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Corso di Laurea Magistrale in Bioingegneria

**Coupling the Bayesian approach and the
swarm technique for an effective
endothelial cell segmentation in specular
images of the cornea**

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A.A. 2013-2014

Data di laurea: 31-03-2014

Alla mia famiglia

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Introduction

The cornea is the most powerful lens of the visual system, whose proper functioning is ensured by good hydration provided by the endothelium of the cornea. The corneal endothelium is the fifth and deepest layer of the cornea. It is a single layer of flat cells, that are rich in mitochondria and with hexagonal shape. They do not possess the ability to reproduce, so the diseases affecting the cell layer permanently damage the endothelium. When some cells die, the neighboring cells modify their morphology expanding and migrating into the cell layer. For these reasons, the morphology of the layer of endothelial cells provides an indicator of the functionality of the tissue.

In particular, there are three parameters used in clinical practice to quantify the conditions of the endothelial layer: endothelial cell density (ECD), polymegethism and pleomorphism or exagonality coefficient. To determine values of these parameters is necessary to make an analysis of the images of the corneal cells. To detect the images of the corneal endothelial layer are used specular or confocal microscope that allow to acquire endothelial images in vivo quickly and without being an invasive examination.

At present, endothelium field analysis is based on a simple visual inspection that uses only a few digital tools to place marks on cells counted to assess cell density. These methods are time-consuming and often they don't allow to perform an actual geometry analysis. Then it is particularly important to study automatic or semi-automatic systems for cell detection and morphological analysis to monitor the health status of endothelial cells.

In the images of the cell field it is difficult to make a correct identification of endothelial cells due to the fact that the cells' boundaries are not clear. Several computer programs are semi-automated, because they allow user to correct by manual adjustment.

The purpose of this work is to provide a completely automated computer program that allows segmenting corneal endothelial cells. In this thesis we used 30 corneal endothelium images, acquired with a specular microscope (*SP-3000P*, *Topcon Co.*).

The main idea, in which is based this program, is to segmented the endothelial images creating a grid of circles. Each circle of this grid is capable to modify its coordinates and radius, in order to detect the cell boundaries.

This thesis adopts a swarm based approach defining a simple entity, that is circle, and a more complex structure, that is the grid. Furthermore, a Bayesian technique is used to define the radius of the circle basing on the mean areas of the endothelial cells. The single circle change its position and size considering only the characteristics of the image. The gray levels of the pixels determine the changes of the circle morphology. Instead, the grid consists of neat lines of circles and it is able to manage the interactions between different circles. Since the endothelium is formed by adjacent cells, the circle grid has to comply with the rule that every circle has no to overlap another. In addition, the environment that the circles move around in, is made up of the image as well as optional resulting output images.

The results of this project show that this approach can give good results if it will be continued properly. The further development of this project is to insert the Bayesian approach. We have to define the probabilities based on the ground truth about some parameters. These make up the Prior Model, which is constituted by some prior information that are used in the algorithm. In fact, the algorithm has to calculate the movement of the circles based on the information provided in the image, which is called Image Model. This information is then integrated with the Prior Model available to determine the final answer of the algorithm.

Because of low quality of the medical images, especially the endothelial images, the previous programs have the difficulty to find out all the cell sides in the endothelial cell field. Even if a cell side is not clear in the image, using this program certainly that side is detected, due to the fact that the interactions between circles and the environment presuppose the existence of a circle side in that position.

Chapter 1

The corneal endothelium

1.1 The cornea

The eye consists of three coats, enclosing three transparent structures. The outermost layer, known as the fibrous tunic, is composed of the cornea and sclera. The middle layer, known as the vascular tunic (or uvea), consists of the choroid, ciliary body, and iris. The innermost is the retina, which gets its circulation from the vessels of the choroid as well as the retinal vessels.

In areas that divide these three coats there are the aqueous humour, the vitreous body, and the flexible lens. The aqueous humour is a transparent fluid that is contained in two areas: the anterior chamber between the cornea and the iris, and the posterior chamber between the iris and the lens. The lens is attached to the ciliary body by the suspensory ligament (Zonule of Zinn), made up of fine transparent fibers. The vitreous body is a clear jelly that is much larger than the aqueous humour present behind the lens, and the rest is bordered by the sclera, zonule, and lens. They are connected via the pupil.

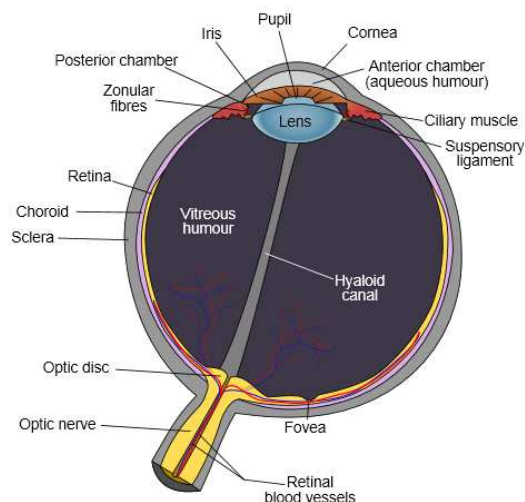


Figure 1.1: the eye structure.

The cornea is a transparent dome-shaped membrane located on the front part of the eye, that covers the iris, pupil, and anterior chamber. The radius of curvature of the cornea is smaller than the eye so it protrudes anteriorly. With the sclera, it forms the fibrous tunic, which are the first and the most external of the three tunics that cover the bulb of the eye. Sclera and cornea are connected together in a region called limbus.

The cornea doesn't have blood vessels; it receives nutrients via diffusion from the tear fluid through the outside surface and the aqueous humour through the inside surface, and also from neurotrophins supplied by nerve fibres that innervate it. In the humans beings, the cornea has a diameter of about 11.5 mm and a thickness of 0.5–0.6 mm in the center and 0.6–0.8 mm at the periphery. While the cornea contributes most of the eye's focusing power, its focus is fixed. The curvature of the lens, on the other hand, can be adjusted to "tune" the focus depending upon the object's distance. The cornea is a very special tissue because of its transparency, avascularity, the presence of immature resident immune cells, and immunologic privilege. A stimulation of the cornea causes an involuntary reflex to close the eyelid, which is called corneal reflex, and it protects the eye from foreign bodies.

The human cornea has five layers. They are listed below, from outside to inside:

Epithelium (1): the most superficial layer of the cornea is the corneal epithelium, about one-tenth of the total thickness of the membrane, that has the function to protect the lower layers from the flow of the tears and bacteria. It is a multilayered epithelium (5-6 layers), with flat cells in the first two layers, multi-faceted in the next two or three, elongated, almost cylindrical, in the last two. Cells, from optically perfect shape, are joined together by tight junctions and those more superficial present numerous microvilli only visible in the electron microscope.

Bowman's membrane (2): it's a thin layer composed of collagen fibrils and its function is to give and maintain the shape of the cornea.

Stroma (3): it consists of 200 layers of flattened plates of type I collagen fibrils called lamellae. In each layer the direction of the collagen fibers is different, to provide maximum mechanical strength.

Descemet's membrane (4): a basement membrane of type IV collagen secreted by the endothelium, located below. It's thin in infancy and increases in thickness in adulthood, with a variable thickness of 4-12 μm .

Endothelium (5): the last layer, facing the anterior chamber. It is a single layer of flat cells and with a hexagonal shape that have nuclei elongated horizontally. Its cells are close to each other through interdigitations that branch off from the lateral portions of their plasma membranes, supported by tight junctions and gap junctions. Their cytoplasm is basophilic due to the development of the rough endoplasmic reticulum, also possess numerous mitochondria, cells are high metabolic activity. Its task is essentially to act as a filter back to the upper layers of the cornea, it is also the main responsible for its hydration.

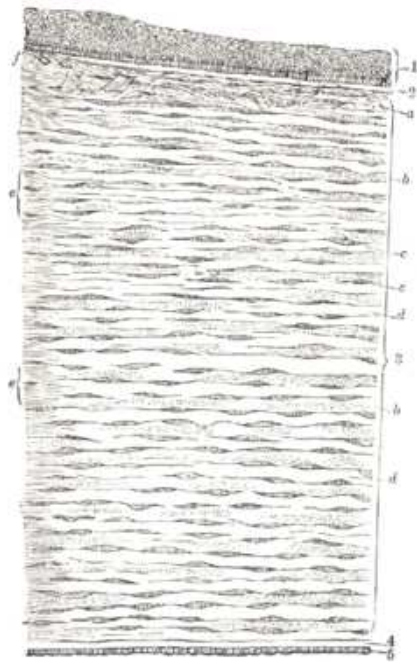


Figure 1.2: the corneal layers.

1.2 The corneal endothelium

The cornea is the transparent front part of the eye and its transparency depends on its hydration level. The hydration remains constant by active water transport, which is performed by the corneal endothelium. The water transport allows leakage of solutes and nutrients from the aqueous humor to the more superficial layers of the cornea while, at the same time, actively pumping water in the opposite direction, from the stroma to the aqueous.

The normal corneal endothelium is a single layer of regularly sized cells with a predominantly hexagonal shape and they are rich of mitochondria. The corneal endothelial cells do not regenerate, unlike the corneal epithelium.

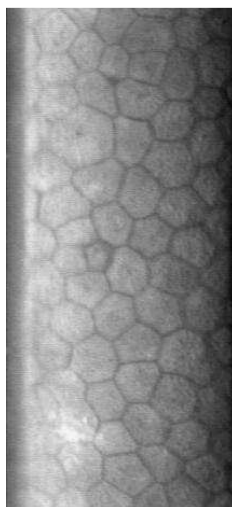


Figure 1.3: hexagonal cells of corneal endothelium visualized by specular microscopy.

They stretch in order to compensate the death of cells that reduces the overall cell density of the endothelium, influencing the fluid regulation. Endothelial cell loss, if sufficiently severe, can cause endothelial cell density to fall below the threshold level that is typically in the range of 500 - 1000 cells/mm². Loss of endothelial cell density is accompanied by increases in cell size variability (polymegathism) and cell shape variation (polymorphism). Deformation and migration of the cells cause a less regular morphology. It is important to distinguish changes in the endothelial field caused by pathologies to changes that are due to aging.

