UNIVERSITÀ DI PADOVA

FACOLTÀ DI INGEGNERIA

ELABORATO

Retinal vasculature analysis:

tuning and optimization for RETCAM images

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CORSO DI LAUREA IN INGEGNERIA BIOMEDICA

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Summary

This work is focused on tuning an algorithm for the automatic detection of the retinal blood vessels. Such optimization and adaptation are aimed to allow the analysis of the vessel network in preterm babies affected by Retinophathy of Prematurity.
In the first chapter, the anatomical structure of the human eye is described and special attention is paid to the retina. The second chapter deals with ROP . The third chapter focuses on the segmentation of retinal images, especially in premature babies, and introduces the RetCam imaging system. In the fourth chapter the applied algorithm is defined, and the changes that have been applied to it are carefully explained. Finally, the fifth chapter presents the search results with the related conclusions and recommendations for the future.

Introduction

In Italy, even if at the present time studies report a low incidence of vision impairments affecting children, such diseases have a severe psychosocial impact.
Low vision that is present at birth or that occurs early in childhood leads to more complex situations, compared with the case of adult patients. Actually, as well as causing partial disability, it also has negative effects on many areas of development and learning.
As far as regards perception, the sense of sight plays a key role in the neuromotor, cognitive and emotional development of children. That is why vision impairments can severely compromise the first phases of interaction with the surrounding environment.
Both in industrialized and developing countries, one of the leading causes of childhood blindness during the first few years of life is represented by ROP, also called Retinopathy of prematurity**.**
The incidence of ROP among extremely preterm infants is increasing as a result of increased survival of this population, in fact infants who survive today, would have not survived long enough to develop ROP in past. After all, there is a renewed interest in this disease.
At the present time, several ophthalmologists are conducting studies on the retinopathy of prematurity, increasing our knowledge concerning this disease. Nevertheless, many aspects of this disease are still poorly understood. For example, it remains unclear why ROP occurs only in a subgroup of preterm babies with a very low birth weight, and not in other infants with the same clinical conditions, or why, after the same medical and surgical treatments, in some cases the retinopathy regresses spontaneously, whiles in other ones it progresses leading to blindness.
Therefore, studying the ROP, its aspects and correlated problems represents an important challenge and a charge for ophthalmologists, who are expected to treat infants who cannot lead a normal life at present.|1|
Due to the complexity of this pathology, the possibility of having an automatic method that provides useful information to the clinicians about the state of the disease, would represent an important aid. Medical observations are subjective and the severity level is given by qualitative assessments and is related only to the experience of doctors: evaluation of the disease progress may vary from doctor to doctor and it is therefore not possible to accurately determine its level of severity.
The *RetCam* is a highly sensitive instrument, that allows rapid capture of eye fundus images, through a optical imaging system, with a lens of 130 degree field of view and an available yellow filter for fluoroangiography and digital imaging. The invention of such system allows overcoming the imperfections of ultrasound technology, namely the influence of the operator. Moreover, the possibility of conducting a fluoroangiographic examination in newborn babies, that was impossible until a short time ago, has laid the foundation for a new diagnostic and clinic assessment of this disease.
With the increasing availability of digital fundus camera, there is a wide consensus of opinion that an automatic analysis of such digital images might, at least partially, relieve ophthalmologists of the burden of retinopathy screening. |2|

Automatic analysis entrusting to the doctor simple operations, allows to automating a subjective process, giving opportunities to obtain repeatable and comparable diagnosis and having regard for the objectivity of that method.
In this work we tuned and optimized algorithms that, basing on RetCam images, automatically track the retina blood vessel network.
The aim of this project is to standardize and make more objective the way to proceed, so that it could give more information to the ophthalmologists, who could decide more easier how to treat the pathology.

Chapter 1

The eye structure and function

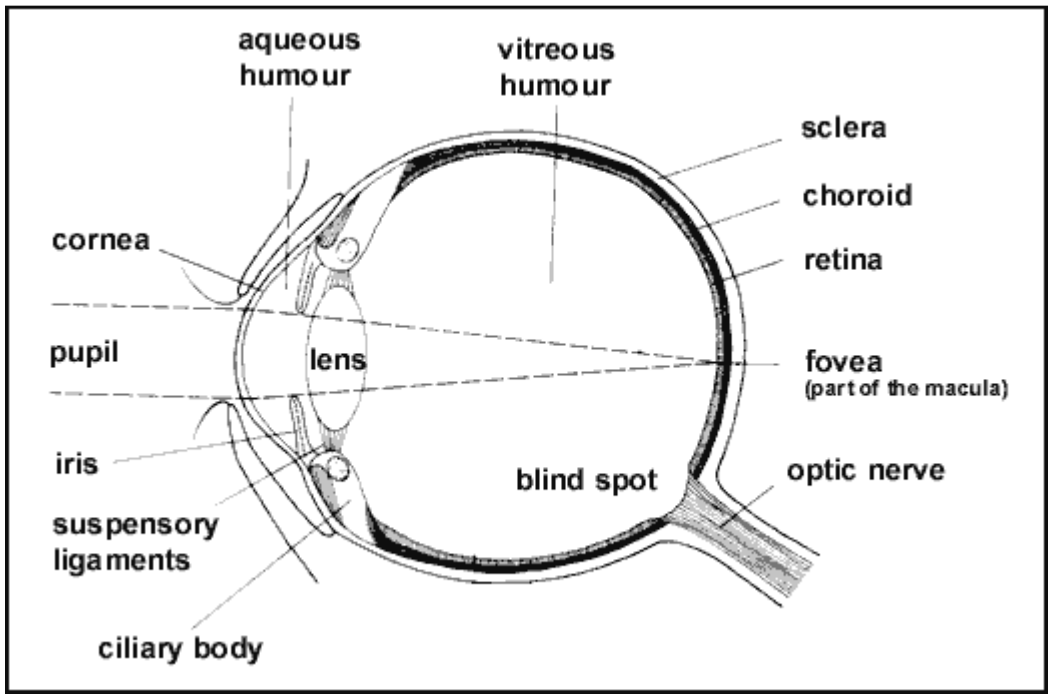


Fig. 1 The eye structure

Eye is like a camera, the external object is seen like the camera takes a picture of any object. Light enters the eye through a small hole called the pupil and is focused on the retina, which is like a camera film. Eye also has a focusing lens, which focuses images from different distances on the retina. The colored ring of the eye, the iris, controls the amount of light entering the eye. It closes when light is bright and opens when light is dim. A tough white sheet called sclera covers the outside of the eye. Front of this sheet (sclera) is transparent in order to allow the light to enter the eye, the cornea. Ciliary muscles in ciliary body control the focusing of lens automatically. Choroid forms the vascular layer of the eye supplying nutrition to the eye structures. Image formed on the retina is transmitted to brain by optic nerve. The image is finally perceived by brain. A jelly like substance called vitreous humor fill the space between lens and retina. The lens, iris and cornea

are nourished by clear fluid, aqueous humor, formed by the ciliary body and fill the space between lens and cornea. This space is known as anterior chamber. The fluid flows from ciliary body to the pupil and is absorbed through the channels in the angle of anterior chamber. The delicate balance of aqueous production and absorption controls pressure within the eye.|3|

