red light falls into the “tissue window”, a wavelength region of low absorption. Two illumination sources were used: (1) a 650 nm, 5 nm full-width at half-maximum (FWHM) diode laser (Sanyo DL3147–060 diode in LT230P-B collimation unit, Thorlabs, with custom controller) in conjunction with a BE-05M beam expander (Thorlabs), and (2) a high-power LED emitter with a center wavelength of 627 nm and 25 nm FWHM (LXHL-LD3C, Luxeon, San José, CA) in conjunction with a collimating lens (1" diameter, $f=25$ mm) and a spatial filter consisting of two lenses (1" diameter, $f=40$ mm) and a mounted, 150 μ m, pinhole (all optical components from Thorlabs unless otherwise noted). Lenses and pinhole were arranged in a manner that both lenses had a common focal plane, and the pinhole was placed in the focal plane. Since a spatial filter rejects light rays that are not parallel to its axis, the output is a highly collimated, but not coherent, beam of light.

The large-diameter collimated beam was then directed onto the sample, immersed in a parallel-wall sample bath (custom made by Vitriforms, Inc). Inside the bath, the sample was supported by nylon ball bearings (Small Parts, Inc.) and rotated by a stepper motor with 0.9° per step attached to the top of the sample bath.

A motorized translation stage (Anaheim Automation) provided the ability to position the sample in axial (Z) direction, and centering of the sample with respect to the beam was possible through a manual translation stage inserted between the Z translation stage and the sample bath.

Light passing through the sample was collected through a second spatial filter (model 910A, Newport) and digitized with a high-resolution CMOS camera (1920 $\times$ 1080 pixels with 12 bits per pixel, Silicon Imaging SI-1920M). With 5 μ m square pixel size, the total active area of the camera was 9.6 $\times$ 5.4 mm.

B. Image Reconstruction

Because the second spatial filter blocks scattered photons, only ballistic photons are collected by the camera. These photons propagate in a straight line, therefore the light intensity at detector k can be calculated from Lambert-Beer's law (Equation 1):

$$I_k = I_{0,k} \exp \left(- \int_s \mu_t(s) ds \right) \quad (1)$$

Here, $I_{0,k}$ is the incident light for detector k , and μ_t is the tissue absorbance along a straight path s . In scattering samples, such as tissue, μ_t is the sum of the scattering component μ_s and the absorbing component μ_a . By taking the logarithm of Equation 1, the projection data g_k at detector k can be represented by Equation 2:

$$g_k = \ln \left(\frac{I_{0,k}}{I_k} \right) = \int_s \mu_t(s) ds \quad (2)$$

From the projection data, the cross-sectional image of attenuation coefficients can be reconstructed using either algorithms like the well-known filtered backprojection (FBP [16]) or by iterative algorithms such as the simultaneous algebraic reconstruction technique (SART [2]). Because of the superior reconstruction quality of SART [2], we focused on this technique to reconstruct cross-sections from our projection data. Generally, the n -th iteration of the SART reconstruction can be represented by Equation 3:

$$\mu_j^{(n+1)} = \mu_j^n + \frac{\lambda}{\sum_i H_{ij}} \cdot \sum_i H_{ij} \left(g_i - \frac{\sum_k H_{ik} \mu_{ik}^n}{\sum_k H_{ik}} \right) \quad (3)$$

Here, μ_j is the j^{th} element of the image matrix (interpreted as a one-dimensional vector of image elements), g is the known projection data (Equation 2), and H_{ij} is the system matrix that describes the geometry-dependent contribution of a ray hitting detector i to the pixel j . λ is an underrelaxation parameter ($0 < \lambda \leq 1$) which allows to improve reconstruction quality at the expense of reconstruction time. In a phantom study, we have demonstrated the superiority of the SART algorithm over FBP, particularly in the presence of areas affected by refractive index mismatch [13]. We have also pointed out that reconstruction time is a concern. For this reason, we focused on a more efficient algorithm known as SART with ordered subsets (OS-SART [15]). It partitions the projection data into disjoint subsets and then uses all the data in a single subset per backprojection. OS-type algorithms are popular because of their order-of-magnitude acceleration over SART with only minimal modification of the SART algorithm. We used OS-SART as presented by Wang and Jiang [24], where the reconstruction equation is now written as Equation 4:

$$\mu_j^{(n+1)} = \mu_j^n + \frac{\lambda}{\sum_{i \in l} H_{ij}} \cdot \sum_{i \in l} H_{ij} \left(g_i - \frac{\sum_k H_{ik} \mu_{ik}^n}{\sum_k H_{ik}} \right) \quad (4)$$

