ADAPTIVE HYBRID SEGMENTATION MODEL BASED ON WATERSHED AND ENHANCED U-NET FOR ENDOTHELIAL HUMAN CORNEA CELLS

AHMED SAIFULLAH SAMI

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy

School of Computing
Faculty of Engineering
Universiti Teknologi Malaysia

DECEMBER 2021

DEDICATION

Dedicated to my beloved parents, wife and my children Murad and Mizhda , whom without their love and support This research would have never been completed.

ACKNOWLEDGEMENT

Thanks to Allah SWT for everything I was able to achieve and for everything I tried but I was not able to achieve. First of all,

I would like to take this opportunity to gratefully acknowledge the wholehearted supervision of Prof. Dr. Mohd Shafry Bin Mohd Rahim during this work. His dedication, skilful guidance, helpful suggestions and constant encouragement made it possible for me to deliver a thesis of appreciable quality and standard.

I would also like to say special thanks to Prof. Dr. Ghazali bin Sulong, whose precious guidance, support and encouragement were pivotal in establishing my self-confidence in this endeavour.

I am forever indebted to my parents for their patience and understanding, alleviating my family responsibilities and encouraging me to concentrate on my study.

Finally and most importantly, I would like to express special thanks to my wife Heleen Yaseen for her support when it was most required. Without her help and Encouragement, this study would not have been completed.

ABSTRACT

Segmentation of the medical image plays a significant role when it comes to diagnosis using a computer-aided system. This study focused on the human corneal endothelium's health, one of the research areas that is particularly interested in human cornea health. Various pathological environments expedite the extermination of the endothelial cells, which abnormally decreases the cell density. Dead cells worsen the hexagonal design. In this study, medical feature extraction was obtained depending on the segmentation of the endothelial cell boundary. The task of segmentation of such objects is considered challenging due to the nature of the image captured during endothelium layer examination by ophthalmologists using confocal or specular microscopy. The resulting image suffers from various issues that affect the image's quality, such as noise, shadow, and blurry image. So, the study's primary goal was to propose and develop an automatic and robust model for the segmentation of endothelial cells of the human cornea obtained by in vivo microscopy and computation of the different clinical features of endothelial cells. A new scheme of image enhancement was proposed, such as The Contrast-Limited Adaptive Histogram Equalization (CLAHE) techniques to enhance contrast to achieve the goal of this study. After that, a new image denoising technique Enhanced Wavelet Transform Filter and Butterworth Bandpass for Segmentation (WTBBS) was employed. Subsequently, brightness level correction was applied by using the moving average filter and the CLAHE to reduce the effects of the non-uniform image lighting produced as a result of the previous step. The primary focus of this study was the segmentation stage. This stage involved precise detection of the endothelial contours. So, a new segmentation model was proposed, which is an Adaptive Hybrid Trainable Model for Segmenting Endothelial Cells (AHTMSEC). The AHTMSEC includes one crucial step: an Artificial Neural Network for Adaptive Segmenting (ANNAS) to identify the complexity of the image and the suitable algorithm. The output of this step was processed using either the Enhanced U-NET Approach for Endothelial Cell Segmentation (EU-NETAECS) or the Trainable Segmentation and Distance Transform (TDWS) to enhance the Watershed Transform for cell segmentation. In the segmentation stage, the shape of the cells was extracted, and the contours were highlighted. This stage was followed by clinical feature extraction and the used of the features for diagnosis. In this stage, several relevant clinical features such as Pleomorphism Mean Cell Perimeter (MCP), Mean Cell Density (MCD), Mean Cell Area (MCA), and Polymegathism were extracted. The role of these clinical features was crucial for the early detection of corneal pathologies and the evaluation of the health of the corneal endothelium layer. Every process was benchmarked against the best and up-to-date segmentation and clinical features detection techniques found in the literature. The existing methods of image enhancement and segmentation have been enhanced considerably via original ideas. Significant contributions of the present study on medical feature extraction based on segmentation were enumerated and ranked from top to bottom according to the degree of importance. The accuracy of the adaptive segmentation model for images classification was 97.5 %. It can be observed that the values obtained using the manual and automated techniques did not exhibit statistically significant differences for any of the five clinical features. The manual and automated processes differences were below 2%, 2%, 1%, 1.5%, and 3.5% for MCD, MCA, Polymegathism, MCP, and Pleomorphism, respectively.

ABSTRAK

Segmentasi imej perubatan memainkan peranan penting apabila diagnosis menggunakan sistem berbantukan komputer dijalankan. Kajian ini memberi tumpuan kepada kesihatan endotelium kornea manusia, salah satu bidang penyelidikan yang memberi perhatian terhadap kesihatan kornea manusia. Pelbagai persekitaran patologi mempercepatkan penghapusan sel endotel yang secara tidak normal mengurangkan kepadatan sel. Sel-sel mati memburukkan reka bentuk heksagon. Dalam kajian ini, pengekstrakan ciri perubatan diperoleh bergantung pada segmentasi batas sel endotel. Tugas segmentasi objek tersebut dianggap mencabar kerana sifat tangkapan imej semasa pemeriksaan lapisan endotelium oleh pakar oftalmologi menggunakan mikroskop confocal atau spekular. Imej yang dihasilkan mempunyai pelbagai masalah yang menjejaskan kualiti imej seperti bercak, bidang gelap dan imej kabur. Oleh itu, tujuan utama kajian ini adalah untuk mencadangkan dan membangunkan model automatik dan teguh untuk segmentasi sel endotel kornea manusia yang diperoleh dengan mikroskopi dalam tubuh dan pengiraan ciri klinikal sel endotel yang berbeza. Skema peningkatan imej baru dicadangkan seperti teknik-teknik Penyamaan Histogram Adaptif Kontra Terhad (CLAHE) untuk meningkatkan kontras bagi mencapai tujuan kajian ini. Seterusnya, teknik denoising imej baru yang dikenali sebagai Penapis Transformasi Wavelet yang Dipertingkatkan dan Laluan Jalur Butterworth untuk Segmentasi (WTBBS) digunakan. Seterusnya, pembetulan tahap kecerahan diterapkan dengan menggunakan Penapis Sederhana Bergerak dan CLAHE untuk mengurangkan kesan pencahayaan imej yang tidak seragam yang dihasilkan sebagai hasil dari langkah sebelumnya. Fokus utama kajian ini adalah peringkat segmentasi. Peringkat ini melibatkan pengesanan kontur endotel yang tepat. Oleh itu, model segmentasi baru dicadangkan, iaitu Model Hibrid Adaptif yang Boleh Dilatih untuk Membahagikan Sel Endothelial (AHTMSEC). AHTMSEC merangkumi satu langkah penting, iaitu Rangkaian Saraf Tiruan untuk Segmentasi Adaptif (ANNAS) untuk mengenal pasti kerumitan imej dan algoritma yang sesuai. Hasil dari langkah ini telah diproses dengan menggunakan kaedah EU-NETAECS atau TDWS. Pada peringkat segmentasi, bentuk sel telah diekstrak, dan kontur telah ditonjolkan. Peringkat ini diikuti dengan pengekstrakan ciri klinikal dan menggunakan ciri tersebut untuk diagnosis. Di peringkat ini, beberapa ciri klinikal yang berkaitan seperti Min Perimeter Sel Pleomorphism (MCP), Min Ketumpatan Sel (MCD), Min Luas Sel (MCA), dan Polymegathism telah diekstrak. Peranan ciri klinikal ini sangat penting untuk pengesanan awal patologi kornea dan penilaian kesihatan lapisan endotelium kornea. Setiap proses telah ditanda-aras mengikut segmentasi terbaik dan terkini serta teknik pengesanan ciri klinikal yang terdapat dalam literatur. Kaedah penambahbaikan dan segmentasi imej yang sedia ada telah dipertingkatkan dengan ketara melalui idea asal. Sumbangan utama kajian ini mengenai pengekstrakan ciri perubatan berdasarkan segmentasi telah disenaraikan dan diberi peringkat dari atas ke bawah mengikut tahap kepentingan. Ketepatan model segmentasi adaptif untuk klasifikasi imej adalah 97.5%. Dapat diperhatikan bahawa nilai yang diperoleh menggunakan teknik manual dan automatik tidak menunjukkan perbezaan yang signifikan secara statistik untuk mana-mana lima ciri klinikal. Perbezaan proses manual dan automatik masing-masing berada di bawah 2%, 2%, 1%, 1.5%, dan 3.5% untuk MCD, MCA, Polymegathism, MCP, dan Pleomorphism.

