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Automated 3-D cell counting method for grading uveitis of rodent eye *in vivo* with optical coherence tomography

Woo June Choi^{1,3}, Kathryn L. Pepple^{1,2}, and Ruikang K. Wang^{1,2,*}

¹Department of Bioengineering, University of Washington, Seattle, WA, USA

²Department of Ophthalmology, University of Washington, Seattle, WA, USA

³School of Electrical and Electronics Engineering, College of ICT Engineering, Chung-Ang University, Seoul, Republic of Korea

Abstract

In preclinical vision research, cell grading in small animal models is essential for the quantitative evaluation of intraocular inflammation. Here, we present a new and practical optical coherence tomography (OCT) image analysis method for the automated detection and counting of aqueous cells in the anterior chamber (AC) of a rodent model of uveitis. Anterior segment OCT (AS-OCT) images are acquired with a 100kHz swept-source OCT (SS-OCT) system. The proposed method consists of two steps. In the first step, we first despeckle and binarize each OCT image. After removing AS structures in the binary image, we then apply area thresholding to isolate cell-like objects. Potential cell candidates are selected based on their best fit to roundness. The second step performs the cell counting within the whole AC, in which additional cell tracking analysis is conducted on the successive OCT images to eliminate redundancy in cell counting. Finally, 3-D cell grading using the proposed method is demonstrated in longitudinal OCT imaging of a mouse model of anterior uveitis *in vivo*.

Graphical abstract

Rendering of anterior segment (orange) of mouse eye and automatically counted anterior chamber cells (green). Inset is a top view of the rendering, showing the cell distribution across the anterior chamber.

*Corresponding author: wangrk@uw.edu.

Supporting Information

Media: Comparison of cell counting on consecutive OCT frames of mouse anterior segment before and after application of the cell redundancy removal method.

Keywords

Uveitis; ocular inflammation; anterior chamber cell; 3-D cell counting; optical coherence tomography

1. Introduction

Uveitis, a form of ocular inflammation, is an important cause of vision loss worldwide [1]. The condition typically involves intraocular structures of the uveal tract including iris, ciliary body, and choroid. Slit lamp examination is the current gold standard for the evaluation of uveitis severity in patients, where visible cells in the anterior chamber (AC) are manually counted and graded with the Standardization of Uveitis Nomenclature (SUN) criteria [2]. However, such method is subjective by examiners and particularly challenging in small animal models of uveitis simply because of the small size of the rodent eye, and further it is susceptible to inter-observer variation [3].

There has been recent interest in the objective assessment of uveitis using optical coherence tomography (OCT). OCT is a non-invasive imaging modality, providing micrometer-resolution, three-dimensional (3-D) imaging of biological tissue at depth of few millimetres [4]. Several OCT research groups have investigated its potential in quantitatively evaluating human uveitis by counting AC cells in anterior segment OCT (AS-OCT) images [6–8]. In the AS-OCT image, the cells were seen as individual hyper-reflective spots [7,8]. A custom software was reported capable of identifying and locating the cells based on their reflectance distribution in the OCT images, allowing for cell counting within a limited field of view. The amount of counted cells were well correlated with clinical grading [6,7]. This OCT image based cell grading enabled quantification of AC inflammation severity. While the OCT approach has been mostly explored for use in human uveitis in clinical practice, there is a relative paucity of literature related to its utility for small animal uveitis. Experimental

rodent models of uveitis have been commonly used to replicate the immunopathogenic mechanisms of human uveitis and test novel uveitis therapies for treatment [9]. Therefore, quantitative evaluation of uveitis in preclinical domain is also valuable and could lead to better understanding of mechanism in human uveitis.

Hence, this Letter first presents an OCT image based automatic AC cell counting method for evaluating uveitis in small animal models. The proposed method is applied to AS-OCT images of an acute uveitis model in mice, allowing for automated cell detection and counting across the inflamed AC *in vivo*.

2. Material and method

For OCT measurement of the ocular tissue, we employed a swept-source OCT (SS-OCT) system with a swept laser operating at center wavelength of 1.3 μ m and with a sweeping rate of 100kHz across a spectral bandwidth of 100nm. The system had spatial (axial $\times$ lateral) resolution of the system of 21 μ m $\times$ 22 μ m in air, respectively, and a measured sensitivity of 105dB. The system description is detailed in a previous literature [10]. For rodent uveitis, we adopted an established experimental model of uveitis that was previously reported [11]. 4-week-old young adult CB57BL/6 mice (N=3) weighing 23~25g were prepared to generate acute anterior uveitis (AAU). AAU was initiated in the right eye of mouse with an intravitreal injection of 5 μ g killed mycobacterium tuberculosis H37Ra antigen (DifcoLaboritories, Detroit, MI) in a phosphate buffered saline (PBS) at day 0. Intraocular inflammation peaks at two days post-injection. OCT imaging was performed before the injection on day 0 and at peak inflammation on day 2. For other mice (N=2), imaging was performed on days 0, 2, 5, 7, 11, and 14. Prior to intravitreal injection, all mice were treated with a subcutaneous injection of 0.1mg mycobacterial extract emulsified in mineral oil on day -7. During injection and OCT imaging, the animals were anesthetized with 2~5% isoflurane inhaled via a nose cone. An OCT volume dataset (512(X) $\times$ 512(Y) $\times$ 2048(Z) voxels) was acquired for each animal at a B-scan rate of 80frame/s, covering the ocular tissue area of 5mm(X) $\times$ 4mm(Y). The animal study protocol was approved by the Animal Care and Use Committee of the University of Washington (animal study protocol #4184-04) and was compliant with ARVO guidelines for use of animals in vision research.