The only difference to conventional SART (Equation 3) is the limitation of the backprojection to a subset l . Further acceleration was achieved by adapting the ray-tracing method proposed by Siddon [22]. Instead of assuming a fixed object (and therefore a fixed reconstruction array) around which the detector system is rotated, we assumed a rotating object inside a fixed array-detector system. This approach allows dramatic reduction of the computational effort spent on the calculation of the system matrix H with only minimal tradeoffs in image quality [8].

C. Reconstruction samples

To validate the scanner's performance, we designed samples using transparent polytetrafluorethylene (PTFE) tubing (Zeus Industrial Products, Inc.). Two types of phantoms were built (Figure 2): The first series of phantoms consisted of a single PTFE tube of 3.4 mm inner diameter and 0.2 mm wall thickness, filled with a sucrose-ink solution. Sucrose solution of 3.8% by weight was prepared, because the 3.8% sucrose minimized refractive index mismatch. To prepare the absorbing sucrose-ink solutions, first 10 μ l of artist's black India ink (Daler-Brownes) was diluted in 10 ml of the 3.8% sucrose solution. Six solutions with different final ink concentrations were prepared by adding 1.5 μ l, 2.5 μ l, 4 μ l, 5 μ l, 5.5 μ l, and 7 μ l of the diluted ink in 1 ml of sucrose solution. The second series of phantoms was built from two concentric tubes, the inner one with 3.4 mm inner diameter and 0.20 mm thickness, the outer one with 4.7 mm inner diameter and 0.25 mm thickness. In this case, 4.5% sucrose was needed to minimize refractive index mismatch. Dilutions of ink at the concentrations described above, but in 4.5% sucrose solution were prepared and used to fill the space between the tubes, while the inner tube was filled with ink-free sucrose solution. All phantoms were immersed in a sucrose solution (3.8% for the single-wall phantoms, 4.5% for the double-wall phantoms) for image acquisition. Tube diameters were chosen to approximate the diameter of tissue-engineered blood vessels (4 mm inner diameter). The camera was oriented such that a projection with 1920 pixels was acquired orthogonal to the axis of rotation. To reduce the reconstruction time and image size, we reduced the number of pixels in each projection from 1920 to 480 using bilinear interpolation. For each reconstruction, we acquired either 200 or 400 projections (at 1.8° and 0.9° angular increment between projections, respectively). Absorbance of each fluid was determined in a Beckman DU520 spectrophotometer in standard methacrylate cuvettes at 620 nm. The PTFE tube was segmented in the cross-sectional images. Mean absorbance values were determined by averaging the reconstructed absorbance (i.e., pixel value) in the reconstructed ink-filled areas, and the mean radius r_M of the tube was computed from the segmented area under the assumption of a near-circular geometry. Recovery of the attenuation coefficient was defined as the difference between absorbance determined by the spectrophotometer and mean absorbance inside the reconstructed PTFE tube normalized by the spectrometer value. A normalized geometrical error was calculated from the difference between mean radius r_M and known tube radius, normalized by the tube radius. Signal-to-noise ratio (SNR) was approximated by dividing the mean pixel value inside the segmented tube by the standard deviation of the pixel values.

RESULTS

Projections of the phantom (no ink) taken with laser and LED illumination are shown in Figure 3. A strong diffraction pattern can be observed with the associated ring artifacts in the reconstruction. The vertical stripes were caused by diffraction at the boundary between sample and liquid media because of the slight mismatch of the refractive index between them. This is a typical phenomenon associated with coherent light sources⁶. No such diffraction pattern was observed with LED excitation. For this reason, all further projections were acquired using LED illumination. Figure 3 also shows that refraction occurs at the edges of the PTFE tube in spite of index matching. Refracted light does not pass the spatial filter. For this reason, the edges of the tube appeared too dark, and absorbance in the reconstruction was overestimated. To compensate for this effect, any pixel that fell below 0.5% of the background intensity was considered invalid and ignored in the reconstruction equation¹³. In addition, the effects of refractive index mismatch were further reduced by performing a blank scan of the tube and using this scan data as the incident intensity I_0 in Equation 2.