TABLE OF CONTENTS

	TITLE	PAGE
	DECLARATION	**iii**
	DEDICATION	**iv**
	ACKNOWLEDGEMENT	**v**
	ABSTRACT	**vi**
	ABSTRAK	**vii**
	TABLE OF CONTENTS	**viii**
	LIST OF TABLES	**xii**
	LIST OF FIGURES	**xiii**
	LIST OF ABBREVIATIONS	**xviii**
	LIST OF SYMBOLS	**xix**
	LIST OF APPENDICES	**xx**
CHAPTER 1	**INTRODUCTION**	**1**
1.1	Overview	1
1.2	Research background	4
1.3	Problem statement	8
1.4	Research Goal	11
1.5	Research Questions	11
1.6	Research Objectives	12
1.7	Research Scope	12
1.8	Research Significances	13
1.9	Research Outline	14
CHAPTER 2	**LITRETURE REVIEW**	**15**
2.1	Introduction	15
2.2	Basic Optic Anatomy and Physiology	16
2.3	Corneal Imaging Techniques	16
	2.3.1 In Vivo Confocal Microscopy (IVCM)	17

	2.3.2	Ocular Coherence Tomography (OCT)	17
2.4	Pre-processing of images		18
	2.4.1	Mathematical morphology	18
	2.4.2	Techniques of noise reduction and contrast enhancement	19
2.5	Segmentation		32
	2.5.1	Segmentation method for cell segmentation and medical feature extraction	33
	2.5.2	Segmentation research trends and issue	58
	2.5.3	Discussion on segmentation method	62
	2.5.4	Medical feature measurement	63
2.6	Summary		65
CHAPTER 3	**RESEARCH METHODOLOGY**		**67**
3.1	Introduction		67
3.2	Research Framework		68
	3.2.1	Phase A: Problem Formulation	70
	3.2.2	Phase B: Design	71
	3.2.3	Phase C: Development	72
	3.2.4	Phase D: Implementation	73
	3.2.5	Phase E: Evaluation	76
3.3	Qualitative Evaluation		77
	3.3.1	Quantitative Evaluation Approach	77
	3.3.2	The Bland and Altman Method for Closeness	78
	3.3.3	Linear Regression for Correlation	79
3.4	Dataset		80
	3.4.1	Dataset_1 (alizarine)	80
	3.4.2	Dataset_2	83
3.5	Summary		85
CHAPTER 4	**IMAGE ENHANCEMENT AND REGION EXTRACTION**		**87**
4.1	Introduction		87

4.2	Contrast-Limited Adaptive Histogram Equalization (CLAHE) Technique	88
4.3	New Denoising Scheme based on Enhanced Wavelet Transform Filter and Butterworth Bandpass for Segmentation (WTBBS)	95
	4.3.1 Moving average filter	106
4.4	Summary	109
CHAPTER 5	**SEGMENTATION OF ENDOTHELIAL CELLS**	**111**
5.1	Introduction	111
5.2	Proposed Framework (AHTMSEC)	111
	5.2.1 ANN for Adaptive segmenting (ANNAS)	113
	5.2.2 Texture features (7 Moments Features)	116
	5.2.3 ANN Classifier	119
5.3	Trainable Segmentation and Distance Transform (TDWS) to enhance the Watershed Transform for cell segmentation	121
	5.3.1 Initial Segmentation	122
	5.3.2 Distance Transform and H-minimum marker for the Watershed transform	124
	5.3.3 Enhanced U-NET Approach for Endothelial Cell Segmentation (EU-NETAECS)	133
	5.3.4 Enhancement of U-Net Compared with Original	134
	5.3.5 Training model	137
	5.3.6 Medical Feature Extraction Step (Clinical Feature Extraction Stage)	141
5.4	Summary	143
CHAPTER 6	**EXPERIMENTAL RESULTS**	**145**
6.1	Introduction	145
6.2	Segmentation Assessment	148
6.3	Performance parameters obtained using the binary classification test.	149
6.4	Automated vs Manual Measurements	151
6.5	Benchmarking	164
6.6	Limitations for the future work	169

6.7	Summary	170
CHAPTER 7	**CONCLUSIONS AND FUTURE WORK**	**173**
7.1	Contributions	176
7.2	Future Direction and Recommendation	178
REFERENCES		**181**
APPENDICES		**191**
LIST OF PUBLICATIONS		**193**

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Most effective image enhancement techniques	28
Table 2.2	Segmentation methods and techniques for corneal endothelial cells	59
Table 3.1	Briefing of operational research design for Phase A	70
Table 3.2	Summary of operational research design for Phase B	71
Table 3.3	Summary of operational research design for Phase C	73
Table 3.4	Summary of operational research design for Phase D	75
Table 3.5	Performance Measures	76
Table 6.1	Adaptive Model Evaluation for two rounds.	149
Table 6.2	R^2 values	155
Table 6.3	3 -6R^2 *values for the linear regressions of the clinical features*	158
Table 6.4	Mean difference, confidence limits, and the parameters for the linear fit	161
Table 6.5	Mean difference, confidence limits, and the parameters for the linear fit based on the CNN model	164
Table 6.6	Performance measurement using manual processes, the CEAS system by Al-Fahdawi et al. (2018) and the proposed model for five clinical features	166
Table 6.7	Performance comparison between manual measurements, the work of (Ruggeri et al., 2010). and the proposed model pertaining to clinical features	167
Table 6.8	Performance comparison between the manual measurements, the work of Scarpa and Ruggeri (2015) and the proposed model pertaining to the clinical features	168
Table 6.9	Performance comparison between the manual measurements, the work of Poletti and Ruggeri (2014) and the proposed model pertaining to five clinical features.	169

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 1.1	Shadow occurrence at the edges of image	9
Figure 1.2	Over segmentation problem	10
Figure 1.3	Under segmentation problem	10
Figure 2.1	Anatomical structure of human eye (Acharya U et al., 2008).	16
Figure 2.2	Frequency partition map for 16-band DFB	19
Figure 2.3	Creation of directional image: a) Boundaries having direction in the range 88.5-90 degrees b) Boundaries having direction in the range 123.5-135 degrees	21
Figure 2.4	Median filter mask – a) for median filter, b), and c for convolution filter (For noise elimination, followed by the use of a 5x5 mask-based convolution filter to obtain optimal results)	21
Figure 2.5	The original image has a jagged profile with immense noise (Fig a). The proposed convolution filter-based mask is used to get a relatively smooth result	22
Figure 2.6	Using filters: a) original (normalized), normalized view after: b) median, c) mask filter, d) median and mask filter	22
Figure 2.7	Examples of original corneal endothelial cell images from: (a) Control subject, (b) Morbidly obese patient, and (c) Diabetic patient showing a lower	24
Figure 2.8	Morphological operator for noise and illumination	26
Figure 2.9	Algorithm1 for detecting human corneal endothelium cells (Vincent and Masters, 1992).	34
Figure 2.10	Cell detection algorithm by Angulo and Matou (2005).	37
Figure 2.11	Demonstrates the detail pertaining to the segmentation Skeletons, watersheds and distance maps for endothelial cell segmentation b by Gavet and Pinoli (2008)	38
Figure 2.12	Watershed based model with fuzzy marker by Tiñena et al. (2009).	39

Figure 2.13	Segmentation with simple algorithm – a) original b) binarized c) segmented image	40
Figure 2.14	Transformation of image from Cartesian domain into quasi polar field	41
Figure 2.15	A block diagram S-PSO for analysing the endothelium cell	47
Figure 2.16	Demonstrates the general work-flow for corneal endothelium image segmentation developed by Fabijanska (2017)	52
Figure 2.17	Cell location for four methods	55
Figure 2.18	Cell count of endothelial images by four methods	55
Figure 3.1	The phases Block diagram of the research design	69
Figure 3.2	Operational diagram design for Phase D	74
Figure 3.3	Demonstration of the F-measure	78
Figure 3.4	Dataset_1 with 30 endothelial images captured by Sony camera (SSC-DC50AP)	82
Figure 3.5	Dataset_2 :sample of 50 endothelial images by (Al-Fahdawi et al., 2018) captured by HRT III RCM	84
Figure 3.6	Images Dataset Examples: A: Confocal Microscopy and B: Specular Microscopy	85
Figure 4.1	CWBM schema	87
Figure 4.2	Block creation by dividing the image into 36 blocks	89
Figure 4.3	Histogram representation for every block of the input image A. B represents the histogram for the border blocks, C represents the histogram for the inner blocks, D represents the histogram for the corner blocks while E shows the histogram for the output image	89
Figure 4.4	Example of histogram equalisation using the CLAHE technique	91
Figure 4.5	Iterative redistribution algorithm	92
Figure 4.6	The effect of CLAHE for two cases, B and D are the filtered images for the original images A and C.	93
Figure 4.7	The CLAHE effective A, B, C and D are the original images, as E, F, G and H are the output images.	94
Figure 4.8	The sub-bands LL, LH, HL and HH	96