The post-natal amount of total cells that composes the endothelium is achieved by the sixth week of gestation, and it's about 390000 to 560000 cells per cornea. Till to the first few years of life the number of cells remains the same, but the spatial density decreases rapidly due to the cornea growing, regardless of the presence of diseases to the tissue. In the adulthood there are no sensible variations to the cornea's dimension, but the density of cells decreases anyway especially after the age sixty. Furthermore, the two eyes have significant differences in endothelial density. However, changes of the endothelial cell field is also due to pathologies specific to the endothelium. Furthermore other ophthalmic pathologies influence the active transport of the corneal endothelium. In addition, there are sufferings related to surgical interventions or pathologies that affect other structures of the eye but they have some effects to the endothelium.

There is no medical treatment that can promote healing or regeneration of the corneal endothelium. All corneal pathologies cause an opacification of the cornea due to severe edema and the only possible solution is corneal transplant.

The corneal transplant is a surgical procedure that requires a donor; the healing time is slower than other body parts due to the cornea's avascular nature, that, on the other hand, allows a very low risk of rejection. Although, the new cornea has to be in good condition in order not to have a rapid destruction of the new transplanted tissue. In case of failure, a second operation would have a worse prognosis than the previous one.

Using tissue engineering could be an alternative choice to the corneal transplant. This procedure consists in to create in vitro the endothelial layer exploiting the endothelial cells' capability to reproduce in vitro. New studies have been done in this field to create new corneal tissue that could be transplant.

1.3 Instrumentation

When the corneal endothelium is damaged, the healing process does not occur with mitosis, but with a process of enlargement of the cells, to fill the holes left by the died ones. Three different classes of instruments perform endothelium analysis *in vivo*:

- Slip Lamp
- Confocal Microscope
- Specular Microscope

The slit lamp is an instrument that uses a high intensity light source that is focused on a thin sheet of light into the eye. The most important field of application is the examination of the anterior segment of the eye including the crystalline lens and the anterior vitreous body. Supplementary optics such as contact lenses and additional lenses permit observation of the posterior segments and the iridocorneal angle that are not visible in the direct optical path. This type of microscope does not allow visualizing an image of the endothelial cell field.



Figure 1.4: INAMI L-0185 Slit Lamp Microscope with Halogen Lamp.

The **confocal microscope** is constituted by a normal transmission electron microscope and an apparatus that has the function to light up and detect the image of a sample illuminated. This instrument uses pinholes positioned on optical planes to select specific focus point. The main advantage of the confocal approach is the use of spatial filtering to eliminate out-of-focus light or flare in specimens that are thicker than the plane of focus; it is used in particular to obtain cornea images.



Figure 1.5: CONFOSCA 3.

The **specular microscope** casts light onto the cornea and captures the image that is reflected from the optical interface between the corneal endothelium and the aqueous humor.



Figure 1.6: Topcon SP-3000P Specular Microscope.

1.3.1 The Specular Microscope

In 1920 Alfred Vogt gave a first description of the structure of the corneal endothelium using a modified slit lamp. In 1968, David Maurice was able to capture an image of a rabbit corneal endothelium using a contact specular microscope. This method was too invasive and different studies permitted to perform changes in order to make the examination less invasive. Laing, Bourne and Kaufman adapt the microscope in order to obtain endothelium images *in vivo*. Over the years, several changes brought to the use of microscopy for diagnostic purposes in clinical practice.

Unlike other techniques in which the incident light passes through the objects, in cell microscopy one observes the light reflected by objects.

A part of the incident light on a smooth surface is reflected with an angle of reflection equals the angle of incidence ($i_1 = i_2$). Another part is refracted at an angle that depends on the angle of incidence and the relationship between different indexes of refraction of the means in contact:

$$n_1 \sin(i_1) = n_2 \sin(i_2)$$

where n_1 is refractive index of the first object and n_2 is refractive index of the second one.

The intensity of the reflected light is:

$$R = \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2}$$

If the surface is not smooth, only a part of the reflected light undergoes a specular reflection with angle of reflection equal to that one of incidence. Indeed, there is a scattering of reflected and refracted light.

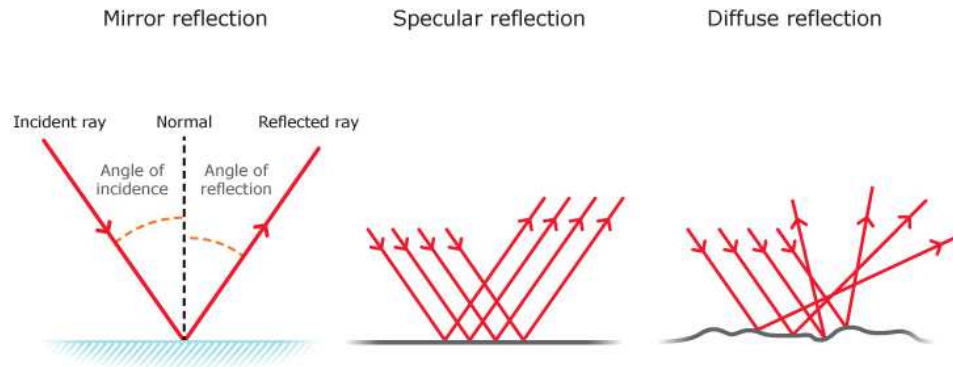


Figure 1.7: reflection on plane surface and on irregular surface.

In the specific case of the cornea most of the light is refracted through the cornea in the aqueous humor, the corneal tissue absorbs a part, a portion is dispersed, and only the remaining part undergoes a specular reflection. The specular reflection is greater as the difference between the indices of reflection of the different interfaces is important. The image of the endothelium is clearer if the incident beam used is narrow, as it has less overlap of the areas crossed by the beam).

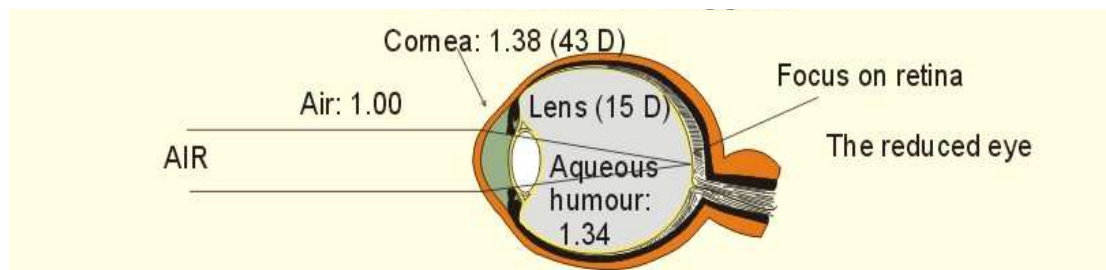


Figure 1.8: refractive indices of the main interfaces involved in the specular reflection.

The first two interfaces (air - the tear film, tear film - corneal epithelium) are very close, then the reflected light is detected as a single signal. The detection system acquires two signals: the first from the two interfaces and the second from the interface corneal endothelium - aqueous humor.

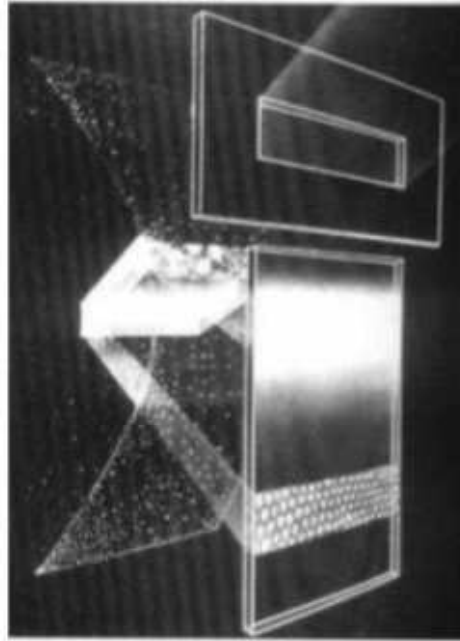


Figure 1.9: illumination and observation system.

Corneal thickness is measured by the reflected light of the first two interfaces, while the image of the endothelium derived from the reflection of the third interface. The reflection of light components, which is not detected by the microscope produces a dark image, while each reflected light to the microscope produces a bright image.

In clinical practice, specular microscopy is the most accurate way to examine the corneal endothelium. The non-contact specular microscope allows a non-invasive examination of the endothelium that lasts a few seconds. The endothelium on the digital image appears as a large mosaic of polygonal cells, mostly hexagonal, where the cell body is clear and the edges appear dark.

1.4 Analysis of the corneal endothelium

The morphology of the endothelium is a very regular hexagonal pattern, which allows the optimal cell packing. Therefore the analysis of the cell field morphology and the evaluation of the deviation from the regular hexagonal pattern can assess the history of corneal endothelium health.

The doctor has the opportunity to assess the state of health of the endothelium observing the scanned image with a specular microscope. However, it is useful to make a quantitative assessment of the image through the estimate of some diagnostic parameters.

In particular, there are three parameters that are used to quantitatively characterize the endothelial cells' condition:

- *endothelial cell density* (ECD): number of cells per mm²;
- *polymegethism*: $\frac{\text{sd (cell's area)}}{\text{mean (cell's area)}}$;
- *pleomorphism*: fraction of hexagonal cells over the total number of cells.

The degree of cellular loss, due to diseases, trauma, chemical toxicity or other causes, can be observed with specular microscopy as a decrease of the cell density (less cells per unit area), an increase in the variation of the individual cells area and a change of the cells' shape.

The main problem to analyze the images that are obtained using specular and confocal microscope is that cell boundaries are not continuous and the contrast is not optimum. When the images belong to pathological subjects, the irregularities are more numerous in both size and shape of endothelial cells. Moreover in these images the brightness is not uniform also due to the presence of pathology.

Human beings have the capability to perceive some structures that do not exist but they are deduced by neighborhood influence. Unfortunately, this ability cannot be automated; for this reason the automatic detection of the endothelial cell boundaries is a very challenging problem.

Chapter 2

Theoretical basis

2.1 Matlab programming

The Matlab language allows you to create programs using both procedural and object-oriented techniques.

2.1.1 Procedural programming

In procedural programs data are used as individual variables or fields of a structure and you can define the operations applied on data as functions, whose arguments are the variables. Programs usually call a sequence of functions, that operate on data performing different operations and then they return modified data. It is very easy to write a program using different functions, however, when the complexity of the program increases, it becomes more difficult to manage them. In these cases, we have to use a different programming approach: the object-oriented programming.

2.1.2 Object-oriented programming

The Object Oriented Programming (OOP) is a programming language that allows you to define objects that are able to interact to each other.