1.1 Retina

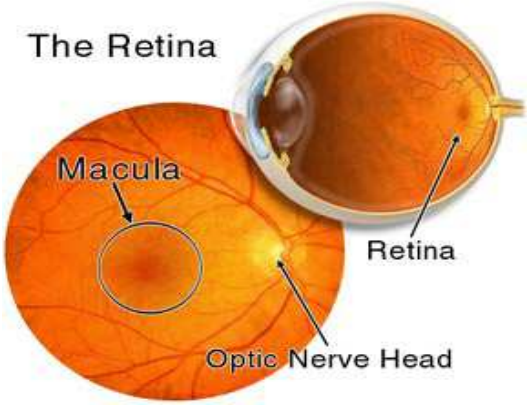


Fig. 2 The retina

The retina is the internal layer of the eye that receives and transmits focused images.
The total retina is a circular disc of approximately 42 mm diameter, in the center of the retina is the optic nerve. From the center of the optic nerve radiate the major blood vessels of the retina. Approximately 17 degrees (4.5-5 mm), or two and half disc diameters to the left of the disc, can be seen the slightly oval-shaped, blood vessel-free reddish spot, the fovea, which is at the center of the area known as the macula by ophthalmologists. A circular field of approximately 6 mm around the fovea is considered the central retina while beyond this is peripheral retina stretching to the ora serrata.|3|
The optic nerve contains the ganglion cell axons running to the brain and, additionally, incoming blood vessels that open into the retina to vascularize the retinal layers and neurons.
The ganglion cells (the output neurons of the retina) lie innermost in the retina closest to the lens and front of the eye, and the photosensors (the rods and cones) lie outermost in the retina against the pigment epithelium and choroid. Rods function mainly in dim light and provide black-and-white vision, while cones support daytime vision and the perception of colours.
Light must, therefore, travel through the thickness of the retina before striking and activating the rods and cones . Subsequently the absorption of photons by the visual pigment of the photoreceptors is first translated into a biochemical message and then into an electrical message that can stimulate all the succeeding neurons of the retina.
The retinal messages concerning the photic input and some preliminary organization of the visual image into several forms of sensation are transmitted to the brain from the spiking discharge pattern of the ganglion cells.
The retina is very complex and contains many nerve cell types; there are many interneurons packed into the central part of the section of retina intervening between the photoreceptors and the ganglion cells.
It is at the culmination of all neural processing that the message concerning the visual image is transmitted to the brain along the optic nerve.

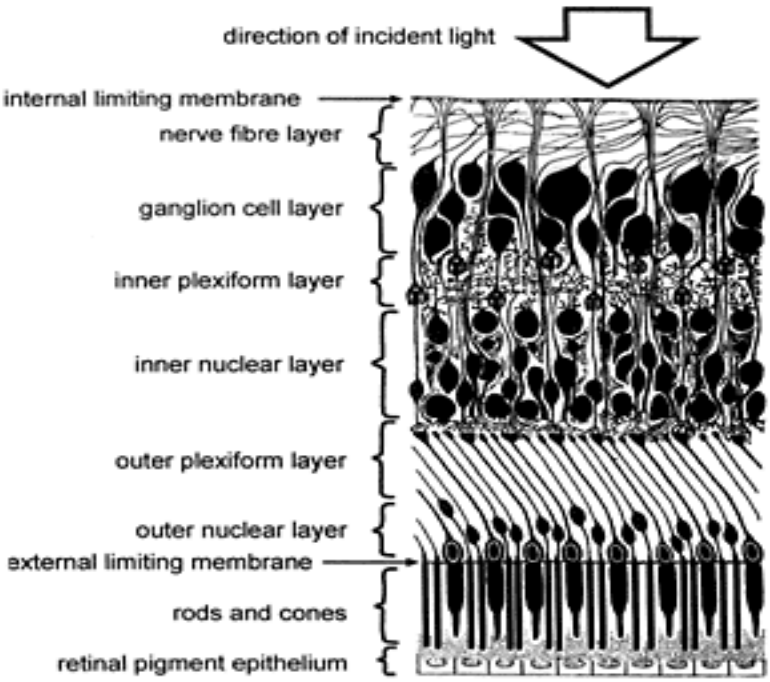


Fig. 3 Vertical section of the retina

1.1.1 Retinal vasculature

Knowledge of physiological retinal vasculature is particularly important because many forms of rethinopathy are caused by an abnormal vascular growth, among these certainly falls Retinopathy of Prematurity.

Central retinal artery is a branch of the ophthalmic artery which penetrates the optic nerve; it vascularizes the retinal membrane.
Several branches run from the central retinal artery and cover almost the whole retinal surface, while the capillary network originate the veins that flow in central retinal vein protruding from the eyeball through the pupil.|4|

1.1.2 The fovea

The fovea is located in the center of the macula region of the retina. It is a depression in the macula and has the highest density of cones; therefore the fovea is the retinal region with the greatest visual activity and is responsible for determining colours.
In normal retinal images it is the darker region and is characterized by the absence of both blood vessels and axons of nerve cells.

1.1.3 The optic disc

The optic disc is the spot on the retina where the optic nerve leaves the eye. There are no sensory cells here, creating a blind spot. Each eye covers for the blind spot of the other eye and the brain fills in the missing information.
The optic disc has a slight central depression and any variation of it may have a great importance from the clinical point of view.

1.2 The choroid

The choroid lies between retina and sclera. The choroid is a soft, thin, brown, extremely vascular layer, lining the inner surface of the sclera. It is composed of layers of blood vessels that nourish the back of the eye. The choroid connects with the ciliary body toward the front of the eye and is attached to the edges of the optic nerve at the back of the eye. The main function of the choroid lies in the blood nourishment of the outer layers of the retina.|4|

Chapter 2

Retinopathy of prematurity (ROP)

2.1 ROP: definition

The retinopathy of prematurity is a multifactorial eye disease which affects babies born before the 37° gestation week; it can be mild and may resolve spontaneously, but may lead to blindness in serious cases.
Prematurity is considered the main cause of ROP's onset because a low gestation conditions the regular development of the retina; as such, all preterm babies are at risk of contracting ROP, and very low birth weight is an additional risk factor. Normally, maturation of the retina proceeds in-utero and at term, the mature infant has a fully vascularized retina. However, in preterm infants, the growth of retinal blood vessels doesn't reach the peripheral area of the retina and this leads to several complications.
In fact, retinal blood vessels begin to grow starting from cells called spindle cells which develop from the optic nerve through the ora serrata. Spindle cells reach the ora serrata at the 29° week of gestation, whereas the blood vessels develop later and they reach the retinal periphery only during the last weeks of gestation.
A relevant condition that allows the migration and maturation process of the spindle cells is the hypoxic environment in the uterus. This hypoxic condition strongly changes after birth influencing the retinal blood vessels development; in fact, the hyperoxigenated blood leads to the formation of dangerous free radicals that decelerate the spindle cells maturation process.
Multiple factors can determine the progression if the disease, including overall health, birth weight, the stage of ROP at initial diagnosis and the presence or absence of "plus disease". Supplemental oxygen exposure, while a risk factor, is not the main risk factor for development of this disease. Restricting supplemental oxygen use does not necessarily reduce the rate of ROP, and may raise the risk of other hypoxia-related systemic complications.|1|
Patients with ROP are at greater risk for strabismus, glaucoma, cataracts and myopia later in life, and should be examined yearly to help prevent and treat these conditions.

