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Longitudinal Analysis of Mouse SDOCT Volumes

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Abstract

Spectral-domain optical coherence tomography (SDOCT), in addition to its routine clinical use in the diagnosis of ocular diseases, has begun to find increasing use in animal studies. Animal models are frequently used to study disease mechanisms as well as to test drug efficacy. In particular, SDOCT provides the ability to study animals longitudinally and non-invasively over long periods of time. However, the lack of anatomical landmarks makes the longitudinal scan acquisition prone to inconsistencies in orientation. Here, we propose a method for the automated registration of mouse SDOCT volumes. The method begins by accurately segmenting the blood vessels and the optic nerve head region in the scans using a pixel classification approach. The segmented vessel maps from follow-up scans were registered using an iterative closest point (ICP) algorithm to the baseline scan to allow for the accurate longitudinal tracking of thickness changes. Eighteen SDOCT volumes from a light damage model study were used to train a random forest utilized in the pixel classification step. The area under the curve (AUC) in a leave-one-out study for the retinal blood vessels and the optic nerve head (ONH) was found to be 0.93 and 0.98, respectively. The complete proposed framework, the retinal vasculature segmentation and the ICP registration, was applied to a secondary set of scans obtained from a light damage model. A qualitative assessment of the registration showed no registration failures.

Keywords

optical coherence tomography; retina; mouse; vessel; segmentation; registration; longitudinal analysis

1. INTRODUCTION

Spectral domain optical coherence tomography (SDOCT)^{1,2} is an optical interference based imaging modality that provides high resolution images of the retina using the back scattering properties of tissues. In addition to being routinely used in the diagnosis and management of a variety of ocular³⁻⁶ and neurological diseases in human subjects,⁷⁻¹¹ it has also begun to find widespread use in the study of animal models of retinal pathologies.¹²⁻¹⁴ Structural changes in animal models were typically studied using *ex vivo* histology. However, the

freezing and sectioning of the tissue are known to introduce artifacts and, more crucially, makes the longitudinal study of animals impossible. SDOCT, being noninvasive, allows for the accurate *in vivo* study of the retina in the same animal for the duration of the study.

Murine models, in particular, find widespread use in the modeling of a variety of retinal diseases.^{15–19} Discerning localized changes in the retina requires that the scans be aligned precisely. This is of interest in studying retinal degeneration models (such as rod degeneration in rd10 mice), where the structural change may be gradual, as well as during the development and testing of protective drug therapies. In murine models, the fovea is absent and the scan acquisition area is determined solely based on the location of the optic disc. This makes the scans prone to significant rotational offsets, frequently larger than those seen in human subjects. Figures 1(a) and (b) show examples of projection images of SDOCT scans obtained from the same mouse 2 days apart. The second scan has a rotational offset of approximately 13°. Typically, local changes are detected by considering sub-regions within the scan area as shown in Figs. 1(c) and 1(d). However, due to the offset between the two scans, the same area will not be analyzed.

Similar problems of accurate alignment in human scans have been addressed previously.^{20,21} The retinal vasculature provide good landmarks that can be used to register the scans.^{22–24} Here, we propose a comparable framework for the longitudinal analysis of mouse OCT volumes. First, the retinal surfaces are segmented using an automated approach.²⁵ Projection images are then created using the segmented surfaces, and a pixel classification approach is used to identify the retinal vessels and the ONH. Deng *et al.*²⁶ showed that the use of features from nine retinal layers can provide the best classification accuracy. However, when the disease causes significant structural remodeling (see Section 2) these nine layers may not be available. Thus, the proposed method only relies on two retinal layers. Next, an iterative closest point (ICP)^{27,28} algorithm, is used to register the segmented vasculature.

2. ANIMAL MODEL AND DATA ACQUISITION

The light damage (LD) model^{19,29} is frequently used to simulate rod degeneration and is achieved by exposing the animals to four hours of cool fluorescent light (3000 – 5000 lux). See²⁵ for specific details of the experiments.

Two sets of mice were used in the experiments. Set 1 consisted of ten female BALB/cJ mice (10 – 12 weeks), where both eyes were scanned at baseline and subsequently on day 4, 6, 8, and 12 post LD. This experiment did not include controls. Eighteen scans (10 pre LD and 8 post LD) selected at random were used to train and test the vessel segmentation. Manual delineations of the vessels and optic nerve head (ONH) were created by manually tracing the structures in GNU Image Manipulation Program³⁰ (GIMP). Set 1 was used to train the classifier, which was used to segment the retinal vasculature and ONH. Set 2 also consisted of ten female mice, five of which were reserved as control animals. SD-OCT scans were acquired from both eyes of the mice on four days—1, 3, 6, and 9 days after light-induced retinal damage. This set was used to test the registration process.

