Rapid, Automated Mosaicking of the Human Corneal Subbasal Nerve Plexus

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***Abstract*— Corneal Confocal Microscopy (CCM) is an in vivo technique used to study corneal nerve morphology. The largest proportion of nerves innervating the cornea lie within the subbasal nerve plexus, where their morphology is altered by refractive surgery, diabetes and dry eye. The main limitations to clinical use of CCM as a diagnostic tool are the small field of view of CCM images and the lengthy time needed to quantify nerves in collected images. Here, we present a novel, rapid, fully automated technique to mosaic individual CCM images into wide-field maps of corneal nerves. We implemented an OpenCV image stitcher that accounts for corneal deformation and uses feature detection to stitch CCM images into a montage. The method takes 3-5 minutes to process and stitch 100 frames on an Amazon EC2 Micro instance. The speed, automation, and ease of use conferred by this technique is a first step towards point of care evaluation of wide-field subbasal plexus maps in a clinical setting.**

Running title - Rapid, Automated Mosaicking of the Subbasal Plexus

Keywords - Corneal Confocal Microscopy; Image Processing; Speeded-up Robust Features; Computer Vision; Dry Eye

List of non-standard abbreviations - Corneal Confocal Microscopy (CCM); Corneal Nerve Branch Density (CNBD); Corneal Nerve Fiber Density (CNFD); Corneal Nerve Fiber Length (CNFL); Corneal Nerve Fiber Tortuosity (CNFT); Laser in situ keratomileusis (LASIK); Speeded-up Robust Features (SURF); Subbasal Nerve Plexus (SBP); Random Consensus Sampling (RANSAC); Application Program Interface (API)

I. INTRODUCTION

The human cornea is densely innervated by branches of the nasociliary division of the ophthalmic (V1) branch of the trigeminal nerve. Nerve fibers enter the cornea radially from all directions, forming a clockwise whorl whose center lies inferonasally [9]. Upon reaching the corneal epithelium, fibers turn 90 degrees and travel anteriorly through the epithelium. The subbasal plexus (SBP) is located immediately posterior to the epithelium and comprises the largest proportion of corneal nerves. The plexus is altered by refractive surgical procedures, such as LASIK [4], as well as epitheliopathies associated with diseases such as diabetes and chronic dry eye. Loss of corneal innervation is accompanied by a host of symptoms, including burning and persistent dryness. These symptoms are believed to occur secondary to post-operative hypoesthesia and interruption of the reflex loop that connects the ocular surface to the tear secreting machinery comprising the lacrimal functional unit [8]. Given that LASIK is one of the most commonly performed vision correction surgeries and dry eye is among the most common conditions for which patients seek eye care, there is significant demand for methods with which to observe and quantify corneal nerves.

In vivo corneal confocal microscopy (CCM) is a technique that offers a novel way to examine corneal innervation. The technique is comparable to in vitro histological techniques (absent staining) and allows for repeated observation of the subbasal plexus over time. CCM is a non-invasive and effective metric for quantification of nerve loss in dry eye and other conditions. In spite of CCM's significant clinical potential for quantifying subbasal nerve morphology, major limitations include the small field of view of CCM images (0.16mm^2), the lack of automation and the lengthy amount of time required for capturing and quantifying images. Recent advancements have been made in mapping SBP nerves on a cornea-wide scale [2], yet, the need for quick, fully automated montaging and quantification remains. We present here a novel technique for wide field mosaicking of the SBP nerves in a human cornea.

II. MATERIALS AND METHODS

A. Image Acquisition

Corneal scans were captured using the Heidelberg Retina Tomograph Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany). Corneal scans were performed independently by an experienced ophthalmologist on four patients without clinical signs of pathology. Individual image sizes were 400 m x 400 m, equivalent to 384 pixels x 384 pixels. Data was uploaded to the software as individual images for quantification or video scans for montaging and quantification.