Timing is one of the important factors that make the treatment successful in ROP, because the disease can advance very quickly and delayed treatment often reduces the chances of success.

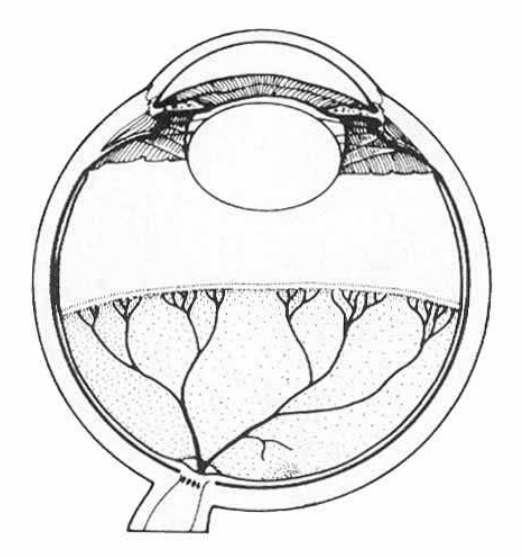


Fig. 4 Stage 1 ROP. Retinal blood vessels fail to reach the retinal periphery and multiply abnormally where they end. After a normal birth, the retinal blood vessels fill the anterior periphery of the retina.

2.1.1 History

During the 1940s and 1950s, ROP, also known as retrolental fibroplasia, was the leading cause of blindness in children in the United States. In 1942, Terry first reported the disease that was published in a report on the histological findings of end-stage cicatricial disease. In 1951, Campbell first suggested that ROP was related to the introduction of oxygen therapy into the newborn nursery, and this was confirmed by Patz.|1| When the concern for possible eye damage induced the reduction in using oxygen, it was no possible to monitor gas in arterial blood, as a result, it had negative effects on premature mortality.|4|
Today, after oxygen therapy has been studied and found not to be the single causative agent, the factors that play a role in the pathogenesis of ROP are still unknown.

2.2 Clinical classification

In 1984, a committee consisting of 23 ophthalmologists from 11 countries formed the International Classification of Retinopathy of Prematurity (ICROP). This new classification system demarcated the location of the disease into zones of the retina (1, 2, and 3), the extent of the disease based on the clock hours (1-12), and the severity of the disease into stages (0-5).|4|

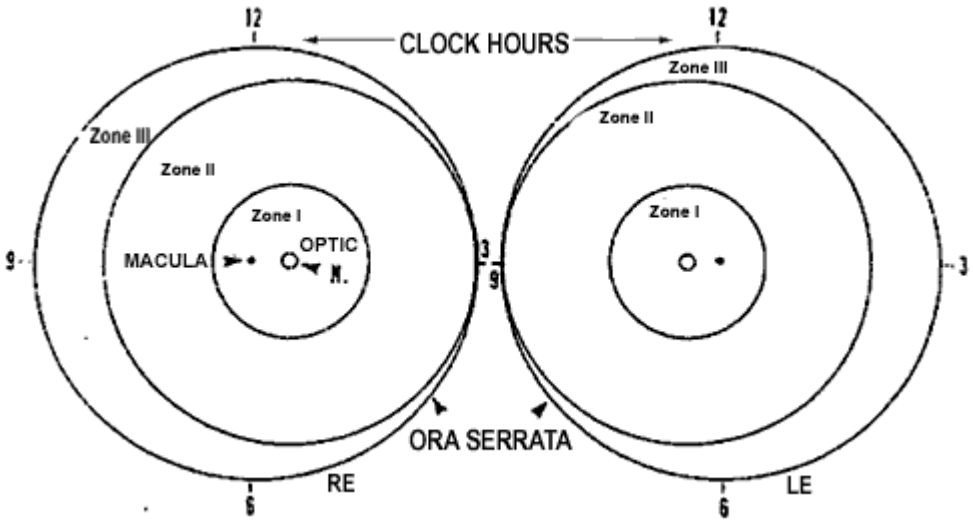


Fig.5 Zones of the retina in ROP

2.2.1 Localization and extent

The location of ROP refers to the location relative to the optic nerve. The retinal vessels normally start their growth at the optic nerve and gradually move toward the edge of the retina. Vessels that are farther from the optic nerve (or the closer to the edge of the retina) are more mature and less concerning. This has been standardized by dividing the retina into three zones. Zone I is an area centered on the optic disc and extending from the disc to twice the distance between the disc and the macula. Zone II is a ring concentric to Zone I which extends to the nasal ora serrata (the edge of the retina on the side of the eye toward the nose). Zone III is the remaining crescent of retina on the temporal (toward the temple) side.

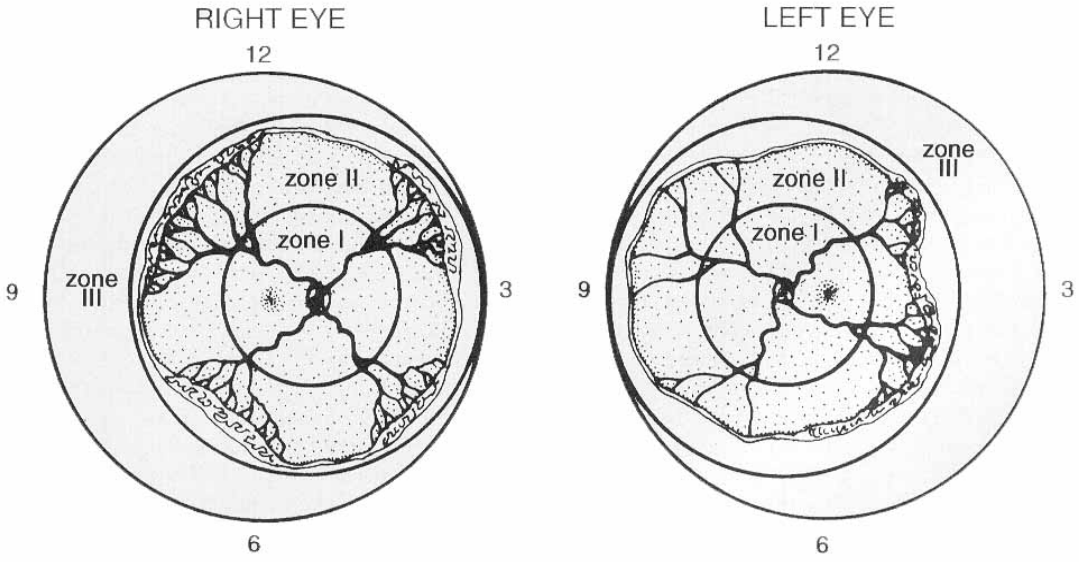


Fig.6 Zone 1 is the most posterior retina, that contains the optic nerve and the macula (zone of acute vision). Zone 2 is the intermediate zone where blood vessels often stop in ROP. Zone 3 is the peripheral zone of the retina, where vessels are absent in ROP, but present in normal eyes.

