Automated detection and cell density assessment of keratocytes in the human corneal stroma from ultrahigh resolution optical coherence tomograms

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Abstract: Keratocytes are fibroblast-like cells that maintain the optical clarity and the overall health of the cornea. The ability to measure precisely their density and spatial distribution in the cornea is important for the understanding of corneal healing processes and the diagnostics of some corneal disorders. A novel computerized approach to detection and counting of keratocyte cells from ultra high resolution optical coherence tomography (UHR-OCT) images of the human corneal stroma is presented. The corneal OCT data is first processed using a state-of-the-art despeckling algorithm to reduce the effect of speckle on detection accuracy. A thresholding strategy is then employed to allow for improved delineation of keratocyte cells by suppressing similarly shaped features in the data, followed by a second-order moment analysis to identify potential cell nuclei candidates. Finally, a local extrema strategy is used to refine the candidates to determine the locations and the number of keratocyte cells. Cell density distribution analysis was carried in 3D UHR-OCT images of the human corneal stroma, acquired in-vivo. The cell density results obtained using the proposed novel approach correlate well with previous work on computerized keratocyte cell counting from confocal microscopy images of human cornea.

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References and links
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1. Introduction

Keratocytes are specialized fibroblasts, located in-between the collagen fibers comprising the matrix of the human corneal stroma [1, 2, 3]. Their major function is to ensure the structural integrity and optical clarity of the stroma. Keratocytes are actively involved in the healing of
the stroma during inflammation and structural injury. Therefore, non-invasive evaluation of the keratocyte cell volume density in health and disease, could lead to better understanding of the healing process and pathological degeneration of the human corneal stroma. In the past, minimally invasive imaging of the human cornea with confocal microscopy was combined with automatic algorithms for evaluation of the keratocyte cell volume density [4, 5, 6, 7]. Although this approach has led to some very interesting and informative results, it has a few major limitations: a) the imaging is partially invasive, since the confocal imaging probe has to come in contact with the corneal tissue, to sustain high lateral and axial imaging resolution; b) the imaged regions are limited to a few square millimeters at the apex of the cornea, because of the design of the confocal imaging probe.

Optical coherence tomography (OCT) is a non-invasive imaging modality [8, 9] that allows for depth-resolved, cellular level resolution imaging of the structural composition of biological tissue at depths of ~1.5 mm below the surface in highly scattering tissue. Anterior segment ultrahigh resolution (< 5μm) optical coherence tomography (UHR-OCT) was developed over the past decade for high speed, in-vivo imaging of the human cornea [10, 11, 12, 13, 14, 15]. The axial UHR-OCT resolution is sufficient for the imaging, identification and even segmentation of the major corneal layers [16, 17].

A representative healthy human cornea image, acquired in-vivo with a state-of-the-art, high speed 1060nm UHR-OCT system [15] is shown in Fig. 1A. The 3 μm axial resolution is sufficient to image the individual corneal layers: epithelium (Ep), Bowman’s membrane (BM), stroma (S) and the Descemet’s - Endothelium complex (DEC), since the endothelium is very thin and cannot be resolved separately with the current axial resolution. The image also shows highly reflective (black) mostly circular features distributed within the stroma (Fig. 1A, red arrows). Fig. 1B shows a representative en-face confocal microscopy image, acquired from a small region in the human corneal stroma, in which keratocyte cells (Fig. 1B, red arrows) appear as highly reflective (whitish) objects on the highly transmissive dark background tissue. By comparing the two images, it is logical to hypothesize that the circular hyper-reflective spots observed in UHR-OCT tomograms of the corneal stroma are reflections from keratocytes. Since the UHR-OCT lateral resolution is ~15 μm, it is not possible to resolve individual keratocyte cells in en-face UHR-OCT images with the current design of the imaging probe. However, if our hypothesis is correct, the number of highly reflective spots per unit volume of tissue, identified from a 3D image set of UHR-OCT tomograms of human corneal stroma should correlate well with the cell number density measured from a 3D set of confocal images of the stroma. Based on this hypothesis, we have developed an automatic algorithm for identification and counting of keratocytes from UHR-OCT corneal tomograms.

Here we present a novel computerized approach to detection and counting of keratocyte cells from UHR-OCT images of the human cornea. The paper is organized as follows. First, the methodology behind the computerized approach for detecting and counting keratocyte cells is described in detail in Section 2. Experimental results and a cell density distribution analysis using 3D sets of human corneal images acquired in-vivo with a research grade high speed, UHR-OCT system are presented and discussed in Section 3. Finally, conclusions are drawn in Section 4.

