



University of Science and Technology
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ERASMUS +

Final Project

Bachelor's Degree in Biomedical Engineering

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DEVELOPMENT OF AN ALGORITHM FOR THE DETECTION OF RETINAL LESIONS

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Kraków, June 2022



ABSTRACT

This thesis is intended to support and complete the development of an algorithm whose purpose is to detect some alterations presented in glaucoma and diabetic retinopathy diseases. Firstly, a study of the anatomy and physiopathology of the eye will be made. The mentioned pathologies and the target alterations of the algorithm will be studied as well. The developed algorithm differentiates healthy from pathological fundus images. The next step is to discover the alterations in the pathological ones. The proposed algorithm, *TFG_code ()* works with nine retinographies with Portable Network Graphics (PNG) and Joint Photographic Experts Group (JPG) image formats which are retrieved from two different online available datasets used for different projects. The results are successful for all those images.

Key words: Retinal lesions, detection algorithm, diabetic retinopathy, glaucoma.



RESUMEN

Esta tesis pretende apoyar y completar el desarrollo de un algoritmo cuya finalidad es detectar algunas alteraciones que se presentan en las enfermedades de glaucoma y retinopatía diabética. En primer lugar, se realizará un estudio de la anatomía y fisiopatología del ojo. También se estudiarán las patologías mencionadas y las alteraciones objetivo del algoritmo. El algoritmo desarrollado diferencia en primer lugar las imágenes de fondo de ojo sanas de las patológicas. El siguiente paso es descubrir las alteraciones en las patológicas. El algoritmo propuesto, *TFG_code ()*, trabaja con nueve retinografías de los formatos de imagen Portable Network Graphics (PNG) y Joint Photographic Experts Group (JPG) que se recuperan de dos conjuntos de datos diferentes disponibles online utilizados para diferentes proyectos. Los resultados son satisfactorios para todas esas imágenes.

Palabras clave: Lesiones de la retina, algoritmo de detección, retinopatía diabética, glaucoma.

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CHAPTER 1. INTRODUCTION

1.1. Purpose of the thesis

The aim of this thesis is to develop an algorithm that detects the presence/absence of retinal lesions provided images of the fundus of the eye, also known as the retina. The algorithm will be realized thanks to self-directed learning and the knowledge base acquired during the four years of the Biomedical Engineering Degree.

Having said this, the main objective of the thesis is clear. Furthermore, the specific objectives that will be achieved through the development of the present thesis are presented below:

- Getting to know MATLAB environment in depth.
- Acquire general knowledge of anatomy, physiology, and physiopathology of the eye.
- Integrate medical and informatics concepts.
- Good handling and application of image processing tools.
- Raise the awareness of the importance of image process in the medical field.

In the past, doctors had to interpret and diagnose diseases of the human body through the signs expressed in its surface. They could also see the inside part only through invasive techniques such as surgeries and biopsies. This meant discomfort for the patient and alteration of the body unnecessarily with the risks that this entails. These reasons were determinant for the appearance of medical imaging.

Medical imaging as it is known today is very helpful in the medical field. It is used in everyday workflow in every kind of healthcare facility. Medical imaging has allowed medicine to improve and provide the patient a more personalized and precise diagnose and treatment. Medical imaging has two main and wide applications:

- Surgical support system.
- Clinical decision/Diagnostic support system.

The algorithm developed in this thesis is part of the diagnostic support system. As part of it, at an idealistic stage, it is a tool to help doctors diagnose retinal lesions.

Nowadays, informatics is in constant improvement. Medical images can be automatically analyzed with the appropriate software. It reduces diagnostic times and so improves the efficiency of health systems. In the vast majority of the human diseases, the early diagnosis and early treatment of them implies better recovery and fewer side effects.

In short, medical imaging processing is continuously refining in order to achieve more realistic images of the human tissues that lead to more precise diagnosis and surgical support.



1.2. Justification of the thesis

The justification of this thesis is academic as well as personal.

Medical imaging is one of the areas of biomedical engineering that most caught my attention. During the four years of my degree, Biomedical Engineering, I have attended numerous subjects that have helped me to achieve the necessary knowledge to process medical images: biomedical images, informatic and networks, basis of diagnosis and treatment in medical-surgical pathology or and biomedical signals, to name a few. Therefore, the decision of the topic was the enthusiasm to finish my studies with a project that allowed me to continue my development in this area.

On the academic level, a thesis is needed in order to finish the Biomedical Engineering Degree. This final project needs to be related to some topic covered thorough the degree. Having these in mind, and, considering my personal preferences as described before, I decided to focus my final project on the medical imaging area.

1.3. Structure of the thesis

This final project is divided into 6 chapters. Each of them, at the same time, is divided into subsections to facilitate the general understanding of the chapter. Each chapter deals with an independent topic from the rest although all of them contribute to the same main objective of the thesis.

Chapter 1. It concludes with this subsection. This chapter serves as an introduction to the final project. The purpose and the justification of the thesis have been set out. Moreover, the structure of the thesis is now described.

Chapter 2. It deals with medical background. Anatomy and physiology of the eye is explained in the first place. Afterwards, some important retinal alterations are set out. This information is important to understand the relevance of the thesis.

Chapter 3. It addresses relevant information about retinal imaging and its processing tools. They have served as a source of learning and inspiration for the development of the algorithm.

Chapter 4. It contains the materials and methods used during this thesis. Techniques used in the algorithm are exhaustively explained.

Chapter 5. It presents the results of the algorithm performance.

Chapter 6. It analyses the results exposed in the previous chapter.

Afterwards, some conclusions will be drawn. Finally, the bibliography will be detailed, and the thesis will be concluded. In addition, the annex includes all the developed MATLAB code.

CHAPTER 2. MEDICAL FRAMEWORK

2.1. Introduction to medical issues

After the first organizational chapter where the layout of the thesis has been established, Chapter 2 is introduced. Chapter 2 is of great importance to understand some concepts treated during the rest of the chapters.

When working on any of the biomedical engineering field two different knowledge areas are fused. These areas are health sciences and engineering. None of them can be underestimated because in the absence of one of them, appropriate medical solutions cannot be conceived. On the one hand, engineering knowledge is the key to solve the problems that posed by medicine. On the other hand, medical knowledge is crucial to entirely identify and understand the mechanisms of pathologies on which action wants to be taken.

Hereinafter, the subsections of Chapter 2 explain important aspects of the eye, the body organ that this final project deals with. The information has been extracted from different sources such as anatomy books and relevant medicine institutions.

Chapter 2 is divided into four subsections, being the first of them this introduction. Secondly, an overview of the human senses is conveyed. In the third place, the anatomy and physiology of the organ of vision is set out. In the fourth place, the retinal alterations which will be discussed in later chapters are described. Finally, more precise information is provided on the target alterations the algorithm will detect.

2.2. Human senses

Sensation/feeling is a mental process that give living beings the awareness of what is happening around them. Human senses are physiological mechanisms of sensation. The purpose of senses is to enable the body to communicate and interact with its surroundings so as to respond appropriately to it and preserve homeostasis [1].

According to Aristotle, in *De Anima*, there are only five types of senses: sight, touch, hearing, taste and smell [2]. They differ from one to another in four criteria. Table 1 shows the four criteria and their content for each Aristotelian sense. Throughout history, there have been many scientists that have claimed to be more than only five senses. Therefore, more than one sense's taxonomy is known [2].

	Vision/Sight	Touch	Hearing	Taste	Smell
Representation	Color, shape and movement in front of our eyes	Temperature, pressure, shape and movement at the surface of our body	Sounds, volume, pitch, objects being struck or vibrated	Flavours in the mouth or on the tongue	Odours located either in the nose or in the air around the nose
Phenomenal character	Visual experiences	Tactile experiences	Auditory experiences	Taste experiences	Olfactory experiences
Physical stimulus	Electromagnetic waves	Mechanical pressure and temperatura	Pressure waves	Chemicals	Volatile molecules
Sense organ	Eyes	Skin	Ears	Tongue	Nose

Table 1. Criteria to differentiate the Aristotelian senses.

Source: [2].

2.2.1. Sensory pathways

Sensory pathways consist of the different anatomical structures that allow the human being to be aware of sensations. Every sensory pathway has these four parts: receptors, sensory neurons, sensory tracts and sensory areas [1].

Receptors. Cells responsible for the detection of the stimulus are usually very specific in the type of physic phenomena they are altered with. Once it has changed its original conditions, an electrical impulse is generated through different mechanisms.

Sensory neurons. As part of the neurological system, their function is to transmit the impulse from the receptor to the central nervous system.

Sensory tracts. In the central nervous system, the impulse is transmitted by white matter neurons of the spinal cord in the first place and, finally, of the brain.

Sensory areas. They are the areas of the brain in which sensory information is processed. Consequently, the human is aware of the sensation. Most of the cases, those areas are in the cerebral cortex.

2.2.2. Characteristics of sensations

Aristotelian senses share some characteristics, known as characteristics of sensations. They are described as follows [1]:

Projection. Sensations, which are recognized in the sensory areas, seem to come from the area where the stimulus has altered the receptors. The projection of sensations can explain the phantom pain. The phantom pain consists of the appearance of sensation in a limb after it has been amputated. This happens because nerves endings still generate electrical impulses and so the information is transmitted to the brain.

Intensity. The degree of the stimulus is reflected on the sensation. The greater degree of stimulation, the more intensity of the sensation is perceived. The stimulus affects different number of receptors depending on the intensity with which it is applied. The number of receptors stimulated is directly proportional to the number of electrical impulses that are going to be transmitted to the sensory areas.

Contrast. The brain tends to compare a new sensation of one type to the previous sensation of the same type. Having this said, make sense to say that sensations can be exaggerated or diminished depending on the current surroundings.

Adaptation. When receptors are continuously over time stimulated with the same grade of intensity, they reduce the number of electrical impulses generated. As a result, human beings become unaware of unremitting stimulus. For example, pressure receptors in the skin adapt very quickly. That is why, most of the time, people are not aware of presence of different items in their bodies, such as watches, clothes, or hats.

After-image. Sometimes sensations can be perceived even though the stimulus over the receptors no longer exists. This happens, for example, when a bright light stimulates the retinal receptors. Electrical impulses continue to be transmitted to the visual area in the cortex until shortly after the stimulation has ceased.

2.3. The eye

The eye is the organ in charge of vision, as reflected in Table 1. Both eyes are in the same coronal and transverse planes. However, they are symmetric respectively from the mid-sagittal plane. Each one receives the name of the half where they are located, right and left [3]. Hereinafter the anatomy and physiology of the eye will be briefly described so as to facilitate the comprehension of this thesis.

2.3.1. Eye's anatomy

The eye consists of the eyeball and the optic nerve. The bone cavities structures that protect the eyeballs are the orbits. The orbits are formed by the lacrimal, maxilla, zygomatic, frontal, sphenoid, and ethmoid bones. They resemble quadrangular pyramids whose bases are directed anterolaterally and their apices, posteromedially. The axis of a quadrangular pyramid is the imaginary line that connect the apex with the middle point of the quadrangle. The orbital axes of both orbits diverge approximately 45° . Nevertheless, the optical axes of both eyeballs are parallel in the primary position of the body. The optical axis is the direction in which the sight is focused [1], [3].