The extent of ROP is described by how many clock hours of the retina are involved.

2.2.2 Staging of the disease

Retinal vascular changes are divided into stages, they are usually used to describe the abnormal vascular response at the junction of the vascularized and avascular retina.

Stage 1

- is characterized by a demarcation line between the normal retina nearer the optic nerve and the non-vascularized retina more peripherally.

Stage 2

- ROP has a ridge of scar tissue and new vessels in place of the demarcation line. The white line now has width and height, and occupies some volume. It may take on a pink color as it becomes more vascularized. Small tufts of new vessels may appear posterior to the ridge.

Stage 3

- ROP shows an increased size of the vascular ridge with growth of fibrovascular tissue on the ridge and extending out into the vitreous. Fibrous scar tissue is beginning to form in this stage, with attachments between the vitreous gel and the ridge.

Stage 4

- it refers to a partial retinal detachment. The scar tissue associated with the fibrovascular ridge contracts, pulling the retina away from the wall of the eye. There may also be an exudation of fluid under the retina, contributing to the detachment. Stage 4 is further categorized depending upon the location of the retinal detachment. In Stage 4A, the detachment does not include the macula, and the vision may be good. In Stage 4B, the macula is detached, and the visual potential is markedly decreased.

Stage 5

- ROP implies a complete retinal detachment, usually with the retina pulled into a funnel-shaped configuration by the fibrovascular scar tissue. Eyes with stage 5 ROP usually have no useful vision, even if surgery is performed to repair the detachment.

In addition, Plus disease may be present at any stage. It describes a significant level of vascular dilation and tortuosity observed at the posterior retinal vessels. This reflects the increase of blood flow through the retina.

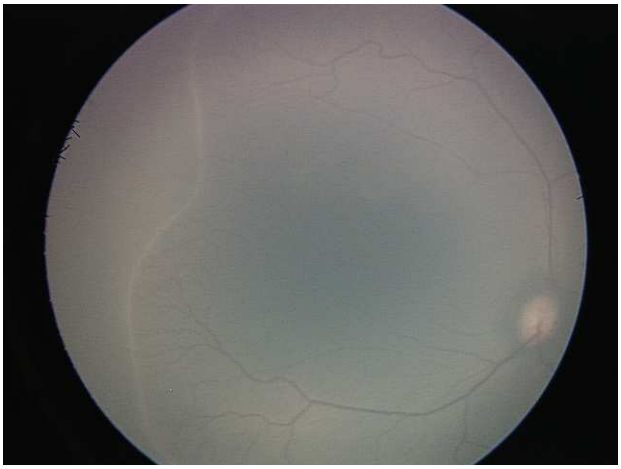


Fig.7 Stage 1

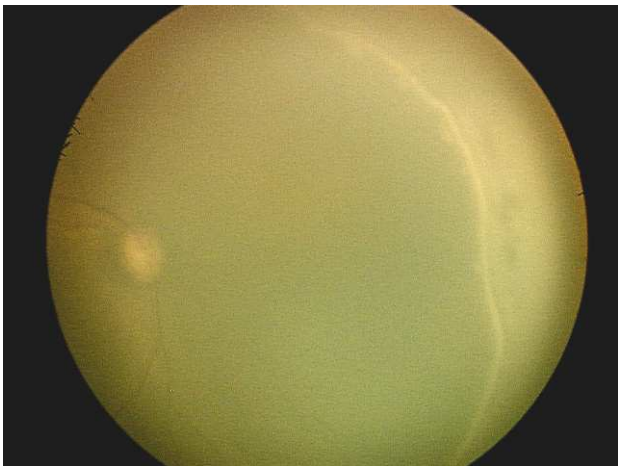


Fig.8 Stage 2

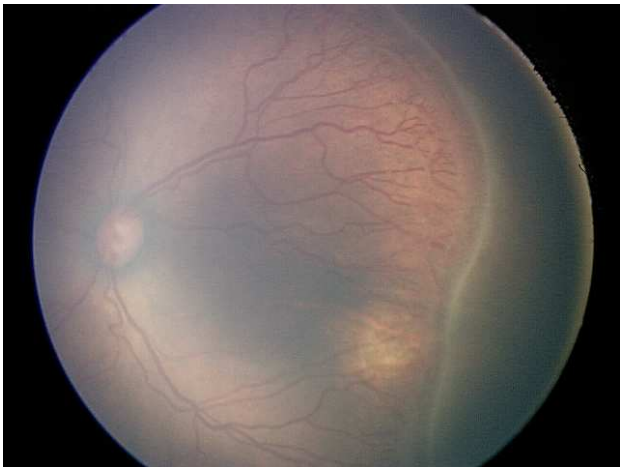


Fig.9 Stage 3

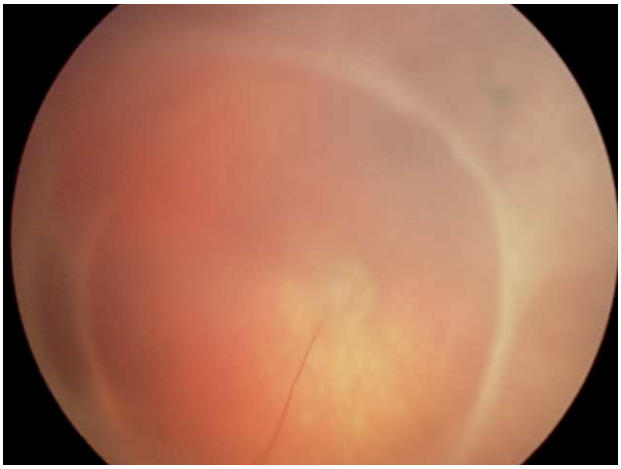


Fig.10 Stage 4

Stages 1 and 2 do not lead to blindness. However, they can progress to more severe stages. Threshold disease is defined as disease that has a 50% likelihood of progressing to retinal detachment, it is considered to be present when stage 3 ROP is present in either zone I or zone II, with at least 5 continuous or 8 total clock hours of disease, and the presence of plus disease. Progression to stage 4 or to stage 5 will result in substantial or total loss of vision for the infant.

2.3 Diagnosis

From the classification, it emerges that the disease develops through a progressive process characterized by stages of different severity. In some cases the disease can spontaneously evolve until a complete healing and a natural blood vessels maturation.
Early diagnosis assumes a strategic role because it allows to control how the disease evolves and decides the best moment for an intervention.|4|
Following pupillary dilation using eye drops, the retina is examined using a special lighted instrument (an indirect ophthalmoscope). The peripheral portions of the retina are pushed into view using scleral depression. Examination of the retina of a premature infant is performed to determine how far the retinal blood vessels have grown (the zone), and whether the vessels are growing flat along the wall of the eye (the stage) or not .

2.3.1 Screening

The American Association for Pediatric Ophthalmology and Strabismus, the American Academy of Ophthalmology and the American Academic of Pediatric related in 1997 a document (reviewed in 2001), in which is stated that every newborn who weighs less than 1500 grams and/or with gestational age less than 28 weeks have to be examinated and screened.
Patients at risk of ROP are periodically controlled following a protocol, in order to localize the lesion and to monitor the evolution of the disease.
Associated with retinopathy of prematurity, a large number of ocular abnormalities may occur. These include myopia, microvascular alterations and peripheral retinoschisi.