Figure 4.9	Sub-bands for the vertical, diagonal, approximation, and horizontal information concerning the image	97
Figure 4.10	WTBBS Schema for Endothelial Cell Segmentation	99
Figure 4.11	WTBBS Algorithm	101
Figure 4.12	The effects of enhanced WT for two cases. A and C are the *CLAHE applying result, while B and D are the filtered images using WT.*	102
Figure 4.13	Shows the output of the enhanced WT: A, B, C, and D are *CLAHE outputs*, while E, F, G, and H are, respectively, the outputs of the enhanced WT filter	103
Figure 4.14	The result of the WTBBS filter for two cases A and C are CLAHE result, while B and D are the images filtered using the WTBBS shema.	104
Figure 4.15	Shows the effects on images of the WTBBS schema: A, B, C and D are *CLAHE outputs*, while E, F, G, and H are WTBBS (Enhanced WT filter and Butterworth Bandpass filter) outputs	105
Figure 4.16	The effects of the moving average and CLAHE filter for two cases. A and C are the WTBBS filter outputs, and the other B and D are the images filtered using the moving average and CLAHE filters	107
Figure 4.17	The output of the moving average and CLAHE filters for two cases. A, B, C, and D are the WTBBS filter outputs, while E, F, G, and H are the images filtered using the moving average and CLAHE filters	108
Figure 4.18	Moving average filter Algorithm	109
Figure 5.1	Block diagram of the AHTMSEC	112
Figure 5.2	Histogram A represents an image of a small cell, while histogram B represents an image of a big cell	114
Figure 5.3	A sample of a3 x3 block for static feature extraction	114
Figure 5.4	ANN for Adaptive Segmenting (ANNAS)	115
Figure 5.5	Adaptive model for segmenting ROI automatically	116
Figure 5.6	Typical neural network topology	119
Figure 5.7	Fundamental structure of an Artificial Neuron	120
Figure 5.8	Initial Level Trainable Cell Segmentation	123
Figure 5.9	A) Endothelial Cells with original greyscale pixels, (B) Binary image of overlapping objects, C) Distance	

	transform of the image, (D) Marker control for the cells, and (D) Watershed transform output.	126
Figure 5.10	Images depicting the output of the TWDS segmentation-based method.	128
Figure 5.11	Voronoi Diagram process output	129
Figure 5.12	Watershed based Model	129
Figure 5.13	Case 1 shows the output of the TWDS segmentation method	130
Figure 5.14	Case 2 shows the output of the TDWS segmentation-based method	131
Figure 5.15	Case 3 shows the output of the TDWS segmentation method	132
Figure 5.16	Enhanced U-NET Approach for Endothelial Cell Segmentation (EU-NETAECS)	136
Figure 5.17	Sample patches and the corresponding actual images	138
Figure 5.18	training architecture of 32 by 32 block size	139
Figure 5.19	Output of the EU-NETAECS for three cases	140
Figure 6.1	General framework for performance evaluation	146
Figure 6.2	The effects of filters that can help ROI segmentation of the Endothelial Cells	147
Figure 6.3	:3-6*Accuracy of the proposed Adaptive model*	149
Figure 6.4	Watershed transform applied on two images A-large cells, C-small cells, and the segmented images B and D.	150
Figure 6.5	Effects of the CNN Model	151
Figure 6.6	Correlation for the density feature for the images segmented using Watershed Transform-based model.	152
Figure 6.7	Correlation for the cell area feature for the segmented images	153
Figure 6.8	Correlation for the cell per feature for the segmented images	153
Figure 6.9	Correlation for the Polymegathism feature for the segmented images	154
Figure 6.10	Correlation for the Pleomorphism feature for the segmented images	154

Figure 6.11	Correlation for the density feature for the images segmented using CNN	155
Figure 6.12	Correlation for the cell area feature for the images segmented using CNN	156
Figure 6.13	Correlation for the CellPer feature for the images segmented using CNN	156
Figure 6.14	Correlation for the Polymegathism feature for the images segmented using CNN	157
Figure 6.15	Correlation for the Pleomorphism feature for the images segmented using CNN	157
Figure 6.16	Bland-Altman scatter plot for the density feature	158
Figure 6.17	Bland-Altman scatter plot for the cell area feature	159
Figure 6.18	Bland-Altman scatter plot for the CellPer feature	159
Figure 6.19	Bland-Altman scatter plot for the Polymegathism feature	160
Figure 6.20	Bland-Altman scatter plot for the Pleomorphism feature	160
Figure 6.21	Closeness for the density feature using CNN	161
Figure 6.22	Closeness for the cell area feature using CNN	162
Figure 6.23	Closeness for CellPer feature using CNN	162
Figure 6.24	Closeness for the Polymegathism feature using CNN	163
Figure 6.25	Closeness for the Pleomorphism feature using CNN	163
Figure 7.1	Output of the image enhancement scheme A) present original image While B) present the enhanced image with clear cell border and clean cell body	176

LIST OF ABBREVIATIONS

ANN	-	Artificial Neural Network
ASF	-	Alternated Sequential Filter
CDF	-	Cumulative Distribution Function
CLAHE	-	Contrast Limited Adaptive Histogram Equalisation
CLSM	-	Confocal Laser Scanning Microscope
CNN	-	Convolution neural network
DFB	-	Direction Filter Banks
DFT	-	Discrete Fourier Transform
EC	-	Endothelial Cells
FD	-	Fractal Dimension
FFT	-	Fast Fourier Transform
HSV	-	Hue Saturation Value
MCA	-	Mean Cell Area
MCD	-	Mean Cell Density
MCP	-	Mean Cell Perimeter
MSE	-	Mean Squared Error
NAV	-	Normalization of the average brightness of the vertical
PDF	-	Probability Density Function
ROI	-	Region of interest
SD	-	Standard deviation
WT	-	Wavelet Transformation

LIST OF SYMBOLS

σ	-	Scaling parameter
	-	Coefficient of determination
α	-	Clip factor
β	-	Clip limit
	-	Bayes threshold
	-	Noise variance
	-	Signal variance without noise.
∇D	-	Morphological sub-geodesic reconstruction

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
Appendix A	Expert Letter for Evaluation Opinion	191
Appendix B	Acceptance Letter	192

CHAPTER 1

INTRODUCTION

1.1 Overview

Segmentation of the medical image plays a significant role when it comes to diagnosis using computer aid system, The fact that endothelial cells lack propagation gives room for the replacement of space and activity of the dead cell by others that that are nearby. Subsequently, the wide variety of cells and their properties by age and pathologies at birth, the count of cells is 6500 cells/mm2, and decreases spontaneously during the lifetime, at 80 is 1700 – 2000 cells/mm2 (Ko et al., 2001). Specifically, Mohd Salih (2011) stated that people whose age lies between 25 to 32, the epithelium cell density is observed to be around 3000-3500 cells/mm2. However, the value is below 2000 cells/mm2 in the elderly population.

Various pathological environments fasten the extermination of the endothelial cells which in turn decreases the cell density in an abnormal manner. Dead cells worsen the hexagonal design. The mutilated endothelial cells can no longer revive back and that gives room for neighboring cells to migrate and expand so that they can fill in the space. The latter results to cell elongation that is unpredictable as well as increase in size and thinning. Cell density is therefore a major parameter when it comes to explaining the health condition attributed to corneal endothelium.

Similarly, Vigueras-Guillén et al. (2018a) indicates that today three parameters are applied when evaluating the health ranking of endothelium. The parameters are polymegethism which is also termed as cell variation, pleomorphism also known as hexagonally and endothelial cell density. Various approaches to separation of every cell found in corneal endothelium‟s image have been generated and they are all giving accurate results. Getting cell contours that are reliable needs manual delineation of the cell boundaries because there are a lot of endothelial cells in every square millimeter and segmenting them manually has proven to be an activity that consumes a lot of time.

Additionally, arrangement of cells in “corneal endothelium” is quite important for the ophthalmologists since it gives essential diagnostic information concerning the status of the cornea health and signs of any disease (Bourne, 2003).

The first method of assessing corneal endothelium dates back to 1920, when Voge first reported a method for examining endothelial mosaics by specular reflection using a slit lamp biomicroscope. In 1968, Maurice2 first reported observing the endothelium at 400x magnification using a specially designed corneal microscope, coining the term mirror microscope. Brown3 described a non-contact mirror microscope in 1970 (American Academy of Ophthalmology, 1997).