A) Layers of the eyeball

The eyeball is a complex layered spherical structure inside of which are located the receptors of the sight sense. It measures approximately 25 mm in diameter. It is suspended inside the orbit since it is attached to six extrinsic muscles and a fascial suspensory apparatus. The muscles have another function. They make possible the coordinate movements of the eyeball achieving a full use of the sense of sight.

The eyeball is composed of three layers as it is schematized in Figure 1. They are the outer fibrous layer, the middle vascular layer, and the inner layer. Each one has different roles to contribute to the sense of vision. There is an external connective tissue layer whose only function is to support it within the orbit. The three layers are explained below in order from outer to inner part of the eyeball [1], [3].

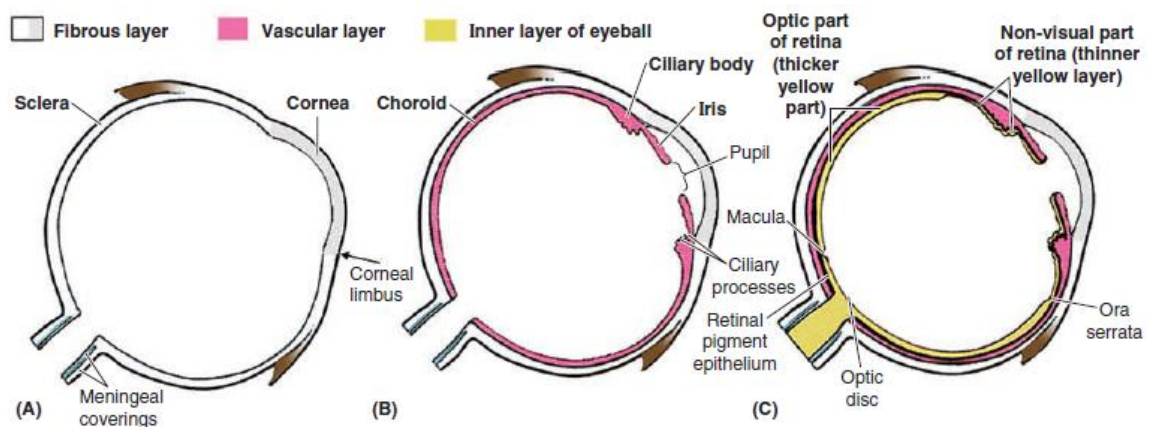


Figure 1. Layers of the eyeball. Superior views of transversely sectioned right eyeball.

A. Outer fibrous layer. B. Middle vascular layer. C. Inner layer.

Source: [3]

Fibrous layer. The fibrous layer Figure 1.A. is made of connective tissue and provides resistance to the eyeball. It also defines the spherical shape. This layer is formed by the sclera and the cornea. The sclera covers the posterior five sixths of the surface of the eyeball. It is the attachment surface for the extrinsic muscles mentioned before and for the intrinsic muscles of the eye, which will be explained later. The sclera is responsible for the white color seen from the outside [3].

The one sixth of the surface left is covered by the cornea. In contrast to the sclera, this structure is transparent and more convex. It is of great importance its transparency because it is the first structure where the electromagnetic waves refract in order to reach the visual receptors appropriately. Another difference between both parts of the fibrous layer is the vascularity: while the sclera is relatively avascular, the cornea is completely avascular to achieve a high degree of transparency [3].

Vascular layer. The vascular layer of the eyeball Figure 1.B. is also called uvea. It contains three differentiate structures: choroid, ciliary body, and iris. The choroid lines most of the sclera. It is highly and tidily vasculated; the vessels are thicker in the outer part and thinner in the inner part.

It supplies nutrients to the adjacent structures which are the sclera in the outer layer and retina in the inner one. The choroid has the highest perfusion rate per gram of all the tissues of the body. Due to the abundance of the vessels, the color of the choroid is reddish brown.

Moreover, the ciliary body is a ring-like structure that connect the other two parts of the vascular layer (choroid and iris). It has a circular muscle which is connected with the lens through suspensory ligaments. The contractions and relaxations of it help to focus the lens [1], [3].

Finally, the iris is a contractile diaphragm made up of two sets of muscle fibers. It also has pigments of different colors due to genetics, and its manifestation is known as the 'eye color'. The iris' central opening is the pupil, the place where light gets inside of the eyeball. The iris is innervated by the oculomotor nerve, and it can be stimulated parasympathetically or sympathetically. The parasympathetic response consists of the contraction of its circular fibers and, as a result, the pupil contracts so that less light goes through it. By contrast, the sympathetic response contracts the radial fibers, the pupil dilatates and more light goes inside the eyeball. These involuntary movements are reflexes that protect the retina from intense light [1], [3].

Inner layer. Lastly, the inner layer of the eyeball is the retina Figure 1.C. It is the sensory neural layer of the eyeball. It covers the posterior two-thirds of the inner surface. The retina consists of two functional parts with different locations in it: the optic one and the non-visual one. The microscopic structure of the retina is represented in Figure 2. The optic part terminates anteriorly along the *ora serrata* which is the irregular posterior border of the ciliary body. It has two layers: a neural layer and a pigmented layer.

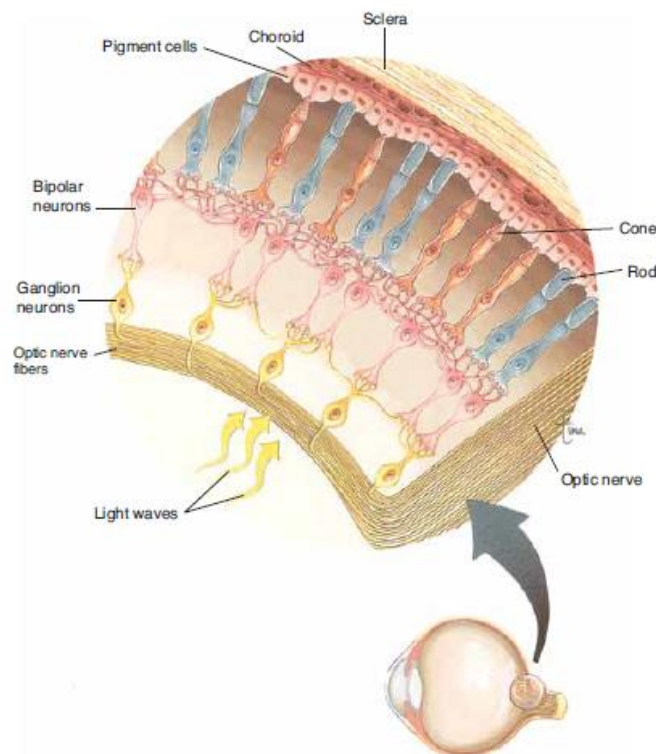


Figure 2. Microscopic structure of the retina around the optic disc.

Source: [1].

The pigmented layer is made of a single layer of cells with the purpose to, along with the choroid, absorb the light so as to reduce scattering of light in the eyeball.

The neural layer consists of the first stages of the visual sensory pathway (receptors and sensory neurons). The light receptors are rods and cones, and they detect the presence of light and colors, respectively. They distribute heterogeneously in this layer, being the rods more abundant towards the periphery of the retina. On the other hand, cones are more copious in the center of the retina increasing in an oval area called the macula lutea. The macula lutea is imaginatively crossed by the optical axis. In the center of the macula there is a depression called the fovea centralis, where the density of cones reaches its peak. The fovea centralis is known as the area of most acute vision [1], [3].

Another important part of the retina is the optic disc. It is located on the optic functional part of the retina. However, the optic disc is insensitive to light. This is due to the fact that it is the place where sensory fibers and vessels convey in order to go through the layers of the eyeball. The optic disc is often known as blind spot for the already explained fact [3].

B) Segments and chambers of the eyeball

To fully understand the eyeball, it can be explained from another perspective. The eyeball can be divided into the anterior and posterior segments.

Anterior segment. The anterior segment is further split into the anterior and posterior chambers. The anterior cavity is between the cornea anteriorly and the pupil and iris posteriorly. The posterior cavity, on the other hand, is limited by the pupil and iris anteriorly and by the lens and ciliary body posteriorly. Both chambers of the anterior segment are occupied by aqueous humor. Aqueous humor is produced in the posterior chamber by the ciliary body, and it goes through the pupil to the anterior chamber. It is clear so as to let the light pass through it and its function is to provide with nutrients the cornea and the iris since they are avascular. Aqueous humor is drained by the limbal plexus. To maintain the pressure of the aqueous humor in the anterior chamber at appropriate levels is of relevant importance to prevent some important diseases such as glaucoma. The pressure of the aqueous humor is also known as intraocular pressure (IOP) [1], [3].

The lens is part of the anterior chamber, between the iris and the posterior chamber, to be more precise. It is a solid clear structure with biconvex moldable shape enclosed in a capsule. Its function is to focus the electromagnetic waves in a suitable way in order to form images true to reality. The shape of the lens can be changed by the action of the ciliary muscles depending on the distance of the objects wanted to be focused. This conscientious process receives the name of accommodation. In presence of parasympathetic stimulation, the ciliary muscles contract and the zonular fibers (they bind these muscles with the capsule of the retina) relax. In consequence, the lens becomes more spherical and near objects can be well focused. On the contrary, in absence of nerve stimulation, the zonular fibers tightened, and the convexity of the lens is reduced. This way distant objects are focused [1], [3].

Posterior segment. This segment also receives the name of vitreous chamber as it is full of vitreous humor. Vitreous humor is a clear semisolid substance for the same reason as the aqueous humor (let the light pass through it). The posterior segment is limited by the lens and the retina. Its function is to maintain the retina in place. Loss of vitreous humor could be the trigger of retinal detachment of the choroid with the vision problems it entails [1], [3].

2.3.2. The optic nerve

The optic nerve is responsible for the transmission of the electrical impulses from the receptors (rods and cones in the retina) to the visual sensory area in the cerebral cortex. Sensory fibers distributed in the optic part of the retina are gathered in the optic disc. The optic nerve begins at the sclera and when it pierces out of the eyeball it becomes myelinated. It exits the orbit via de optic canals and through sensory tracts it reaches the cerebral cortex [3].

2.3.3. Refractive path to the retina

Refraction is the change of pathway that a wave undergoes when it passes from one medium to another with different refractive indices. It only occurs if the wave is obliquely incident on the separating surface of both medias and if the two media have different refractive indices.

In this case, the light waves refraction in the eye, there are four refractive media. All of them, as expressed previously are clear so that most of the light passes through them and the receptors in the retina can receive the most information possible. The four refractive media are cornea, aqueous humor, lens and vitreous humor in this order. Most of the refraction is produced in the first stage, in the interface between the cornea and aqueous humor. However, as the cornea always has the same degree of convexity, another stage of refraction is needed to focus appropriately objects in the near and far field of view. The lens is the structure that bends conscientiously. This way the electromagnetic waves finally go through the vitreous humor and strike in the retina. Figure 3 is a representation of the refractive path to complement this information [3].

