Quantifying structural distortions in retinal tissue before and after injury

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***Abstract*—Quantifying the deformation of retinal tissue after injury is crucial in understanding and characterizing the functional effect of cellular changes. We present a novel method to compute multiple quantitative measures from a single set of retinal images collected with a confocal microscope. We automatically detect nuclei within the outer nuclear layer (ONL) in these images and based on the results, we also automatically measure the thickness of the ONL, the local cell density and the distortion indices within the ONL. These measurements not only verify previous conclusions about retinal restructuring after detachment, but also provide biologists with significant new information about the regional responses within the layer of photoreceptor nuclei.**

I. Introduction

Modelling biological changes (i.e. injury or disease) or characterizing a phenotype using cell biology techniques often require measurement from many tissue samples. Paradoxically, the number of samples needed for analysis is often limited. Thus maximizing the amount of information attainable from a single sample is very important, as it would reduce the reliance on sample size. Furthermore, correlating measurements of different phenomena from a single sample can reveal important relationships among the phenomena that may not have been apparent previously.

To this end, this paper demonstrates the capability of our system to extract multiple biologically meaningful measurements from images of mouse retinal tissues. Here, fluorescent images of tissue from normal (control) and detached mouse retinas were analyzed. The resulting quantitative measurements corroborate the cellular changes previously observed to occur following retinal detachment in both human and animal models [1].

II. Materials

Experimental retinal detachments were performed on C57BL/6J mice. These eyes were injected with bromodeoxyuridine for other experiments performed not described here. Mice were sacrificed on the third day following detachment. Their eyes were harvested, fixed, dissected, embedded, and sectioned. The retinal sections were stained with various primary antibodies followed by secondary antibodies conjugated to the fluorophore cy-3 and then mounted on slides. The sections were imaged on an Olympus Fluoview laser scanning confocal microscope. Images were collected with an approximately 5-20% overlap. For this study, the background fluorescence was used to acquire the image since only the retinal layers and cell bodies were of interest.

III. Quantitative Image Analysis of Retinal Tissues

The number and density of photoreceptor nuclei within the outer nuclear layer (ONL) is directly correlated to visual function [1]. We designed a nucleus detector that automatically detects nuclei within the ONL. The automated nucleus detector approximates the number determined by manual counting with an average error of < 4%. Refer to [2] for more details.

In addition to providing the cell count within the ONL, the detected nuclei are used to obtain measurements such as layer thickness and cell density profiles along the ONL. The ONL region is approximated using morphological operators based on the detected nuclei locations and photoreceptor size. A skeletal backbone or median of the resulting region is generated and used as a reference frame for subsequent measurements. The thickness profile is calculated as the width of the ONL along each backbone location. Similarly, the density profile is computed as the number nuclei inside a region surrounding each backbone location (highlighted region in Fig. 1 (a) and (b)) divided by the area of that region. Refer to [3] for more details.

Thickness and density profiles provide information about the regional responses in the ONL. In order to quantify a global structural distortion of the ONL, we compute *a distortion index* of the thickness profile that is defined as

$$d_{thickness} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(t_i - m_T)^2} \Big/ m_T ,$$

where $m_T = mean\{t_i\}$ and t_i = thickness at location $(x_i , y_i) \in$ *points on the median curve of the ONL*. Similarly, we can compute a distortion index for the density profile. The more structural changes in the ONL, the larger the distortion indices. The distortion index is useful to compare the structural distortion among tissues from two or more different experimental conditions.

IV. Experimental Results

We apply the nucleus detector to mosaics consisting of 13 overlapping images of control and 10 overlapping images of 3-day detached mouse retina. These images are acquired with 5 to 20% overlap in order to align multiple images automatically as described previously [4]. By using mosaics, we can count

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photoreceptor nuclei over a large area of the mouse retina without sampling within the tissue. Figure 1 (a) and (b) show examples of retinal tissues with overlaid detected nuclei centers. ONL thickness and density profiles for each image are also shown in Figure 1 (c). After detachment, the profiles of thickness and density vary irregularly. The changes in the profiles provide the information about the structural changes in the layer. For example, the peaks in the thickness profile indicate the locations where the retina has been folded by cellular movement, death or growth. To quantify the structural distortion, we compute distortion indices of thickness and density profiles from each image. The distortion index of the thickness profile is increased from 0.16 to 0.28 after detachment. The distortion index of the density profile is also increased from 0.03 to 0.05 in response to detachment. To measure the global change in ONL thickness and local density, we calculate the mean and standard deviation (Figure 1 (d) and (e)). The control retina has 37626 nuclei/mm^2 and the detached retina has 40826 nuclei/mm^2. Both the average ONL thickness and local density are greater in the 3-day detached retina with 55.66 μm and 38856 nuclei/mm^2 (52.94 μm and 37091 nuclei/mm^2 in the control retina). Even though the average thickness and local cell density do not change significantly, the distortion indices of thickness and density profiles indicate significant structural distortions in response to retinal detachment. This information can not be extracted from traditional morphometric analysis.