2.3.2 Prevention and treatment

The use of increasingly sophisticated incubators and the monitoring of the oxygen pressure in arterial blood has reduced the incidence of diseases in premature infants; however timing is one of the most important factors that make the treatment successful in ROP, because the disease can advance very quickly (Rush Disease).|5|

The only effective approach to reduce or stop the development of ROP is surgical intervention to extinguish the seeds of growing abnormal vessels in the eye. The various possible treatments depend on the stage of the disease:

- Cryotherapy was the original mode of treatment (since the 1970s). The procedure may be completed with general or topical anesthesia. It involves approximately 50 applications of a freezing probe under direct visualization with cryo applications to the avascular retina anterior to the fibrovascular ridge. The most common complications include intraocular hemorrhage, conjunctival hematoma, conjunctival laceration, and bradycardia.
- Laser surgery (eg, xenon, argon, diode) has been shown to be as effective as cryotherapy for ROP. The systemic adverse effects have significantly decreased, the ocular tissues are less traumatized, posterior zone 1 disease is treated easily, general anesthesia is not necessary and there is less incidence of late complications. Complications include corneal haze, burns of the iris, cataracts, and intraocular hemorrhages.
- Scleral buckling surgery is usually performed for stages 4 and 5. It is a procedure that places a band around the globe of the eye, this brings the retina back into contact with the inner layers of the eye.

In more advanced cases, where there is a total retinal detachment, a vitrectomy can be performed. This, in a good percentage of cases, con lead to a complete retinal reattachment although functional results are not satisfactory.

Chapter 3

Retinal images

3.1 Acquisition of retinal images

Until a few years ago the importance of viewing the map with information about retinal morphology of the macular region, optic nerve and retina as a whole was entirely left to the examination of fluorescein angiography and in part to the perimeter. The fluorescein angiography is a photograph of a particular photographic instrument equipped with a filter capable of imparting the retinal image on photographic film obtained by introducing a fluorescent liquid injected into a vein in your arm. This examination is often not well accepted by patients because pupil dilatation is required to its full availability, and it is clear that this condition is not always easy to obtain among infant patients.
In the last twenty years new techniques have been developed, that allow the acquisition of images with increasing precision, in order to analyze the retinal degeneration.|5|
Around 1990, the first digital fundus camera appeared. It allows non-invasive acquisition of digital images of the fundus, which can be printed and stored on computer for further analysis and comparison over time.
Using a fundus camera, an image of the fundus oculi is acquired. The visible part consists of the retina with its vascular network, the macula and the optic nerve head.
Choroidal vessels are not usually visible in an image taken with a fundus camera, but if the pigmented epithelium is very lightly pigmented, as in case of ROP, the retina becomes almost transparent and the choroid becomes visible.
The usefulness of these new technologies in infants and children is noticeable, because those patients have reduced capacity to collaborate.
However, the fundus images are dependent on the type of instrument used and on the particular subject being photographed, because the major vessels and the optic disc are different from individual to individual.

3.2 RETCAM

The hallmarks of ROP are usually located in the most peripheral area of the eye; in fact, this is the zone in which retinal blood vessels grow during the last weeks of gestation.

The digital technology enables to obtain images that give information about the entire extension of the lesion, because of the wide RetCam field of view.
The RetCam is a unique integrated system that combines first-ever bedside wide-angle viewing, full resolution image selection from real-time digital video with a comprehensive relational database, it allows also the fluorescein angiography. The availability of an immediate image means that precise diagnostic comparisons can be made over time, so this system is ideal for primary care hospitals; hence images can be transmitted for consultation or remote screening.
In case of ROP, wide field imaging capabilities allow visualization of disease status. From initial screening through treatment and follow-up; case management is facilitated with side-by-side comparison of photo documentation and patient data. In this way, ophthalmologists are able to perform a more exhaustive and objective analysis of the patient condition.

RetCam technology represents a fundamental device in ROP care and enables to obtain more accurate and standardized diagnosis and decisions.

3.2.1 Drawbacks

The first important aspect to consider is that timing is extremely important to treat ROP, ophthalmologists must quickly decide which treatment has to be performed, and to do that they need proper information.
The analysis of RetCam images is a time consuming and subjective issue because ophthalmologists have to evaluate a larger number of images.
In addition to this, images captured by RetCam present differences compared to those of adults acquired by any fundus camera. The main difference is that RetCam images have a wide field of view (120°-130°) whereas fundus camera images usual ly have a field of view which doesn't exceed 60°.
In order to diagnose the presence of ROP, the possibility of analyzing images of a large area of the eye is indispensable, although this leads to quality loss in acquired images.
RetCam images have a low level of resolution if compared to those by any fundus camera, moreover infants eyes present a very flimsy and transparent retina. Choroidal vessels are often easily confused as retinal vessels.
Imaging of the retina in premature infants is considerably more challenging than in adult subjects because many factors, including small pupils and difficulties in examining premature babies, combine and limit the quality of the final images.

Chapter 4

Automatic detection of retinal vessels

Due to the complexity of ROP images, making a decision towards the cure of the disease and at the same time avoiding or reducing difficult interventions, is a challenging task. The possibility of having an automatic device to provide useful information to the clinician about the actual state of the eye under exam would represent an important aid and would improve the quality in treating premature infants.
On the one hand the realization of a software that automatically detects the stage of ROP basing on RetCam images, would standardize and make more objective the way to proceed and would give more information to the ophthalmologists that could decide more easier how to treat the pathology; on the other hand RetCam images present, in general, a low level of resolution that makes their automatic analysis challenging.
The aim of this project is to adapt and optimize some algorithms originally developed for standard retinal images |6| in order to make them perform efficiently and robustly on RetCam images.

4.1 Algorithm for the image tracking of adults

Here is a summary of the operation of the functionally of the original algorithms.

The image is considered as a weighted un-oriented sparse graph where each node represents a pixel. Graphs describe the adjacency among pixels in the images and they are aimed to identify retinal vessels. In fact, vessels are minimum cost paths connecting remote nodes.
As first step, luminance and contrast drifts are removed, then a seed point extraction identifies a set of points used as starting nodes for simultaneous searches, seed points should be as spread-out as possible in the image. When two search frontiers meet, the computed shortest path is recorded; new paths are found by iterating the procedure, until the entire vessel network is reconstructed. Then, in order to cover the unexplored region with low-contrast vessels and overcome the intrinsic inability of the algorithm to find circular paths, a custom fixing procedure is run. Actually, it is noteworthy that every new path found is accepted only if the average gray level of its pixels significantly differs from the average value of the preprocessed image.|2| |6|

4.2 Methods

4.2.1 Introduction

As already mentioned above, the images taken into account have a lower resolution compared to those of a normal fundus of an adult. Moreover, the retinal blood vessels show different features. Furthermore, in case of a too sensitive detection of the vessels, retinal vessels of newborn babies can be mistaken for the underlying choroidal vessels.
Having carefully examined the features of such images, it stands to reason that the "standard" algorithm with its parameters is not applicable to the automatic analysis of retinal vessels in newborn babies.