Later in 1975, Laing demonstrated a clinically useful microscope capable of imaging the endothelium at 200x magnification. Shortly after, Bourne and Kaufman (1976) reported the results of a photographic flash that allows for clearer photos. The introduction of clinically useful endothelial cell microscopy in 1975 dramatically increased clinical and basic scientific research on the corneal endothelium. Prior to this period, the inner layer of the corneal endothelium was known to be important in maintaining corneal clarity, but the poor regenerative capacity of humans was not well understood. Between 1975 and 1978, several clinical studies using a mirror microscope suggested that certain intraocular events, such as vitreous-endothelial contact, result in corneal endothelial depletion, resulting in trauma or endothelial contact.

Corneal endothelial cells are currently being clinically evaluated in vivo using imaging techniques such as specular microscopy (SM) and confocal microscopy (Yao et al., 2019). These microscopic techniques allow in vivo visualization of corneal endothelial cells to examine cell morphology and health. Unfortunately, the field of view (FOV) of these microscopy techniques is limited by both confocal synchronization and corneal curvature. In addition, MS is most commonly used in clinical practice to characterize epithelium and endothelium, but it is not possible to evaluate all layers of the cornea. IVCM, on the other hand, requires eye contact to achieve a high numerical aperture that helps break down a single endothelial cell. This can lead to patient discomfort, damage to the corneal surface, and an increased risk of corneal infections and abrasions. While Bizheva et al. (2017) showed that Optical coherence tomography (OCT) enables non-contact in vivo 3D imaging for anterior ocular imaging with spatial resolution down to the intracellular level which provides deep penetration of the signal into the core tissue, allowing all layers to be viewed at the same time. The axial resolution of OCT depends on the center wavelength and bandwidth of the light source, but the lateral resolution depends mainly on the objective of the imaging. Therefore, OCT does not require high NA unless very high lateral resolution is required. Various types of OCT systems have been demonstrated in transverse and anterior corneal endothelial cell imaging, including spectral domain OCT (SD) (Ang et al., 2016), full field OCT (FF) and the domain of Gabor (Mazlin et al., 2018). to augment. GD (Yao et al., 2019). The lateral resolution of all systems has been reported to be approximately 2 μm in tissue, resulting in a very limited field of view and depth of field (DOF). As a result, the system was very sensitive to rapid eye movements, Fast SDOCT (Tan et al., 2018) was able to show a successful representation of corneal endothelial cells in small FOVs during in vivo imaging. To date, OCT has failed to frontal visualize corneal endothelial cells in vivo to allow quantitative analysis . More importantly, the spatial resolution required for in vivo OCT imaging of anterior corneal endothelial cells is still unknown.

Despite of long history of endothelial imaging, but yet there lack debatable precise fully-computerized means that help in calculating the cell borders and successfully performing assessments and quantitative assessments of the characteristics. Notable inter and intra-observer disparities can still be seen (Hoppenreijs et al., 1996). Kitzmann et al. (2005) confirmed there are a number of tools which are available and can be used to assessing the density of the cell and endothelium‟s morphometry. Both non-contact specular and confocal microscopes give quality images from the peripheral and central cornea. Besides, Salvetat et al. (2011) states that the non-contact confocal microscopy is the current modality which in as much as it gives the same quality like the other microscopes, generates a huge field of view. That means, the medical feature for extraction by use of the image processing forms provide tremendous assistance when getting correct diagnosis of the cornea health. The latter increases the accuracy and saves on time. Analysis of the said parameters can also be brought out spontaneously by use of a diagnosis model that is computer aided. The model should also be fully automatic from the instance of capturing the image using a medical tool and the examination given for diagnosis by an optometrist.

Huang et al. (2018) depicted that physical representation of cells is a task that is quite labor-intensive. The provided software by microscope manufacturers for segmenting the cells has insubstantial performance. Such integrated software has indicated the erroneousness of the automated analyses when compared with expert commentary and that calls for a model that is fully automatic. The study focuses on creating such a model through development and enhancement of image processing techniques so that the current challenges which exist in measurement and segmentation of cornea endothelial cells can be dealt with.

1.2 Research background

Medical imaging encompasses the technologies that are employed in viewing the human body with the aim of monitoring, diagnosing or treating medical conditions. This is basically aimed at obtaining an inside image of the bodily structure in a manner that is non-intrusive as potential. Medical imaging has emerged as one of the commonly used methods of laboratory test that is going through changes in the past decade. There has been a rapid advancement in this area, thereby leading to the development of more accurate and less intrusive devices

Much of the current study attributed to division of the cell that attempts to come up with a model that is fully automatic and one that will cater for detection of cells and quality of the image. That is because of image"s intensity and many numbers of region of interests ROI. An example can be found in the early works of Nadachi and Nunokawa (1992) who used morphological thinning and scissoring to rectify the medical features. Lost boundaries are then edited physically whilst a (Vincent and Masters, 1992) work histogram was derived from calculating cell size and the number of neighbors for every cell. The derivation gave quantitative information pertaining the cornea heath. The histogram resulted from using a dome extractor in marking cell edges and applied marker-driven watershed segmentation to get binary images. Both were semi-automatic, which needs the manual editing to complete segmentation

In order to deal with the challenge in Angulo and Matou (2005) and Gavet and Pinoli (2008), a proposal for constraining the watershed segmentation through the distance map was made. A slightly contrasting method was suggested by Bullet et al. (2014), who came up with watersheds on the map and divided the fused cells by use of Voronoi diagrams. Nevertheless, as it can be seen in Gavet and Pinoli (2014), the methods are receptive to the setting of the parameter and therefore requires research before the prime results are derived.

Arguably, Selig et al. (2015) has come up with a proposal of using stochastic watershed so as to avoid the interaction between the user, change of parameters and the empirical setting. While Dagher and El Tom (2008) made use of the watershed contours in initializing many balloon snakes. A comparable method was suggested by Charłampowicz et al. (2014), where the various active contours for the snakes continues to evolve from circular sections derived through thresholding.

Foracchia and Ruggeri (2000) and Ruggeri et al. (2010) have taken advantage of shape modeling technology using the prior knowledge incorporated into the Bayesian analysis framework (Foracchia and Ruggeri, 2007). This approach is based on using neural networks to make classification of the cells in the cell body, marking every pixel as the cell vertex or the body by the use of vector machines (Poletti and Ruggeri, 2014), and growing number of vertices hence coming up with a normal hexagons into the boundaries of the cell by the use of genetic algorithm (Scarpa and Ruggeri, 2016b). The researcher seeks to develop an accurate, reliable, and fully automatic model capable of segmenting the endothelium cell. The researcher also tackles some significant issue that was a severe challenge to achieve their goal. They impose during their work to solve the problems such as the artifact that the microscopic may produce during the acquisition, which includes noise, bluer and uneven illumination, especially at the border of the image due to the nature of the cornea endothelium layer, besides, the mechanism of capturing and the reflection of light.

Most of the studies start to enhance images before the segmentation phase using a sophisticated pre-processing tier and scheme, which significantly influences segmentation accuracy. In some models, post-processing also was required. One of the dedicated pre-processing models was introduced by Khan et al. (2006) , which involves using the bandpass filter to the image‟s input. An illumination that is not uniform is see when the content having low frequency is dealt with through the lower region of the sub band.

Also Sharif et al. (2015) noted that the noise of high frequency is taken care of by the band pass's upper sub band section. During his analysis of state of the artworks done by others, the noise type of such image can be classified into two types photon noise and read noise which called Poisson and Gaussian such that the Photon noise result from the emission (and detection) of the light itself generated by microscopy This follows a Poisson distribution, while Read noise, arising from inaccuracies in quantifying numbers of detected photons. This follows a Gaussian distribution for which the standard deviation changes with the local image brightness. Thus almost every study concerning endothelial cell segmentation consists of first-processing treatment followed by binarization just before the segmentation phase. In some research, post-processing was needed to overcome some unwanted result from segmentation process such over-segmentation or under segmentation or disconnected marker the determined cell boundary which effect feature analysis and extraction as an example the study Vigueras-Guillen et al. (2019) which apply three post-processing method to improve the CNN model base segmentation outcome firstly.