B. Montage Generation

We integrated a high-level image-stitching pipeline available through an online computer vision library [13] to achieve rapid, automated montaging of SBP nerves. This stitcher mosaics images in two steps: image registration and mosaic composition. The image registration step identifies descriptors by finding unique features in an image using

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SURF. SURF first locates interest points; pixels that are brighter than their neighbors or located at an intersection of edges. The pixels surrounding each interest point are described by a unique feature vector. Feature vectors of interest points in one image are then mapped to key points in other images by both comparing their feature vectors and their relative location. RANSAC is used to estimate homology between the images and ensure that the feature matching is accurate.

The distance between each point in one image and its corresponding points in other images would be the same if the pictures were flat. However, due to ridge-like deformations of the convex cornea, there are discrepancies between the displacement vectors. From these discrepancies, a transformation model that maps all points on one image to another is computed. The pre-processed images are warped and merged using the transformation model. Exposure discrepancies and seams are removed and the finalized mosaic is displayed to the user.

C. Vector Quantification

The vector quantification pipeline preprocesses the images to achieve maximum accuracy during the final nerve detection phase. The images are first passed through a Laplacian of Gaussian filter to remove noise from the tissue surrounding the nerves. The resulting image is then passed through a Harris Corner filter to reinforce the edges of the nerve fibers. The final preprocessing phase uses a customized ridge detector for identifying the edges of nerve fibers and normalizing the color of nerve fibers to ensure accurate detection.

To facilitate accurate detection of nerve fibers, a simple binarization of the preprocessed image is conducted using a threshold derived from the nerve fiber normalization process. The scores from the Harris Corner Filter and binary image are used to create vector descriptions of the nerves. From each point in the binary image, fixed-length lines are drawn in all directions and a score is generated for each line based on correlation with the Harris Corner filter's output. The scores of parallel lines are also considered. The line with the highest score is selected as a vector and the process is repeated recursively to generate vectors from the points. The resulting vectors are added to an R-tree, a data structure used for spatial analysis of nerve fibers. Overlapping trees are merged if the distance between them is below a threshold, or if the nerve's orientation intersects another nerve's orientation within a certain distance. The vector trees of the images are resolved into a single tree that contains every unique vector that traces a nerve fiber (Figure 1). This tree will ultimately be used to quantify CNFL, CNFD, CNBD and tortuosity. CNFL is the composite length of all nerve vectors. CNFD is obtained by dividing the number of trees greater than a certain size (determined relative to the image size) by the area. CNBD is obtained by dividing the number of nerve branchings (nodes with more than one child) by area. Tortuosity is calculated by averaging the angle between vectors that compose nerves. While beyond the scope of the mosaicking algorithm presented here, further refinement of the specific methods used to generate these quantitative readouts of nerve morphology are ongoing and the topic of future investigation.

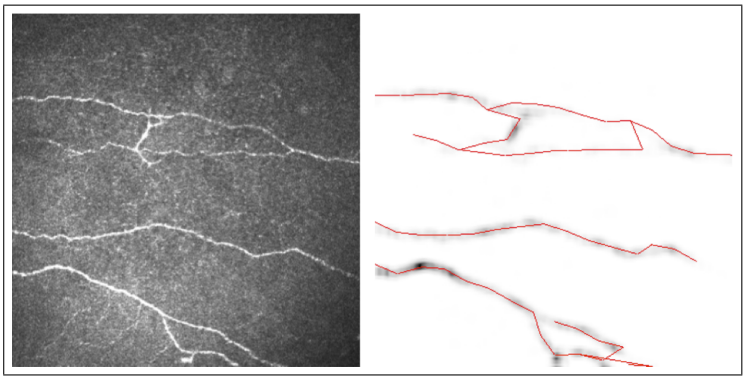

Fig. 1. Raw image input (left) and processed output (right). Processing pipeline traces vectors onto nerve fibers for quantification.