Only through a preliminary study and after having familiarized with the code, it stands out that the most important steps of this code are: the seed points finding procedure, the exploring procedure. The changes made to the algorithm concern just these two phases.
It is necessary to clarify that such changes are not aimed to reshape drastically the code, but to optimize and adapt it, in order to analyse retinal images, obtained with RetCam. The code was optimized by modifying the most important parametres, ensuring that their values are corrected for the analised images.
There is another important remark connected to this analysis, namely that the images of infants with ROP are very diverse. The features of the images are strictly connected to the different stages of the disease. That is why it is difficult to propose universal solutions for the retinal vessel tracking.

4.2.2 Assessing changes

As regards the detection of a set of points (seed points), from which the tracking step will start, since the whole point of the sparse tracking procedure is to ensure that even non connected vessels can be tracked, these seed points should be as spread-out as possible in the image. Therefore, the seed points finding algorithm is applied to regularly spaced horizontal and vertical lines of pixels. |7|
Firstly, it was necessary to evaluate and determine the grid in which such points had to be arranged. A grid crowded with too many rows and columns would have slowed down the software and increased the possibilities of detecting false seed point (hence, false vessels). On the other hand, if the distance between rows and columns was too wide, only the major vessels could have been detected. Therefore a halfway solution seemed to be the most convenient. Compared with the original algorithm, the solution found allows a distance between seed points slightly higher or equal to nine.

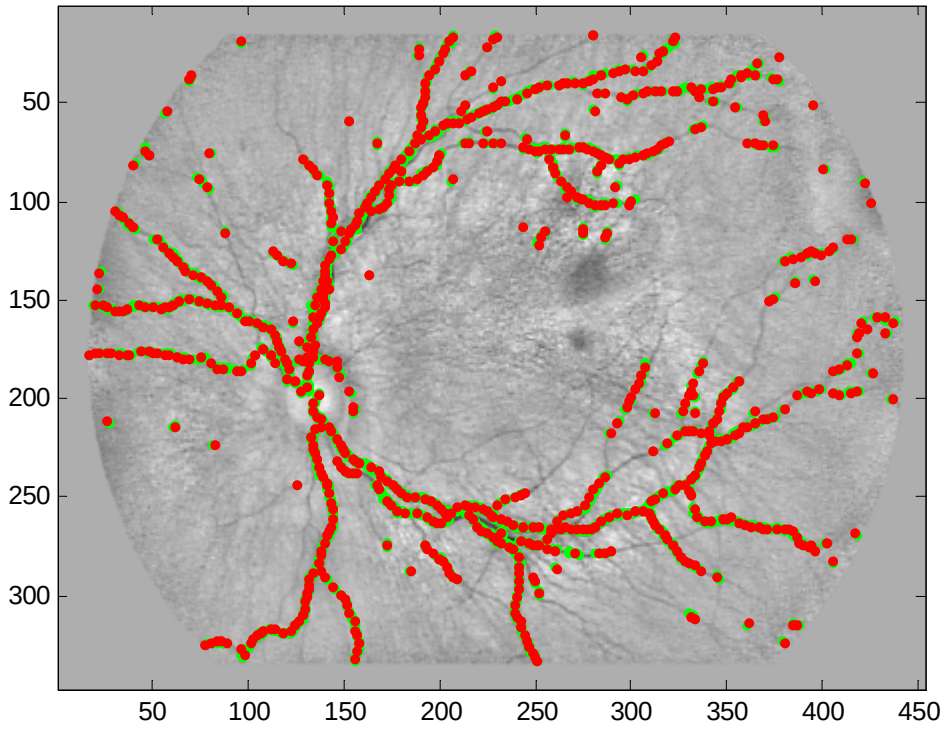


Fig. 11 With a distance of 2, seeds are too thick.

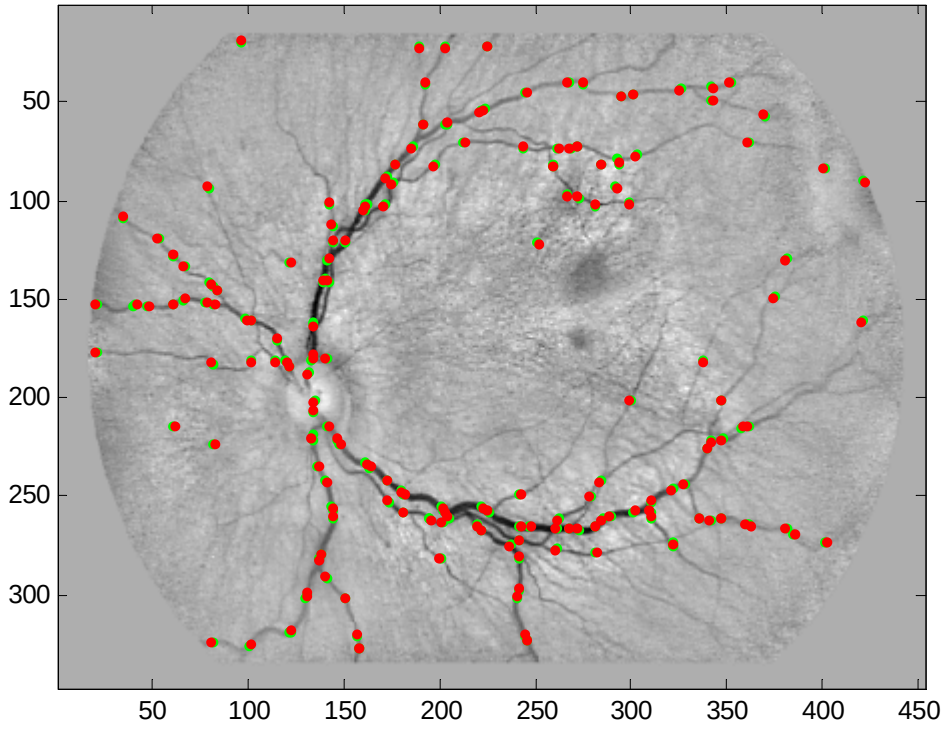


Fig. 12 Seed points are too distant from each other.

Every single row or column is processed as a monodimensional signal, to which a moving average filter to reduce noise is applied.|7| Moreover morphological operations are used in order to better distinguish the vessels. In fact, vessels may present a central reflex that makes the algorithm trace two sides separately, indicating the presence of two vessels instead of one, or viceversa. Moreover, by substracting to each pixel the average of the neighbouring, it is possible to detect the prospective edge of the vessel, since the contrast between vessel and background is enhanced.
This solution is noteworthy: actually, it was given more importance to the sensitivity of the seed finding procedure (detected vessels over true vessels) than to its specificity (not detected vessels over “non-vessels”). In fact, the effectiveness of the algorithm is not undermined by the presence of wrong seed points but rather by their lack in critical regions of the image.|2|
As a result, the number of seed points is strictly connected to the value of the reference threshold: the higher is the value, the more visible is the colour contrast between vessel and background. On the contrary, by decreasing the threshold, it is possible to detect the smallest vessels (with a lower contrast), but there is the risk of retaining many false positives, i.e. seed points that are located on the background or the choroid.
Let μ and σ be respectively the mean and the standard deviation of an extracted profile. Let $T=f(\mu, \sigma)$ be the threshold over which seeds are extracted on profiles resulting from matched filtering. The two parameters μ and σ allows to control two different situations: in the first case, when the peaks of the profiles are aligned the parameter μ must be given a greater importance, whereas in the second case, when those peaks are isolated, it is necessary to give more importance to σ. In the case of preterm babies, the standard deviation plays a predominant role. In fact, the more it increases, the more the darkest vessels stand out, while the choroid background becomes less important.
The optimization of such parameters represents the main step of this process. In fact, a right and accurate detection of the seed points assures a detailed analysis of the retinal vessel network.