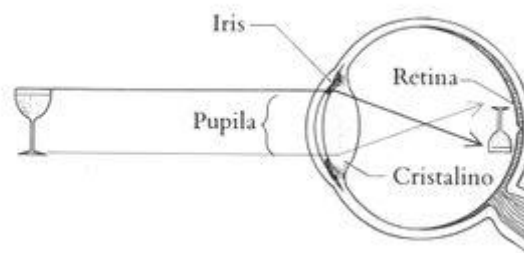


Figure 3. Refractive path in the eye.

Source: [4]

In the retina, the image is projected upside down because of the nature of the refractive path. Therefore, it is the brain that is in charge of processing the information so as to turn the image the right way up.

2.3.4. Physiology of vision

When the light strikes the retina, both rods and cones are stimulated. Immediately chemical reactions begin within these cells in order to generate electrical impulses. In rods, the protein rhodopsin breaks down to form the protein scotopsin and a derivate of vitamin A, retinal. During these chemical reaction, electrical impulses are generated [1].

Chemical reactions in cones also involve retinal. It is believed that there are three types of cones to differentiate the three primary colors: red absorbing, blue absorbing, and green absorbing cones. Depending on the wavelength of the light that strikes the cones each type of cones will be stimulated with different strength and that will be reflected on the electrical impulses each type of cones generate [1].

2.4. Retinal diseases

Clinical manifestations, also knowns as clinical picture, are the patient's signs and symptoms related to a certain disease. A disease can be express not only in the organ or system where it is generated, but also it can manifest anywhere in the body. The knowledge of the clinical picture of a disease is of great importance to speed up the diagnosis, and therefore the treatment, of a disease.

Nowadays, many retinal alterations are known. It is important to diagnose them with swiftness because in many cases advanced stages can result in the loss of vision. Retinal alterations can be either signs of a specific disease or not. This section is dedicated to a short review of two of the main reasons for blindness among adults aged 50 years and older in 2020, according to a survey methodology, the Rapid Assessment of Avoidable Blindness (RAAB) [5]. These retinal diseases can be diagnosed via retinal image analysis.

2.4.1. Glaucoma

Intraocular pressure (IOP) in an adult person oscillates in its normal values between 12 and 22 mmHg. Values of IOP above 22 mmHg are considered higher than normal [6]. The pressure of aqueous humor can increase because of failures in its drainage. If IOP of the anterior segment increase, so does the pressure in the posterior segment.

Glaucoma is a disease where the optic nerve is damaged because of the higher pressure that its fibers are subjected. If the compression persists over time these cells die, and the nerve cannot

transmit the electrical impulses the visual receptors generate [7]. Some risk factors of glaucoma are the following [7]:

- Age over 40.
- Family medical records of glaucoma.
- High eye pressure.
- Myopia and hyperopia.
- Long-term steroid medications.
- Diabetes, migraines, high blood pressure and poor blood circulation.

2.4.2. Diabetic retinopathy

According to the World Health Organization [8], diabetes is a chronic, metabolic disease characterized by elevated blood glucose levels, or glycemia. Over time it leads to serious damage important structures of the body such as heart, blood vessels, eyes, kidneys, and nerves.

Diabetes retinopathy is an eye disease caused by diabetes. Diabetic retinopathy affects, to be precise, retinal blood vessels. Blood vessels in the retina can be altered, partially disrupted, or created. Alteration means that they can be stretch so that their diameter is higher, and, as a result leak blood or they can reduce their diameter so that the blood cannot pass through with the same ease. There are two main stages of diabetic retinopathy. Non-proliferative diabetic retinopathy can degenerate into proliferative diabetic retinopathy if the first one is not well treated in time [9].

Non-proliferative diabetic retinopathy (NPDR). It is quite common among diabetic patients and their vision is blurry. Tiny blood vessels can respond to the disease in two different ways: swell or occlusion. When tiny blood vessels swell, so does the macula. This state of the macula is called macular edema and it can be the reason of vision loss. On the other hand, when the vessels close off, the macula suffer from macular ischemia. In consequence, exudates can form in the retina and affect the vision.

Proliferative diabetic retinopathy (PDR). This is the more advanced stage of the disease. It is characterized by the neovascularization, which is the development of new blood vessels. These new structures are more fragile than usual, and they often bleed into the vitreous. Due to little bleeding the person affected might see a few dark floaters. However, if the bleeding is abundant, it could block vision completely.

2.5. Algorithm target alterations

The proposed algorithm, as described later, detects some alterations that relate with the diseases previously exposed. This subsection is intended to explain those alterations and help the reader to identify them in a fundus image. It is important to point out that the presence of this alterations do not unequivocally confirm the corresponding disease. On the contrary, further exploration and confirmation is needed.

2.5.1. Cup to disc ratio

Cup to disc ratio, CDR, is a measurement that help ophthalmologists to diagnose and assess the progression of glaucoma. Optic disc can be either flat or have a certain amount of normal cupping. However, with the increase of intraocular pressure characteristic of glaucoma disease, there is often a pathological cupping of the optic disc [10].

$$CDR = \frac{disc_{diameter}}{cup_{diameter}}$$

Mathematical expression 1. Cup to disc ratio. Diameters.

CDR is calculated as described in Mathematical expression 1. To calculate this ratio, two parameters are needed: optic disc diameter and optic cup diameter. The normal CDR is less than 0.5. Consecutively, when CDR exceeds this value, it can be an indicative of glaucoma. In Figure 4 are the optic disc and cup perimeters highlighted and two arrows are indicating the diameter of them.

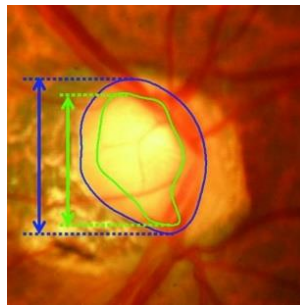


Figure 4. Disc and cup areas marked.

Source: [11].

In the development of the algorithm the CDR is not calculated as in Mathematical expression 1. Instead of the diameters of these structures, the areas of them are used to calculate the ratio. It is more robust in this way, because, as the perimeters are not perfect circumference or ellipses, the diameters vary depending on the inbounds or outbounds of the perimeter in the measurement place. The relation of area and diameter led to Mathematical expression 2.

$$CDR = \sqrt{\frac{disc_{area}}{cup_{area}}}$$

Mathematical expression 2. Cup to disc ratio. Areas.

2.5.2. Microaneurysms

Microaneurysms are local disturbances or outbounds of the retinal blood vessels. They are often the earliest sign of diabetic retinopathy [12]. These protuberances could lead to leakage and retinal edema. In a fundus image such as Figure 5 they look like red dots scattered in all the retinal area.

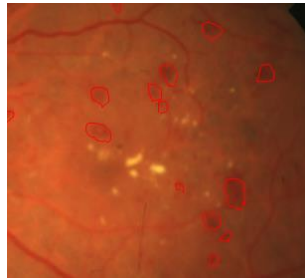


Figure 5. Fundus image with microaneurysms highlighted.

Source: [13].

2.5.3. Exudates

Exudates are lipid residues that leak from the damaged blood vessels, often capillaries. They are characteristic of more diseases than diabetic retinopathy. That is why it is not the only feature searched in the proposed algorithm. In Figure 6, and in every fundus image containing exudates, they are presented as white little regions dispersed in the retina.

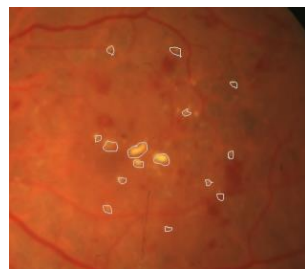


Figure 6. Fundus image with exudates highlighted.

Source: [13].

CHAPTER 3. RETINAL IMAGING AND RETINAL IMAGE PROCESSING

3.1. Retinal imaging

The existence of retinal imaging has contributed to the development of medicine in the area of ophthalmology. It forms part of the non-invasive medicine. It is crucial that the eye remains untouched and unaltered while trying to identify or assess the development of pathologies or diseases. Otherwise, it will cause deterioration in the vision organ. The storage of this image allows the ophthalmologists to analyze them without the patient presence and makes possible the posterior processing of them.

The first step in retinal imaging was the invention of the ophthalmoscope by von Helmholtz in 1851 [14]. Two years later, in 1853 the first drawings of retina were published by the Dutch ophthalmologist van Trigt [14]. With the time, inspection and evaluation of the retina with ophthalmoscope, whose technical name is funduscopy, became a routine for medicine professionals [14].

More than 10 years later, in 1886, the first fundus image was taken by Jackman and Webster [15]. Later in 1926 the first fundus camera was commercialized [15]. These devices receive the name of retinograph and the procedure and the images taken by it, retinographies. Retinography, is therefore, used to name the technique used and the resulted digitalized and colored images.

A major limitation of retinographies is that they are 2 dimensions representations of the fundus of the eye while the retina has changing thickness, that is 3 dimensions. That is the reason why later other techniques appeared such as Optical Coherence Tomography (OTC) [14].

In daily routines, ophthalmologists check the conditions of the retina with non-invasive procedures that are safe for the patients and themselves and cost-effective. Direct verification with ophthalmoscope is the standard. However, if some alteration is perceived by the professional, he/she can determine the realization of more complex techniques such as retinography and OTC for the posterior analysis of the images.

3.2. Retinal image processing

As the informatics developed through the years, so did the tools used for image processing in all fields, including healthcare. The autonomous segmentation and classification of medical images makes the healthcare sector more effective so that costs and waiting lists and are reduced. The earlier any pathology is detected, the earlier it can be overcome and, consecutively, side effects reduced.

Retinal image processing started with the first publication of a method for retinal vessel segmentation by Matsui et al. Their approach was based on mathematical morphology, and they used fluorescein angiograms, where vessels contrast more than in a normal retinography [14].



Over the years, more informatic techniques have been applied to healthcare images. The field had a huge change in the 1990s with the introduction of digital filter-based image procedures. The number of publications rapidly increased [14].

The latest informatic improvement that is revolutionizing the sector is the artificial intelligence (AI). AI implies the computer capacity of learning, assimilating, and acting in accordance, activities related to living beings [16]. One of the models for manipulation of AI is neural networks. There have been so many advances in medical imaging with them and it is a research field little exploited so far. Therefore, artificial intelligence is the state of the art in terms of retinal image processing.

An application where processing of the medical images is hugely useful is the population screening procedures. Medical screening is a strategy based on the systematic application of techniques to direct preventive detection of first signs or stages of diseases. During the course of the screening, a lot of information is gathered. In medical imaging, it means that hundreds or thousands of images need to be storage and then analyzed. The one-by-one image examination of those files would mean a lot of resources both of medicine professionals, time, and money. By the use of automated techniques in image processing the process is optimized in all those aspects.

All in all, medical image processing improves healthcare sector and life expectancy of patients that beneficiate from it. Image processing techniques can be considered clinical decision/diagnostic support systems. It is, therefore, a very promising area of research.

CHAPTER 4. MATERIALS AND METHODS

4.1. Main tool

MathWorks is an American privately held corporation specialized in producing mathematical computing software for engineers, scientists, mathematicians, and researchers. Its most famous products are MATLAB and Simulink.