The first step in OOP is to identify all the objects the programmer wants to manipulate and how they relate to each other. Once an object has been identified, it is generalized as a class of objects. A class consists in: attributes, that define the characteristics or properties of the objects by invoking the class, and methods, that are procedures that operate on attributes. The constructor is a part of the class that initializes values of the attributes. An object is an instance of a class. It is equipped with all the attributes and methods defined by the class, and acts as a provider of "messages" (methods) that the executable code of the program (procedures or other objects) can be activated on request. An area of memory identifies an object, its attributes are stored in it and the value of the latter determines the internal state of the object. Certain kinds of Matlab objects are handles that are references to objects. Instantiate an object means to allocate

memory and initialize it if necessary according to the specifications defined by the class. A part of the program that uses an object is called client.

A programming language is called “object oriented” when it allows you to implement three mechanisms using the native syntax of the language: encapsulation, inheritance and polymorphism. The encapsulation consists in the separation of interface of a class from the corresponding implementation, so that the client of an object of that class can use the first, but not the second. The inheritance allows the derivation of new classes from those already defined creating a hierarchy of classes. This kind of class through inheritance (subclass), keeps the methods and attributes of the classes from which it is derived (superclasses or parent classes), and can also define their own methods or attributes, and redefine the code some of the methods that are inherited through a mechanism called overriding. This gives the possibility to reuse the code. Polymorphism allows you to write a client that can make use of objects of different classes, but with the same common interface, at runtime, the client will trigger different behaviors without prior knowledge of the specific type of the object passed to it.

The advantages of the object oriented programming are an increased understanding, an easy management of projects in order to have better control over related data, in comparing to the use of functions, and moreover the use of classes allows to reuse the code for other purposes.

Some mechanisms included in the management of the objects causing an overhead in terms of time and memory, which, in certain circumstances, can lead to problems of efficiency. Another disadvantage is the OOP greater structural complexity compared to procedural languages, in view of the limitations introduced to track the object paradigm.

2.2 The segmentation

The segmentation consists in dividing the image into significant regions. In general, the segmentation allows to change the image into something that is more meaningful and easier to analyze. Segmentation is one of the most difficult goals to achieve in image processing and it is used to locate objects and edges. There are many methods for segmenting images and the three main algorithms are: thresholding, region growing and splitting and merging. The thresholding technique applies a threshold T to divide the pixels belonging to the object to segment and the pixels belonging to the background. The region growing is a method that starts with one or more pixels to which adds neighboring pixels that comply with a predefined criterion of similarity. Instead region splitting and merging splits the image into sub-regions, which are then further subdivided or combined to satisfy a predetermined criterion of similarity. However, medical images are subject not only to shape and position variation, but also to several types of noise, so the classical methods fail to give satisfactory results. Because of its robustness and stability, the technique of the swarm would be ideal in clinical environment.

2.2.1 The swarm based approach

Medical images are very difficult to be segmented because of anatomical shapes variability and complexity and data limitations, in addition to the typical data problems of noise, aliasing, etc...

The swarm approach is very flexible and it is based on designing an agent and defining some rules in order to obtain a specific emergent behavior. The image is the environment in which an agent moves and interacts with image pixels and neighboring agents. The interaction can occur between different agents, between an agent and the entire swarm or through the environment.

An agent is an entity that can have some memory cells and it interacts with the image pixels and its neighboring agents to determine its movement in the next iteration. The most simple agent is a single point, that reads the pixel value at its current position, and that is equipped with a single memory cell, in which is stored the last seen pixel value. The further sophistication is to consider an agent with physical extension, where the memory has to remember good positions and directions. The agent has to make decisions based on this memory.

A useful example to understand the swarm method is the Resnick's Termites ⁽¹⁾. A population of termites moved small twigs around, using three rules:

- Wander around aimlessly, until bumping into a wood chip.
- If bumping into a chip, and not carrying, pick it up.
- If bumping into a chip, and carrying, put it down.

In this way the pixels got a structure of the human corneal endothelium. The pattern can change if few rules and another swarm are added. The termites and the other swarm can be called workers and builders.

The workers are little entities that have the only information about their location in the image. In this context, the twigs are interpreted as the image pixels so workers can carry a twig (this means that the value of the pixels is changed from zero to one) or put down it, then they turn around and move around the image. If they have a twig for a long time, they throw it away and a new twig is created. The builders are bigger than the workers and they move around considering a larger area of the image. They reject other builders during the construction of a nest, which consists on a ring of twigs. The value of the pixels are considered as the weight of the twig. The workers can only put a twig down next to the heavier one. If there are not any workers in the area, the builder remains stationary during the building of a nest. Instead, if twigs are already present in the area, other twigs are added to close the ring.

2.2.2 Local Operators on spatial domain

Spatial domain techniques operate directly on the pixels of an image. The local operators on spatial domain transform an image $f(x, y)$ in an image $g(x, y)$, in which each pixel of g is determined by a mask S_{xy} around of the corresponding pixels of f . For this reason, these are called local operators. The process consists on moving the center of the filter mask from point to point in an image. At each point (x, y) , the result of the filter at that point is the sum of products of the filter coefficients and the corresponding neighborhood pixels in the area spanned by the filter mask (Figure 2.1). The shape of the mask that is used determines the kind of filtering that is applied.

These operators have the function to reduce noise, increase contrast and extract the contours.

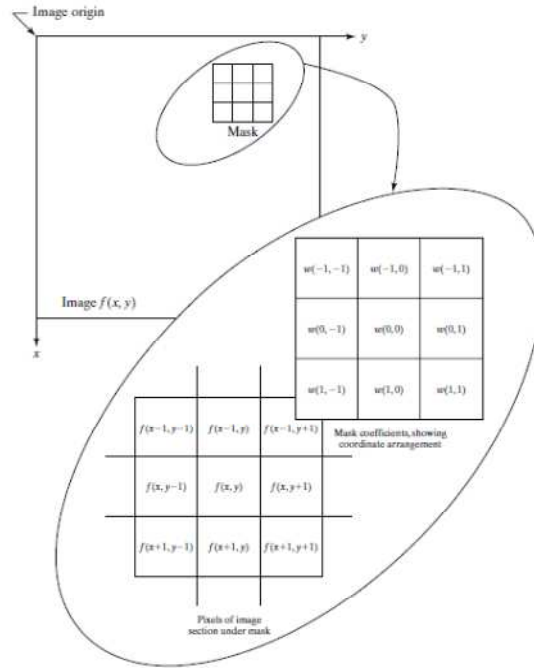


Figure 2.1: the mechanics of linear spatial filtering using a 3x3 mask.

A filter is applied on an image using the correlation or the convolution operation. If the mask used has the size $m \times n$, where m and n are odd integers $m = (2a+1)$, $n = (2b+1)$, the correlation is defined as:

$$g(x, y) = \sum_{s=-a}^a \sum_{t=-b}^b w(s, t) f(x + s, y + t)$$

$$g(x, y) = w(x, y) \circ f(x, y)$$

The convolution is the same process in which the mask w is rotated by 180° prior to passing it by f .

$$g(x, y) = w(x, y) * f(x, y) = \sum_{s=-a}^a \sum_{t=-b}^b w(s, t) f(x - s, y - t)$$

The local operators that use convolution on discrete domain are spatial filters that can perform a smoothing operation, that is able to reduce noise but it blurs the image, or they can increase the image contrast with a sharpening operation.

For pixels that are located in the edge of the image it is necessary to establish conventions for the definition of the surroundings, which is located partly outside the image, when the mask is close to the edge. One of the solutions to this problem is the zero padding. This technique consists in padding the image before the filtering. It can be added some rows and columns with a constant value (0 for instance) or to replicate the neighbor pixels.

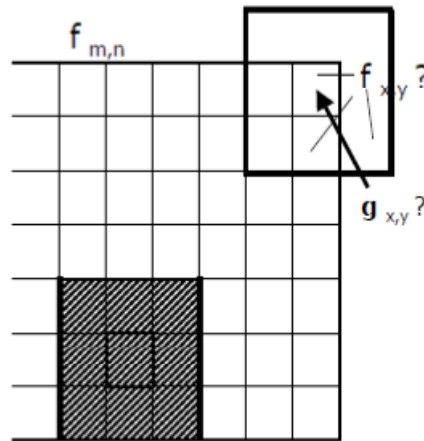


Figure 2.2: effects at the edges of the image.

Smoothing filters are simply averaging filters, which reduce the evidence of the details in the image. They are also called low-pass filters, because they leave unchanged low frequencies but they attenuate high frequencies, which correspond to image details.

Averaging filters replace each pixel the average of the pixel values in the neighborhood defined by the mask. In this way the differences of the gray levels of adjacent pixels are reduced. Also the abrupt transitions are decreased reducing noise, but also the contours are smoothed. A simple mask 3x3 that makes an averaging operation can be defined as:

$$\frac{1}{9} \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} H(u,v)$$

One of the most important local operators are differential operators that not only improve the image but also they extract image edges. A contour can be defined as a set of points that represent the maximum variation of light intensity along the perpendicular direction to the tangent at a point of the same contour. The points of maximum

brightness variation can be considered as the maximum of the first derivative or as the zero of the second derivative.

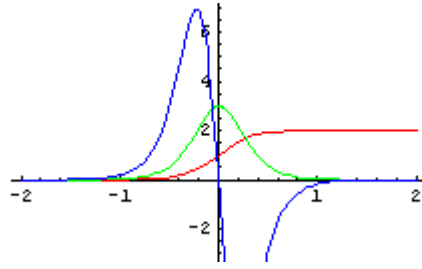


Figure 2.3: edge (red), the first derivative (gray) and the second derivative (blue).

Usually the derivatives are approximated in the discrete field as differences between adjacent pixels.

$$\frac{\partial f}{\partial x} = f(x + 1) - f(x) \qquad \frac{\partial f}{\partial y} = f(y + 1) - f(y)$$

$$\frac{\partial^2 f}{\partial x^2} = f(x + 1) + f(x - 1) - 2f(x)$$

$$\frac{\partial^2 f}{\partial y^2} = f(y + 1) + f(y - 1) - 2f(y)$$

The first derivative has the property to produce larger edges than the second derivative a stronger response to ramp edges. The second derivative has the characteristic to be sensitive to fine detail and for this reason it accentuates the noise, it also produces a double contour to step changes. In addition, the sign of the second derivative can be used to determine the type of the edge transition.

2.3 The Ground Truth

The project, that is illustrated in this work, has the purpose to segment endothelial images of the cornea. To make a comparison of results, it is used an image, which is called *ground truth* (Figure 2.4). After the acquisition of the endothelial images by means of specular microscope, the ground truth is created tracing manually cell boundaries or using specific programs basing on the endothelial tissue, in which cell shape, size and the thickness are observed. The ground truth is a binary image where the cell edges are white and the cell bodies are black.