2. Methods

To evaluate the effectiveness of the computerized keratocyte cell detection and counting approach, it was applied to multiple 3D sets of human corneal images acquired in-vivo with a research grade high speed, UHR-OCT system operating in the 1060nm wavelength region. The basic design and detailed description of the system was published previously [15], however, the light source and the camera were recently updated to improve the axial resolution in the
Fig. 1. A representative UHR-OCT cross-sectional image of healthy human cornea acquired in-vivo (A). Ep - epithelium, BM - Bowman’s membrane; S - stroma; DEC - Descemet’s - Endothelium complex layer. Red arrow mark highly reflective spots in the stroma. A representative confocal en-face image of healthy human cornea (B). Red arrows mark keratocyte cells.

human cornea and the image acquisition rate. Briefly, the upgraded UHR-OCT system used in this study is based on a spectral domain design, that utilises a fiber-based Michelson interferometer interfaced to a super-luminescent diode (Superlum Ltd., $\lambda_c = 1020\text{nm}$, $\delta\lambda = 110\text{nm}$, $P_{\text{out}} = 10\text{mW}$). A corneal imaging probe comprised of 3 achromat doublet lenses (Edmund Optics) and a pair of galvanometric scanners (Cambridge Technologies) was designed for in-vivo imaging of the human cornea. The UHRCT system provided $\sim 3\mu\text{m}$ axial and $\sim 15\mu\text{m}$ lateral resolution in corneal tissue. Corneal tomograms were acquired with an InGaAs linear array, 1024 pixel camera (SUI, Goodrich) with 92kHz readout rate, that was interfaced with a high performance spectrometer (P&P Optica). The UHR-OCT system provided 95dB SNR for 1.3mW optical power incident on the human cornea.

Volumetric images ($512 \times 512 \times 512$) were acquired in-vivo from a region in the corneal of a healthy human subjects, located just below the corneal apex to void strong back-reflection imaging artefacts. The imaging procedure was carried out in accordance with the University of Waterloo ethics regulations. The images were oversampled by at least 50% in x and y direction of the scan, to avoid gaps between sequential A-scans or B-scans.

The proposed computerized keratocyte cell detection and counting approach consists of four main stages: i) despeckling, ii) thresholding, iii) cell candidate selection, and iv) cell identification. A flowchart of the proposed approach is shown in Fig. 2. In the despeckling stage, the corneal OCT data is processed using a modified general Bayesian speckle reduction technique [23] to significantly reduce speckle while preserving important details. In the thresholding stage, a thresholding strategy is applied to the despeckled OCT data to suppress features with similar morphological characteristics as keratocyte cells that have fundamentally different reflectivity characteristics. In the candidate selection stage, given the thresholded data, potential candidates are identified via a second-order moment analysis, which provides a reliable indication of uniqueness that is characteristic of keratocyte cells. The immediate peaks of the saliency measure are selected as keratocyte cell candidates. In the cell identification stage, the
Fig. 2. Step-by-step flowchart of the novel cell detection and counting approach

De-Speckle

Thresholding

Cell Candidate Selection

Cell Identification

Is Pixel intensity > Th

yes

Pi = Th

no

Pi = Pi

Is Local Maxima?

no

No Cell Detected

yes

No Cell Detected
final set of keratocytes are determined (and subsequently counted) via a non-maximum sup-
pression strategy, which effectively eliminates redundant candidates representing the same cell
within proximity of each other. A detailed description of each of the four stages is provided in
the subsequent sections.

2.1. Stage 1: despeckling

One of the major challenges when dealing with OCT data is the presence of speckle, which is
an inherent characteristic of all imaging systems based on interferometric detection. The noise
component of speckle results in an overall grainy appearance of images acquired using OCT,
which can have an significant impact on the accuracy of feature detection and analysis using
such data. As such, an important first step is to reduce the presence of speckle noise in OCT
data while preserving the fine details needed for accurate analysis.

One approach to speckle reduction is to make fundamental design changes can be made to the
OCT imaging system. Such changes include the use of a partially spatially coherent broadband
light source [18], frequency compounding via the use of two incoherence interferometric sig-
nals [19], and angular compounding [20, 21], and spatial diversity [22]. However, such design
changes can result in increased system complexity and imaging time, and the amount of speckle
reduction is relatively limited. Therefore, in the proposed approach, an algorithmic approach to
speckle reduction was utilized.

