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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 min to process and stitch 40–100 frames on an Amazon EC2 Micro instance. The speed, automation and ease of use conferred by this technique is the first step toward point of care evaluation of wide-field subbasal plexus (SBP) maps in a clinical setting.

Keywords: computer vision; corneal confocal microscopy; dry eye; image processing; speeded-up robust features.

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Q3: Author designator 5 has been given for Nancy A. McNamara but the corresponding affiliation is missing. Hence it has been deleted. Please check and confirm

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 inferiorly [15]. Upon reaching the corneal epithelium, fibers turn 90° 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 laser *in situ* keratomileusis (LASIK) [4], as well as epitheliopathies associated with diseases such as diabetes and chronic dry eye [10, 12]. Loss of corneal innervation is accompanied by a host of symptoms, including burning and persistent dryness. These symptoms are believed to occur secondary to postoperative hypoesthesia and interruption of the reflex loop that connects the ocular surface to the tear secreting machinery comprising the lacrimal functional unit [13]. 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 way to examine corneal innervation. The technique is comparable to *in vitro* histological techniques (absent staining) and allows for repeated observation of the SBP over time. CCM is a noninvasive and effective metric for quantification of nerve loss in dry eye and other conditions [23]. Despite CCM's significant clinical potential for quantifying SBP nerve morphology, major limitations include the small field of view of CCM images (0.16 mm^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, 8, 19, 22, 24], 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.

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Materials and methods

Image acquisition

This retrospective study was conducted under the Proctor Foundation's Committee for Human Research protocol. Corneal sequence scans were captured using the Heidelberg Retina Tomograph (HRT) 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. Anesthetic drops were applied to the patient's cornea, and gel was placed inside a small cup that was then attached to the HRT. The cup prevented contact between the gel and the patient's cornea. Scan time was 5 min per eye, with a frame rate of eight frames per second. An external fixation light was used during imaging. Individual image sizes were 400 µm × 400 µm, equivalent to 384 pixels × 384 pixels. Scans were uploaded to the software as individual images for quantification or video scans for montaging and quantification. Each mosaic was assembled from a single sequence scan.

Montage generation

We integrated a high-level image-stitching pipeline available through the OpenCV library [14] 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 speeded-up robust features (SURF) [3]. 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. Random consensus sampling (RANSAC) [9] is used to estimate homography between the images and to ensure that the feature matching is accurate.

Because of ridgelike deformations of the convex cornea, there are discrepancies between the displacement vectors of matched points. From these discrepancies, a transformation model that maps all points on one image to another is computed. The preprocessed images are warped and merged using the transformation model. Exposure discrepancies and seams are then removed.

The technique is hosted on a web server, allowing users to upload multiple images or an audio-video interleaved video file. These images are sent to the server where they are stitched together and processed. The user can then examine the processed image and view results in a web-based interface. The code used for this web-based montage process is available online at the following link: https://github.com/techlabeducation/ImageProcessing1.1.

Results

Evaluation of the efficacy of any montaging program is challenging, as no standard method exists by which to compare manual and automated mosaics [19]. The montaging software described here was tested on four previously recorded data sets, each consisting of 40–100 sequential scans. The most recently published comparable approach took 10 min to montage 100 frame sequence scans [8]. The mosaicking algorithm proposed here takes 3–5 min to process and montage 40–100 frame video sequences (Figure 2) and removes erroneous frames automatically. Morphologic parameters for Figure 2 including corneal nerve fiber length (CNFL), corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fiber tortuosity (CNFT) have been obtained using manual tracing with CCMetrics [5–7, 17, 18, 21] and displayed in Figure 3.

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.