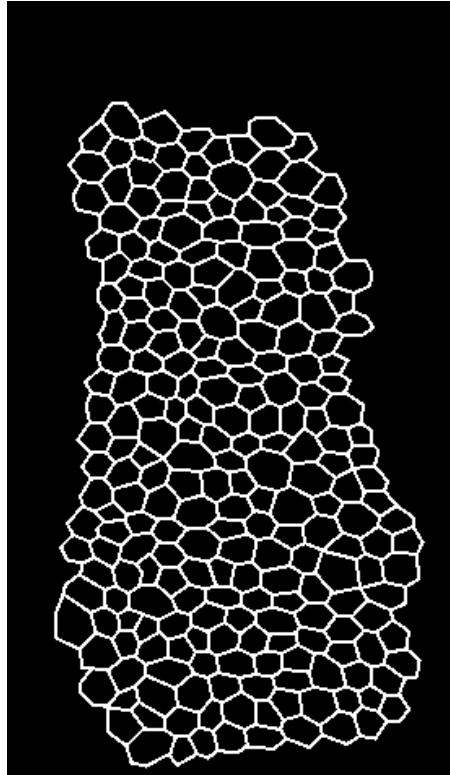


Figure 2.4: the ground truth image acquired by Topcon SP-3000P.

Chapter 3

The corneal endothelial image analysis

At present, the analysis of the corneal endothelial images is performed using manual or semi-automatic methods. The main purpose of research is to implement a method able to detect corneal endothelial cells automatically. A computerized method capable of segmenting endothelial cells is very important to estimate the clinical parameters (**cell density, pleomorphism, polymegethism**). These parameters are used to diagnose some diseases affecting the cornea.

The images of cornea have the advantage of a limited range of possible image configurations. Therefore, the possible appearance of the endothelium field is relatively constant, even if it belongs to people that are affected by some corneal disease. The methods that deal with this kind of images have the purpose to detect characteristics that remain steady. On the other hand, the method of image capture could represent a problem. The imaging devices impose some limits that prejudice the quality of corneal images.

Manual analysis is still very used in medical environments. The main problem of manual segmentation is the limited number of cells that is possible to evaluate (about 10-20). The consequence is the impossibility to derive useful information regarding the state of health of the endothelium field. The clinical experts are able only to count the number of cells without considering their morphology and this operation requires a lot of time. Moreover this approach is not accurate because of the quality of images and the fact that cells are counted viewing the video screen or the microscope.

The advancement of technology has led to the development of some semi-automatic systems able to reduce the processing time. In recent years these systems are improved, however they still need manual corrections.

3.1 The Image segmentation

In this thesis we propose a global approach capable of segmenting images of corneal endothelial cells. The performance of this method is assessed by computing on a set of 30 original images and 30 manual images that are the ground truth images (Figure 3.1-3.2).

The manual images were obtained defining manually cell boundaries inside a ROI (Region of Interest). The ROI is manually defined in order not to consider the peripheral regions, where there is low image quality. The ROI is a region in which the edges are clear enough to allow a reliable detection of cell contours and it covers an area of about 0.1 mm^2 that includes an average of 220 cells. The cell contours are defined manually using a public-domain image manipulation program (GIMP).

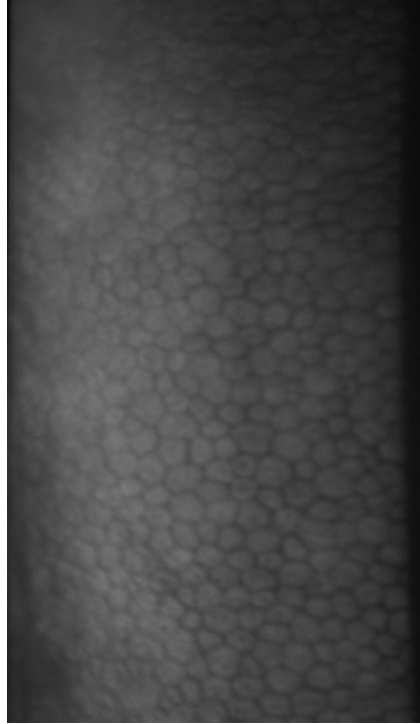


Figure 3.1: corneal endothelium image.

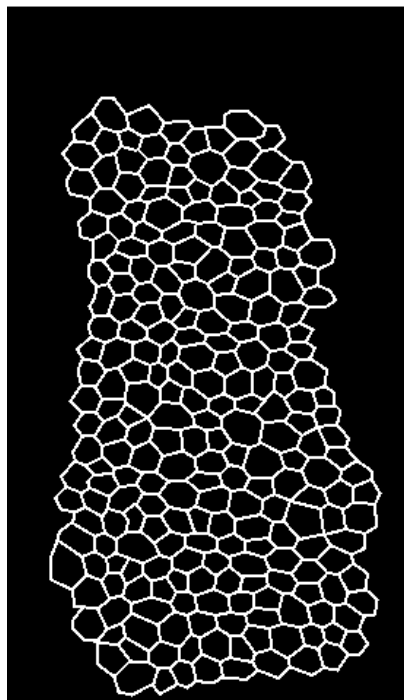


Figure 3.2: the ground truth image.

The images were acquired with a specular microscope (*SP-3000P, Topcon Co.*) from health and pathological subjects. They are 240 x 480 pixels grayscale images, that covers an area of 0.25 x 0.5 mm².

The basic idea, on which the whole program is based, is the creation of a grid of circles overlapped on the image to be segmented. Then, these circles will change their morphology, in order to capture the edges of the endothelial cells of the cornea. If it were a three-dimensional image, it is as if the circles were marbles that must be placed in the valleys (light areas) of the lattice cell.

Applying the method of swarm, a class Circle was created to define the easiest agent that is used to find the cell boundaries. The coordinates of center and the radius define the circle. They must be able to move and change their size. To achieve this goal, we created some methods that evaluate the movement of a single circle. The class Circle defines movements that are based on the gray level of the area covered by each circle.

One of the methods of the class has the purpose to calculate the barycenter of the area inside the circle basing on the gray levels of the image.

The first way, that we proved to implement the barycenter of the circle, was based on the mathematical formula of the barycenter for a discrete system of N points is:

$$R = \frac{m_1 r_1 + m_2 r_2 + \dots + m_N r_N}{M} = \frac{\sum_{i=1}^N m_i r_i}{\sum_{i=1}^N m_i}$$

where $M = m_1 + m_2 + \dots + m_N$ is the total mass of the system and r_i are radius vectors with respect to the reference system used.

We adapted this equation to our field of application assuming the gray levels as masses. In this way, we created a repulsive force that is capable of rejecting the black pixels, especially if they are located near the circle center. The barycenter would be sometimes too high, so it is necessary to use a threshold, in order to impose a little movement to the circle coordinates in each iteration. This method to estimate the barycenter, that defines the circle movement for next iteration, works well when the circle center is located far from cell boundaries (Figure 3.3-3.4), instead it does not give good results if the circle center is near a cell contour (Figure 3.5).

Baricenter Test

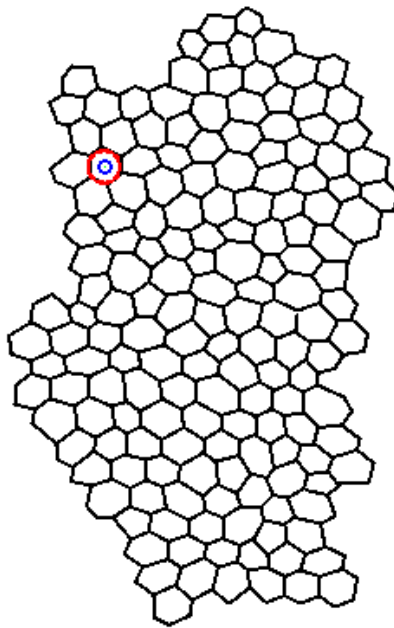


Figure 3.3: repulsive force when circle center is in good location on manual image.

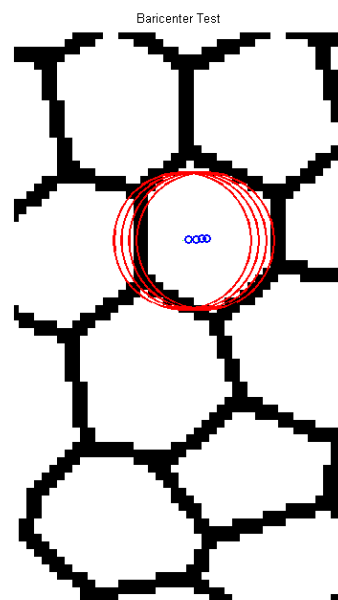


Figure 3.4: zoom of the circle movement in each iteration on manual image.

Baricenter Test 1

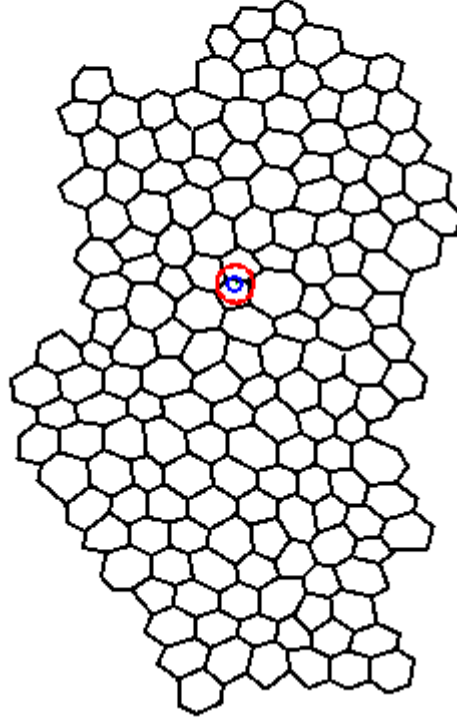


Figure 3.5: repulsive force when circle center is in bad location on manual image.

Therefore, we estimated the barycenter considering an attractive force toward the white pixels using intensity transformations.

The power transformation is an image enhancement technique (Figure 3.6). One of these types is the power function that allows to obtain an image in which light region get dark. Instead, the square transformation lightens the image.