MATLAB is the abbreviation of *MaTrix Laboratory*. It is a programming platform with which different systems and products can be analyzed and designed with the purpose of transforming the world. MATLAB high-performance language is matrix based so it allows a natural expression of computational mathematics. The typical uses of MATLAB include data analysis, algorithms development, and creation of models and applications [17].

MATLAB has a vast function library to facilitate the user's work quickly and efficiently. These functions include computational algorithms which include from elementary functions like sum, sine, and complex arithmetic to more sophisticated functions like Fourier transforms. MATLAB capabilities can be extended thanks to the toolboxes which can be installed.

During the development of the proposed algorithm, MATLAB was the programming platform used. It has been the most used tool during the Biomedical Engineering Degree at UPV and therefore there is an ease of working with its interface. Special mention might be given to 'Image Processing Toolbox'. It includes a lot of image processing techniques functions that speed up the process of creating image processing algorithms.

4.2. Images format: Portable Network Graphics

Portable Network Graphics, most commonly known as PNG, is a file format used for lossless image compression [18]. It was developed by an Internet working group headed up by Thomas Boutell that came together in 1994 to work in the project of creating a new file format. PNG's lossless compression rate (using Deflate Algorithm) is 10% superior to GIF file format. Lossless compressions make it perfect for storing digital images with the purpose of editing. PNG is one of the file formats of the input images of the algorithm developed.

The PNG format has the following features:

- It supports translucent pixels. One color pixel can be converted to transparent. The degree of transparency, opacity, can be controlled.
- PNG images can be saved using true color, palette, and grayscale format.
- It offers the possibility to use more than 256 colors.

4.3. Images format: Joint Photographic Experts Group

Joint Photographic Experts Group, which abbreviation is JPEG or JPG, is the other file format used in the proposed algorithm. JPEG and PNG are the two file formats most used on the web.

JPG images take up very little storage space and they are quick to upload and download. Its main advantage over the rest of file formats is capacity to compress. Nonetheless, during the compression some redundant information is deleted from the file permanently.

4.4. Imaging processing techniques included in the algorithm

For the development of the proposed algorithm, different techniques of image processing have been studied in depth theoretically and practically. This subchapter is devoted to the explanation of the main techniques used so that the whole comprehension of the algorithm is conceivable.

Firstly, several intensity transformations are explained. Secondly, related pixel operations are described. Finally, segmentation is clarified. Watershed technique is the most important segmentation technique in this algorithm.

4.4.1. Intensity transformations

This group of transformations can be easily identified by the following characteristic. They are punctual operators, that is to say that the updated value of one pixel only depends on its previous value. The intensity transformations included in the proposed algorithm are brightness adjustment, contrast adjustment, shade correction, thresholding, complementary image, and arithmetic operations between two images.

A) Brightness adjustment

Brightness of an image manifests itself through the intensity of the pixels. The intensity of pixels varies between 0 and 1 or 0 and 255, corresponding 0 and 255 to white color in gray scale images. It can be easily modified by adding or subtracting some constant B to the average of the pixel intensities in the image, as expressed in Mathematical expression 3. The result in the histogram is the displacement of itself. Therefore, pixels that were previously close to black or white color can be saturated to black or white, respectively.

$$im_{new} = im + B$$

Mathematical expression 3. Brightness adjustment.

Figure 7 shows a piece of created code in MATLAB. The process to adjust brightness is described. There, im_g is the target image of this transformation. The brightness will be decreased in dif_media intensity unities, difference between the mean value of the input and reference images.

```
% Adjustment of the brightness with the reference image.
dif_media = media_im - media_im_ref;
im_g = im_g - dif_media;
```

Figure 7. MATLAB code. Brightness adjustment.

Source: TFG_code().

B) Contrast adjustment

Contrast of an image is also known as dynamic range. It is the difference between the maximum and minimum value of pixel intensity in the image. In this way, the more difference, the more contrast. It also can be modified with simple pixel by pixel operations, as can be deduced from Mathematical expression 4. When the factor C is over 1, the contrast is enhanced. Otherwise, contrast is reduced, and consecutively, there is less difference between a pair of pixels brightness.

$$im_{new} = C * (im - im_{average}) + im_{average}$$

Mathematical expression 4. Contrast adjustment.

MATLAB has an own function to adjust the contrast of an image, $imadjust()$. Figure 8 presents an example of the instructions that lead to contrast adjustment in the proposed code.

```
% The green channel of the RGB color space is the one that shows the best
% contrast for red lesions. This will be the one used.
im_g = im (:, :, 2);
im_g = imadjust(im_g);
media_im = mean2(im_g);
```

Figure 8. MATLAB code. Contrast adjustment.

Source: TFG_code().

C) Shade correction

During the acquisition of medical images there can be problems because of the non-uniform background illumination. If it is not corrected, it can lead to misdiagnoses, specially through image processing. The solution is shade correction. The background illumination can be estimated with different techniques. In the proposed algorithm it is done with a gaussian filter, which removes high frequencies from the image. More about gaussian filters will be explained afterwards. Finally, the background of the image is subtracted from the original image. Mathematical expression 5 represents what has been explained.

$$im_{new} = im - background$$

Mathematical expression 5. Shade correction.

Figure 9 corresponds to a portion of the *TFG_code()* function where the shade correction method is developed. Firstly, a median filter is applied, and the background is obtained. Then, after some change of image format and contrast adjustment, the background is subtracted from the original image. The sum of 128 intensity units is to solve the problem of negative subtraction.

```
% Shade correction. We should create a median filter and subtract it from
% the image. A mask will be applied before subtraction operation. The
% problem with subtraction is the negative saturation. Values bellow 128
% will be negative differences.
im_sc = medfilt2(im_g,[40 40]);

im_g = int16(im_g);
im_g = imadjust(im_g);
im_sc = int16(im_sc);
im_sc = imadjust(im_sc);
im_corrected = imsubtract(im_g,im_sc) + 128;
```

Figure 9. MATLAB code. Shade correction.

Source: *TFG_code()*.

D) Thresholding

Thresholding is a very useful processing technique that aims to obtain binary images in which only pixels with a certain intensity value (or inside a range of intensity values) in the original image are set to 1 and the rest of them are set to 0. Typically, these binary images are also known as masks. There are simple, double or semi thresholds. In the proposed algorithm, only simple thresholds are applied for example to obtain a mask of the retinal vessels. Mathematical expression 6 represents the thresholding technique where pixels over 200 of brightness will be set to 1 in the mask.

$$mask = im > 200$$

Mathematical expression 6. Thresholding.

Figure 10 gathers some lines of the code of *segmentation_disc()* in which the last one shows how the threshold is carried out. In this case, the threshold of the image *imR* is the pixel intensity where the 99.06 % of the pixels have a lower intensity.

```
% Threshold image.
hist = imhist(imR);
[height,width] = size(imR);
npixel= height*width;
imhist_norm = hist/npixel;
hist_acum = cumsum(imhist_norm);
P = 0.04;
rmax = find(hist_acum>=(1-P),1,'first');
imThres=imR>rmax;
```

Figure 10. MATLAB code. Thresholding.

Source: *segmentation_disc()*.

E) Complementary image

The complement of an image contains the exact same information as the previous image. The histogram of the complementary is inverted so that those pixels that were white are now black, as deduced from Mathematical expression 7. The same happens with the intermediate pixels of the gray scale. This transformation can be used to modify an image before using it in another preprocessing techniques such as opening or watershed.

$$im_{complement} = 255 - im$$

Mathematical expression 7. Complementary image.

MATLAB has an own function created specially to achieve the complementary image, Figure 11. In consequence, *imcomplement()* is used to reduce the complexity of the developed code.

```
negativo_imu = imcomplement(imu);
```

Figure 11. MATLAB code. Complementary image.

Source: segmentation_cup().

F) Arithmetic operations between two images

Sometimes, it is opportune to make some arithmetic pixel by pixel operations between two images. In order to do so, the images must have the same size in terms of rows and columns. The most typical operations are sum, subtraction and multiplication. Multiplication is represented in Mathematical expression 8.

Shade correction is one type of images subtraction where two images are involved, but because of its importance in the algorithm it has been explained in more depth. Moreover, multiplication of two images is used in the algorithm to multiply a mask with an image.

$$im_{new} = im * mask$$

Mathematical expression 8. Pixel by pixel multiplication.

Figure 12 presents a piece of the code where two images are multiplied to obtain a third image. The multiplication can be done as expressed on Mathematical expression 8. However, MATLAB has an own function to do so, *immultiply()*.

```
% Shade correction. We should create a median filter and subtract it from
% the image. A mask will be applied before subtraction operation. The
% problem with subtraction is the negative saturation. Values below 128
% will be negative differences.
im_sc = medfilt2(im_g, [40 40]);
mask = im_g > 30;
im_sc = immultiply(im_sc, mask);
```

Figure 12. MATLAB code. Pixel by pixel multiplication.

Source: TFG_code().

4.4.2. Related pixel operators

Modifications of images through related pixel operator are more complex than intensity transformations. In contrast to intensity transformations, related pixel operators consider the intensity of the pixel whose intensity is being modified as well as the others of the pixels with which they share some characteristic. The related pixel operators included in the proposed algorithm are opening, closing, top hat, geodesic distance, and morphologic reconstruction. They are going to be explained based in binary or gray scale images.

A) Opening

Opening transformation γ has many applications in image processing. Previously to image transformation, a structuring element must be wisely created. In the resulted image those bright areas, where the structuring element does not fit, are erased. However, the rest of the images does not vary. The infinite size and shape possibilities of the structuring element makes opening operation greatly powerful. In the algorithm, it is used, for example, to detect vessels in different orientations. Mathematical expression 9 consists of the concatenation of two basic morphological operators. First an erosion operation ε and then a dilatation δ .

$$\gamma(im) = \delta(\varepsilon(im))$$

Mathematical expression 9. Opening operation.

Figure 13 shows a portion of *find_lines()* code where the opening operation is carried out. It is done through the MATLAB function *imopen()*. It is used to find those lines oriented in certain directions. The function *find_lines()* is called in the function *vesselDetection()*.

```

% There will be an opening operation for every search direction. The
% structuring element is different in each operation. N is the number of
% orientations in which the lines are going to be searched. The search
% angles are considered equispaced in the semicircumference.
equiesp = 180/(N-1);
aperturas = zeros(filas,columnas);
]for i = 1:N % for every search direction
    direc_busc = equiesp*(i-1); % search direction
    SE = strel('line',tam,direc_busc);
    aperturas(:, :, i) = imopen(imInput, SE);
-end

```

Figure 13. MATLAB code. Opening operation.

Source: *find_lines()*.

B) Closing

Closing operation φ is substantially similar to opening one. Its main difference is that in the resulted image from closing operation those areas where the structuring element does not fit are erased whereas the rest of the image is not modified. It can be expressed as a dilatation δ followed by an erosion ε as in Mathematical expression 10.

$$\varphi(im) = \varepsilon(\delta(im))$$

Mathematical expression 10. Closing operation.

