Scalable Interactive Analysis of Retinal Astrocyte Networks

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1 Introduction

Retinal astrocytes are one of two types of glial cells found in the mammalian retina. In mice, these highly planar cells are located in the innermost retinal layer termed the nerve fiber layer and are robustly stained using anti-glial fibrillary acidic protein (GFAP). We sought to develop an in-depth visual analysis of the astrocyte distribution across the entire retina. Using laser scanning confocal microscopy, whole retinal datasets were captured at high resolution and subsequently assembled into seamless montages using the bio-imaging software *Imago* [1]. This produces very large images for quantitative and qualitative analysis. Retinal astrocytes are then segmented using a Random Walk method previously described [2].

We previously developed a system [3] to visualize segmentation results and analyze cell distributions. Continued use of the system and users' feedback revealed a critical need to create an updated system in which retinal datasets are viewed at full resolution (0.31 μm/pixel). Additionally, a need for interactive segmentation parameter choices and more comprehensive visual analysis tools was identified. The challenges for these improvements range from the size of the data to the speed of the algorithms involved.

Here, we address all those challenges and report progress on the analysis tools implemented, enabling insights to be communicated both visually and quantitatively.

2 The Visualization System

To address the above challenges, we re-implemented the interactive visualization as follows.

2.1 Image pyramid cache

A major hurdle in visualizing retinal data sets consists in the sheer image dimensions of the microscopy output. Resulting montages are as large as 300 megapixels. We designed and implemented an image pyramid system to be flexible without any size constraints. We store our patches in separate small files organized in a directory structure according to scale factors. The system supports arbitrarily large images and is tolerant against individual file corruptions, using a simple redundancy scheme.

To support fast switching among different pre-rendered segmentation results, the system caches recently-viewed patches in memory until they are 30 seconds[1] older than the most recently-loaded patch. For smooth navigation, low resolution patches are always ready for display while the high resolution version is loading.

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[1] value empirically determined in viewing experiments

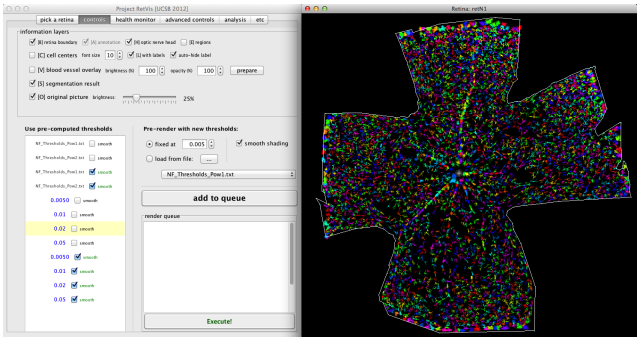


Figure 1: A full retina and the control panel.

2.2 Multithread processing

To improve system performance from our previous version, we parallelized our computation using Java thread pools. In most cases, a large amount of computation occurred in independent repetitive tasks across multiple cells. Typically, our system spawns a pool of N threads where $N = 1.5\times$ *(# of CPU cores)*. A number of threads higher than the number of cores produces a faster result because threads can become intermittently idle, yielding execution time to more active tasks.

This change greatly improved the performance in the following areas: pre-computed segmentation of cells in an entire data set (4,500 cells) is now completed in 2 hours compared to 2-3 days in the previous version. Pre-rendering of a full-resolution whole retina using new segmentation parameters and constructing its image pyramid is accomplished in approximately 4 minutes. Performing region-based analysis requires less than 10 seconds when analyzing up to 50,000 regions.

2.3 Visual analysis tools

Investigators can switch on/off several layers of information: original image, segmentation results (pre-computed for arbitrary parameter sets), blood vessels, nuclei locations, and annotations to highlight, e.g., the optic nerve head or the outer retina boundary. Fig. 2 shows the original image (left), segmented cells with a high segmentation threshold (middle), and low threshold (right).

Users can divide areas into square regions (Fig. 3), or by concentric circles around the optic nerve head (Fig. 5). Sizes are under user control. Region-based analysis can be done on the following quantities: number of cells; density (number of cells per area); distance from the center of optic nerve head; minimum distance from retina's border; area; and perimeter.

A central visualization option for analysis results consists in overlaid heat maps using custom color coding. Heat maps can be displayed in solid color or blended with user-selected transparency over the retina image to show context, as shown in Fig. 3. The heat map color transfer function can be displayed as a continuous or customizable step function. For a quantitative analysis, the user can export all values to a spreadsheet in MS Excel .xls format.