The SDOCT scans were acquired on a BiopTigen SDOCT ophthalmic imaging system XHR 4110 (Leica Microsystems Inc. IL, USA). A rectangular scanning mode ($1.4 \times 1.4 \times 1.6$ mm, 1000 A-scans $\times$ 100 B-scans with 2048 voxels per A-scan, and a pixel size of $1.4 \times 0.78 \mu\text{m}$) was used, with ten B-scans acquired at each location. The ten B-scans were averaged in order to improve the signal-to-noise ratio. Figure 2(a) shows a B-scan from a control mouse, while Figs. 2(b) and 2(c) show B-scans from an LD mouse (Set 2) acquired on day 3 and day 9 post LD, respectively.

3. METHOD

As indicated in Fig. 3, the method consists of two main components—the segmentation of the retinal vasculature and the subsequent registration of longitudinal scans.

3.1 Vasculature Segmentation

The scans were first segmented using ASiMOV,²⁵ an automated method that segments ten retinal surfaces in the normal SDOCT volumes and six surfaces in the LD scans. Initially, in post LD mice, the swelling in the tissue obscures the ONL as depicted in Fig. 2(b). As the swelling reduces (days 6 to 9), the ONL becomes more visible (see Fig. 2(c)).

The shadows cast by the vasculature are clearly visible near the photoreceptors, but the vasculature can also be visualized in other retinal layers. Within the retinal nerve fiber ganglion cell complex (RNFGC) and the inner plexiform layer (IPL), for instance, the vessels appear quite bright. Deng *et al.*²⁶ utilized and trained a classifier using features obtained from nine retinal layers in order to segment the retinal vasculature. However, in LD scans, certain layers are completely missing and the outer nuclear layer (ONL) is only visible once the swelling has reduced. To address this issue, we trained a random forest³¹ on features obtained from only two projection images. The first is created within the IPL from a region $15 \mu\text{m}$ in thickness below the RNFGC. The second projection image is created using the outer retina, defined by the top of the outer plexiform layer (OPL) and the Bruch's membrane (BM). Figures 2(d) and 2(e) show the two projection images of the inner and outer retina in the control mouse scan, respectively. Similarly, the projection images obtained from the day 3 and day 9 scans of the LD mouse are shown in Figs. 2(f)–(g) and Figs. 2(h)–(i), respectively.

The features consisted of a Gabor filter bank ($\sigma = \{25, 35, 45, 60 \mu\text{m}\}$, $\theta = \{0, \pi/4, \pi/2, 3\pi/4\}$ radians), first and second order Gaussian derivatives ($\sigma = 7, 15, 25, 45 \mu\text{m}$), and mean and standard deviation of the intensity within a specified window (7×7 , 11×11 , 13×13 pixels). The random forest (RF) consisted of 50 trees, with the number of features selected at each level in the trees set to 10. The classifier was trained to detect three classes, namely the vessels, the optic nerve head and the background. Note that the training of the RF was conducted using manual tracings on the 18 scans selected from Set 1.

3.2 Registration of Longitudinal Scans

The left eyes from Set 2, which were imaged at four time points, were first segmented using ASiMOV²⁵ and the retinal vasculature maps were then obtained using the pixel classification approach. Note that the RF used at this stage was trained on all 18 available

scans from Set 1. Small connected components were removed to filter out the noise in the pixel classification result. Next, the vessel maps associated with the same mouse from timepoints 2–4 were registered to the baseline scan using an ICP^{27,28} algorithm. Figure 4 shows an example of the registration process. The retinal vasculature from the two scans shown in Fig. 4(a) and 4(b) are segmented using the proposed pixel classification approach. The output from this step is converted into point clouds as shown in Figs. 4(c)–(d). Figure 4(e) shows the two point clouds overlaid after the registration process. This particular pair had a rotational offset of approximately 26° .

4. EXPERIMENTS AND RESULTS

4.1 Vessel Segmentation

The vessel and optic disk segmentation was validated using Set 1 in a leave-one-out experiment. The RF was trained on seventeen datasets and tested on the eighteenth. The area under the curve (AUC) was found to be 0.93 and 0.98 for the vessels and the optic disc, respectively. Figure 5(a) and 5 (b) shows the projection images obtained from a normal mouse scan. The corresponding manual tracing and the pixel classification result of the vasculature and the ONH are shown in Figs. 5(c) and 5(d), respectively. An example of the segmentation in an LD scan acquired 12 days post LD is shown Figs. 5(e)–(h). Despite the extremely noisy input (Figs. 5(e)–(f)), the vessel and ONH segmentation are of good quality.