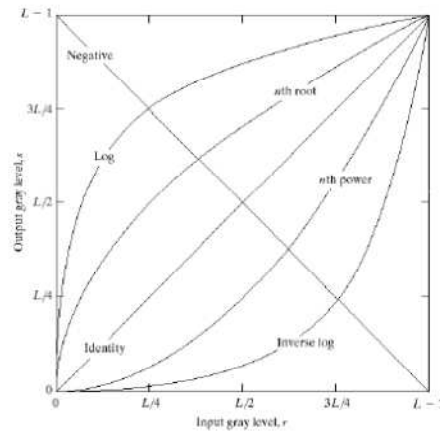


Figure 3.6: some gray level transformation functions.

We calculated the barycenter using the 2th power of the gray levels, in order to get the brightest pixels more noticeable, and the square root. However, these forces can find the cell boundaries only if the initial circle has a good position on the image.

Furthermore, we proved another approach to the problem. We used a smoothing filter, in particular a circular averaging filter (Figure 3.7). This kind of filter allows to obtain an output image with smoothed gray levels.

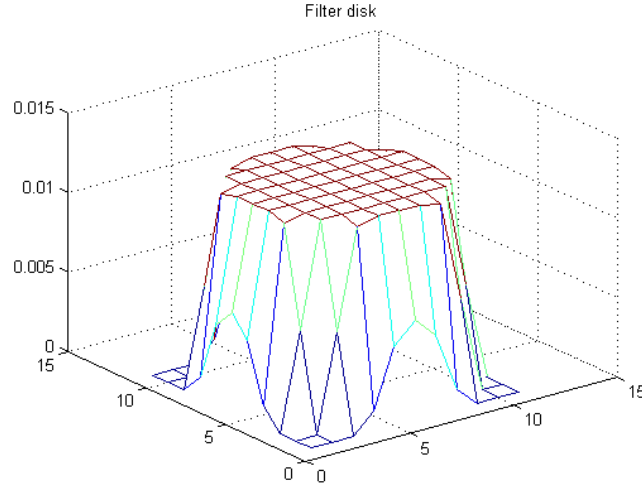


Figure 3.7: circular averaging filter (disk filter).

The gray levels in the blurred image grow gradually up to reach the maximum intensity value, which theoretically corresponds to the center of the cell. We considered the 2th power of the gray levels in the area covered by the circle, in order to amplify the response of the light pixels. Then, we selected the maximum gray levels and we changed the circle center from its position to the nearest maximum location that was found. This solution worked well enough, but it was not stable, because there could be always a maximum in which the circle center can move.

Therefore, to create a more stable method to find the barycenter of the circle, we used the differential operator of the first derivative. First of all, we used the same smoothing filter that was used in the previous approach to obtain a distribution of the gray levels that permits to identify the lightest region inside the circle.

We created some images that allowed us to test this method. In figure 3.8 we can see the action of the smoothing filter on the test image:

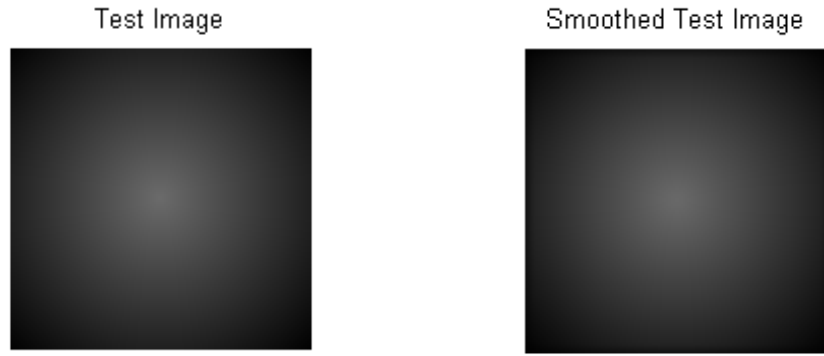


Figure 3.8: test image (left); test image after smooth filtering (right).

Then, we calculated the first derivative on the smoothed image. The barycenter was found searching the coordinates in which the first derivative was maximum, that is the point in which the first derivative had a change of sign both along the coordinate x and y (Figure 3.9).

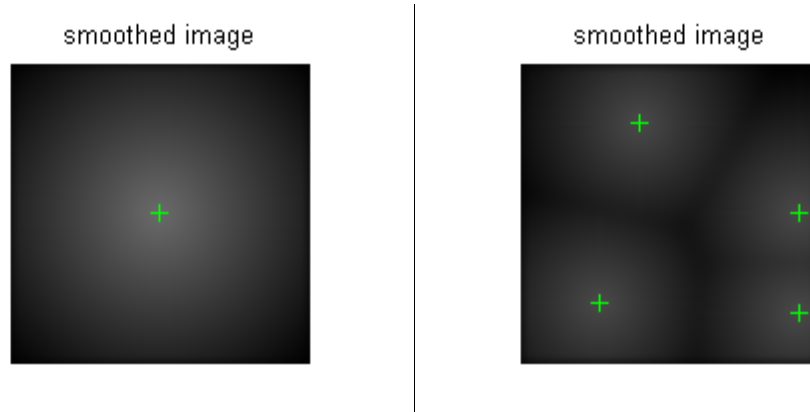


Figure 3.9: smoothed image with a cross where the first derivative is maximum (left); smoothed image with four maximum of the first derivative (right).

When we applied the circle on the test images, the circle center moved toward the nearest point where the first derivative was the highest (Figure 3.10).

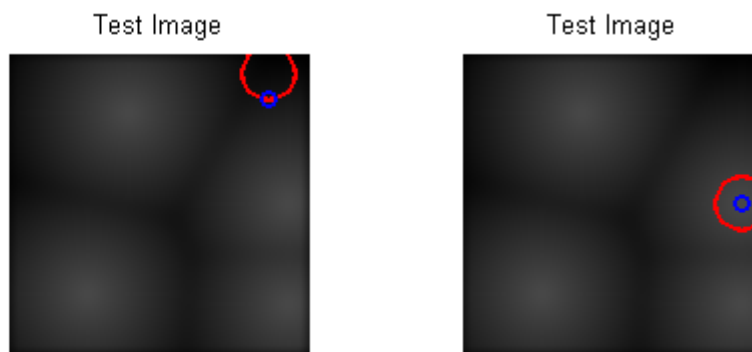


Figure 3.10: test image, after smooth filtering, with the initial position of the circle (left); test image, after smooth filtering, with the final position of the circle (right).

We applied this new approach to the corneal images and we obtained good results (Figure 3.11).

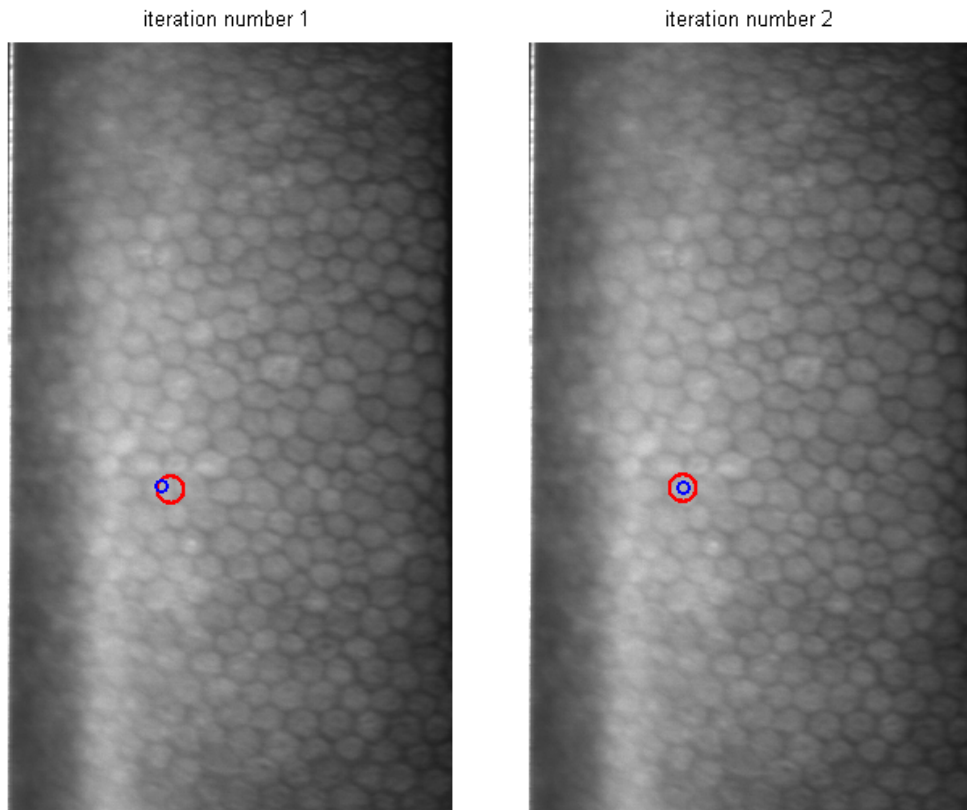


Figure 3.11: derivative approach on endothelial images.

Another important change of the circle is to assess the possibility to enlarge or reduce its radius. Indeed, the circle could be at the center of a cell of the endothelial field, but it could be smaller than the cell size. It is useful to enlarge the circle considering the gray levels of the pixels that belong to the space near the circle boundaries. We considered a bigger and a smaller annulus than the initial circle and we calculated the mean of the gray levels of these three areas. To decide if it was useful to enlarge the initial circle or to reduce its radius, it was evaluated where the mean of the gray levels was minimum (Figure 3.12). In the test image we can see that the circle increased its radius up to reach the cell edges where the black pixels were responsible for the decrease of the mean of the image intensity.