Furthermore, it is done by applying Biomarker estimation from edge images, using Fourier analysis, and finally using characteristic S.D. To improve edges of the cell, image enhancement was performed by authors due to specific artifact existed in the image obtained by confocal or specular microscopic used in accession of medical and biomedical facts lead to segmentation issues that are more profound. Such include the divergent noises that are associated such as Poissonian, Rician, Speckle and Gaussian noise (Meziou et al., 2011). To evaluate and measure the amount of noise in such images and the study showed Gaussian noise is one of the common noises encountered, Poisson noise features confocal microscopy as a result of complex appearances of the cell, analogous of power and the information pertaining to the gradient was located from the photon variables that follows statistics from Poisson (Young, 1996). Subsequently, Sheppard et al. (2006) presented the sources of noise as end results of the size of the pinhole, form of detection, and the imaging data on the ratio of noise to signal.

Also, another artifact affects the quality of images; this artifact is the uneven illumination due to light focusing on the work of Habrat et al. (2016). The problem of distribution of brightness was treated by adjusting the brightness levels in rows and columns. More investigation presented in chapter 2 about the methods and techniques used to solve such artifact, which is combined with noise and dark edges of the images.

The region of interest (ROI) is often segmented manually by a properly trained expert. When manual segmentation is done, multiple subjective measurement decisions could be involved, and such decisions may cause an increase in the probability of intra- and inter-observer flaws. When such errors occur in terms of judging endothelial cells, the consequences can be severe in positions of missed chances (false negatives) and false anxieties (false positives). It has been noted by some medical practitioners that raising false alarm due to erroneous judgement is highly unacceptable.

Thus, it is crucial to develop automatic solutions because they facilitate speedy analysis, while minimizing the problems of intra- and inter-observer variation.

1.3 Problem statement

Generally, there are many research gabs associated with automatic medical image analysis, and most of these gabs are presentd because of the nature of the imaging modality. Such that there is no fully automatic model that is able to deal with different features that images contains which influence in the results in very challenging technical issues. Even though, there are different techniques that have been developed for the analysis of endothelial cells images as well as segmentation of ROI, there are so many limitations in terms of technical challenges associated with the extant solutions (at least until now). A summary of such challenges is given as follows:

1- The noise caused by image acquisition, and the removal of such noise using traditional filters may be a difficult task, as important information of image may be removed together with the noise.

2- Shadow is a continuous occurrence happening in the most medical images. Such shadows often happen in the cases of images. Which increases the difficulty of segmentation because of the unclear region with weak details, that may lay across the ROI (see figure1.1).

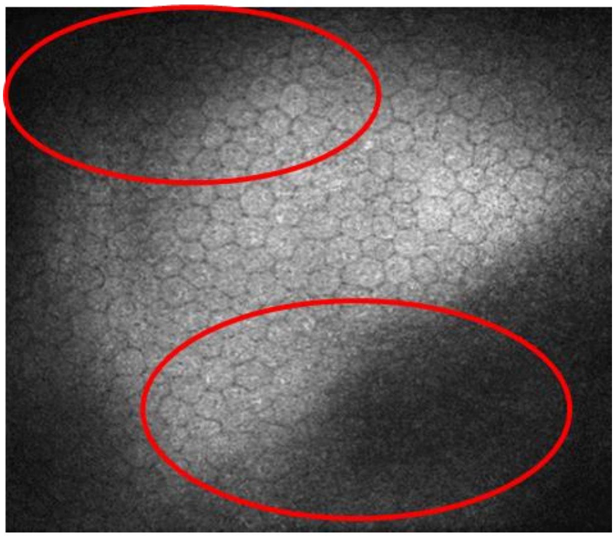

Figure 1.1 Shadow occurrence at the edges of image

Majority of the problems associated with medical image involve one or two ROI, but in this study a large number of cells are included in the dataset which is used. The cells are separated by poor border, thereby leading to great difficulty in the segmentation of the images.

3- Not only do humans possess different complex shapes of soft tissues in their eyes, but these tissues are also different because of displacements of the human eye during the acquisition of the image. Which cause an blurred images due minor movement of the eye during examination

4- The contrast of such images seems to be low, as they possess unclear boundaries with diverse objects existing. There is similarity between the values of pixel intensity within the boundary region, and this in turn increases the difficulty of identifying the specific border among the ROI (Over-segmentation problem), and for the training human expert. Over-segmentation accrue when the segmentation method extracts the ROI and this ROI include part of the background. In such cases, there will be failure of conventional boundary-identifying methods depending on gradient data.

A B

Figure 1.2 Over segmentation problem

5- Lack of homogeneity in the ROI implies that areas with different textures may be present in the boundaries of the ROI. In an event that there is inhomogeneity within the ROI, the expert may be confused about the actual ROI and other areas outside the ROI, which may in turn lead to under-segmentation. Under-segmentation involves the erroneous exclusion of parts of the ROI from the final segmentation results. As shows the figure 1.3. the same cell has different texture. When apply segmentation technique the whole cell can"t be segmented correctly

(A) (B)

Figure 1.3 Under segmentation problem

1.4 Research Goal

The main goal of the study is to propose and develop a totally automatic, robust and real-time model for the segmentation of endothelial cells of the human cornea obtained by in vivo microscopy and computation of the different clinical features of endothelial cells to achieve the goal first the visual quality of the images should be improved by reducing their unwanted degradations and enhancing their poor contrast. Such Improvements in quality will enhance the overall image which will contribute to accomplish the main goal of this study which is segmentation to obtain accurate medical information this will be achieved using image enhancement methods A pre-processing scheme of methods has been proposed in this research to obtain a decent image quality to highlight cell border furthermore two segmentation method was proposed to achieve accurate and precise cell segmentation, all those method will serve the purpose of clinical feature extraction which will be used by expert for better diagnosis of the medical condition of endothelium layer. The main differences of this study when compared to recent research the study direction toward finding the overall solution for different type of images modalities such that it extract the medical information regardless what type of image was obtain for endothelial cells by constructing a method that able to treats images with small or large cells

1.5 Research Questions

The main research questions of this study are determined as the following:

1- How to enhance uneven illumination that occur in the endothelial cell images?

2- How to reduce and remove the noise that exists without affecting vital information such as cell border?

3- How to accurately segment the border of multiple ROI without overlapping? with each other or with the cell itself such as over and under-segmentation?

4- How to measure the cell shape and size in the whole or part of the images to extract clinical features?

1.6 Research Objectives

To achieve research goals a logical objective was determined as follow:

1- Apply a new pre-processing scheme which involves the reduction of noise and enhancement of input image quality as well as highlight the border of the ROI.
2- Build an adaptive segmentation model to distinguish between images with certain quality in the datasets used in this research , due to the microscopic used , two kind of image exist such as small cell images and large cell images .
3- Develop a new segmentation method that involves the precise detection of endothelial contours to extract the shape of the cells which present the contours

1.7 Research Scope

In this study, In order to achieve the objectives of this research, it is essential to highlight the study scope such that the study focusses on endothelial cell images of human cornea which were obtained by either non-contact confocal or specular microscopy such images need to be enhanced by reducing the noise and uneven illumination enhancement as a pre-processing stage other artefact in not considered due to the impact of such noise and contrast on the segmentation stage ,the image that study will focus on consist of Two dataset which will be used in these research consist of 80 images with ground truth which was extracted by expert the main method depend on Segmentation will be used for cell shape and size detection no classification of certain disease will be made only medical feature extraction will be measured such as as Pleomorphic Mean Cell Perimeter (MCP), Mean Cell Density

(MCD), Mean Cell Area (MCA), and Polymegathism to achieve accurate segmentation two main process was used which consist of trainable Watershed and enhanced CNN base algorithm in addition ,the measurement and evaluation will be compared with the ground truth of manual segmentation for the specific datasets finally the Benchmarking of the proposed model will be made comparing with four authors working on the same datasets that this study rely on. all methods and techniques been chosen depending on the research gabs recommended by other researcher through investigation of recent works done such that pre-processing scheme was proposed due artefacts that exists in images and the effect of such scheme on the resulting images also the both segmentation methods were chosen according to previous studies that proved the effectiveness of both methods when dealing with such type of images, also both method were not saturated and need major improvement to achieve precise segmentation

1.8 Research Significances

The methods proposed in this research will provide an enhanced visual quality for endothelial cell images by using appropriate contrast enhancement, also new demonising method will deals with the sensitivity of such images in addition the denoising method will affect the images so additional enhancement will be needed. These topics are highly important, not only in the imaging area, but also in the medical field, as mentioned by the previous published articles, books and conferences over the last few years. Moreover, these enhancement will play a remarkable role in the segmentations stage

The needs of a full automatic segmentations methods in the field of cornea health which can process confocal and specular images effectively and efficiently while preserving their important details is fundamental to provide visually improved images helping specialists to provide an accurate diagnosis of diseases. Therefore, the proposed model tackle the up to date issue in those kind of image modalities and the need of reliable and accurate measurement is the major demands by experts due to intensive and time consuming manual analysis of such medical cases this study will contribute, By finding the proper solution which help the researcher and expert to step up in these field

1.9 Research Outline

The findings of this study are presented in this research thesis which is organized into seven chapters as outlined in the following:

i. Chapter 1 presents an introduction to the proposed research, formulates the research problem and discusses the aims, motivation and scope of this study.
ii. Chapter 2 provides a detailed review about the significant contributions in both pre-processing and segmentation field, and different methods are synthesized. and analysed of previous studies which carried out to enhance and segment the cell boundary
iii. Chapter 3 outlines the proposed research methodology, describes the quality measurement metrics employed, details the benchmarking process and explains the different image datasets used in this research.
iv. Chapter 4 presents the proposed methods in detail by mentioning their concepts, mathematical equations to provide a full understanding about how these methods function.in term of image enhancement to highlight cell border
v. Chapter 5 the proposed segmentation scheme will be detailed step by step , such as all related algorithm will be discussed in logical and sequential order to present the prcess of endothelial cell segmentation and present the way each algorithm works
vi. Chapter 6 discusses the results realized by application of the proposed methods.
vii. Chapter7 Lastly, Chapter seven concludes the study by listing the major achievements and provides recommendations for future studies.