III. Results

Evaluation of the efficacy of any montaging program is challenging, as no standard method exists by which to compare manual and automated mosaics [11]. The montaging software described here was tested on four previously recorded data sets, each consisting of up to 40 sequential scans. The resulting mosaics were qualitatively compared to the results of the most recently published approach [2], which took 27 minutes to montage 61 frame video sequences [16]. Total processing time per patient for the previous method included 20 minutes for manual removal of erroneous tissue [16]. The mosaicking algorithm proposed here takes 3-5 minutes to process and montage 100 frame video sequences (Figure 2) and removes erroneous tissue automatically. The resulting stitch will ultimately be used to quantify CNFL, CNFD, CNBD and CNFT.

IV. Discussion

Creating mosaics of the SBP has evolved over the past decade from manual assembly of corneal maps to automated mosaic generation by improving image capture and accuracy. However, the advances realized through automation have still not been able to reduce image processing time for practical point of care use.

The first reference for creating mosaics by Patel et al [9] in 2005 described how montages of subbasal plexus images were manually created and quantified. Subsequent studies also used a manual montaging technique to map the cornea in keratoconus [10] and post-LASIK [12] patients. Yet, early imaging techniques were limited by the long duration of time required for capturing images and the lack of automation.

In 2010, Zhivov et al [15] described a real-time, automated montaging process using a modified HRT imaging modality, which required an expert ophthalmologist to operate it. This web-based CCM montage process is still available today and its rapid image acquisition time (<3min) was an important

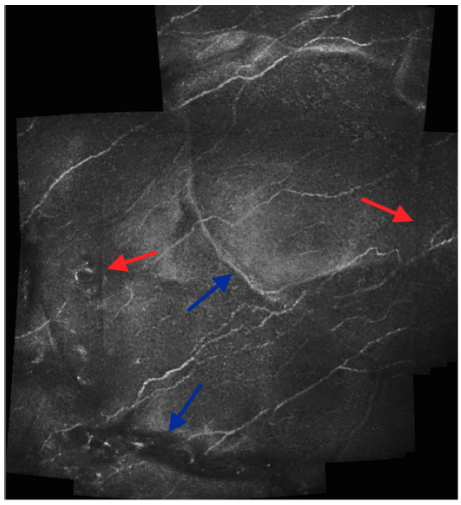

Fig. 2. Stitch of 100 images (4.52 minutes on an Amazon EC2 micro instance). Distance between a point in one image and its corresponding points in other images would be the same if the pictures were flat. However, due to ridge-like deformations of the convex cornea, there are discrepancies between the displacement vectors (red arrows). Artifact is also seen (blue arrows).

step towards creating wide-field maps. Limitations of this imaging program included the need for multiple attempts to effectively generate the montages [3]. Additionally, the time required for quantification of images was too long for clinical point of care use. Similarly in 2012, Turuwhenua [14] et al described a feature-based montaging method similar to the stitching pipeline presented here. The method was particularly effective since it did not falsely match images, and rejected those that did not meet the parameters of the stitch. Once again however, the amount of time dedicated to offline post-processing and mosaic generation (1.5 - 3 hours) was a major limitation.

Lum et al [7] were the first to use wide-field montages to observe changes in SBP nerve morphology following use of orthokeratology lenses. Scanning for this method took about 10 minutes to complete. Montages were manually generated from 400-700 images using Photoshop and used to observe loss of the whorl pattern of the SBP and an increase in tortuosity.

More recently, Efron et al [6] described a mosaicking technique using the HRT's video (sequence) mode followed by semi-automated tiling with Image-Pro Plus 7 software. While this technology greatly advanced the capability of SBP imaging, the blending process between images resulted in reduced montage quality due to loss of details. Subsequently, in 2014 Ziegler et al [16] used a large-scale reconstruction method, first described by Allgeier et al [2], to facilitate reconnection of nerve fibers and subsequent analysis using Mathematica 9.0.1. This offline method provided enhanced image quality and achieved superb accuracy while greatly reducing the time required for mosaic generation. Montaging 61 images took nearly 27 minutes, resulting in a total image processing time of 3 hours, thus limiting the feasibility of use in a clinical setting [16].