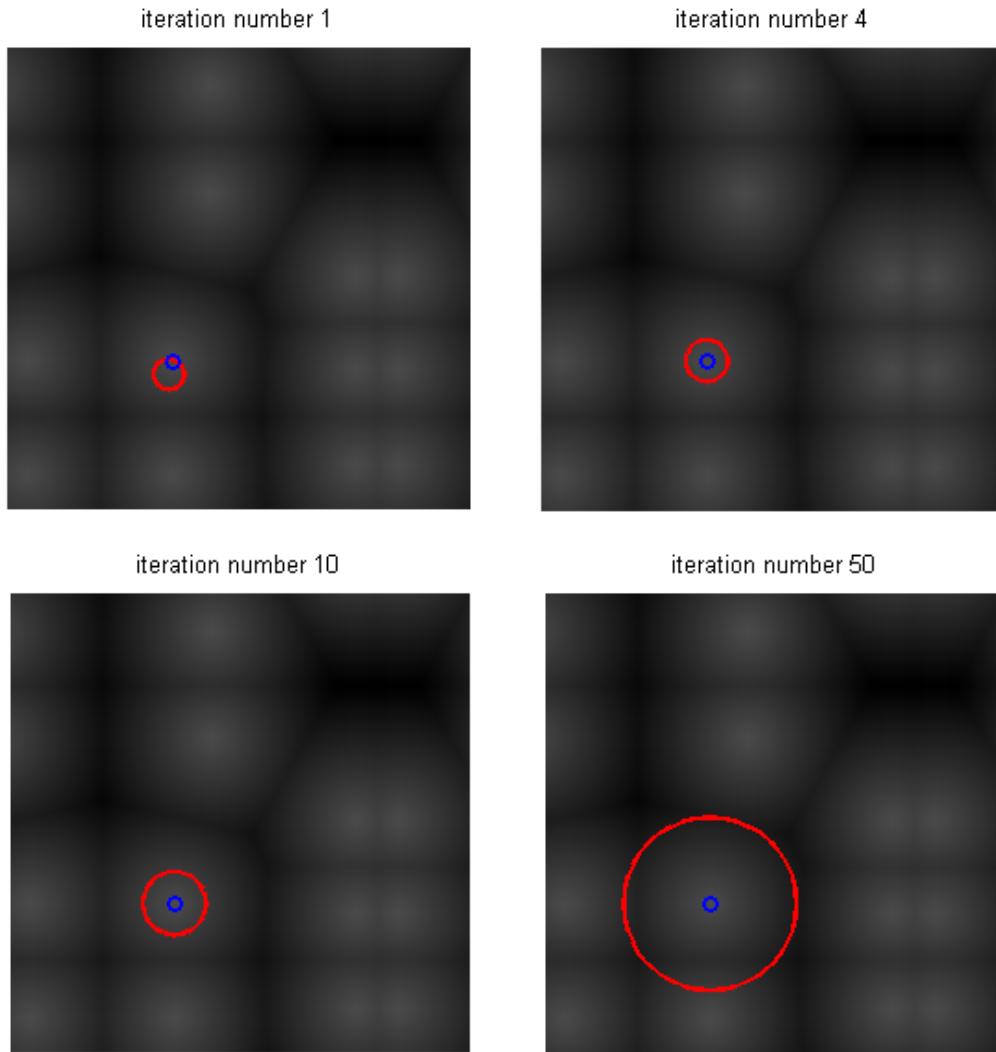


Figure 3.12: enlargement of the circle on test image after 4, 10, 50 iterations in order to catch the cell boundaries.

Then we built a grid as a set of circles that was positioned in staggered rows to have the least area among different circles (Figure 3.13). According to the swarm approach, this structure represents the builder of the program. In the grid, the forces exerted by the other circles change the position of the single circle.

manual image with grid

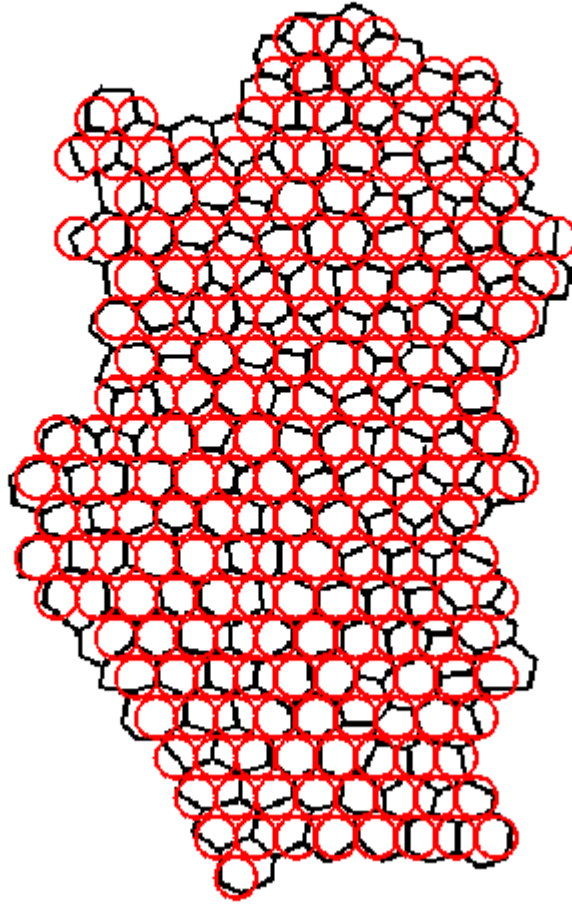


Figure 3.13: circle grid overlapped manual image.

Two different circles that belong to the grid don't have to overlap because they have to find the boundaries of two different cells. Therefore, it is calculated the distance between the circle centers, if it is lower than the sum of radius of the two circles, the circles are moved in order to be adjacent each other. The movements for each iteration are little, because the system has to consider different changes of position that are due to different image features. In Figure 3.14 it is possible to observe the circle movement on the ground truth image to visualize better the interactions between circles.

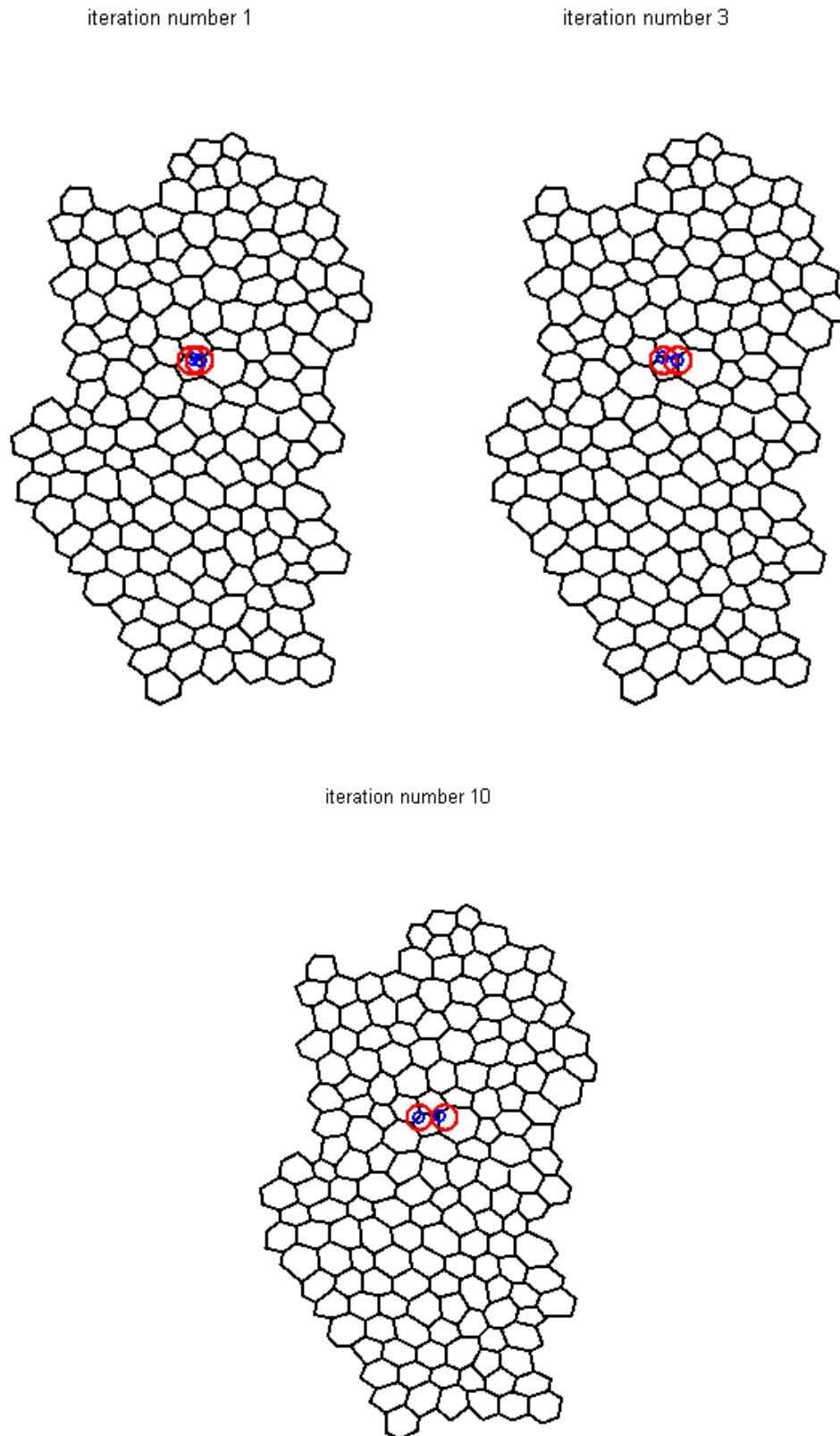


Figure 3.14: circle movement due to the interaction between two circles on manual image.

Using the class circle and the class grid, the image segmentation was made considering a Region of Interest (ROI) in the corneal endothelial image. This ROI was defined

based on the region of the ground truth image in order to make a comparison between the manual approach and this completely automatic approach.

In the ROI the grid of circles was constructed that, according to the rules defined in the methods of the classes, changed their radius and the coordinates of their center to detect the boundaries of endothelial cells. In order to obtain a global approach, the various movements, both the motion based on the image and the motion based on the interactions between different circles, is applied after their evaluation for all the circles that belongs to the grid. In this way, the method is totally independent from the starting point and previous decisions evaluated in the program.