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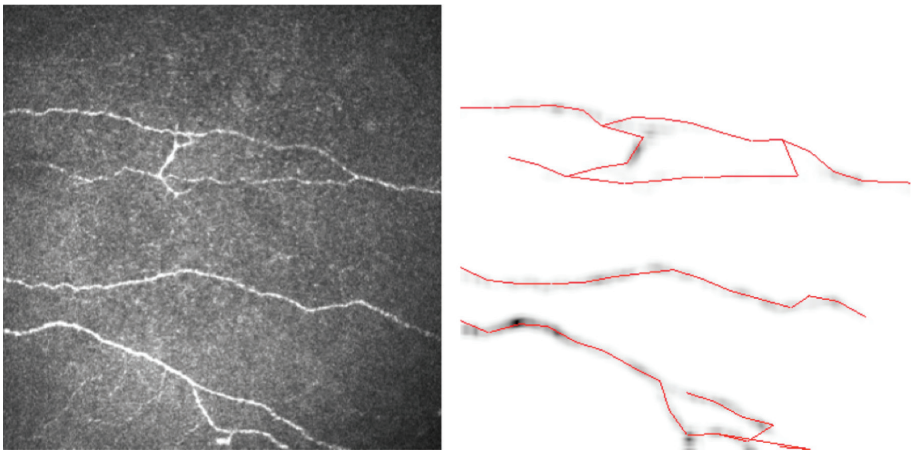

Figure 1: Raw image input (left) and processed output (right).
Processing pipeline traces vectors onto nerve fibers for quantification and is a future direction of the mosaicking software presented here.

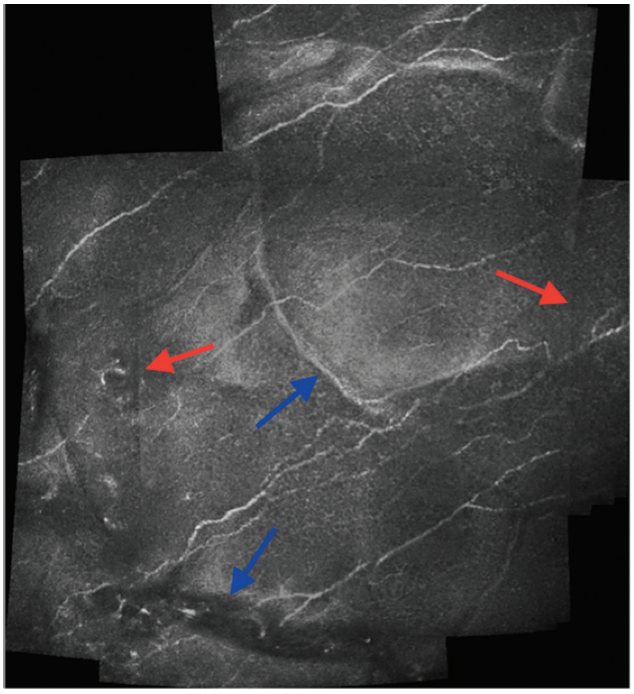

Figure 2: Stitch of 100 images (4.52 min on an Amazon EC2 micro instance).
Distance between a point in one image and its corresponding points in other images would be the same if the pictures were flat. However, because of ridgelike deformations of the convex cornea, there are discrepancies between the displacement vectors (blue arrows). Artifact is also seen (red arrows). Scale: 522×569 px.

CNFL	CNFD	CNBD	CNFT
5018	30	9	13.824

Figure 3: Results of manual quantification of Figure 2 using CCMetrics. CNFL (mm), CNFD (no. fibers per mosaic), CNBD (no. branches per mosaic) and CNFT are shown.

The first reference for creating mosaics by Patel et al. [15] in 2005 described how montages of SBP images were manually created and quantified. Subsequent studies also used a manual montaging technique to map the cornea in keratoconus [16] and post-LASIK [20] patients. Yet, early imaging techniques were limited by the long duration of time required for capturing images and the lack of automation. Lum et al. [11] 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 approximately 10 min to complete. Montages were manually generated from 400 to 700 images using Photoshop and used to observe loss of the whorl pattern of the SBP and an increase in tortuosity.