In order to perform the closing operation, MATLAB has its own function, `imclose()`, as presented in Figure 14. The parameters needed to conduct the operation are the image where it will be applied and the morphological structuring element that is going to be used.

```
% Removal of vessels. Structuring element bigger than the smallest vessel
% to remove.
SE=strel('square',25);
imc = imclose(imR,SE);
```

Figure 14. MATLAB code. Closing operation.

Source: segmentation_disc().

C) Top hat

Top hat is a morphological filter, as opening and closing. It is used to detect little bright areas in binary or gray scale images. In other words, it is the subtraction of the image obtained with opening filtering to the original image, as expressed in Mathematical expression 11. In the new image, there is not any influence of the background so the target areas can be easily identified.

$$im_{new} = im - \gamma(im)$$

Mathematical expression 11. Top hat operation.

Top hat operator has its dual version which is captured in Figure 15. First, the closing function is carried out. As the resulted image of the closing operation has always the same or higher value of intensity pixel for each pixel in reference to its previous value, the dual top hat operation consists of the subtraction of the original image to the output of the closing operation. In this way, the resulted image will have always positive values (or 0).

```
% Removal of the background. Structuring element bigger than the thinnest
% blood vessel. The final image is a mask of the removed vessels.
SE = strel('square',12);
cierre = imclose(imInput,SE);
tophat_dual = mat2gray(cierre-imInput);
```

Figure 15. MATLAB code. Dual top hat operation.

Source: vesselDetection().

D) Geodesic distance function

Geodesic distance function is applied to binary images and the resulted images are always gray scale images. The intensity values of the output image rather than representing structures, they have meaning concerning geodesic distances. In the image obtained, the more brightness of a pixel, the farther that pixel is from the nearest edge. Figure 16 represents the binary input image and the output image of the function described. It is calculated through successive erosions where the input image of an erosion operation is the output image of the previous erosion. The pixels erased in the different erosions receive increased values of brightness.

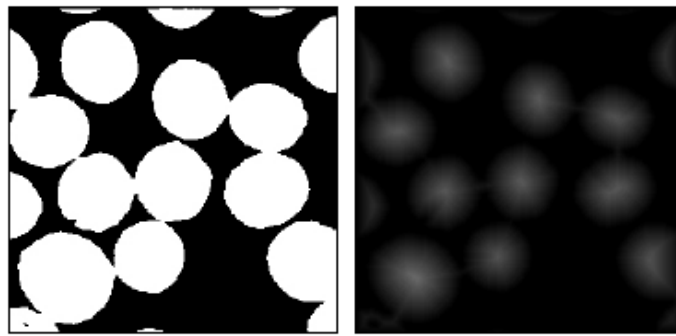


Figure 16. Output of geodesic distance function.

Source: [19]

Figure 17 shows the own MATLAB function to calculate geodesic distances to the nearest edge. `bwdist()` is used in order to obtain the internal marker to the watershed which will be the brightest point in the image, corresponding in most of the cases to the center of the optic cup and disc.

```
% Internal marker to watershed. It will be a binary image where the only
% pixel set to 1 is the brightest pixel in the image.
negativo_imu = imcomplement(imu);
d= bwdist(negativo_imu); % distance to the edge
m_pixel=max(max(d));
mark_int=d>m_pixel-1;
```

Figure 17. MATLAB code. Geodesic distance.

Source: `segmentation_cup()`.

E) Image reconstruction

Reconstruction of an image can be also recognized as region growing operator. To understand this operator is important to define the images used in the operation. Image reconstruction requires two input images: marker and image. Sometimes, instead of image it can be named as mask because reconstruction is typically done with binary images. However, it can be also applied to gray scale images. The marker will always be a binary image where some special pixels are set to 1.

The aim of region growing operator is to reconstruct the structures from the input image whose at least one of its pixels matches the marker image, Mathematical expression 12. It consists of successive multiplications of both images. The result is dilatated and another time multiplied by the original image. When the multiplication does not change anymore, it means it is the output reconstructed image.

$$im_{new} = reconstruction(im) = \begin{cases} marker = \delta(marker) * im \\ repeat\ until\ idempotence \end{cases}$$

Mathematical expression 12. Image reconstruction.

MATLAB has the *imreconstruct()* function which allows the reconstruction process not to be developed for each programmer. The parameters that it needs are the marker and the mask, as expected from the above explanation. In Figure 18 the original pathologies will be grown thanks to image reconstruction where the mask is the preprocess image, and the marker is the binary image where the white zones are part of red lesions.

```
% Image reconstruction. The aim is to grow the original pathologies. The  
% threshold image is the marker in the reconstruction.  
im_red_lesions = im2int16(im_red_lesions);  
imRecons = imreconstruct(im_red_lesions, im_novessels);
```

Figure 18. MATLAB code. Image reconstruction.

Source: *TFG_code()*.

4.4.3. Segmentation

Segmentation consists of the division of an image in different regions with significance. Each region is characterized by one or more aspect such as brightness intensity or color and will be identified by a label. The segmentation process can be divided into two steps. Firstly, the method of segmentation is applied to the original image. Afterwards, the image regions are labeled. Segmentation is greatly helpful to detect some region of interest in an image. There exist different types of segmentation techniques.

In the developed algorithm, watershed is the one used. It is based on considering the image as a topographical relief where local maximums are considered as mountain tops and local minimums, valleys. Watershed divides the images into zones of influence of each local minimum simulating a flooding process.

The main problem is the abundance of local minimum pixels due to noise of the image. The solution is to impose the 'valleys' through a binary image. Figure 19 shows the watershed method with imposed valleys referred to as marker. One region per marker is created. This improvement of the method is also implemented in the proposed algorithm.

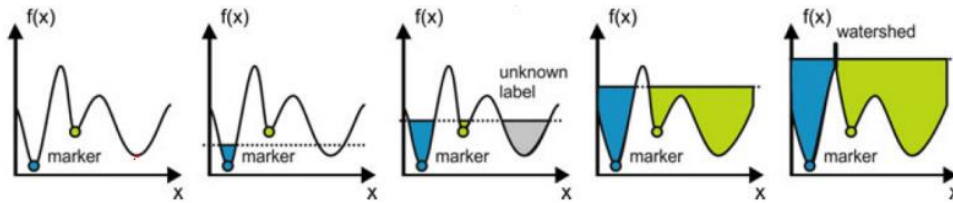


Figure 19. Watershed segmentation method.

Source: [19]

Figure 20 shows the code for two of the MATLAB functions that enables the watershed segmentation to be performed. *imimposemin()* is used in order to impose the minimum values (or valleys) in the image desired to segmentate so as to prevent the problem previously described. Finally, the *watershed()* function returns the image segmented in as many regions as the minimum points imposed.

```
% Imposition of minimum values in the gradient image.
img2= imimposemin(img,mrk);

% Watershed function.
imout= watershed(img2);
```

Figure 20. MATLAB code. Watershed segmentation.

Source: *segmentation_cup()*.

4.5. Algorithm description

This subchapter will be dedicated to the description of the MATLAB code that make up the whole algorithm, whose name is *TFG_code()*. The algorithm is divided into three main parts followed by the display code part. Each part corresponds to one of the three target alterations described in the subchapter 2.5. The objective of the algorithm is to detect these three alterations. Therefore, it serves as diagnostic support system.

4.5.1. Cup to disc ratio calculation

For this section of the algorithm it has been necessary to create two additional functions, *segmentation_cup()* and *segmentation_disc()*. They are quite similar to each other. They prepare an internal and external marker where the internal one is a point in the center of the cup and disc (same point in both cases) and the external one is a circumference with center the internal marker. These are the minimum points imposed to improve the result of the watershed segmentation. Once the optic disc and cup are segmented, CDR is calculated.

The workflow established in *TFG_code()* for the calculation of the CDR can be graphically described in Figure 21. A deeper explanation is needed to ensure the fully understanding of the steps followed.

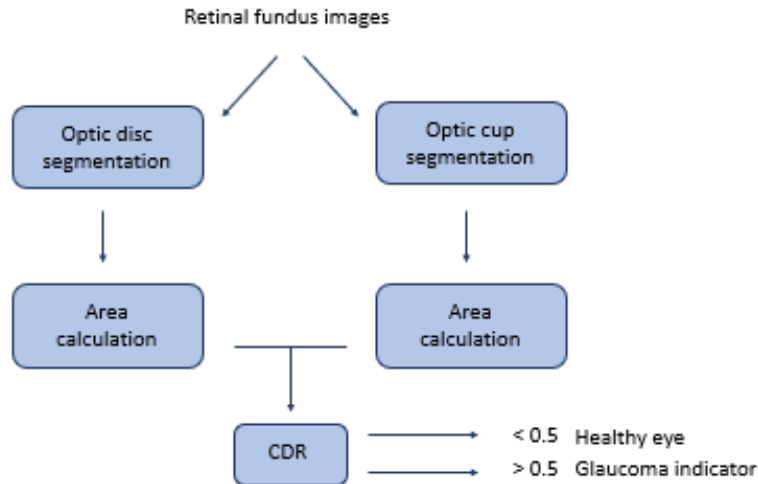


Figure 21. Workflow in *TFG_code()* to determine CDR glaucoma indicator.

Source: Own development.

Firstly, retinal fundus images are the input of two of the functions developed during this thesis, *segmentation_cup()* and *segmentation_disc()*. Once these areas, optic cup and disc, are segmented, the next step is to calculate the area of each target zone. With this information it is possible to conduct Mathematical expression 2 with which the CDR is obtained. Figure 22 is the code where the areas and the CDR are obtained.

```

% Calculation of the cup to disc ratio (CDR).
pix_disc = bwarea(imout);
pix_cup = bwarea(imout_CUP);
CDR = sqrt(pix_cup/pix_disc);
  
```

Figure 22. MATLAB code. Calculation of CDR.

Source: *TFG_code()*.

The last step occurs during the displaying of the message box. An *if* loop is created so that the message is different depending on the CDR value. If it is above 0.5, then the image corresponds to a possible glaucoma patient. Otherwise, the message informs that the value is in physiological levels.

4.5.2. Microaneurysms detection

Firstly, the algorithm reads and stores the green channel of the RGB color space. Secondly, shade correction is applied to the image in order to remove the background from the image. Thirdly, exudates are removed from the image so that they do not interfere in the further processing. For the same purpose, the retinal vasculature is removed. To do so, an additional function has been created, *vesselDetection()*. The next step is the reconstruction of the image via region growing method where the image is the image from the last stage, and the marker is the threshold image of the same one. Finally, this image is overlapped on the input image so that microaneurysms can be easily recognized.

The workflow of this part of the algorithm is contemplated in Figure 23. Further explanation of the workflows will be provided.