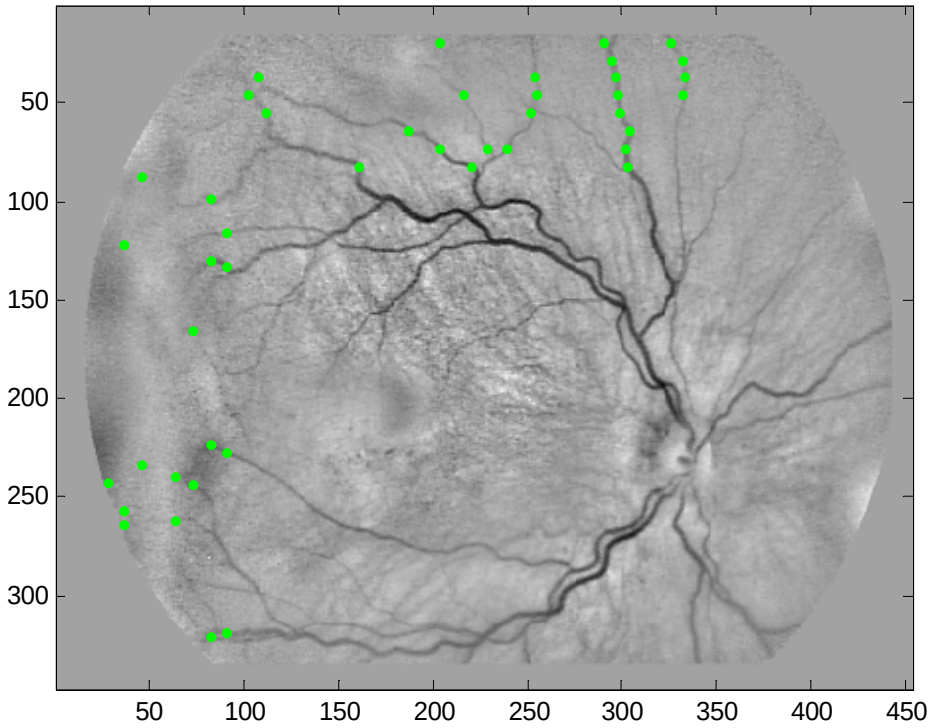


Fig.13 Differently oriented grids allow locating seed points over differently oriented vessels

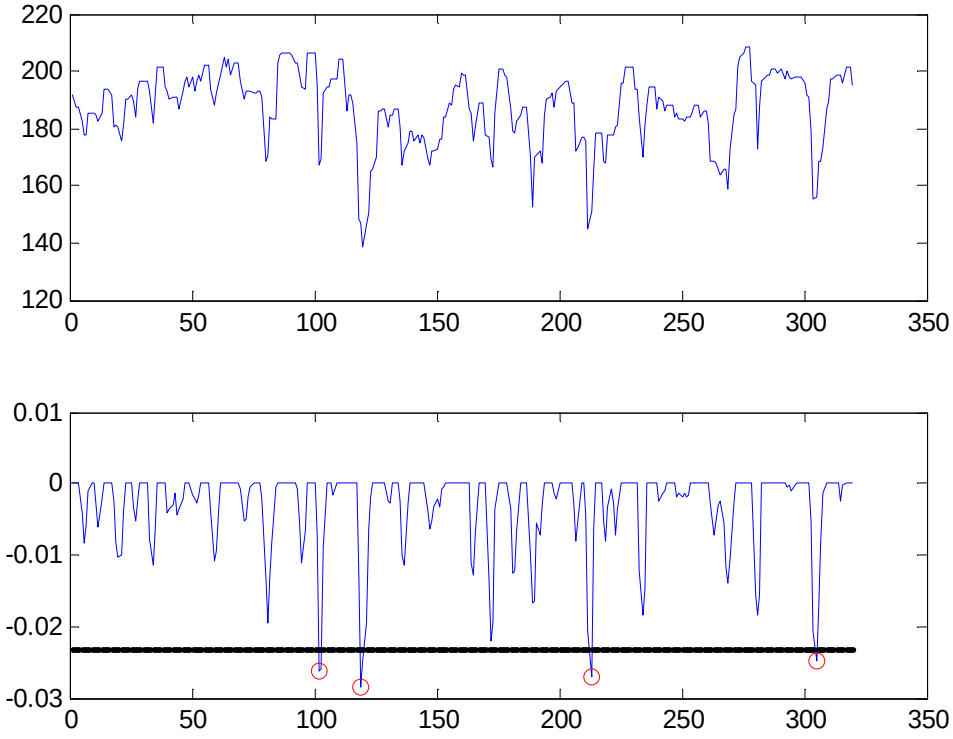
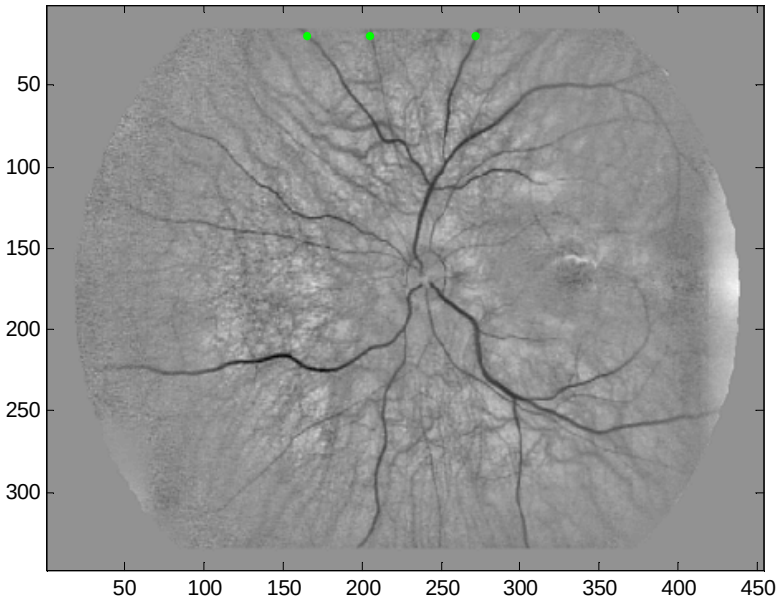
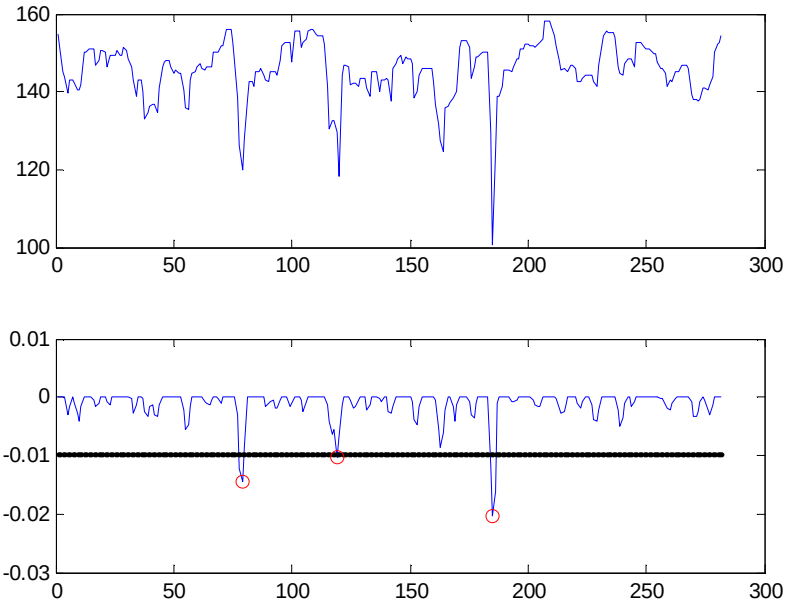
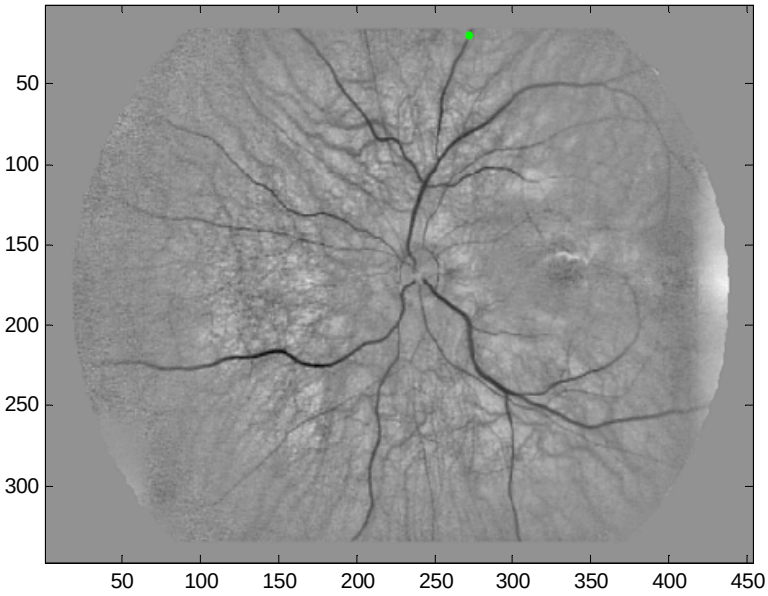