Taking an algorithmic approach as opposed to a hardware design change approach, in the
despeckling stage of the proposed approach, the corneal OCT data is processed using a modified
general Bayesian speckle reduction technique [23]. This algorithmic strategy was chosen as it
was shown in research literature to achieve improved speckle reduction and detail preservation
when compared to popular algorithmic speckle reduction methods such as the versatile wavelet-
based method proposed by Pizurica et al. [24], the anisotropic diffusion method proposed by Yu
and Acton [25], and the Type II Fuzzy anisotropic diffusion method proposed by Puvanathasan
and Bizheva [26].

The modified general Bayesian speckle reduction technique used can be described as fol-
lows. Let \( M(\mathbf{x}) \), \( I(\mathbf{x}) \) and \( N(\mathbf{x}) \) be random variables representing the measured data, noise-
free data and speckle noise of unknown distribution at pixel \( \mathbf{x} \) respectively. Furthermore, let
\( m = \{ m(\mathbf{x}) | \mathbf{x} \in X \} \), \( i = \{ i(\mathbf{x}) | \mathbf{x} \in X \} \), and \( n = \{ n(\mathbf{x}) | \mathbf{x} \in X \} \) be realizations of \( M, I \), and \( N \)
respectively. Given that speckle in OCT arises from the constructive and destructive interferences
of the backscattered signal from biological issues [27], it can be modeled as having a mul-
tiplicative relationship with the noise-free data, dependent on the wavelength of the imaging
beam and the imaged object’s details [28],

\[ m(\mathbf{x}) = i(\mathbf{x}) \cdot n(\mathbf{x}). \] (1)

This can be transformed into an additive relationship via a logarithmic transform, given the
expression

\[ \log m(\mathbf{x}) = m(\mathbf{x}) = \log [i(\mathbf{x}) \cdot n(\mathbf{x})] = \log \{ i(\mathbf{x}) \} + \log \{ n(\mathbf{x}) \} = i(\mathbf{x}) + n(\mathbf{x}). \] (2)

The general Bayesian least squares estimate of the noise-free data \( \hat{i}(\mathbf{x}) \) can be expressed
as [23]

\[ \hat{i}(\mathbf{x}) = \exp \left[ \int p(i(\mathbf{x})|m(\mathbf{x}))i(\mathbf{x})di(\mathbf{x}) \right]. \] (3)

The posterior distribution \( p(i(\mathbf{x})|m(\mathbf{x})) \) used in Eq. 3 is computed using a non-parametric
Gaussian Parzen window estimation strategy based on pixels taken from the surrounding \( K \times K \).
neighbourhood in logarithmic domain:

\[
\hat{p}(i(x)|m(x)) = \frac{p^*(i(x)|m(x))}{\int p^*(i(x)|m(x)) di(x)},
\]

where \( p^* \) is defined as

\[
p^*(i(x)|m(x)) = \frac{1}{\sqrt{2\pi}} \sum_k \exp \left( -\frac{1}{2\sigma} (m(x) - m(x_k))^2 \right) \exp \left( -\frac{1}{2}(i(x) - m(x_k))^2 \right),
\]

where \( k \) represents the \( k^{th} \) pixel in the local neighbourhood. Based on testing, a 11 \( \times \) 11 neighbourhood was found to produce strong results. Examples of the despeckled corneal OCT data is shown in Fig. 3.

Fig. 3. Original (A) and despeckled (B) human corneal UHR-OCT images.

2.2. Stage 2: thresholding

An important issue in keratocyte cell detection and counting that must be tackled is the presence of physiological features as well as artifacts that share similar circular morphological characteristics as keratocyte cells when imaged using OCT. To differentiate such features and artifacts from the keratocyte cells, one is motivated to take the underlying reflectivity characteristics into account. Since the nuclei of keratocyte cells are highly reflective, it was observed that keratocyte cells appear as circular hyper-reflective spots in UHR-OCT tomographs. Therefore, one can make use of the underlying reflectivity characteristics to separate keratocyte cells (which have very high reflectivity) from similarly shaped features and artifacts (which possess noticeably lower reflectivity).