V. Conclusion

In this paper, we present a novel method to compute multiple quantitative measures from a single set of confocal microscope images of retinas: total cell counts, thickness and density profiles, the distortion indices of the profiles, the average thickness, and the average local density. We demonstrate that the tools developed here provide the biologists with a new way to interpret and characterize the structural differences of retinal tissue under different experimental conditions.

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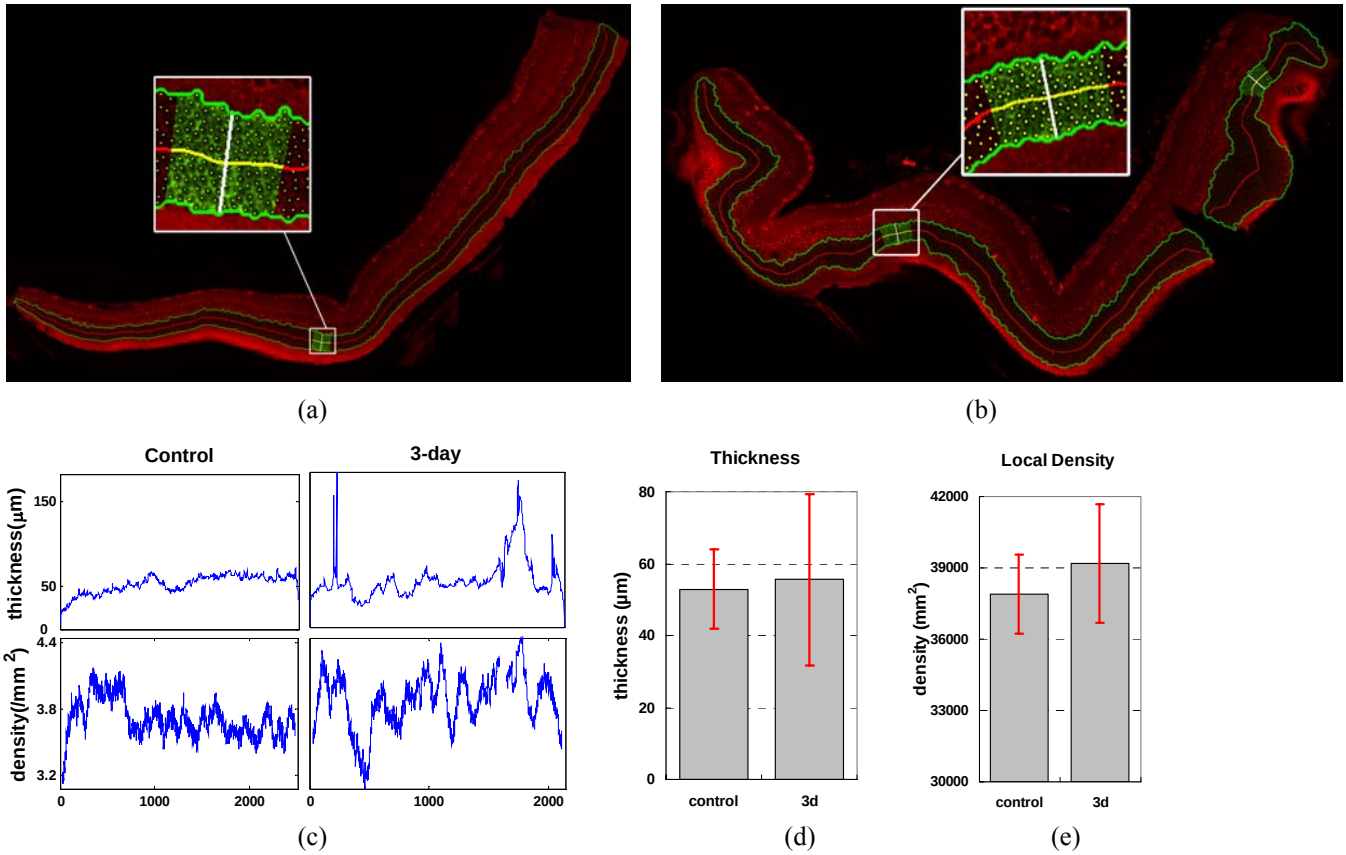

Figure 1. (a) Confocal image of a control mouse retina. The boundary and median curve of the ONL are displayed, and detected cell centers are marked. (b) Confocal image of a 3-day detached mouse retina. (c) Thickness profiles (top) and density profiles (bottom). (d) Average thickness of control and 3-day detached retina. The thickness of the control and detached retina are 52.94 μm (σ = 11.04) and 55.66 μm (σ = 23.75) respectively (p = 0.00001 <0.05). (e) Average local densities of the control and detached retina are 37090.8 nuclei/mm^2 (σ =1652.58) and 38856 nuclei/mm^2 (σ = 2487.53) respectively (p = 6.59E-120 <<0.05).