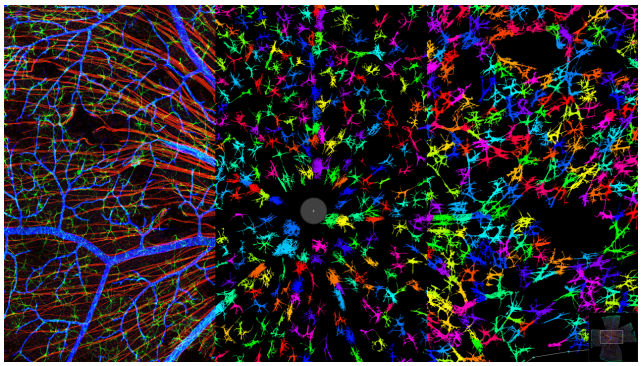
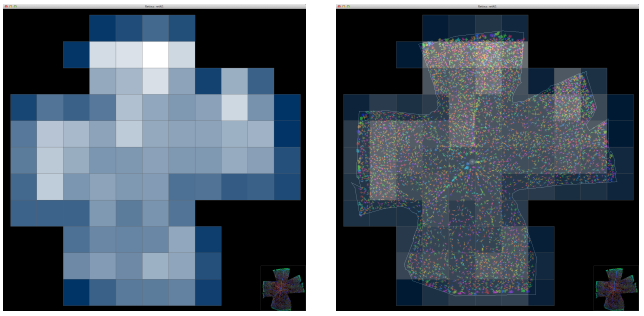


Figure 2: A composite image showing three views of a retina.



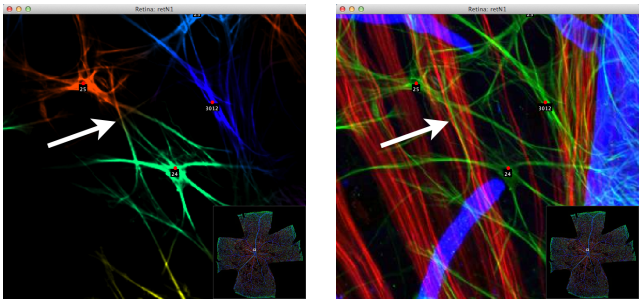
(a) opaque (b) 50% transparency

Figure 3: Heat map of number of cells in 500 μm^2 square regions

3 Sample use cases

We present three scenarios where our tool was used to analyze large scale retinal datasets.

3.1 Visual inspection of segmentation result



(a) segmented astrocytes (b) original picture

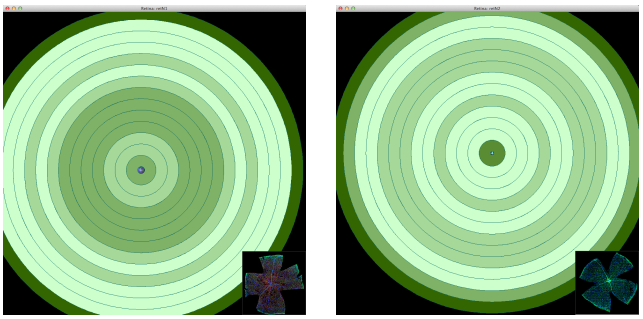
Figure 4: An incorrect splitting of an astrocyte process.

Use of our tools by experts in the field of retinal cell biology helped verify that the employed semi-automatic segmentation procedure yields plausible results. Cells that do not lie entirely on blood vessels appear to be more accurately segmented. Our users arrived at this conclusion by carefully reviewing both the segmentation results and the original image.

A few cells exhibit re-occuring inaccuracies. A process of a cell that reaches into the body of another cell is occasionally segmented in half instead of being assigned to just one of the cells. For example, the process in Fig. 4 (arrow) should be relegated to cell #25 (the upper cell).

3.2 Analyzing the distribution of astrocytes in a retina

We can divide the retina into circular regions around the optic nerve head and paint the regions according to statistics of interest. In Fig. 5, we encode the number of cells per area as the brightness of a region. The colors are discretized into 5 levels to see the contrast more clearly. A tentative observation gained from initial use of our visualization system is that there are two regions of increased astrocytic density.

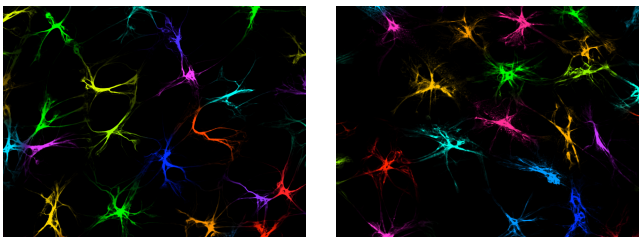


(a) normal retina #1 (b) normal retina #2

Figure 5: Brighter regions have more cells per area.

3.3 Comparing healthy vs. detached retinas

Using full resolution images, differences between healthy and purposefully-compromised retinas can be compared in detail. As shown in Fig. 6, astrocyte morphologies in a detached retina appear to be more ragged and elongated, warranting further investigation.



(a) normal retina (b) detached retina #2

Figure 6: Comparing two retinas

4 Conclusions

With an image pyramid technique and multithread processing, we redesigned and improved a visualization system with integrated analysis tools for large retinal astrocyte datasets. Future development will focus on the implementation of new analytic tools and automatic detection of relationships among quantities.

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References

[1] http://www.mayachitra.com/imago.

[2] V. Ljosa and A. Singh. Probabilistic segmentation and analysis of horizontal cells. In *Data Mining, 2006. ICDM '06. Sixth International Conference on*, pages 980 –985, 2006.

[3] P. Suwannatat, G. Luna, B. Ruttenberg, R. Raviv, G. Lewis, S. Fisher, and T. Hollerer. Interactive visualization of retinal astrocyte images. In *Biomedical Imaging: From Nano to Macro, 2011 IEEE International Symposium on*, pages 242 –245, 2011.