Based on these observations, in the thresholding stage, the following thresholding strategy is applied to the despeckled OCT data \( i(x) \) to suppress features with similar morphological characteristics as keratocyte cells but have fundamentally different reflectivity characteristics:

\[
i_{\text{Th}}(x) = \begin{cases} 
\alpha, & \text{if } i(x) \geq \alpha \\
i(x), & \text{if } i(x) < \alpha
\end{cases}
\]
where $\alpha$ indicates the reflectivity threshold. To obtain the reflectivity threshold, the reflectivity statistics of imaged keratocyte cells was automatically learned from a set of training data of keratocyte cells identified by a trained expert from imagery captured using the same instrumentation. Based on the learned reflectivity statistics of imaged keratocyte cells, the reflectivity threshold was selected as the median of the statistical distribution, which provides a reasonable choice for the threshold. An example of the thresholded data is shown in Fig. 4.

Fig. 4. An example of the thresholded data.

2.3. Stage 3: cell candidate selection

Given that nuclei of keratocyte cells are highly reflective, while the surrounding collagen fibers are of much lower reflectivity, keratocyte cells can be viewed as circular points of high saliency within the data where there is considerable change in reflectivity when compared to the surrounding regions. Motivated by this observation, in the cell candidate selection stage, given the thresholded data $i_{Th}(x)$, we first compute the corresponding second-order moment matrix $\Phi_{Th}$:

$$\Phi_{Th} = \begin{bmatrix}
\langle \{\Delta x i_{Th}(x)\}\{\Delta x i_{Th}(x)\}\rangle & \langle \{\Delta x i_{Th}(x)\}\{\Delta y i_{Th}(x)\}\rangle \\
\langle \{\Delta y i_{Th}(x)\}\{\Delta x i_{Th}(x)\}\rangle & \langle \{\Delta y i_{Th}(x)\}\{\Delta y i_{Th}(x)\}\rangle 
\end{bmatrix}$$  \hspace{1cm} (7)

where $\Delta x$ and $\Delta y$ denote the reflectivity gradient in the x and y-directions, and angular brackets denote Gaussian averaging respectively. This second-order moment matrix $\Phi_{Th}$ characterizes the reflectivity changes in various directions of the data.

Based on the computed second-order moment matrix $\Phi_{Th}$, the potential candidates are identified via a second-order moment analysis using the Noble saliency measure [29] (denoted as $\rho_{Th}$), which provides a reliable indication of the reflectivity changes when compared to surrounding regions that is characteristic of keratocyte cells:

$$\rho_{Th}(x) = \frac{\det(\Phi_{Th})}{\text{trace}(\Phi_{Th})}$$  \hspace{1cm} (8)
where \( \text{det} \) represents the determinant of the second-order moment matrix:

\[
\text{det}(\Phi_{\text{Th}}) = \langle \{ \Delta x \text{Th}(x) \}^2 \rangle \langle \{ \Delta y \text{Th}(x) \}^2 \rangle - \langle \{ \Delta x \text{Th}(x) \} \{ \Delta y \text{Th}(x) \} \rangle^2
\]

and \( \text{trace} \) represents the trace of the second-order moment matrix:

\[
\text{trace}(\Phi_{\text{Th}}) = \langle \{ \Delta x \text{Th}(x) \}^2 \rangle + \langle \{ \Delta y \text{Th}(x) \}^2 \rangle
\]

An example of the saliency map computed from the thresholded data is shown in Fig. 5. The immediate peaks of the saliency measure are selected as keratocyte cell candidates.

![Saliency Map](image)

Fig. 5. An example of a saliency map computed from thresholded data.

2.4. **Stage 4: cell identification**

Given the set of keratocyte cell candidates, it is important to ensure that the same keratocyte cell is not represented by multiple keratocyte cell candidates marked on the same OCT B-scan, which can lead to inaccurate cell counting results due to counting the same cell multiple times. This is accomplished in the cell identification stage of the proposed approach through the use of non-maximum suppression [30] to effectively eliminate redundant candidates representing the same cell within proximity of each other. An illustrative example of the non-maximum suppression strategy is shown in Fig. 6. Scanning through the set of keratocyte cell candidates, only the candidates with the highest saliency value within a local neighbourhood are selected as part of the final set of keratocyte cells used for counting. By only selecting those with the highest saliency value within a local neighbourhood, the redundant candidates representing the same cell but have lower saliency values are eliminated, hence avoiding reduced counting accuracy due to counting the same cell multiple times. Based on testing, a 11 × 11 local neighbourhood was found to provide strong results.