Figure 4 shows reconstructed cross-sections (slice 270 out of a stack of 1080, arbitrarily chosen) for six different ink concentrations. Consistent with findings reported on a simulation phantom

¹³, the OT system underestimated the attenuation coefficients. However, a very high correlation between absorbance as determined by spectrometry and absorbance determined with the OT system ($R^2 = 0.99$) was found (Figure 5). In all cases, the normalized geometrical error was less than 5%. Figure 6 shows the reconstruction of two concentric tubes with the space between the tubes filled with ink. It becomes evident that the tubes are not exactly concentric, which can be attributed to inaccuracies in manufacturing the phantom. Visual inspection confirmed the inhomogeneous thickness of the ink layer. The reconstructions in Figure 4 and Figure 6 show prominent artifacts. In the center of the ink-filled space (Figure 4), a small region of apparent low absorbance exists. This artifact may have been caused by specular reflection of the beam between the tube surface and the planar glass, leading to higher incident intensity in the center. In Figure 6, the top right part of the ring is shifted towards the center, reminiscent of a reconstruction artifact that appears when the center of rotation does not coincide with the center of the detector system.

The ability of the OT system to acquire three-dimensional data is demonstrated in Figure 7. The entire volume of 5.4 mm height was acquired within one revolution of the sample. The collected dataset consisted of 1080 slices with 400 projections and 1980 pixels per projection. It was first scaled down to 120 slices (480 pixels per projection) for reconstruction and subsequently to $120 \times 120 \times 120$ voxels for rendering. The isosurfaces visible in Figure 7 correspond to the transitions from the low-absorption fluid to the more strongly absorbing ink.

Our implementation reduced reconstruction time per slice from 500 seconds (100 iterations of SART) to 90 seconds (OS-SART with 5 iterations and 20 subsets). Reduction of the number of projections from 400 to 200 reduced reconstruction time to 46 seconds, but decreased SNR by 23%. Further reduction of the number of projections was considered unacceptable since SNR decreased markedly at 100 projections (60% reduction), and decreased even further with 50 projections. Reconstruction time of the FBP algorithm was 10 seconds with a 60% lower SNR than OS-SART. Different numbers of subsets (10 subsets with 10 iterations and 5 subsets with 20 iterations) did not change SNR more than 1%. An overview of the performance and computational time of the reconstruction methods is provided in Table 1.

DISCUSSION

This study shows the feasibility of a fast volumetric optical transillumination tomography scanner. With one single revolution of the sample, an axial section of the sample that has the same height as the image sensor is imaged. Key components include the parallel-window sample bath, a motor to rotate the sample inside the bath, and a spatial filter to block scattered light from the sample. These components were used in the first-generation device presented earlier ¹⁰. The new design, however, differs in several fundamental ways. First, we used an expanded beam to capture a two-dimensional projection of the sample. As a consequence, the translational motion of the source/detector system relative to the sample is no longer needed, and the new device only has a motor for sample rotation Θ and coarse positioning in axial direction. The low number of optical and mechanical components makes this device unparalleled in simplicity and cost efficiency. Furthermore, this is to our knowledge the first time that a light-emitting diode (LED) has been used as the source of collimated light. One other device was reportedly equipped with LED ²⁰. However, the LED in this device were used as idealized point sources for a 64-pixel projection acquisition. With the corresponding low resolution, the device was primarily aimed at educational purposes. The resolution of the system presented in this study, on the other hand, is determined by several factors including the diffraction limit of the collimating lenses, pin hole size and the refractive index match between sample and immersing liquid (mismatch can cause the diffraction at the boundary), and the image detector pixel size. In addition, the LED source is not perfectly collimated. This would necessitate an infinitely small pinhole in the source spatial filter. We compromised

between incident intensity and blur by using a 150 μm pinhole. Angular scatter of almost 0.002° is accepted into the filter due to the diffraction limit, which would correspond to cross-talk between about 20 neighboring pixels. With a smaller pinhole and a stronger source, cross-talk could be reduced. Second, additional blurring is caused by the admission of photons that underwent small angular deflections by scattering into the second spatial filter. The 150 micron pinhole is relatively large and was necessitated by the difficult alignment procedure. The device could be further improved by employing preassembled and adjusted spatial filters with a correspondingly smaller pinhole. As presented here, we estimate the true resolution of the device to be between 50 and 100 microns.