Figs. 1(a) and 1(b) show representative OCT cross-sectional images of the mouse eyeball at day 0 and day 2, describing the full-length intraocular anatomy. On day 2, the eye (Fig. 1(b)) demonstrated features of inflammation when compared to the baseline (Fig. 1(a)), including AC cells (arrows in (c)), an enlargement of a box in (b)), pupillary membrane (PM) in the AC and turbidity of a vitreous body. These signs of inflammation were confirmed by a histologic section on day 2 (Fig. 1(d)), indicating that OCT can detect the presence of inflammation in the rodent eye [11].

In order to detect and count AC cells from the OCT images, a computerized method is proposed here, which consists of five main steps: (i) Despeckling, (ii) Binarization, (iii) Removal of objects outside AC, (iv) cell candidate determination, and (v) cell counting. Figure 2 illustrates the step by step procedures for the proposed approach. The anterior segment was first cropped from the OCT cross-section of eyeball (a left top in Fig. 2), and

then despeckled using a 3×3 kernel median filtering to smoothen rough speckle textures (i). In binarization step (ii), a binary thresholding was applied to the despeckled image with a pre-defined reflectivity threshold value, $BG + 2(BG_STD)$ [5], where BG is an average signal level of background (air) and BG_STD is a standard deviation of the background region. To remove the AS structures (cornea, iris, and lens) in the binary image, flood-fill operation was applied to fill the gaps in the binary objects, and then all the objects connected to the image border were cleared (iii). In the step of identifying cell candidates (iv), we made the assumption that the aqueous single cells in AC can be differentiated from the other AC interior features (e.g., pupillary membranes, cell clusters, and blood vessels detached from iris) based on leukocytes having a spheroidal shape [8]. With this assumption, the objects were estimated using a simple metric to evaluate its roundness: $4\pi \times \text{area} / \text{perimeter}^2$. Given the average leukocyte diameter of $10\mu\text{m}$ [12] and a specified beam spot size of $25\mu\text{m}$, an effective cell diameter can be calculated to be $25\mu\text{m}$, and thus the cell area in the OCT image was calculated $491\mu\text{m}^2$ which is more than 10 pixels ($1\text{pixel} = 48.5\mu\text{m}^2$). The metric value for roundness was empirically defined from 0.8 to 1.6 in our study, where the value of 1 indicates that the object is circular. If the AC objects were met this roundness range (yellow colored values on objects in (iv)), they were judged as the potential cell candidates. Finally, the selected cell candidates were counted (encircled with white circles in (v)) and the counted cell number was recorded. The counts were displayed on the OCT image frame along with the frame number (v).

Counting free floating cells on the individual OCT images provides good information regarding inflammation in one cross-section within the AC. However, this single cross-section (snapshot) may not be accurate in representing the level of inflammation in the entire AC. Error can be introduced by regional differences in the distribution of inflammatory cell [6]. One way to solve this problem is to apply the cell counting method to the 3-D OCT dataset of the entire AC. However, there may be a possible redundancy issue in the cell counting, i.e., cells can be repeatedly counted on adjacent frames due to high sampling density in OCT acquisition, resulting in an overestimation of the cell counts. As an example, Fig. 3 illustrates the problem of cell redundancy in a series of OCT images. Three AC cells (white arrows) are visible in the first frame (#247). In the two subsequent frames (#248 and #249) the far left and right cells in the first frame are still seen at the same pixel location. Therefore, if cell counting was performed on these three images, the same two cells would have been counted three times, each artificially increasing the total cell number. This error can also be seen with a new cell that is first detected in frame #248 (yellow arrow). The $t\#$ and $d\#$ in the frames indicate the elapsed time and displacement from the first frame.

To compensate for the false positive counting due to the redundancy, we developed a simple redundancy removal method, in which the centroid of the cells identified above was utilized. Briefly, after adjacent two cross-sectional OCT frames were processed with the procedures described in Fig. 2, the centroids of the isolated cells in each frame were calculated. Then, correlation of the centroids between the adjacent frames was evaluated. Assume that (x, y) is the centroid coordinates in the first frame, and (x', y') is that of the adjacent frame. If $|x - x'| + |y - y'|$ is smaller than 12, the cells corresponding to the two centroids were then regarded as the identical cell. The value 12 is a maximal amount of pixel shifts that was

empirically set considering the cell motion and the bulk tissue motion of the mouse eye such as breathing. However, the bulk tissue motion or aqueous humor flow may displace the cell in vertical axis (z-direction) during B-scan, leading to deviation of the centroids of the cell over the threshold set, which may give rise to positive false cell count. The increase of OCT B-scan rate may reduce the centroid mismatch between adjacent frames by fast sampling of the cell motion. This procedure was sequentially repeated for all the consecutive frames in the 3-D datasets, finally resulting in the isolated cells without redundancy.