REFERENCES

Abdul Khalid, N. E., Ibrahim, S., and Manaf, M. (2011). Brain abnormalities segmentation performances contrasting: Adaptive network-based fuzzy inference system (ANFIS) vs K-nearest neighbors (k-NN) vs fuzzy c-means (FCM). In N. Mastorakis, and V. Mladenov (Eds.), *Proceedings of the 15th WSEAS International Conference on Computers* (pp. 285-290). World Scientific and Engineering Academy and Society.

Acharya U, R., Ng, E. Y. K., and Suri, J. S. (Eds.). (2008). *Image modeling of the human eye*. Artech House.

Al-Fahdawi, S., Qahwaji, R., Al-Waisy, A. S., Ipson, S., Ferdousi, M., Malik, R. A., and Brahma, A. (2018). A fully automated cell segmentation and morphometric parameter system for quantifying corneal endothelial cell morphology. *Computer Methods and Programs in Biomedicine*, *160,* 11-23. https://doi.org/10.1016/j.cmpb.2018.03.015

American Academy of Ophthalmology (1997). Corneal endothelial photography: Three-year revision. *Ophthalmology*, *104*(8), 1360-1365. https://doi.org/10.1016/S0161-6420(97)30134-1

Ang, M., Konstantopoulos, A., Goh, G., Htoon, H. M., Seah, X., Lwin, N. C., Liu, X., Chen, S., Liu, L., and Mehta, J. S. (2016). Evaluation of a micro-optical coherence tomography for the corneal endothelium in an animal model. *Scientific Reports*, *6,* 29769. https://doi.org/10.1038/srep29769

Angulo, J., and Matou, S. (2005). Automatic quantification of in vitro endothelial cell networks using mathematical morphology. In J. J. Villanueva (Ed.), *Proceedings of the 5th IASTED International Conference on Visualization, Imaging, and Image Processing, VIIP 2005* (pp. 51-56). ACTA Press.

Ansari, R. (1987). Efficient IIR and FIR fan filters. *IEEE Transactions on Circuits and Systems*, *34*(8), 941-945. https://doi.org/10.1109/TCS.1987.1086224

Bizheva, K., Tan, B., MacLelan, B., Kralj, O., Hajialamdari, M., Hileeto, D., and Sorbara, L. (2017). Sub-micrometer axial resolution OCT for in-vivo imaging of the cellular structure of healthy and keratoconic human corneas. *Biomedical Optics Express*, *8*(2), 800-812. https://doi.org/10.1364/BOE.8.000800

Bland, J. M., and Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, *327*(8476), 307-310. https://doi.org/10.1016/S0140-6736(86)90837-8

Bourne, W. M. (2003). Biology of the corneal endothelium in health and disease. *Eye*, *17*, 912-918. https://doi.org/10.1038/sj.eye.6700559

Bourne, W. M., and Kaufman, H. E. (1976). Specular microscopy of human corneal endothelium in vivo. *American Journal of Ophthalmology*, *81*(3), 319-323. https://doi.org/10.1016/0002-9394(76)90247-6

Bullet, J., Gaujoux, T., Borderie, V., Bloch, I., and Laroche, L. (2014). A reproducible automated segmentation algorithm for corneal epithelium cell images from in vivo laser scanning confocal microscopy. *Acta Ophthalmologica*, *92*(4), e312-e317. https://doi.org/10.1111/aos.12304

Charłampowicz, K., Reska, D., and Boldak, C. (2014). Automatic segmentation of corneal endothelial cells using active contours. *Advances in Computer Science Research*, *11*, 47-60.

Chiu, S. J., Li, X. T., Nicholas, P., Toth, C. A., Izatt, J. A., and Farsiu, S. (2010). Automatic segmentation of seven retinal layers in SDOCT images congruent with expert manual segmentation. *Optics Express*, *18*(18), 19413-19428. https://doi.org/10.1364/oe.18.019413

Chiu, S. J., Toth, C. A., Rickman, C. B., Izatt, J. A., and Farsiu, S. (2012). Automatic segmentation of closed-contour features in ophthalmic images using graph theory and dynamic programming. *Biomedical Optics Express*, *3*(5), 1127-1140. https://doi.org/10.1364/boe.3.001127

Corbin, J. M., and Strauss, A. (1990). Grounded theory research: Procedures, canons, and evaluative criteria. *Qualitative Sociology*, *13*, 3-21. https://doi.org/10.1007/BF00988593

Costa, A. F., Humpire-Mamani, G., and Traina, A. J. M. (2012). An efficient algorithm for fractal analysis of textures. *2012 25th SIBGRAPI Conference on Graphics, Patterns and Images* (pp. 39-46). Conference Publishing Services. https://doi.org/10.1109/SIBGRAPI.2012.15

Couprie, M., and Talbot, H. (2013). Distance, granulometry, skeleton. In L. Najman, and H. Talbot (Eds.), *Mathematical morphology: From theory to applications* (pp. 263-289). Wiley. https://doi.org/10.1002/9781118600788.ch10

Dagher, I., and El Tom, K. (2008). WaterBalloons: A hybrid watershed Balloon Snake segmentation. *Image and Vision Computing*, *26*(7), 905-912. https://doi.org/10.1016/j.imavis.2007.10.010

De Carlo, T. E., Romano, A., Waheed, N. K., and Duker, J. S. (2015). A review of optical coherence tomography angiography (OCTA). *International Journal of Retina and Vitreous*, *1*, 5. https://doi.org/10.1186/s40942-015-0005-8

Fabijanska, A. (2017). Corneal endothelium image segmentation using feedforward neural network. In M. Ganzha, L. Maciaszek, and M. Paprzycki (Eds.), *Proceedings of the 2017 Federated Conference on Computer Science and Information Systems* (Vol. 11, pp. 629-637). Polish Information Processing Society. https://doi.org/10.15439/2017F54

Fabijańska, A. (2018). Segmentation of corneal endothelium images using a U-Net-based convolutional neural network. *Artificial Intelligence in Medicine*, *88*, 1-13. https://doi.org/10.1016/j.artmed.2018.04.004

Fabijańska, A. (2019). Automatic segmentation of corneal endothelial cells from microscopy images. *Biomedical Signal Processing and Control*, *47*, 145-158. https://doi.org/10.1016/j.bspc.2018.08.018

Foracchia, M., and Ruggeri, A. (2000). Cell contour detection in corneal endothelium in-vivo microscopy. *Proceedings of the 22nd Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, *2*, 1033-1035. https://doi.org/10.1109/iembs.2000.897902

Foracchia, M., and Ruggeri, A. (2003). Corneal endothelium analysis by means of Bayesian shape modeling. *Proceedings of the 25th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, *1*, 794-797. https://doi.org/10.1109/iembs.2003.1279884

Foracchia, M., and Ruggeri, A. (2007). Corneal endothelium cell field analysis by means of interacting Bayesian shape models. *29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 6036-6039. https://doi.org/10.1109/IEMBS.2007.4353724

Gavet, Y., and Pinoli, J.-C. (2008). Visual perception based automatic recognition of cell mosaics in human corneal endothelium microscopy images. *Image Analysis and Stereology*, *27*(1), 53-61. https://doi.org/10.5566/ias.v27.p53-61

Gavet, Y., and Pinoli, J.-C. (2014). Comparison and supervised learning of segmentation methods dedicated to specular microscope images of corneal endothelium. *International Journal of Biomedical Imaging*, *2014*, 704791.

https://doi.org/10.1155/2014/704791

Grimes, W. S. N. (2016). *Image processing and analysis methods in quantitative endothelial cell biology* [Doctoral dissertation, University College London]. http://discovery.ucl.ac.uk/1529859/

Habrat, K., Habrat, M., Gronkowska-Serafin, J., and Piórkowski, A. (2016). Cell detection in corneal endothelial images using directional filters. In R. S. Choraś (Ed.), *Advances in Intelligent Systems and Computing Vol. 389* (pp. 113-123). Springer. https://doi.org/10.1007/978-3-319-23814-2_14

Hertz, J., Krogh, A., and Palmer, R. G. (1991). *Introduction to the theory of neural computation*. CRC Press. https://doi.org/10.1201/9780429499661

Hiroyasu, T. *et al.* (2013) 'Extracting rules for cell segmentation in corneal endothelial cell images using GP', *Proceedings - 2013 IEEE International Conference on Systems, Man, and Cybernetics, SMC 2013*. IEEE, pp. 1811–1816. doi: 10.1109/SMC.2013.305.

