OPTICAL COHERENCE TOMOGRAPHY FOR DIMENSIONAL METROLOGY OF LAB-ON-A-CHIP DEVICES

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ABSTRACT

Here we present a new metrology tool for the determination of the dimensions of channels from final product lab-on-a-chip devices. Using Optical Coherence Tomography (OCT) areas of up to 2 mm x 2 mm were imaged in less than 1 second, without the need of introducing fluorescent molecules in the channels, and the images analysed with in-house written ImageJ macros. The results obtained with OCT for the height and width of channels are in agreement with results obtained with confocal fluorescence microscopy, used in this study as the reference measurement.

KEYWORDS: Dimensional Metrology, Optical Coherence Tomography (OCT), Poly(methyl methacrylate) (PMMA), Confocal Fluorescence Microscopy

INTRODUCTION

Standardization of lab-on-a-chip technologies is increasingly becoming an area of interest within this field. Some of the parameters of interest stated in the literature and roadmaps are the microchannels’ internal dimensions, the external device geometry and autofluorescence [1, 2]. Different applications will require different materials. Each material will require a different set of parameters to be measured for standardization purposes. Biological applications in lab-on-a-chip have surged in the last years and therefore parameters of materials commonly used for these applications (usually polymeric materials) could well dictate the kind of standard testing that will be developed. Polymeric materials mostly used for biological applications in microfluidic systems are: polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA) and polycarbonate.

Techniques that have been traditionally used in the characterization of microelectronic devices could be used, to some extent, to characterize lab-on-a-chip devices. However, these techniques have a number of limitations when probing microfluidic internal structures. For example, scanning probe methods (e.g., atomic force microscopy, AFM) have restricted access to corners of certain features in these devices. In addition, AFM can only be used with preassembled devices and not final products. Optical techniques, for example interferometric microscopy, can generate 3-dimensional images of microchannels provided that the lid is optically transparent or no lid has been placed to seal the device. Overall, optical techniques use UV-Visible spectrum light sources, which generates more scattering compared to techniques using near-infrared (near-IR) wavelengths. In general, the available tools used, so far, to measure dimensional properties of microelectronic devices provide limiting capabilities for the dimensional measurements of lab-on-a-chip final product internal structures. Therefore, a new approach to advance dimensional metrology in lab-on-a-chip final products is needed.

Optical coherence tomography (OCT), a technology commonly used to image sub-surface structures in vivo [3], is based on optical interferometry principles. OCT uses a broadband near-infrared (NIR) source to produce images (Figure 1) in the x- and z-axes (A-scan). A 3-dimensional image, or tomogram, is created when a set of A-scans are combined into a B-scan along the y-axis and assembled into a volumetric image (Figure 1). Scattering is less significant in the NIR region than in the ultraviolet or visible region, therefore allowing OCT to have improved imaging capabilities when compared to other optical techniques. OCT also has a larger sub-surface depth of imaging (up to around 2 mm) and fast scans of few tens of kilohertz for A-scans. Here we present an approach using OCT combined with an in-house written ImageJ Macro to analyze OCT x-z plane images from PMMA devices.

EXPERIMENTAL

A PMMA 4-channel device with dimensions of 50 µm high and 50 µm wide was imaged (empty channels) using spectral domain OCT (SDOCT). The SDOCT system was operated at a center wavelength of 840 nm. SDOCT images were acquired from each area with 200 linear B-scans at 1000 A-scans per B-scan (see Figure 1 and Figure 2). Confocal fluorescence microscopy images were obtained by filling the channels with a fluorophore (Dronpa) and then taking xz cross-sectional scans of the regions of interest (Figure 3).

RESULTS AND DISCUSSION

PMMA lab-on-a-chip devices were imaged using OCT operated in the spectral domain. An example of an OCT image (A-scan) is illustrated in Figure 2 along with the processing steps we used to extract the data from the images. The microchannel surfaces (specifically the top and bottom sides) are observed in Fig. 2A. This image shows an A-scan from where the dimensions of the channels (height and width) can be obtained. A Region of Interest (ROI) is defined by delimiting an area around the top and bottom lines (square). Fig. 2B is an enlarged image of the raw intensity image from the delimited ROI. Fig. 2C displays ROI lines (top and bottom channel surfaces) after a median filter with a 5-pixel radius
Figure 1: Depiction of OCT imaging. A) The channel’s top and bottom sides are observed in this A-scan. The insert shows specifically the top and bottom sides of the channel. B) The internal structures (microchannels) are imaged by scanning the xz plane (A-scan) producing a cross-section image (as shown in A). A B-scan is acquired when the A-scans are moved in the y direction. C) A top view of a B-scan when completed. The images are reconstructed so that the volume of the sample is observed. The arrows at the top of the image point at the edges delimiting the width of the top side of the channel, whereas the arrow heads at the bottom of the image point at the bottom side of the channel.

Figure 2: Processing steps for the determination of the channel dimensions from an OCT A-scan. A) Raw image of the full field of view of an A-scan. The region of interest (ROI) is indicated with the square box. B) An enlarged view of the raw intensity data of the ROI. C) Image in B after processing with a median filter with a 5-pixel radius. D) Image in C after highlighting the intensities above the autothreshold (red). The processing is completed when the intensities above the autothreshold are drawn over the image (Fig. 2D, red areas). The dimensions (height and width) of the channels can be measured using the areas above the autothreshold. All A-scans (xz planes) can then be calculated to obtain the average height and width for each scanned area.

The dimensions measured with confocal fluorescence microscopy are sensitive to the threshold used when processing the images. The threshold was determined by finding the regions at the edge of the images where the change in fluorescence (signal/background intensity) was the largest (Figure 3A and 3C). The processing first required a median smoothing. Then, a filtering process using a Sobel edge detector was carried out. After that, the regions with the largest gradient magnitude were selected. Figure 3 illustrates a mean intensity of 89.3, suggesting an appropriate threshold of 89. Figure 3D examines the sensitivity of the measured channel area to intensity threshold. Both analysis, image gradients and the automated thresholding approach, produce similar thresholds in the regions where the dimensional measurements have smaller sensitivity to changes in threshold.

Figure 4 shows the average height and widths from the 194 replicates obtained from two areas of the device. In this case the measured heights were below the expected value (50 µm) and the expected average widths were above the expected value (50 µm). Of the two measurements, OCT was closer to the expected height as well as to the expected width (48 µm and 56 µm, respectively).
CONCLUSION

This work demonstrates that OCT can be used to image the internal structures lab-on-a-chip devices, specifically in polymeric materials (PMMA). The measured dimensions (height and width) compares with other well-known techniques such as confocal fluorescence microscopy. This new approach could allow for the future development of test standards, which could further the development of standardization of important parameters in lab-on-a-chip devices.

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REFERENCES


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