Refractive index mismatch is a challenge always associated with optical transillumination tomography, reported by our group^{10,12,26} and by others^{4,9,20}. It is not possible to obtain a straight photon path in all cases, particularly when the incident beam intersects with the sample surface at a low angle. In the case of cylindrical samples (such as tissue-engineered blood vessels or the phantoms used in this study), refraction takes place predominantly at the edges, leading to highly overestimated absorption in these regions. We successfully applied a correction that was proposed earlier in a simulation study¹³, but artifacts caused by reflection and refraction can still be seen in the reconstructed images. Furthermore, this study confirms systematic underestimation of the optical absorbance seen in the simulation study¹³. Image contrast is a result of the optical absorbance. Strongly over- or underestimated absorbance values are indicators of acquisition errors and consequently incorrectly reconstructed cross-sections. Highly overestimated absorbance occurs, for example, when refractive index mismatch is not accounted for¹³. For this reason, we have used fluids of known absorbance to qualitatively estimate the influence of refraction and reflection. Particularly the comparison to ink absorbance determined by a photospectrometer (Figure 5) with a regression slope of 0.8 indicates that the image reconstruction predominantly reflects true material properties and not artifacts.

Scattering is a phenomenon fundamentally different from reflection and refraction because the direction of the photon after scattering is random. With a low probability, scattered photons may randomly assume a direction parallel to the optical axis as well. In this case, they will pass the spatial filter and contribute to image formation. However, since scattering is a random event, this will lead to a low level of homogeneous haze. In our reconstructions (Figure 4 and Figure 6), haze was not noticeable.

It remains the subject of advanced studies whether additional correction steps (e.g. raytracing or two-pass reconstruction) can further improve image quality in the presence of refractive index mismatch. In spite of this challenging problem, this study has demonstrated that optical transillumination tomography has the potential to recover the spatial distribution of the absorbance and provide an estimate of geometry. Applied to a typical tissue-engineered blood vessel with a wall thickness of 300 to 500 μm , the cross-section of the wall would be covered by 15 to 25 pixels. This corresponds to a discretization error of less than 7%. The Gaussian blur introduced by pixel cross-talk due to the large pinhole size (full-width-half-maximum of 50 to 100 μm) is an additional source of inaccuracy. While details smaller than 50 μm cannot be identified as a consequence of the Gaussian blur, appropriate thresholding allows at least good recovery of the geometry. The measurement of tissue thickness inhomogeneities and perfusion tests associated with vessel dilation therefore appears possible within the discretization error.

A related technique is the microscope-based optical projection tomography (OPT), introduced by Sharpe et al²¹. For OPT reconstruction, the sample is immersed in an index-matching fluid and rotated inside the focused beam of light of a microscope. In contrast, the transillumination tomography system introduced in this study uses a broad beam of collimated light. Therefore,

OPT uses a translation-rotation scanning mechanism similar to our first-generation device¹⁰. The extraordinary high image quality of the OPT technique can be explained in part by the use of a focused beam, but mainly by the use of Murray's Clear as immersion fluid. Murray's Clear penetrates into the tissue and changes its refractive index and scattering properties. This solution cannot be used in applications for tissue-engineered blood vessels since it changes the mechanical properties. Furthermore, the authors of the OPT study do not share detailed information on reconstruction technique and scan time, although a reconstruction time of 2 to 5 hours was given on a sample comparatively smaller than the one used in this study. The fundamental difference to our technique of broad-beam acquisition is the ability of the large-diameter collimated beam to acquire a projection of a three-dimensional volume rather than a single voxel. Therefore, the major advantage of the large-size projection technique is the comparatively fast acquisition speed when acquiring 3D volumetric scans. Typical cameras acquire images at a rate of 10 to 60 frames per second. With the camera used in this study and 200 projections, one full revolution would take less than 20 seconds and provide a 5 mm axial section as illustrated in Figure 7. This is balanced by the relatively slow reconstruction process of iterative methods. At 50 μm slice thickness, the accelerated OS-SART method used in this study would still require 1 ½ hours to reconstruct the volume. Several avenues for improvement are possible. Computer clusters built on multicore CPUs could provide one to two orders of magnitude of speed improvement. Also, it is possible to employ the computer's graphics engine for fast mathematical operations, potentially achieving another order-of-magnitude acceleration²⁵. Finally, for some applications, the lower SNR of filtered backprojection may be acceptable, at least for a fast preview. In that case, reconstruction time matches acquisition time. It appears feasible at this time to balance acquisition speed with reconstruction speed.