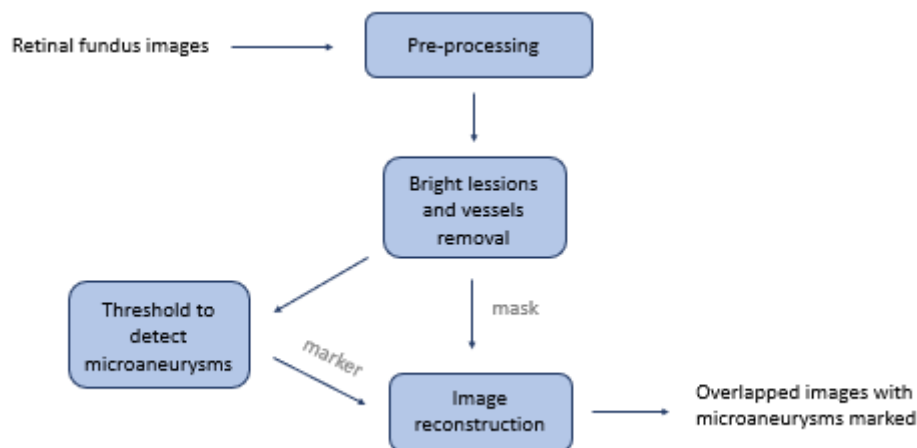


Figure 23. Workflow in *TFG_code()* to detect microaneurysms.

Source: Own development.

The retinal fundus images are the entry information for this part of the code. Immediately, some preprocessing is needed. The green channel of the RGB color space is the one that shows the best contrast for microaneurysms. In consequence, this channel will be the image now. The next step is to adjust the brightness of the image. To do so, one image, *diabetic_09*, which was chosen arbitrarily, is used as a reference to this adjustment. Afterwards, the shade correction operation is carried out in order to not have the interference of the background of the image.

The next step is subdivided into the removal of the bright lesions, Figure 24, and the removal of the vasculature, Figure 25. Having this done, these structures will not further interfere in the detection of microaneurysms. In both cases, two *for* loops are needed in order to browse through all the pixels in the images. Moreover, one *if* loop contains the condition which will be evaluated in the pixels. Depending on its suitability the corresponding pixels will be either set to 128 or maintain its previous intensity value.

```
% Bright lesions removal. Values bellow 128 have an intensity lower than
% the background (red lesions and vasculature). Values higher than 128 will
% be set to 128 for the removal of bright lesions.
im_lesions_remove = im_corrected;
[rows,cols] = size(im_corrected);
]for i = 1:rows
]   for j = 1:cols
       if im_corrected(i,j)>128
           im_lesions_remove(i,j) = 128;
       end
   end
end
end
```

Figure 24. MATLAB code. Bright lesions removal.

Source: TFG_code().

```
% Remove the vasculature. The rows and cols remain the same.
imOut = vesselDetection(im_lesions_remove,mask);
im_novessels = im_lesions_remove;
]for i = 1:rows
|   for j = 1:cols
       if imOut(i,j) == 1 % if the pixel is from the mask.
           im_novessels(i,j) = 128;
       end
   end
end
end
```

Figure 25. MATLAB code. Vasculature removal.

Source: TFG_code().

After that, this image is stored in order to serve later as the mask of the reconstruction operation. At the same time, the image obtained after the pre-processing stage, is further processed with the objective of acquiring a binary image to function as the marker of the reconstruction process.

The image obtained after the removal of the vasculature is threshold in order to set to 1 some of the pixels that correspond to microaneurysms. This threshold can be conducted because these lesions have the brightest pixel values at this stage. As a result, the marker of the reconstruction is prepared.

Finally, the image reconstruction operation is carried out and the image displayed have the lesions overlapped over the original image.

4.5.3. Exudates detection

In this case, the algorithm reads and stores the luminance channel of the HSL color space. To obtain it, `rgb2hsl()` function has been used [20]. The purpose of this image is to be further process to, finally, serve as the marker in the region growing operation. On the other hand, the mask will be the green channel of the original RGB color space. Eventually the image from the reconstruction is subtracted from the green channel, threshold and overlapped as in the microaneurysms case.

The workflow of this piece of algorithm code is presented in Figure 26. Further explanation of the workflows will be provided to gain a full understanding of the code.

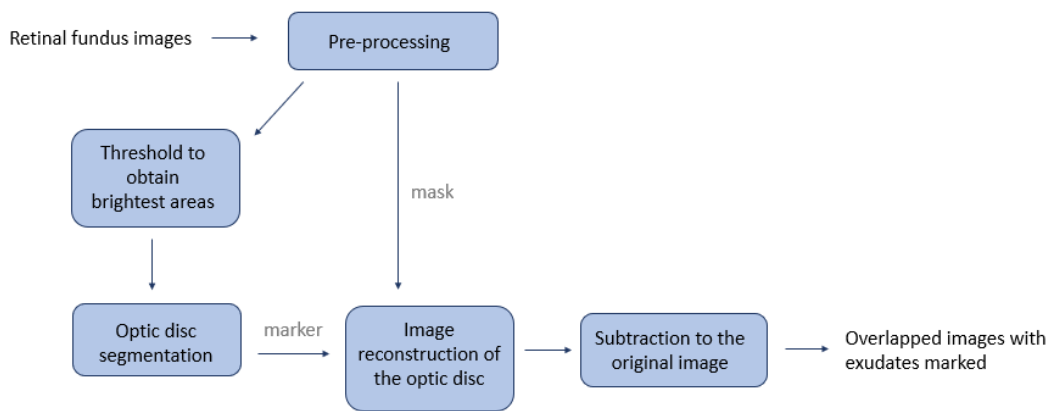


Figure 26. Workflow in `TFG_code()` to detect exudates.

Source: Own development.

The same fundus images that were input in glaucoma and microaneurysms code parts, are here the input as well. In this case, the channel which provides the best contrast to bright lesions is the luminance channel from the HSL color space. So, the preprocessing stage consists of the calling to `rgb2hsl()` function [20] and the election of the appropriate channel, Figure 27.

```

im_2hsl = im2double(im);
hsl=rgb2hsl(im_2hsl);
[~,L,~] = imsplit(hsl);
  
```

Figure 27. MATLAB code. Luminance channel of HSL color space.

Source: `TFG_code()`.

Moreover, the morphologic reconstruction will be conducted with the green channel of RGB color space of the original image as the marker. In consequence, in the pre-processing stage it is obtained and stored for later usage.

Meanwhile, some other instructions are being performed. The luminance image is threshold in order to obtain a mask where the optic disk and bright lesions are set to 1 and the rest of the pixels to 0. Later on, the segmentation of the optic disc through an opening operation where the structuring element is bigger than the biggest bright lesion and smaller than the optic disc results in the marker of the segmentation where there are only pixels of the optic disc set to 1.



With the above-mentioned data, it is possible to carry out the reconstruction of the optic disc. The next stage is to subtract it from the original image. The resulted image does not have optic disk. Consequently, all the pixels whose brightness is above a threshold correspond to exudates.

Eventually, these lesions are overlaid over the original image and are displayed thanks to the display part of the algorithm.

4.5.4. Results display

The last part of the code is also important. The performance of the algorithm is of no use if the results cannot be easily seen and interpreted by the user. The results display section tries to leave no room for doubt.

When *TFG_code()* is ran, the output is in form of images and box messages. The MATLAB functions used are *imshow()*, *subplot()* and *msgbox()*. In the first place, some icons that will be later on used in the displayed box messages are read and stored.

Afterwards, come the calls of the functions name above in order to display the different elements. The first image that appears displays the input image. Secondly, there is a figure with two images which are the overlapped microaneurysms and exudates, if any. Finally, three box messages emerge, each of which informs about the presence of microaneurysms, exudates and high CDR value.

This is the end of the function *TFG_code()*. During its performance more functions have been called. Some of them are also new developed code. To complete this thesis, all the created code is attached in the Annex.

CHAPTER 5. RESULTS

The algorithm *TFG_code()* has been proved to work correctly for nine fundus images. This chapter is subdivided into 3 subsections. Firstly, it is important to point out the sources where this images were extracted. Afterwards, the results are split in the two diseases addressed by the algorithm.

5.1. Image sources

The nine images with which the algorithm has been verify are fundus images of the retina. There are two different types of image formats, described in subchapters 4.2. and 4.3. Moreover, there are three different groups of images in terms of physiopathology: glaucoma images, diabetic retinopathy images and healthy retinal images.

On the one hand, the three glaucoma images are in JPG format. They were extracted from High Resolution Fundus Database [21]. It has been established by a collaborative research group to support comparative studies on automatic segmentation algorithms on retinal fundus images. The gold standard data is generated by a group of experts working in the field of retinal image analysis and clinicians from the cooperated ophthalmology clinics.

On the other hand, there are 3 diabetic retinopathy images and 3 healthy retinal images. They are in PNG format and were selected from STARE Dataset [13]. The project is intended to automatically process retinal images so that the program can determine diagnosis. The STARE Project was initiated in 1975 in U.S. and through the years over thirty professionals of different disciplines such as medicine and engineering have contributed to the project.

5.2. Glaucoma results

The algorithm detects the glaucoma images successfully. A message box informs the user about the CDR calculated and distinguish between a normal and pathological CDR. As previously mentioned in subchapter 2.5.1., there is high possibility of glaucoma when CDR exceeds 0.5.

*The code *TFG_code()* is run for all the nine images that are studied in this thesis. The glaucoma results are displayed in Table 2*

Table 2. The results of CDR for the three glaucoma images were above 0.5, as expected. Therefore, the patients to which these images belong, require of further analysis, or diagnose techniques. Moreover, for the diabetic retinopathy and healthy images this value does not reach that decision threshold.

IMAGE NAME	CDR	GLACUOMA INDICATOR
06_g	0.5237	Yes
08_g	0.62289	Yes
10_g	0.5529	Yes
diabetic_09	0.43288	No
diabetic_136	0.41166	No
diabetic_342	0.38426	No
normal_243	0.32993	No
normal_245	0.42342	No
normal_255	0.47817	No

Table 2. Glaucoma results.

Source: Own development.

Hereafter some figures corresponding to *08_g* image are presented in order to serve as an example of the algorithm performance. Figure 28 is the first output image the algorithm returns, and it corresponds to the input image. Afterwards, Figure 30 which is a message box appears so as to inform of the CDR value and point out that it is a high value. In case of the CDR being in normal values, the output will be different. In particular, Figure 29 is the message box displayed when the input image is *diabetic_09*.

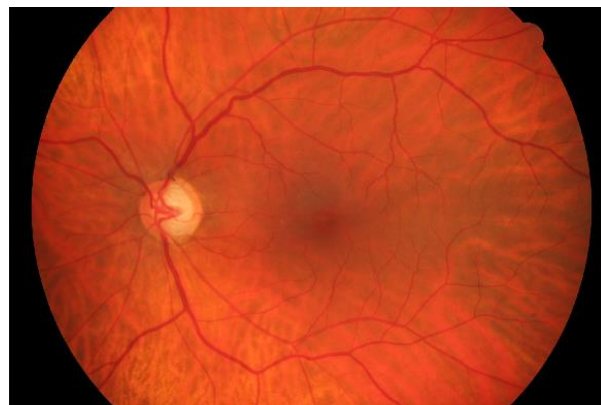


Figure 28. Input glaucoma image to *TFG_code()*.

Source:[21].

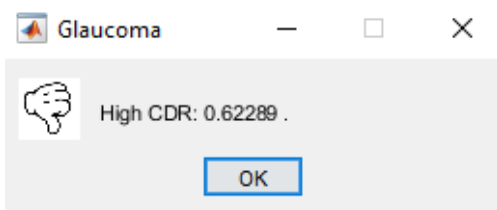


Figure 30. Message box of *TFG_code()*. High CDR.