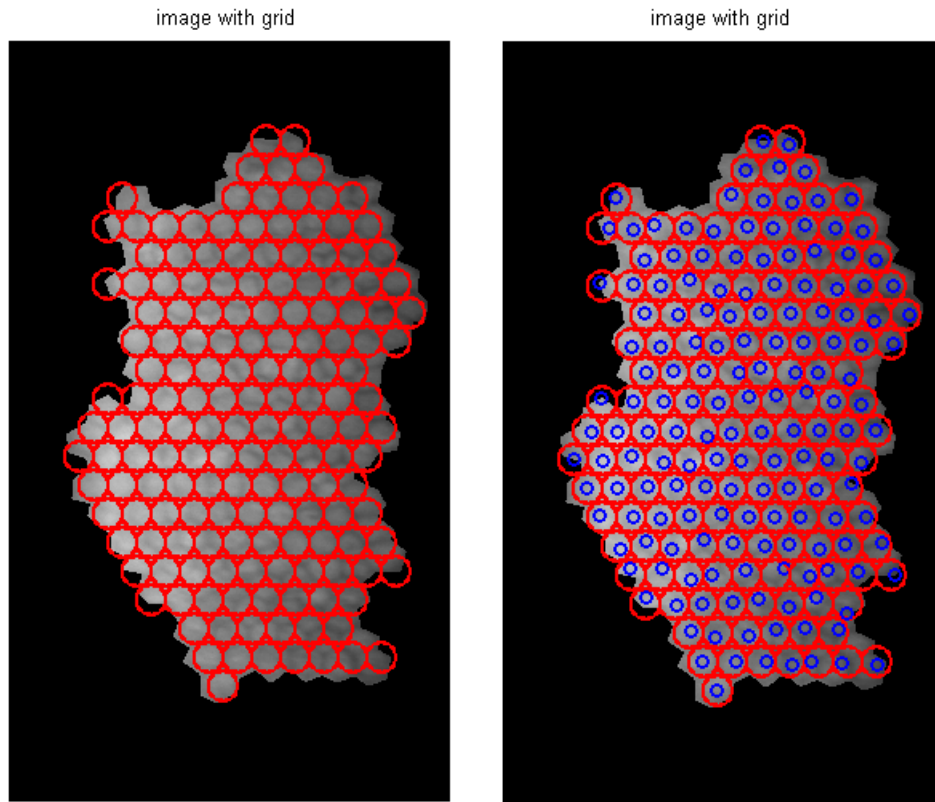


Figure 3.15: the ROI in the original image with circle grid (left); the circle grid with the barycenter (right).

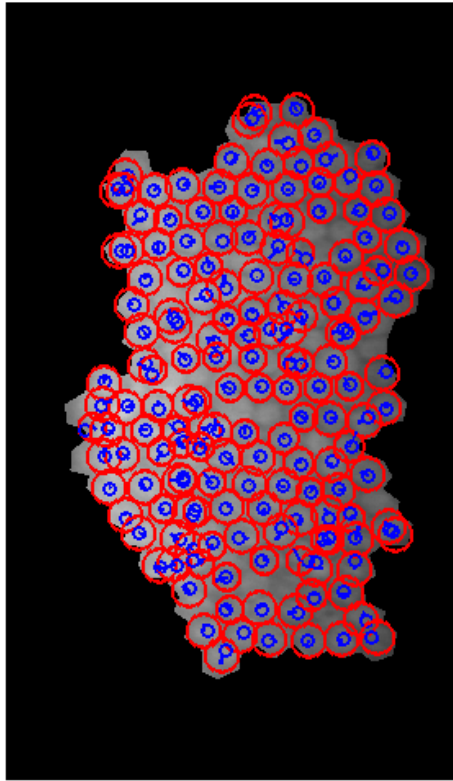


Figure 3.16: modified circle grid after few iterations.

Chapter 4

Evaluation of the image analysis

We had defined the initial methods that build the main program structure to segment endothelium images. Therefore, we can prove this new approach and we can evaluate the initial results of this program, but we have to consider this results as partial. Being the initial part of the program, we have to consider only if this model have the right potentialities to continue the studies using the idea of a circle grid to detect endothelial cells.

4.1 Binary classification

The term classification indicates the assignation procedure of an instance to a particular group, depending on the characteristics of the instance itself. A generic classifier makes a classification mapping a set X of instances in another set Y of labels. To every instance is assigned a label if the instance has some properties, verified by the classifier. The classifier is binary if the labels that can be assigned to every instance are only two. A very common example of binary classifier is every medical test that, analysing human biological samples, provides an indication of the patient's health state. In these cases, the set of instance consists on the patients to be tested, and the two labels are usually named positive or negative to indicate respectively an abnormal or a normal state. The classifier marks an instance with one of the two labels usually depending on a value of a parameter related to the instance itself. The classifier use a threshold value called cut-off value: the instances with a parameter value above the cut-off value are marked with a label, the other ones with the other label. In the example of the medical test, the two categories in which each patient can result into depends on a concentration of substance present on the biological sample. A concentration above a cut-off value indicates that the patient is diseased, in the other case it's in health. More often there are two different cut-off values, in order to say that the patients with concentration between the two values are in health and in the other cases are diseased. In every case, the classification is binary.

It's necessary to distinguish from the label given by the classifier and the actual state of the instance classified. The classifier acts depending on only the settled cut-off value; that is, the assignment of the label positive or negative by the classifier to an instance is a prediction of the instance's actual state; so the classifier states that the instance has a

probability to be actually positive or negative. In the medical example, the result of a clinical test indicates the health state of a patient with a certain probability, and sometimes other exams are necessary to assure that the result of the first test corresponds to the real condition of the patient. Performing the classification of a set X of instances, both the positive and negative labeled instances tend to distribute normally around the mean parameter value that characterize each of the two groups. If, ideally, the two distributions would be separated, it would be possible to give to the classifier a cut-off value able to detect correctly all the instances: that is to say that the instances that are actually positive are classified as positive, and the instances that are classified as negative are actually negative (Figure 3.1a). But always happens that the two distributions overlap themselves: in this case there is not a cut-off value that makes the classifier able to separate absolutely the two categories of instances (Figure 3.1b). This implies that there will be instances classified as positive that really are negative, instances classified as negative that are really positive and instances correctly classified.

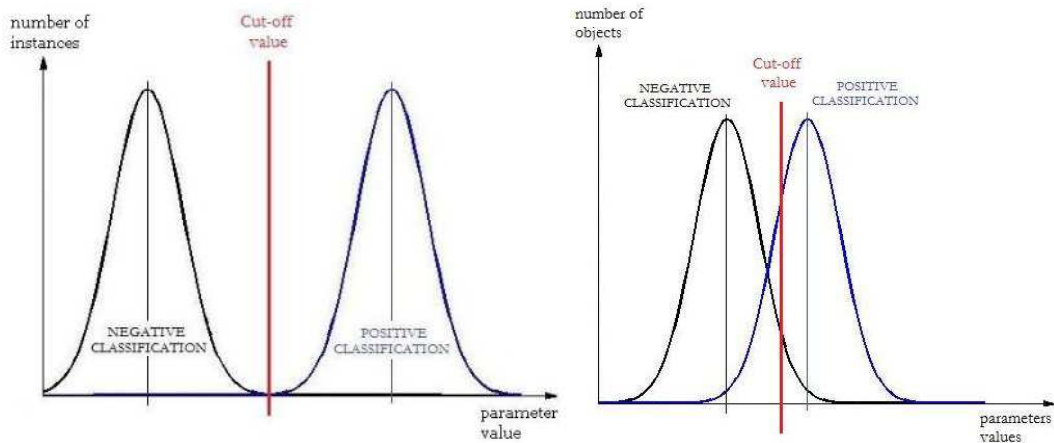


Figure 4.1: (a) an ideal wide separation between the distributions allow to determinate an absolute cut-off value; (b) the overlap of the distributions doesn't allow to determine an absolute cut-off value.

In Figure 4.1b, assuming that an instance with parameter greater or equal the cut-off value is labeled as positive and the other negative, the area under the curve of the negative classification at the right of the cut-off value is the number of instances labeled as positive but that they are really negative; similarly the area under the curve of the positive classification that is at the left of the cut-off value constitutes the number of instances labeled as negative but they are really positive. So every instance classified, compared with his real state can constitute one of the four sequent cases:

- true positive (TP): the instances in input of the classifier that are really positive and the classifier marks as positive;
- false positive (FP): the instances in input of the classifier that are really negative and the classifier marks as positive;
- true negative (TN): the instances in input of the classifier that are really negative and the classifier marks as negative;

- false negative (FN): the instances in input of the classifier that are really positive and the classifier marks as negative.

These results can be summarized on a contingency table:

		actual value		total
		<i>p</i>	<i>n</i>	
prediction outcome	<i>p'</i>	True Positive	False Positive	<i>P'</i>
	<i>n'</i>	False Negative	True Negative	<i>N'</i>
total		<i>P</i>	<i>N</i>	

Table 4.1: contingency table of binary classification results.

The comparison between the test result and the true value allows us to estimate three parameters: sensitivity, specificity and accuracy.

$$\text{Sensitivity} = \frac{TP}{(TP+TN)}$$

$$\text{Specificity} = \frac{TN}{(TN+TP)}$$

$$\text{Accuracy} = \frac{TP+TN}{(TP+TN+FP+FN)}$$

The sensitivity is the portion of positives correctly classified, while the specificity measures the proportion of true negatives correctly classified. A test is very sensitive if it has a low proportion of false negatives, while it is much more specific than the lower the false positives. The accuracy is the ability of a test to provide values corresponding to the real ones.

4.2 Classification and analysis of images

After applying the algorithm for the segmentation of corneal endothelial images, we had to evaluate the sensitivity, specificity and accuracy of this approach.

First of all, we isolated the centers of the circles found. We calculated the Euclidean distance transform and then we applied the watershed. The technique of the watershed allows to detect the contours of the regions (Figure 4.2).