In 2010, Zhivov et al. [24] described a real-time, automated montaging process using a modified HRT imaging modality, which required an expert ophthalmologist to operate it. This CCM montage process is still available today and its rapid image acquisition time (<3 min) was an important step toward creating wide-field maps. Limitations of this imaging program included the need for multiple attempts to effectively generate the montages. Additionally, the time required for quantification of images was too long for clinical point of care use. Similarly in 2012, Turuwhenua et al. [22] described a feature-based montaging method similar to the stitching pipeline presented here. The method was particularly effective because it did not falsely match images and rejected those that did not meet the parameters of the stitch. Once again

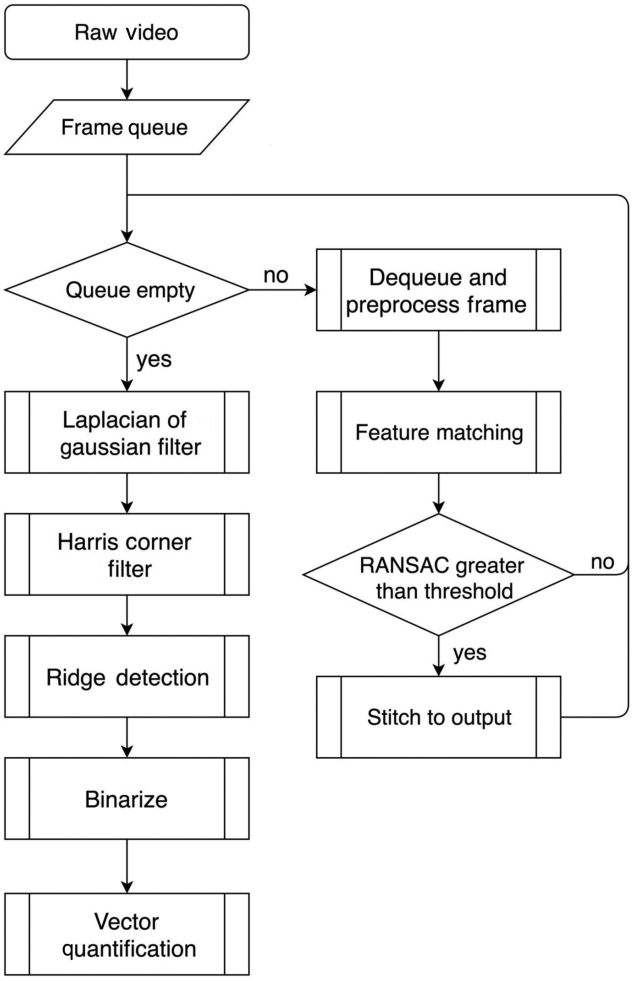

Figure 4: Image processing pipeline for stitching and quantification of images.
SURF first locates interest points. Pixels around the interest point are described by a feature vector. Feature vectors in one image then mapped to feature vectors in other images, and RANSAC is used to ensure accuracy. Discrepancies 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.

however, the amount of time dedicated to offline post-processing and mosaic generation (~1.5–3 h) was a major limitation.

Subsequently, in 2014 Ziegler et al. [25] used a large-scale reconstruction method, first described by Allgeier et al. [1], 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. Yet the method took 27 min to montage 61 images, resulting in a total image processing time of ~3 h and limiting the feasibility of use in a clinical setting [25].

A separate 2014 publication by Poletti et al. [19] 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 s 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 the OpenCV stitching pipeline [14] to montage corneal nerves. This pipeline 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.

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 application programming interface 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 [25] to correct for motion-induced distortion and generate three-dimensional corneal maps. Advancements in minimizing image acquisition time [2] could be combined with the technology to create a clinically applicable, cornea-wide quantification method.

One of the major limitations of the current method is the lack of automated quantification. However, significant advancements have been made in our ongoing effort to automate vector quantification. In this process, the image is first preprocessed through a sequence of filters designed to reduce noise and reinforce nerve fibers, at which point the nerves can then be easily vectorized. However, because this vector quantification pipeline still requires human supervision and correction, it represents the direction of the future work presented here.

Advantages of our analysis technique include the fully automated platform that is rapid and permits image processing and mosaic generation 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 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 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.

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