Source: *TFG_code()*.

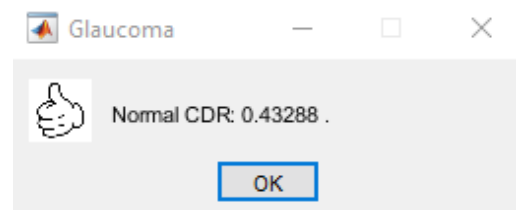


Figure 29. Message box of *TFG_code()*. Normal CDR.

Source: *TFG_code()*.

5.3. Diabetic retinopathy results

To indicate the presence of microaneurysms and exudates the algorithm displays one figure, which contains two images, where they are marked (in case they are detected) and two message boxes informing of the presence or absence of them.

When the function *TFG_code()* is run the instructions start working and the mentioned figures and message boxes appear. These results could be interpreted without the message boxes because they point out something the user can see in the images. However, to avoid confusion, they inform about the existence/non-existence of microaneurysms and exudates. Diabetic retinopathy results can be shown in Table 3. The algorithm determines the presence of both retinal alterations in the three images that presented diabetic retinopathy. On the other hand, there are no alterations detected on the images that do not correspond with the mentioned disease, as expected.

IMAGE NAME	MICROANEURYSMS	EXUDATES
06_g	No	No
08_g	No	No
10_g	No	No
diabetic_09	Yes	Yes
diabetic_136	Yes	Yes
diabetic_342	Yes	Yes
normal_243	No	No
normal_245	No	No
normal_255	No	No

Table 3. Diabetic retinopathy results.

Source: Own development.

Hereinafter, the output images of the code corresponding to diabetic retinopathy will be presented. To present an example, *diabetic_09* image is used. Figure 31 is the image corresponding to the raw image that the algorithm receives. Figure 32 shows the same image as previously, but this time the alterations related with diabetic retinopathy are highlighted. Microaneurysms are presented in red, and exudates are shown in blue.

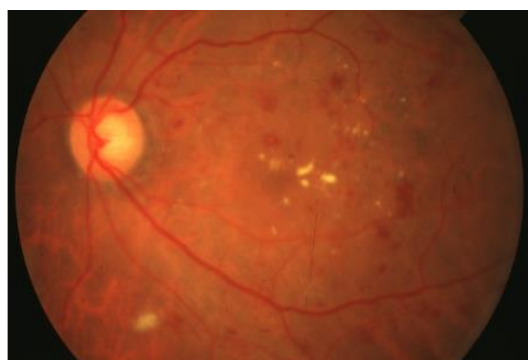
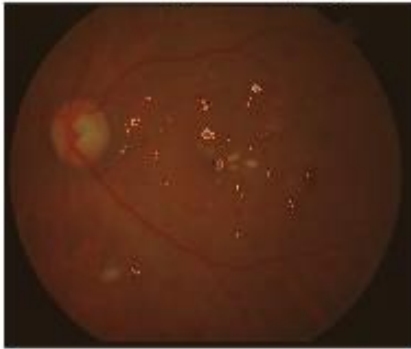


Figure 31. Input diabetic retinopathy image to *TFG_code()*.

Source: *TFG_code()*.

Microaneurysms highlighted



Exudates highlighted

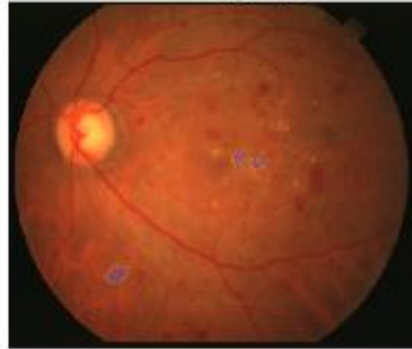


Figure 32. Output images of `TFG_code()`.

Source: `TFG_code()`.

To conclude, the message boxes appear during the algorithm performance. For the example image, *diabetic_09*, Figure 33 and Figure 34 inform of the presence of both, as it was previously clarified. In the contrast, when there are no alterations detected, the message boxes are different. Figure 36 and Figure 35 correspond to the output of the image *normal_243*.

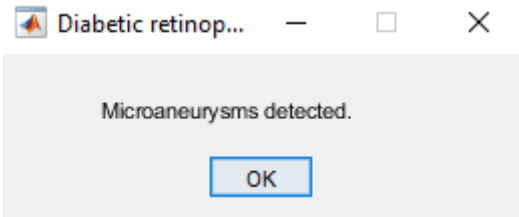


Figure 33. Message box of `TFG_code()`.
Microaneurysms detected.

Source: `TFG_code()`.

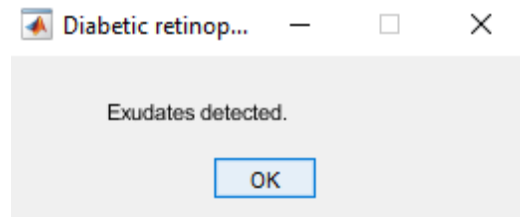


Figure 34. Message box of `TFG_code()`. Exudates detected.

Source: `TFG_code()`.

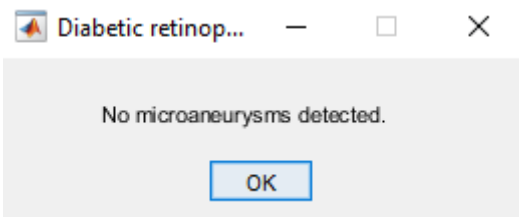


Figure 36. Message box of `TFG_code()`. No microaneurysms detected.

Source: `TFG_code()`.

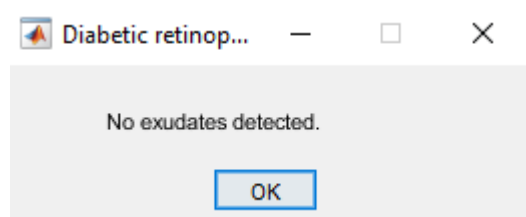


Figure 35. Message box of `TFG_code()`. No exudates detected.

Source: `TFG_code()`.

CHAPTER 6. DISCUSSION OF THE RESULTS

As it was presented in Chapter 5, the algorithm *TFG_code()* was capable of detecting corresponding alterations in the nine selected images. Healthy retinal images are the control samples in this project. They show that when there is no alteration in the retina, the code does not detect anything. However, the algorithm does not have a perfect execution, it has some weak points as well.

In order to present some results in which the weak points are exhibit, Figure 37 is displayed. This fundus image is one of the three diabetic retinopathy images. It can be easily observed that there are microaneurysms and exudates accentuated. Nonetheless, if the images are exhaustively analyzed, some alterations unmarked can be observed in both images. The code has detected some of the alterations, but not all of them. This happens because the morphology or intensity of the pixels that form them are slightly different from the ones the algorithm is capable to spot.

But since this algorithm is intended to serve as a diagnostic support system, it is not of great importance that the algorithm leaves some undetected. After processing the images, the next step will be providing the information to doctors so that they can make a final diagnosis.

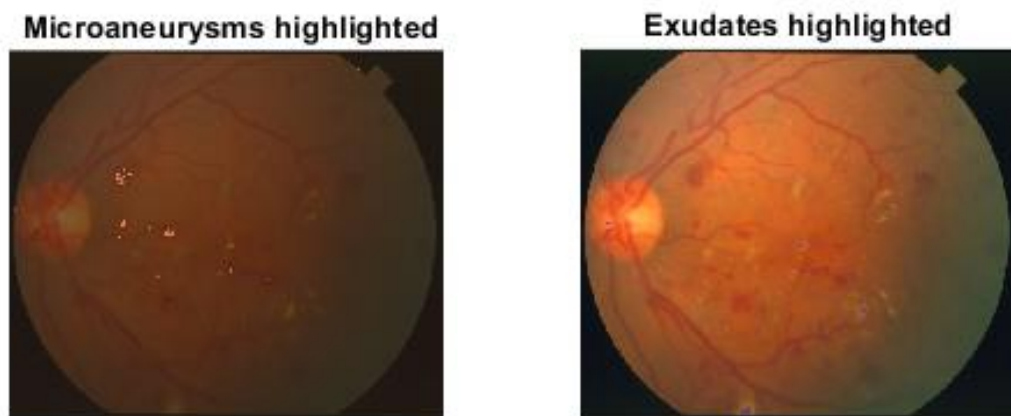


Figure 37. Output images of *TFG_code()*. Weak points of the code.

Source: *TFG_code()*.

CONCLUSIONS

This thesis marks the end of the Biomedical Engineering Degree. Some conclusions will be drawn from the present project, and it will be concluded. During the development of both the written project and the algorithm many of the knowledge acquired through the four years of university have been integrated and further growth.

The main objective of the thesis has been successfully achieved as the algorithm developed manages to warn of the presence of microaneurysms, exudates and high CDR. Furthermore, during this thesis, all specific objectives have been accomplished as well. Before starting to work with MATLAB, there was a period of familiarization training. Nonetheless, many obstacles have appeared as the code was created. For this reason, the MATLAB learning process was constant.

In parallel with this, it was important to get general knowledge of the medical framework concerning the eye. With this purpose, some anatomy and physiopathology books, referenced in the bibliography, were explored.

Having both the technological and medical information in mind, the project could start to be shaped. The integration of these areas was crucial to use the image processing tools to solve a medical problem. This is the goal of biomedical engineering in a nutshell.

Moreover, this project has place value on the importance of medical image processing as *TFG_code()* can be used as a clinical decision/diagnostic support system. As previously mentioned, these systems improve healthcare sector in some aspects, and life expectancy of patients that beneficiate from it because of the earlier intervention.

Additionally, this project could be further developed in the near future. One good improvement that could elevate it to another state would be the introduction of artificial intelligence. With it, the algorithm would certainly improve refine its performance. The weak point pointed out in the previous chapter could be overcome by this step forward. To conclude, this thesis paves the way for the possible development of a program implemented in health systems.

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ANNEX

This annex is intended to complement the thesis to which it is attached. The MATLAB code done for the thesis will be presented. The main function is *TFG_code()*. When it is run, the output images and message boxes shown in the thesis appear.

During the performance of *TFG_code()* more functions are called. Most of them are from MATLAB, the 'Image Processing Toolbox' of MATLAB or from File Exchange of MathWorks. Nonetheless, there are some other functions that have been developed. They were crucial for the development of the algorithm. These functions are also included in this annex.