4.2 Registration of the Longitudinal Scans

In order to qualitatively validate the registration process, the left eyes of the 10 mice (Set 2) imaged at times points 2–4 were registered to the corresponding baseline scan using the ICP algorithm. Since five mice were control mice, the registration evaluation set consisted of 15 control mouse scan registrations and 15 LD scan registrations. No registration failures were found.

Examples of the registration results in a control and LD mouse are shown in Fig. 6. Figures 6(a)–(d) show the projection images of SDOCT scans obtained from an LD mouse at the four time points, respectively. Figure 6(e) shows the point cloud of the segmented vasculature of the baseline scan. Figures 6(f)–(h) shows the point clouds of time points 2–4 before registration. The final result after registration is as shown in Figs. 6(i)–(k).

5. CONCLUSIONS & DISCUSSION

We have presented a framework for the longitudinal analysis of murine SDOCT volumes. The method utilizes a machine learning approach for the classification of the vasculature and ONH in projection images obtained from the SDOCT volumes. In particular, the method only utilizes two projection images and the AUC obtained using this approach provides similar results to those obtained by the previous approach²⁶ that relied on features obtained from nine retinal layers. It is likely that this is due to the redundancy present in these projection images. However, this current study included diseased scans from a model with significant structural remodeling. The rod degeneration characteristic of the LD model generates extremely noisy projection images, particularly at the Bruch's membrane, which typically is the region where the vessel shadows are most distinctly visible. Despite this, the

performance of the vessel segmentation does not suffer and yields results similar to those obtained using only healthy animals.²⁶

The subsequent alignment of the SDOCT volumes allows for the retinal thickness maps to be aligned prior to longitudinal analysis, thereby allowing for the detection of localized structural changes. The vessel segmentation in the LD scans can sometimes be noisy, but despite this the registration does not fail. In the control mouse example shown in Fig. 4, the source scan (Fig. 4(b)) is approximately 26° offset with respect to the baseline scan (Fig. 6(a)). Such large offsets are not typically seen in human scans, but can occur frequently in animal scans. Thus, the registration method needs to be robust and capable of handling large rotational and translational offsets.

Ideally, we would like to gauge the impact of the alignment on the thickness reproducibility but unfortunately, repeat scans were not acquired on either Set 1 or Set 2. However, given the approach's ability to handle noisy data and significant transformations we are confident that the method will improve thickness reproducibility in murine SDOCT volumes.

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Figure 1.

Visualization of the retinal vasculature in projection images of SDOCT scans obtained from the same mouse at (a) baseline and (b) day 2 of the study. (c) and (d) show the same projection images with a grid overlaid to indicate the sub-regions that are typically studied in thickness maps.

Figure 2.

Central B-scans from (a) control mouse (b) an LD mouse at day 3 post LD and (c) an LD mouse at day 9 post LD. Projection images from the (d) IPL and the (e) outer retina of the control mouse scan. Projection images from the (f) IPL and the (g) outer retina of the LD mouse at day 3. Projection images from the (h) IPL and the (i) outer retina of the LD mouse at day 9.

Figure 3.

Flowchart illustrating the basic steps of the proposed longitudinal analysis framework. The retinal vasculature of each scan is segmented using a pixel classification approach. The generated vasculature from two scans can then be registered using an ICP algorithm.

Figure 4.

Outer retina projection images from a (a) baseline scan and (b) time point 4 scan (day 9) from a control mouse. Point cloud of the segmented vasculature obtained for the (c) baseline scan and (d) the time point 4 scan. (e) Overlay of the two point clouds after ICP registration.

Figure 5.

Projection images from an LD normal mouse scan created at the (a) IPL, and (b) the outer retina. (c) Corresponding manual segmentation of the vasculature (red) and the ONH (yellow), and (d) automated segmentation results of the vasculature and the ONH. The last row shows similar images obtained from an LD mouse scanned 12 days post LD.

Figure 6.

Projection images from an LD mouse at (a) baseline, (b) day 3, (c) day 6, and (d) day 9.

Point cloud of (e) baseline scan, (f) day 3, (g) day 6, and (h) day 9 before registration. Point cloud of (i) day 3, (j) day 6, and (k) day 9 after registration to the baseline.