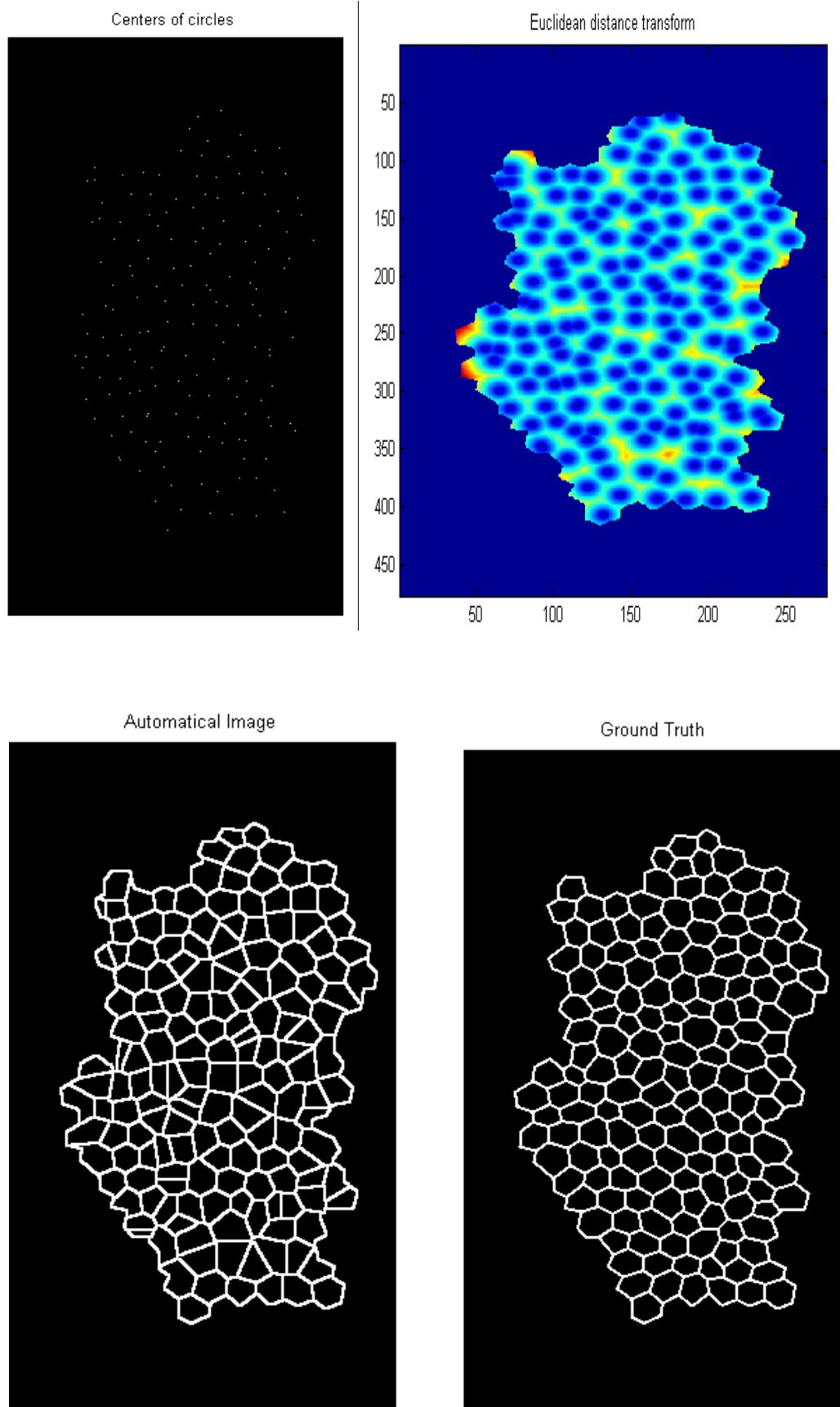


Figure 4.2: (a) image with the centers of the circles; (b) Euclidean distance transform applied to the image of centers; (c) automatic image; (d) the ground truth image.

This image was compared with the ground truth to evaluate the number of true positive, false positive, true negative and false negative:

- a true positive is each pixel that in both the classified image and the ground truth is part of a feature (white);
- a false positive is each pixel that in the classified image results part of a feature (white) but it's not in the ground truth (black);
- a true negative is each pixel that in both the classified image and the ground truth is not part of a feature (black);
- a false negative is each pixel that in the classified image doesn't result part of a feature (black) but it's so in the ground truth (white).

Each automatical image were compared with the corresponding Ground truth in order to calculate the sensitivity, specificity and the accuracy (Table 4.2).

Image Number	Sensitivity	Specificity	Accuracy	Cell_a	Cell_g
1	0.4200	0.7835	0.6993	215	241
2	0.4321	0.7726	0.7007	214	197
3	0.4611	0.7878	0.7191	247	227
4	0.4376	0.7852	0.7110	242	225
5	0.4461	0.7837	0.7109	228	219
6	0.4331	0.7812	0.7127	212	162
7	0.4107	0.7728	0.6931	233	235
8	0.4575	0.7901	0.7168	201	203
9	0.4598	0.7863	0.7139	163	163
10	0.4360	0.7845	0.7100	185	172
11	0.4157	0.7732	0.6913	179	186
12	0.4183	0.7794	0.6965	230	255
13	0.4713	0.7650	0.7247	172	57
14	0.4065	0.7780	0.6932	240	267
15	0.4353	0.7739	0.7092	248	173
16	0.4722	0.7589	0.7286	228	41
17	0.4108	0.7819	0.6919	86	107
18	0.4434	0.7746	0.7128	206	141
19	0.4486	0.7609	0.7126	126	53
20	0.5944	0.7704	0.7523	121	17
21	0.4321	0.7770	0.7077	204	170
22	0.3878	0.7692	0.6783	228	278
23	0.4368	0.7782	0.7073	164	134
24	0.4697	0.7947	0.7266	245	223
25	0.4091	0.7720	0.6914	241	250
26	0.4327	0.7790	0.7034	197	198
27	0.4822	0.7910	0.7280	240	197
28	0.4674	0.7860	0.7279	238	161
29	0.4370	0.7808	0.7047	188	189
30	0.4154	0.7760	0.6999	190	173

Table 4.2: the sensitivity, specificity, accuracy and the number of cells detected automatically and the number of cell in the ground truth.

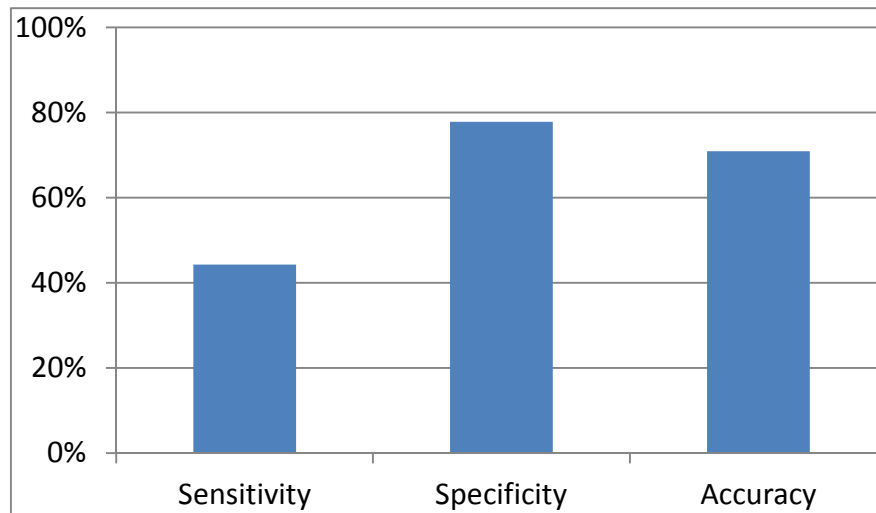


Figure 4.3: histogram of the mean values of sensitivity, specificity and accuracy.

As the histogram of the mean values shows, the sensitivity, specificity and accuracy are not optimal. The sensitivity should be over 60%, instead our program has the ability to recognize a low portion of true positives. The program is specific enough because the specificity is near the optimal value, that is 80%. Also the accuracy has a mean value about 80%. However they are acceptable values because we have to consider that this project is only the main structure on which a Bayesian approach will be based. The Bayesian technique is able to give some prior information that could improve the algorithm performances. The idea is to evaluate some probabilities about some features of the endothelial image. This probabilities make up a Prior Model that can be used, in conjunction with information provided from the image itself, to determine the displacements at each iteration of the grid circles.

Then we calculated the mean absolute error and the relative error in the number of the cells that were detected in comparison to the number of cells that were in the ground truth image.

Mean absolute error = 38

Mean relative error = 21%

These values could be improved using two methods in the class grid that allow you to remove and add a circle considering the interactions of the individual circle with other circles and with the image. In this way, the number of cells would be more accurate.

To summarize, this project has allowed us to try a segmentation algorithm that uses circles instead of polygons. The circles were found to be easier to manipulate since have only two variables, the radius and the center. This allowed us to simplify the code and make it more controllable.

Even if the results don't have optimal values, this program allow us to build the structure of an approach that ensures a good control on the program and that could have good results in the segmentation of the endothelial cells images, if it will be developed

with the Bayesian method. This project had the ability to clarify the movements that circles can make to segment the image, and to define the important characteristics to define in the Prior Model. The swarm technique ensures a perfect control over the code so that it can be easily modified and improved using other approaches. In this way, further development of this program will be implemented easily.

Conclusions

The purpose of this thesis project is to build the initial structure of an algorithm able to perform fully automatic analysis of images of the cornea endothelium.

As already explained in the previous chapters, in the medical field are evaluated clinical parameters able to discriminate images of healthy patients from those of sick patients. At present, the current analysis is carried out with manual or semi-automatic methods that require much time and are unreliable. Therefore, it is fundamental to create an automatic algorithm that allows to define these clinical parameters quickly and reliably.

The work uses a swarm approach for the construction of the grid of circles. The swarm theory is extremely suitable for the analysis of medical images precisely because of their variability and the various types of noise present in them. It's a very robust and stable method that allows an extreme control over the algorithm. This approach considers small entities and more complex structures. The individual entities have to comply with some rules, we have to define the interactions between small entities that make up the most complex structure and, finally, we have to evaluate the interactions with the surrounding environment. This structuring algorithm provides excellent control of the entire project.

The project creates a grid of circles that has the ability to change its structure for the segmentation of the mosaic of the underlying endothelial cells. Indeed, each circle, which is the easiest entity, has the possibility, through the definition of some methods, to change the coordinate of its center and the radius value to fit the shape of the cell. The grid is the most complex structure that controls the interactions between circles. Then, there is an overall assessment of the grid with the external environment, which, in our case, is the image itself.

Because of the poor quality of endothelial images of the cornea, cell sides are often unclear and so they are difficult to detect automatically. An important advantage of this algorithm is that the use of a grid of circles allows to detect also those sides of cells that are poorly defined in the image.

For the construction of the grid of circles has adopted the object-oriented programming in Matlab. This method of programming allows you to follow the conceptual framework of the swarm approach and it ensures excellent versatility of the project with other types of object-oriented programming such as C + +.

It is also a flexible program that could be reused for other different purposes. It is only necessary to modify or create some methods in order to capture the features that are interesting. As a result, the structure of the algorithm remains the same, but some changes are able to make the program suitable to solve other kind of problems related to the image segmentation.

Comparing the images obtained by applying the program and the ground truth, the image parameters, that are sensitivity, specificity and accuracy, were calculated. These parameters appear to be low compared to the desired values, but we have to consider that these values are only partial results. Indeed, this thesis aims to create the initial structure for a larger project that includes a Bayesian approach for the segmentation of this kind of images. The further development of this project is to introduce a prior model, that can provide prior information that has to integrate with information given by the image itself. The prior model will have some probabilities related to some characteristics of the endothelial cells in the image, that will be used in the algorithm to make decisions during the iterations. Surely the development of this program with a Bayesian approach will allow to obtain very satisfactory results.

Another next improvement could be defining some methods that are able to remove and add new circles. This type of methods should consider the entire grid and they should evaluate the interactions between circles and between the grid and the image. These methods should decrease the error related to the number of cells that the program is able to detect, contributing to the improvement of the program performances.

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