3. Results

Fig. 4(a) shows cell counts in four successive images of the total 512 OCT frames before and after application of the redundancy removal method. The counted cells are encircled as white circles. Without correction, all cells in the AC were counted regardless of the frames whereas with correction, the counted cells in the current frame were excluded in next frame (Media). Color dotted circles in the frames represent that if the cells are counted in one frame, they are excluded in the adjacent frames. For comparison, the cell counts for all the frames are plotted as a histogram in Fig. 4(b), where the cell counts before correction is exaggerated due to erroneous accumulation in the cell counts. Consequently, the cell redundancy leads to significant measurement error in the total AC cell number (2981), which was almost 2.6 times greater than the corrected ones (1129) in this case (Fig. 4(c)). The cell counts by our algorithm was 2% less than the result by human grading as a reference (done by experienced ophthalmologist). This indicates a 98% precision rate of our method to the gold standard for cell grading. By using the compensated method, the actual free floating cells present in the whole AC could be counted as well as its distribution in the AC could be identified in three dimensions. Fig. 4(d) displays the counted AC cells (green) together with a reconstructed anterior segment structure (orange). An inset shows the cell distribution within the AC. The uneven distribution of cells has been reported previously and may be due to the combined effect of the aqueous circulation [6] and gravitation of the AC cells.

We applied the 3-D cell counting method to the mouse model of uveitis for longitudinal evaluation of inflammation. The AAU was induced in two animals, and OCT measurements were performed over a period of two weeks. Fig. 5(a) shows OCT cross-sections of the anterior segment of the mouse eye on day 0, 2, 5, 7, 11, and 14, respectively. The animal demonstrated peak inflammation on day 2 that appeared resolved by day 14, which is consistent with our previous result on this uveitis model [13]. The cell counts per frame (Fig. 5(b)) and the cell population images (Fig. 5(c)) provide quantitative and comprehensive information over the course of AC inflammation. Both animals demonstrated a dramatic increase in total cell counts at day 2 (Fig. 5(d)), and then declined at day 5. It was surprising that the cell number increases again on day 7 and remains elevated above baseline through day 14. Prior studies of this model in rats reported spontaneous resolution by day 14 [13], so this rebound inflammation may represent a new finding in mice that deserves further mechanistic investigation. Variability in the level of ocular inflammation between two animals may be due to significant variation in preparation of the mycobacterial extract despite exhibiting the common trend in the inflammation process.

In summary, we have developed a novel OCT image analysis tool for automated detection and counting of cells within whole anterior chamber. The importance of the 3-D cell counting method has been highlighted with a mouse model of uveitis and imaged non-invasively by SS-OCT. The cell counting results suggest that AC cell grading using the OCT approach can be beneficial to longitudinally evaluate ocular inflammation in individual animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

OCT cross-sections of a mouse eyeball on (a) day 0 and (b) day 2. (c) A zoom-in view of a box in (b), showing inflammatory cells (arrows) in the anterior chamber (AC). (c) Day 2 histology confirms the presence of AC cells (black arrows).

Figure 2.

Step process for AC cell grading. The cell grading is performed on an AS-OCT image (left top) with following procedures: (i) Despeckling, (ii) Binarization and followed by flood-fill operation, (iii) Removal of AS objects, (iv) AC objects roundness estimation, and (v) AC cell counting. The counted cells are marked with white circles and its counts is recorded and displayed on the image frame.

Figure 3. AC cells in the consecutive OCT images. The two of three cells (white arrows) in the first frame (#247) and one cell (yellow arrow) in the second frame (#248) are repeatedly observed in the subsequent frames.

Figure 4.

(a) Comparison of AC cell counts in the consecutive OCT images before and after application of the cell redundancy removal method. The counted cells in each frame are encircled with white circles. Compared to before correction, the cell counts after correction were significantly reduced by excluding counting of the cells counted in the prior frames (Media 1). Color dotted circles indicate the cells presented in the prior frames. (b) A histogram representing cell number per frame. (c) Total number of cells counted for all 512 OCT frames. (d) A rendering of the counted cells (green) and the anterior segment structure (orange). An inset shows the cell distribution in the AC structure. N: nasal, T: temporal, S: superior, I: inferior. Scale bar: 1mm.

Figure 5.

Longitudinal OCT measurements and AC cell quantification in single animals. (a) Representative AS-OCT cross-sections on day 0, 2, 5, 7, 11, and 14, and corresponding AC cell number per frame (b) and 3-D cell distribution in AC (c). (d) Time course change in the total cell number in the whole AC.