Honda, H. (1983). Geometrical models for cells in tissues. *International Review of Cytology*, *81*, 191-248. https://doi.org/10.1016/S0074-7696(08)62339-6

Hoppenreijs, V. P. T., Pels, E., Vrensen, G. F. J. M., and Treffers, W. F. (1996). Corneal endothelium and growth factors. *Survey of Ophthalmology*, *41*(2), 155-164. https://doi.org/10.1016/S0039-6257(96)80005-1

Huang, G., Liu, Z., Van Der Maaten, L., and Weinberger, K. Q. (2017). Densely connected convolutional networks. *2017 IEEE Conference on Computer Vision and Pattern Recognition*, 2261-2269. https://doi.org/10.1109/CVPR.2017.243

Huang, J., Maram, J., Tepelus, T. C., Sadda, S. R., Chopra, V., and Lee, O. L. (2018). Comparison of noncontact specular and confocal microscopy for evaluation of corneal endothelium. *Eye and Contact Lens: Science and Clinical Practice*, *44*, S144-S150. https://doi.org/10.1097/ICL.0000000000000362

John, T. (Ed.). (2010). *Corneal endothelial transplant DSEAK, DMEK and DLEK*. Jaypee Brothers Medical Publishers.

Katafuchi, S., and Yoshimura, M. (2017). Convolution neural network for contour extraction of corneal endothelial cells. *Thirteenth International Conference on Quality Control by Artificial Vision*, *103380*, 103380L. https://doi.org/10.1117/12.2264430

Khan, M. A., Khan, M. K., Khan, M. A. U., and Lee, S. (2006). Endothelial cell image enhancement using decimation-free directional filter banks. *2006 IEEE Asia*

Pacific Conference on Circuits and Systems, 884-887. https://doi.org/10.1109/APCCAS.2006.342183

Kiran Kumar, K. V., and Srinivasa, G. (2018). Comeal Endothelium cell segmentation and count using K-means and watershed algorithms. *2018 Second International Conference on Advances in Electronics, Computers and Communications, 1-7.* https://doi.org/10.1109/ICAECC.2018.8479526

Kitzmann, A. S., Winter, E. J., Nau, C. B., McLaren, J. W., Hodge, D. O., and Bourne, W. M. (2005). Comparison of corneal endothelial cell images from a noncontact specular microscope and a scanning confocal microscope. *Cornea*, *24*(8), 980-984. https://doi.org/10.1097/01.ico.0000159737.68048.97

Ko, M.-K., Park, W. K., Lee, J. H., and Chi, J. G. (2001). A histomorphometric study of corneal endothelial cells in normal human fetuses. *Experimental Eye Research*, *72*(4), 403-409. https://doi.org/10.1006/exer.2000.0964

Leibowitz, H. M., and Waring, G. O. (1998). *Corneal disorders: Clinical diagnosis and management*. Saunders.

Marquardt, D. W. (1963). An algorithm for least-squares estimation of nonlinear parameters. *Journal of the Society for Industrial and Applied Mathematics*, *11*(2), 431-441. https://www.jstor.org/stable/2098941

Masters, B. R., and Farmer, M. A. (1993). Three-dimensional confocal microscopy and visualization of the in situ cornea. *Computerized Medical Imaging and Graphics*, *17*(3), 211-219. https://doi.org/10.1016/0895-6111(93)90045-O

Mazlin, V., Xiao, P., Dalimier, E., Grieve, K., Irsch, K., Sahel, J.-A., Fink, M., and Boccara, A. C. (2018). In vivo high resolution human corneal imaging using full-field optical coherence tomography. *Biomedical Optics Express*, *9*(2), 557-568. https://doi.org/10.1364/BOE.9.000557

McCarey, B. E., Edelhauser, H. F., and Lynn, M. J. (2008). Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices and new intraocular drugs and solutions. *Cornea*, *27*(1), 1-16. https://doi.org/10.1097/ICO.0b013e31815892da

Meyer, F. (2012). *The watershed concept and its use in segmentation : A brief history*. 1-11. https://arxiv.org/pdf/1202.0216.pdf

Meziou, L., Histace, A., Precioso, F., Matuszewski, B. J., and Murphy, M. F. (2011). Confocal microscopy segmentation using active contour based on alpha (α)-divergence. *2011 18th IEEE International Conference on Image Processing*, 3077-3080. https://doi.org/10.1109/ICIP.2011.6116315

Mohd Salih, P. A. K. (2011). Corneal endothelial cell density and morphology in normal Malay eyes. *The Medical Journal of Malaysia*, *66*(4), 300-303. http://www.e-mjm.org/2011/v66n4/Corneal_Endothelium.pdf

Montseny, E., and Alvarez, S. (2005). Basic macrotextures structure detection in corneal images using fuzzy techniques. *2005 Annual Meeting of the North American Fuzzy Information Processing Society*, 162-167. https://doi.org/10.1109/NAFIPS.2005.1548526

Nadachi, R., and Nunokawa, K. (1992). Automated corneal endothelial cell analysis. *Proceedings Fifth Annual IEEE Symposium on Computer-based Medical Systems*, 450-457. https://doi.org/10.1109/CBMS.1992.245000

Navab, N., Hornegger, J., Wells, W. M., and Frangi, A. F. (Eds.). (2015). *Medical image computing and computer-assisted intervention - MICCAI 2015: 18th international conference proceedings, part III*. Springer. https://doi.org/10.1007/978-3-319-24574-4

Park, S. -I., Smith, M. J. T., and Mersereau, R. M. (1999). A new directional filter bank for image analysis and classification. *1999 IEEE International Conference on Acoustics, Speech, and Signal Processing, 3*, 1417-1420. https://doi.org/10.1109/ICASSP.1999.756247

Patel, D. V, and McGhee, C. N. (2013). Quantitative analysis of in vivo confocal microscopy images: A review. *Survey of Ophthalmology*, *58*(5), 466-475. https://doi.org/10.1016/j.survophthal.2012.12.003

Patton, M. Q. (2002). *Qualitative research and evaluation methods.* Sage Publications, Inc.

Piórkowski, A. and Gronkowska-Serafin, J. (2011). Selected issues of corneal endothelial image segmentation. *Journal of Medical Informatics and Technologies*, *17*, 239-246.

Piorkowski, A., Nurzynska, K., Boldak, C., Reska, D., and Gronkowska-Serafin, J. (2015). Selected aspects of corneal endothelial segmentation quality. *Journal of Medical Informatics and Technologies*, *24*, 155-164.

Piórkowski, A., Nurzynska, K., Gronkowska-Serafin, J., Selig, B., Boldak, C., and Reska, D. (2017). Influence of applied corneal endothelium image segmentation techniques on the clinical parameters. *Computerized Medical Imaging and Graphics*, *55*, 13-27. https://doi.org/10.1016/j.compmedimag.2016.07.010

Poletti, E., and Ruggeri, A. (2014). Segmentation of corneal endothelial cells contour through classification of individual component signatures. In L. M. Roa Romero (Ed.), *XIII Mediterranean Conference on Medical and Biological Engineering and Computing 2013,* (Vol. 41, pp. 411-414). Springer. https://doi.org/10.1007/978-3-319-00846-2_102

Reinhard, T., and Larkin, F. (Eds.). (2013). *Corneal disease: Recent development in diagnosis and therapy*. Springer. https://doi.org/10.1007/978-3-642-28747-3

Reska, D., Jurczuk, K., Boldak, C., and Kretowski, M. (2014). MESA: Complete approach for design and evaluation of segmentation methods using real and simulated tomographic images. *Biocybernetics and Biomedical Engineering*, *34*(3), 146-158. https://doi.org/10.1016/j.bbe.2014.02.003

Ruggeri, A., Scarpa, F., De Luca, M., Meltendorf, C., and Schroeter, J. (2010). A system for the automatic estimation of morphometric parameters of corneal endothelium in alizarine red-stained images. *British Journal of Ophthalmology*, *94*(5), 643-647. https://doi.org/10.1136/bjo.2009.166561

Ruggeri, A., Grisan, E. and Jaroszewski, J. (2005) ‘A new system for the automatic estimation of endothelial cell density in donor corneas’, *British Journal of Ophthalmology*, 89(3), pp. 306–311. doi: 10.1136/bjo.2004.051722

Salerno, M. *et al.* (1998) ‘A new CNN based tool for an automated morphometry analysis of the corneal endothelium’, in *1998 Fifth IEEE International Workshop on Cellular Neural Networks and their Applications. Proceedings (Cat. No. 98TH8359).* IEEE, pp. 169–174.