A separate 2014 publication by Poletti [10] et al outlined a method consisting of four steps; computation of the score matrix, "nerveness" evaluation, mosaic building and custom blending. The methodology is similar to our approach, taking 400-700 seconds to mosaic 50-80 images. This technique has significant potential for clinical use if integrated within a user-friendly interface.

Here, we have combined previous mosaicking techniques with automated stitching software (Figure 3) to montage corneal nerves [16]. The OpenCV stitching pipeline [13] used in our technique confers a high degree of specificity, including only related images to each stitch. The software is also among the few online methods available, and is unique in its ability to create montages from video sequences and quantify image files within a single interface. The technique is hosted on a secure web server, allowing users to visualize results in a wide field and provides point of care capability for clinical use.

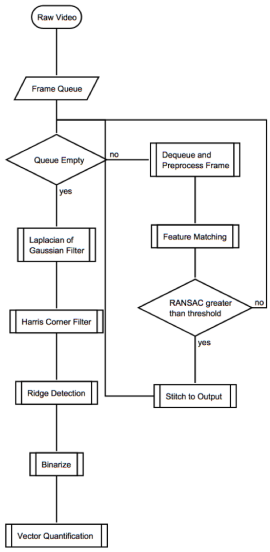

Fig. 3. Image processing pipeline for stitching and quantification of images. SURF first locates interest points. Pixels around the interest point are described by a feature vector. Feature vectors in one image then mapped to feature vectors in other images, and RANSAC is used to ensure accuracy. Discrepancies between the vectors are warped and merged using a transformation model. Both discrepancies and seams are removed and the resulting stitch may be used in quantification.

A. *Advantages*

Automation is an evolving part of modern medical practice, especially in radiology and pathology. The results of automated processes continue to be validated in the professional setting where advancements in the speed and accuracy of imaging and analytical techniques are steadily improving. Software dedicated to the quantitative analysis of corneal nerves is likely to become an important component for assessing ocular surface health in the clinic.

Here, we present the first iteration of a new software, that embodies several important facets of a clinically-applicable analysis method of corneal innervation using CCM. We used

an existing web transport layer (Firebase) to input images and communicate the quantified result through a user-friendly web interface, allowing a single web server to instantly provide results to a clinician without any complicated setup procedure. The Firebase API provides a standard way for developers of future imaging technology to automatically send imaging data to the image processing server and retrieve results. Additionally, our software could be used in conjunction with existing image registration algorithms [16] to correct for motion-induced distortion and generate three-dimensional corneal maps. Advancements in minimizing image acquisition time [3] could be combined with the technology to create a clinically applicable, cornea-wide quantification method.

Advantages of our analysis technique include the fully automated platform that is rapid, and permits image processing, mosaic generation and quantification within minutes. Its speed is due to the use of feature-based stitching, rather than the pixel-to-pixel comparisons used previously. This addresses the main deterrents to clinical use of CCM mosaics - speed, automation and ease of use. The software does not require an experienced ophthalmologist nor a computer programmer to montage and quantify the data. To our knowledge, this is the first technique that is both simple and user-friendly enough to be used by ancillary personnel.

The major limitation of this first-generation software lies in the vector quantification step. The montaging is highly accurate even with low image quality but the current software can only quantify high-quality single images. This drawback is evident when the perimeter of a montage is sometimes detected as a nerve fiber, or when bright artifacts that lie close together are reconnected as nerves. It is feasible to overcome this limitation through the use of previously described automated quantification [5] or manual quantification by an expert user. Thus, the results of our work provide an important step toward developing an accurate, repeatable, rapid and easily computed method of corneal nerve analysis using CCM that may ultimately be incorporated for point of care use in the diagnosis and management of ocular surface disorders affecting corneal innervation.

ACKNOWLEDGMENT

This research was partially funded by a grant from the Quinnipiac University School of Medicine and National Eye Institute grant NEI EY016203. Imaging was performed at the Francis I. Proctor Foundation by Drs. Jeremy Keenan and Salena Lee. Ayush Jain and Kalen Frieberg worked on the stitching algorithms and the user interface.

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