TFG_code()

```
function TFG_code(im)

% Read the input image and display.
im = imread(im);
figure
imshow(im)
title('Input image')

%
% -----  DIABETIC RETINOPATHY ALGORITHM: RED LESIONS
% (MICROANEURYSMS) .
%

% Read 'diabetic_09.png' image. It will be the reference for
adjusting the
% brightness of the rest of the images.
ref = imread('diabetic_09.png');
ref_g = ref (:,:,2);
ref_g = imadjust(ref_g);
media_im_ref = mean2(ref_g);

% The green channel of the RGB color space is the one that shows the
% best contrast for red lesions. This will be the one used.
im_g = im (:,:,2);
im_g = imadjust(im_g);
media_im = mean2(im_g);

% Adjustment of the brightness with the reference image.
dif_media = media_im - media_im_ref;
im_g = im_g - dif_media;

% Shade correction. We should create a median filter and subtract it
% from the image. A mask will be applied before subtraction
% operation. The problem with subtraction is the negative saturation.
% Values bellow 128 will be negative differences.
```

```
im_sc = medfilt2(im_g,[40 40]);
mask = im_g>30;
im_sc = immultiply(im_sc,mask);

im_g = int16(im_g);
im_g = imadjust(im_g);
im_sc = int16(im_sc);
im_sc = imadjust(im_sc);
im_corrected = imsubtract(im_g,im_sc) + 128;

% Bright lesions removal. Values bellow 128 have an intensity lower
% than the background (red lesions and vasculature). Values higher
% than 128 will be set to 128 for the removal of bright lesions.
im_lesions_remove = im_corrected;
[rows,cols] = size(im_corrected);
for i = 1:rows
    for j = 1:cols
        if im_corrected(i,j)>128
            im_lesions_remove(i,j) = 128;
        end
    end
end

% Remove the vasculature. The rows and cols remain the same.
imOut = vesselDetection(im_lesions_remove,mask);
im_novessels = im_lesions_remove;
for i = 1:rows
    for j = 1:cols
        if imOut(i,j) == 1 % if the pixel is from the mask.
            im_novessels(i,j) = 128;
        end
    end
end

% Matched filter. With it, the image approaches the morphology of red
% lesions. They will be easier to detect.
h = fspecial('gaussian',11,1);
im_filtered=imfilter(im_novessels,h,'symmetric');

% Threshold to detect red lesions.
im_filtered = im2uint8(im_filtered);
im_red_lesions = im_filtered > 60;
im_red_lesions = imcomplement(im_red_lesions);

% Image reconstruction. The aim is to grow the original pathologies.
% The threshold image is the marker in the reconstruction.
im_red_lesions = im2int16(im_red_lesions);
imRecons = imreconstruct(im_red_lesions, im_novessels);

% Image with red lesions highlighted.
imRecons = im2uint8(imRecons);
overlap =
labeloverlay(im,imRecons,'Colormap','copper','Transparency',0.4);
figure
sgtitle('Diabetic retinopathy detection')
```

```

subplot(1,2,1)
imshow(overlap)
title(' Microaneurysms highlighted')

%
% -----  DIABETIC RETINOPATHY ALGORITHM: BRIGHT LESIONS (EXUDATES).
%
% The luminance channel of the HSL color space is the one that shows
% the best contrast for optic disc.
im_2hsl = im2double(im);
hsl=rgb2hsl(im_2hsl);
[~,L,~] = imsplit(hsl);

% Threshold to get a mask of the optic disk and bright lesions.
% Luminance channel goes from 0 to 1.
threshold = L > 0.9;

% Open operation to obtain the marker of the reconstruction. The
% structuring element must be bigger than the biggest bright lesion
% and smaller than the optic disk.
SE = strel('disk',6);
im_mask_disc = imopen(threshold,SE);

% Morphologic reconstruction to obtain the full optic disk.
im_gb = im (:,:,2);
im_mask_disc = im2uint8(im_mask_disc);
im_disc = imreconstruct(im_mask_disc,im_gb);
im_nodisc = imsubtract(im_gb,im_disc); % everything above 0 are
exudates.

% Mask of bright lesions.
mask_bright = im_nodisc > 35;

% Image with bright lesions highlighted.
overlap_b =
labeloverlay(im,mask_bright,'Colormap','parula','Transparency',0.4);
subplot(1,2,2)
imshow(overlap_b)
title('Exudates highlighted')

%
% -----  GLAUCOMA ALGORITHM.
%
% Segmentation of the optic cup.
[imout_CUP, mark_int] = segmentation_cup(im);
imout_CUP=label2rgb(imout_CUP);
imout_CUP = imout_CUP(:,:,1);
imout_CUP = imfill(imout_CUP,'holes');

% Segmentation of the optic disc.
imout= segmentation_disc(im, mark_int);
imout=label2rgb(imout,'gray');
imout = imout(:,:,1); % no matter what channel it is.

```

```
% Calculation of the cup to disc ratio (CDR).
pix_disc = bwarea(imout);
pix_cup = bwarea(imout_CUP);
CDR = sqrt(pix_cup/pix_disc);

%
% ----- RESULTS DISPLAY
%

% Read the icons.
mal = imread('mal.png');
bien = imread('bien.png');
gris = imread('gris.png');

% Microaneurysms results.
imRecons = sum(imRecons(:));
if imRecons > 9000000 && imRecons < 100000000
    msgbox('Microaneurysms detected.', 'Diabetic
retinopathy', 'custom', gris);
else
    msgbox('No microaneurysms detected.', 'Diabetic
retinopathy', 'custom', gris);
end

% Exudates results.
mask_bright = sum(mask_bright(:));
if mask_bright > 90 && mask_bright < 400
    msgbox('Exudates detected.', 'Diabetic
retinopathy', 'custom', gris);
else
    msgbox('No exudates detected.', 'Diabetic
retinopathy', 'custom', gris);
end

% CDR results.
if CDR > 0.5
    msgbox('High CDR: '+ string(CDR)+ ' .', 'Glaucoma', 'custom', mal);
else
    msgbox('Normal CDR: '+ string(CDR)+ '
.', 'Glaucoma', 'custom', bien);
end
```


vesselDetection()

```
function vesselMask = vesselDetection(imInput,extMask)

% Removal of the background. Structuring element bigger than the
% thinnest blood vessel. The final image is a mask of the removed
% vessels.
SE = strel('square',12);
cierre = imclose(imInput,SE);
tophat_dual = mat2gray(cierre-imInput);

% Clean more the background with morphologic reconstruction. The last
% step is to delete possible error sources.
referencia = double(tophat_dual);
marcador = find_lines(tophat_dual, 12, 70); % find_lines function
imRecons = imreconstruct(marcador,referencia);
extMask = double(extMask);
imRecons = imRecons.*extMask;

% Final mask of the retinal vessels
imRecons = mat2gray(imRecons).*255;
imRecons = uint8(imRecons);
umbral = graythresh(imRecons); % threshold
vesselMask = imbinarize(imRecons,umbral);
```

find_lines()

```
function imOut = find_lines(imInput, N, tam)

% This algorithm find lines in the input image.
imInput = double(imInput);
imInput = mat2gray(imInput);
[filas,columnas] = size(imInput);

% There will be an opening operation for every search direction. The
% structuring element is different in each operation. N is the number
of
% orientations in which the lines are going to be searched. The
search
% angles are considered equispaced in the semicircumference.
equiesp = 180/(N-1);
aperturas = zeros(filas,columnas);
for i = 1:N % for every search direction
    direc_busc = equiesp*(i-1); % search direction
    SE = strel('line',tam,direc_busc);
    aperturas(:, :, i) = imopen(imInput,SE);
end

% The output image is the maximum of the opening operations.
imOut = max(aperturas, [], 3);
```

segmentation_cup()

```
function [imout, mark_int] = segmentation_cup(imin)

% Green channel of RGB color space.
imR=imin(:,:,2);

% Removal of vessels. Structuring element bigger than the smallest
% vessel to remove.
SE=strel('square',25);
imc= imclose(imR,SE);

% Threshold image.
hist = imhist(imc);
[height,width] = size(imc);
npixel= height*width;
imhist_norm = hist/npixel;
hist_acum = cumsum(imhist_norm);
P = 0.003;
rmax = find(hist_acum>=(1-P),1,'first');
imThres=imc>rmax;

% The biggest object is the objective. Binary image where the object
% of biggest area will be the positive class.
CC=bwconncomp(imThres); % finds connected components
stats= regionprops(imThres,'Area'); % measures the area
n_pixels=cellfun(@numel,CC.PixelIdxList); % pixel number of numel
matrix
for i=1:length(n_pixels)
    imThres(CC.PixelIdxList{i})=0; % black pixels
end
[max_area,idx]=max(n_pixels);
imThres(CC.PixelIdxList{idx})=1; % white pixels
imu=imThres;

% Internal marker to watershed. It will be a binary image where the
% only pixel set to 1 is the brightest pixel in the image.
negativo_imu= imcomplement(imu);
d= bwdist(negativo_imu); % distance to the edge
m_pixel=max(max(d));
mark_int=d>m_pixel-1;

% External marker to watershed.
bwin= mark_int;
stats= regionprops(imu,'Area');
area_imu= stats.Area; % area of the biggest object
r= sqrt(area_imu/pi);
mark_ext=circumference(bwin,r);

% Final marker to watershed.
mrk=mark_int|mark_ext;
```

```
% Gradient of the image.
SE = strel('disk',1);
img=imdilate(imc,SE)-imerode(imc,SE);

% Imposition of minimum values in the gradient image.
img2= imimposemin(img,mrk);

% Watershed function.
imout= watershed(img2);
```

circumference()

```
function bwout=circumference(bwin,r)

% function that returns a binary image of the same size as bwin
% showing a circle of radius r centred at the point containing bwin.
[M,N]=size(bwin);
[yc,xc]=find(bwin);
[x,y]=meshgrid(-(xc-1):(N-xc),-(yc-1):(M-yc));
bwout=(x.^2+y.^2)<=(2*r)^2);
bwout=bwmorph(bwout,'remove');
```

segmentation_disc()

```
function imout = segmentation_disc(imin, mark_int)

% Red channel of RGB color space.
imR=imin(:,:,1);

% Removal of vessels. Structuring element bigger than the smallest
% vessel to remove.
SE=strel('square',25);
imc = imclose(imR,SE);

% Threshold image.
hist = imhist(imR);
[height,width] = size(imR);
npixel= height*width;
imhist_norm = hist/npixel;
hist_acum = cumsum(imhist_norm);
P = 0.04;
rmax = find(hist_acum>=(1-P),1,'first');
imThres=imR>rmax;

% The biggest object is the objective. Binary image where the object
% of biggest area will be the positive class.
CC=bwconncomp(imThres); % finds connected components
stats= regionprops(imThres,'Area'); % measures the area
n_pixels=cellfun(@numel,CC.PixelIdxList); % pixel number of numel
% matrix
```



```
for i=1:length(n_pixels)
    imThres(CC.PixelIdxList{i})=0; % black pixels
end
[max_area,idx]=max(n_pixels);
imThres(CC.PixelIdxList{idx})=1; % white pixels
imu=imThres;

% External marker to watershed.
bwin= mark_int;
stats= regionprops(imu,'Area');
area_imu= stats.Area; % area of the biggest object
r= sqrt(area_imu/pi);
mark_ext=circumference(bwin,r);

% Final marker to watershed.
mrk=mark_int|mark_ext;

% Gradient of the image.
SE= strel('disk',1);
img=imdilate(imc,SE)-imerode(imc,SE);

% Imposition of minimum values in the gradient image.
img2= imimposemin(img,mrk);

% Watershed function.
imout= watershed(img2);
```