Salvetat, M. L., Zeppieri, M., Miani, F., Parisi, L., Felletti, M., and Brusini, P. (2011). Comparison between laser scanning in vivo confocal microscopy and noncontact specular microscopy in assessing corneal endothelial cell density and central corneal thickness. *Cornea*, *30*(7), 754-759. https://doi.org/10.1097/ICO.0b013e3182000c5d

Sanchez-Marin, F. J. (1999). Automatic segmentation of contours of corneal cells. *Computers in Biology and Medicine*, *29*(4), 243-258. https://doi.org/10.1016/S0010-4825(99)00010-4

Scarpa, F, and Ruggeri, A. (2016a). Automated morphometric description of human corneal endothelium from in-vivo specular and confocal microscopy. *The 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 1296-1299. https://doi.org/10.1109/EMBC.2016.7590944

Scarpa, F., and Ruggeri, A. (2015). Segmentation of corneal endothelial cells contour by means of a genetic algorithm. *Ophthalmic Medical Image Analysis International Workshop 2*, 25-32. https://doi.org/10.17077/omia.1023

Scarpa, F., and Ruggeri, A. (2016b). Development of a reliable automated algorithm for the morphometric analysis of human corneal endothelium. *Cornea*, *35*(9), 1222-1228. https://doi.org/10.1097/ICO.0000000000000908

Selig, B., Vermeer, K. A., Rieger, B., Hillenaar, T., and Luengo Hendriks, C. L. (2015). Fully automatic evaluation of the corneal endothelium from in vivo confocal microscopy. *BMC Medical Imaging*, *15*, 13. https://doi.org/10.1186/s12880-015-0054-3

Sharif, M. S., Qahwaji, R., Hayajneh, S., Ipson, S., Alzubaidi, R., and Brahma, A. (2014). An efficient system for preprocessing confocal corneal images for subsequent analysis. *2014 14th UK Workshop on Computational Intelligence*, 1-8. https://doi.org/10.1109/UKCI.2014.6930188

Sharif, M. S., Qahwaji, R., Shahamatnia, E., Alzubaidi, R., Ipson, S., and Brahma, A. (2015). An efficient intelligent analysis system for confocal corneal endothelium images. *Computer Methods and Programs in Biomedicine*, *122*(3), 421-436. https://doi.org/10.1016/j.cmpb.2015.09.003

Sheppard, C. J. R., Gan, X., Gu, M., and Roy, M. (2006). Signal-to-noise ratio in confocal microscopes. In J. B. Pawley (Ed.), *Handbook of biological confocal microscopy* (pp. 442-452). Springer. https://doi.org/10.1007/978-0-387-45524-2_22

Siddiqi, K., Lauzière, Y. B., Tannenbaum, A., and Zucker, S. W. (1998). Area and length minimizing flows for shape segmentation. *IEEE Transactions on Image Processing*, *7*(3), 433-443. https://doi.org/10.1109/83.661193

Soille, P. (2004). *Morphological image analysis: Principles and applications*. Springer. https://doi.org/10.1007/978-3-662-05088-0

Sonka, M., Hlavac, V., and Boyle, R. (2007). *Image processing analysis and machine vision* (3rd ed.). CL Engineering.

Takeda, H., Farsiu, S., and Milanfar, P. (2006). Robust kernel regression for restoration and reconstruction of images from sparse noisy data. *2006 International Conference on Image Processing*, 1257-1260. https://doi.org/10.1109/ICIP.2006.312573

Tan, B., Hosseinaee, Z., Han, L., Kralj, O., Sorbara, L., and Bizheva, K. (2018). 250

kHz, 1.5 µm resolution SD-OCT for in-vivo cellular imaging of the human cornea. *Biomedical Optics Express*, *9*(12), 6569-6583. https://doi.org/10.1364/BOE.9.006569

Tavakoli, M., and Malik, R. A. (2011). Corneal confocal microscopy: A novel non-invasive technique to quantify small fibre pathology in peripheral neuropathies. *Journal of Visualized Experiments*, *47*, e2194. https://doi.org/10.3791/2194

Tiñena, F., Sobrevilla, P., and Montseny, E. (2009). On quality assessment of corneal endothelium and its possibility to be used for surgical corneal transplantation. *2009 IEEE International Conference on Fuzzy Systems*, 1326-1331. https://doi.org/10.1109/FUZZY.2009.5277395

Vecchi, M., Braccio, L., and Orsoni, J. G. (1996). The Topcon SP 1000 and Image-NET systems: A comparison of four methods for evaluating corneal endothelial cell density. *Cornea*, *15*(3), 271-277. https://doi.org/10.1097/00003226-199605000-00008

Vigueras-Guillén, J. P., Andrinopoulou, E.-R., Engel, A., Lemij, H. G., van Rooij, J., Vermeer, K. A., and van Vliet, L. J. (2018a). Corneal endothelial cell segmentation by classifier-driven merging of oversegmented images. *IEEE Transactions on Medical Imaging*, *37*(10), 2278-2289. https://doi.org/10.1109/TMI.2018.2841910

Vigueras-Guillén, J. P., Engel, A., Lemij, H. G., van Rooij, J., Vermeer, K. A., and van Vliet, L. J. (2018b). Improved accuracy and robustness of a corneal endothelial cell segmentation method based on merging superpixels. In A. Campilho, F. Karray, and B. ter Haar Romeny (Eds.), *Image Analysis and Recognition* (pp. 631-638). Springer International Publishing. https://doi.org/10.1007/978-3-319-93000-8_72

Vigueras-Guillen, J. P., van Rooij, J., Lemij, H. G., Vermeer, K. A., and van Vliet, L. J. (2019). Convolutional neural network-based regression for biomarker estimation in corneal endothelium microscopy images. *The 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 876-881. https://doi.org/10.1109/EMBC.2019.8857201

Vincent, L. M., and Masters, B. R. (1992). Morphological image processing and network analysis of cornea endothelial cell images. *Image Algebra and Morphological Image Processing III*, *1769*, 212-226. https://doi.org/10.1117/12.60644

Yao, X., Devarajan, K., Werkmeister, R. M., dos Santos, V. A., Ang, M., Kuo, A., Wong, D. W. K., Chua, J., Tan, B., Barathi, V. A., and Schmetterer, L. (2019). In vivo corneal endothelium imaging using ultrahigh resolution OCT. *Biomedical Optics Express*, *10*(11), 5675-5686. https://doi.org/10.1364/boe.10.005675

Zuiderveld, K. (1994). Contrast limited adaptive histogram equalization. In P. S. Heckbert (Ed.), *Graphics gems* (pp. 474-485). Academic Press. https://doi.org/10.1016/B978-0-12-336156-1.50061-6

LIST OF PUBLICATIONS

Indexed journal

1. Sami, A. S., Shafry, M., Rahim, M., Ahmed, F. Y. H., & Sulong, G. B. I. N. (2019). A Review Study of Methods Utilized For Identifying And Segmenting The Brain Tumor From MR Imageries. *Journal of Theoretical and Applied Information Technology*, 97(11), 2969–2987. www.jatit.org **(Indexed by SCOPUS)**
2. Acceptance letter from the Journal of Intelligent Systems (JISYS) (2021) by Degruyter which entitled *"Trainable Watershed-based Model for Cornea Endothelial Cells Segmentation"* **(Indexed by web of science)**

Non-indexed Journal

1. Abrahim, A. R., Rahim, M. S. M., & Sami, A. S. (2020). Image Splicing Forgery Detection Scheme Using New Local Binary Pattern Variant. *Academic Journal of Nawroz University*, 9(3), 208–215.