In the context of imaging methods for tissue-engineered blood vessels, optical transillumination tomography offers very low cost, simple equipment and an extraordinarily high acquisition speed for 3D volumetric scans. Compared to other methods, such as confocal imaging or optical coherence tomography, it provides lower resolution and lower SNR. Consistent with our earlier study¹⁰, large defects could be determined, but smaller defects, while still relevant in the production of tissue-engineered products, would not be detectable. For this reason, optical transillumination tomography can be envisioned to find its primary application in biomechanical testing, for example, the measurement of vessel dilatation in response to pressure.

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Figure 1.

Sketch of the optical transillumination tomography system. The sample is immersed in index-matching fluid inside a parallel-window sample bath, where the sample can also be rotated by an angle Θ . The sample is illuminated by collimated light, and any light not absorbed or scattered by the sample is collected by the CMOS detector through a spatial filter. The spatial filter rejects scattered photons, i.e. photons that deviate from their original direction. The sketch indicates the two alternative modes of illumination, a diode laser with beam expander, and a LED with a second spatial filter to create a collimated beam of light.

Figure 2.

Sketch of the single-wall (A) and the double-wall (B) phantom with their respective idealized reconstructed cross-sections. In the cross-sections, black indicates a clear fluid with no absorption, while grey indicates a fluid with absorbing or scattering properties. A higher ink concentration (and its associated higher absorbance) would lead to a lighter grey color. Yellow (light grey) rings indicate the thin refracting interface between fluid and tube walls. The tube material itself is non-absorbing and therefore shown in black.

Figure 3. Projections of a PTFE tube in index-matching fluid. Panel A was acquired using laser illumination and shows strong diffraction artifacts that will be visible as ring artifacts in the reconstruction. Panel B was acquired using collimated LED light. There are no noticeable diffraction artifacts.

Figure 4.

Reconstructed cross-sections of a single PTFE tube filled with different concentrations of black ink (from left to right and top to bottom: 1 μ l, 2.5 μ l, 4 μ l, 5 μ l, 5.5 μ l, and 7 μ l of 1:1000 ink dilution in 1ml of sucrose). The tube can be identified as a ring of high absorbance (white). Particularly at low ink concentrations, a dark region becomes visible in the center which can be attributed to a specular double-reflection between bath and tube. The scale bar represents 1 mm, and the grayscale bars show absorbance values in units of 10^{-3} cm^{-1} .

Figure 5.

Correlation between mean ink absorbance determined by optical tomography and absorbance determined by spectroscopy. While the OT system underestimates the absorbance, a high correlation ($R^2 = 0.99$) between the two methods exists.

Figure 6. Reconstruction of one cross-sectional slice of two concentric PTFE tubes with the space between the tubes filled with absorbent diluted ink. The scale bar represents 1 mm, and the grayscale bar shows absorbance values in units of 10^{-3} cm^{-1} .

Figure 7.

Surface rendering of a 5.4 mm section of the phantom. This 3D volume was originally acquired with 1080 1D projections of 1920 pixels and subsequently scaled down to 120 voxels in each dimension. This reconstruction example illustrates the key advantage of the broad-beam acquisition system to obtain a complete 3D tomographic scan in one revolution of the sample. This image corresponds to the sketch in Figure 2.

Table 1

Comparison of SNR and reconstruction time for different reconstruction algorithms and parameters. Reconstruction was based on 400 projections unless otherwise stated.

Algorithm / parameters	Reconstruction time per slice (seconds)	SNR
FBP	10	8.2
SART	550	13.9
OS-SART, 20 iterations, 5 subsets	320	13.8
OS-SART, 5 iterations, 20 subsets	92	13.7
OS-SART, 5 iterations, 20 subsets, 200 projections	46	10.5
OS-SART, 5 iterations, 20 subsets, 100 projections	24	8.2