3. **Results and discussion**

To test the effectiveness of the proposed computerized keratocyte cell detection and counting approach, three different 3D stacks of UHR-OCT corneal tomograms were processed using the
Fig. 6. Illustrative example of non-maximum suppression strategy. Scanning through the set of keratocyte cell candidates, only the candidates with the highest saliency value within a local neighborhood are selected as part of the final set of keratocyte cells used for counting. For example, in the bottom-right yellow square, while there are two immediate peaks (i.e., with saliency values of 5 and 6), only the point with saliency value of 6 is selected as part of the final set of keratocyte cells as they both represent the same keratocyte cell.

novel approach. Keratocyte cell density analysis was carried out in a region of the stroma corresponding to a 1mm × 1mm surface area, centered around the apex of the volumetric OCT image of the cornea. Fig. 7 shows a representative original UHR-OCT tomogram of healthy human cornea (Fig. 7A) and the corresponding image with marked identified and counted keratocyte cells (Fig. 7B, yellow squares).

Fig. 7. A representative unprocessed UHR-OCT image of healthy human cornea (A). Same image with identified and counted cells, marked with yellow squares (B).

To evaluate the spatial distribution of keratocyte cells in axial direction within the chosen tissue volume, the stroma was segmented in depth into 9 layers of equal thickness and cells were counted in each layer. Fig. 8 shows the keratocyte cell number density as a function of depth.
in the stroma for three different sets of 3D UHR-OCT tomograms acquired from three human subjects. Consecutive B-scans (2D cross-sectional frames) in the 3D OCT image stack were separated by a step of \(\sim 10\mu m\). Considering that the lateral imaging resolution was \(\sim 15\mu m\) to \(20\mu m\) within the corneal stroma, our imaging data was oversampled by a factor of 30-50%. This ensured that we did not miss any cells in the stroma. To ensure that we did not count the same cell multiple times, cells that appeared at the same location \((x,y)\) in frames \((n-1), n\) and \((n+1)\) were counted only once. The final volumetric cell count was determined by calculating the number of detected cells per \(mm^2\) for each B-scan, and summing over the number of B-scans. As a final step, the cell number density in \(mm^3\) was computed by considering the thickness of the stroma, which was determined during the layer segmentation stage of the proposed algorithm.

These results presented in Fig. 8 suggest that the keratocytes number density is slightly higher in the anterior part of the stroma and that it decreases monotonically toward the posterior end of the stroma. This result correlates well with the overall trend observed in similar studies that were conducted in-vivo on a large group of subjects with clinical confocal microscopy [4, 5, 6, 7]. In terms of the average keratocyte cell number density, different confocal microscopy
studies have reported somewhat different values and fairly large uncertainties for data acquired from small groups of healthy subjects. Specifically, the study in [4] showed mean keratocyte cell number density of 47,100 cells / mm$^3$ in the healthy anterior stroma, with the number decreasing to 27,900 cells / mm$^3$ in the posterior stroma. The study in [7] showed a similar trend of decrease of keratocyte cell number density from the anterior to the posterior end of the stroma, though the absolute values for the mean keratocyte cell number density, measured in the selected group of healthy subjects was slightly lower: approximately 35,000 cells / mm$^3$ in the anterior stroma and 24,000 cells / mm$^3$ in the posterior stroma. The keratocyte cell number densities determined with our novel algorithm from UHR-OCT images of the healthy stroma agree very well with the published data. We also observed significant subject-related variation in the total cell number density, for the 3 subjects imaged in our study (data presented in Fig. 8).

In this study we adopted an indirect method of verifying the performance of the newly developed keratocyte cell counting algorithm, specifically designed for UHR-OCT images of the cornea, by comparing the OCT results acquired from a limited number of subjects with published results obtained from confocal microscopy studies of the human cornea. In the future, we plan to adopt a more direct approach by imaging the same area of the human cornea with OCT and confocal microscope and comparing the cell density in the chosen tissue volume. The study will also include a large number of healthy subjects to explore the effects of age, gender and race on the measured keratocyte cell density with the healthy human corneal stroma.

4. Conclusions and future work

In this work, a novel computerized approach was introduced for the purpose of robust keratocyte cell detection and counting from UHR-OCT images of the human cornea. Experimental results obtained using the proposed approach on human corneal UHR-OCT images acquired in vivo from healthy subjects compared well with published results from similar studies conducted with confocal microscopy. Non-invasive keratocyte cell number density evaluation could be a very useful tool in clinical studies of healing processes in the cornea for cases of corneal scarring, infections and surgeries. Further work involves a more comprehensive analysis that will compare keratocyte cell density measurements from the same location in the cornea with both UHR-OCT and confocal microscopy in a large group of healthy subjects.

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