Fig.13 (a) Profile on which seed points are extracted.
Fig.13 (b) After matched filtering, thresholding provides the set of points suggesting the presence of vessel.




160
140
120
100
0
50
100
150
200
250
300
0.01
0
-0.01
-0.02
-0.03
0
50
100
150
200
250
300

(a)


50
100
150
200
250
300
50
100
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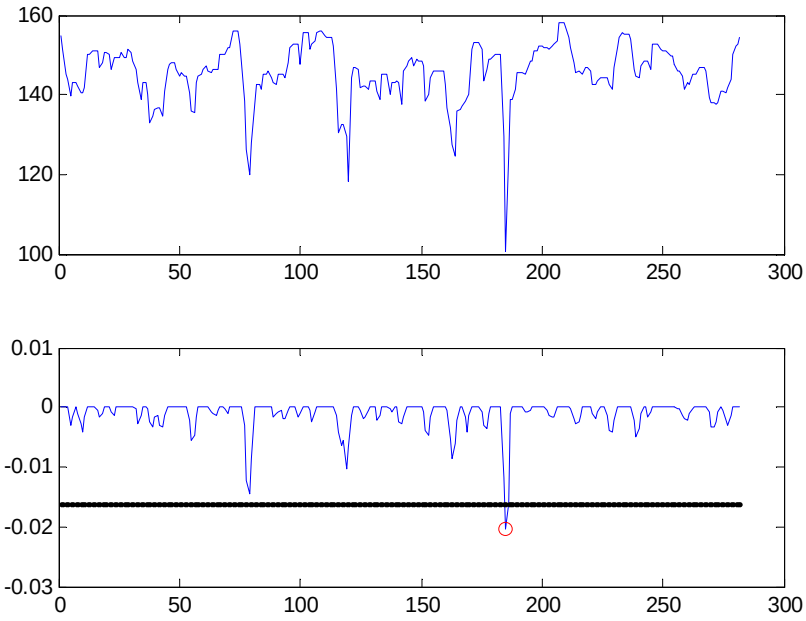
200
250
300
350
400
450



(b)

Fig. 14 In case b, if more importance is given to variance, only one peak instead of three can be registered, though being the starting image the same.

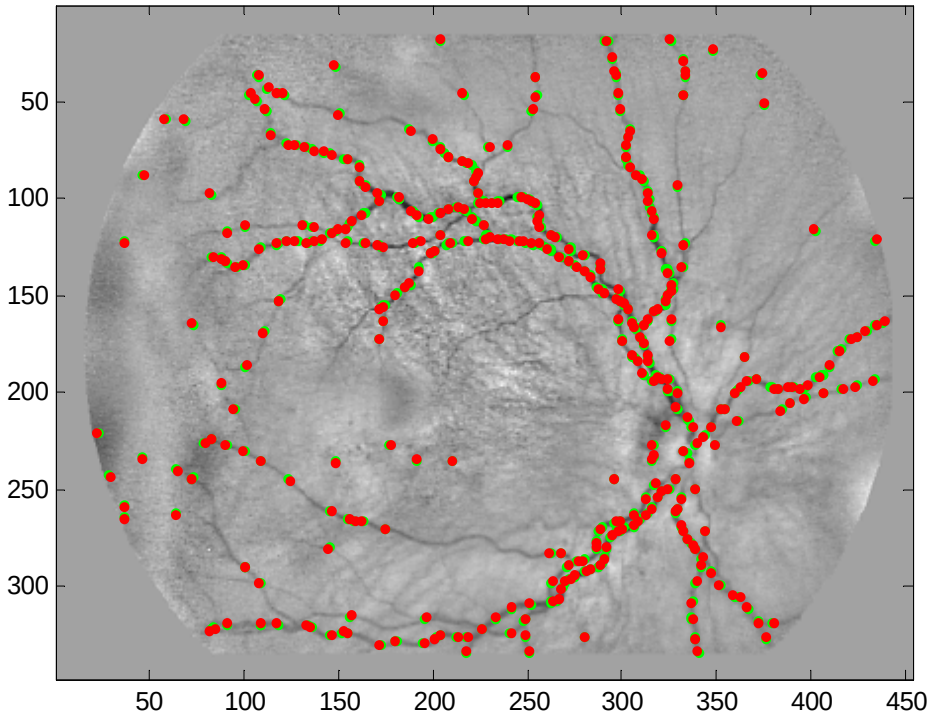
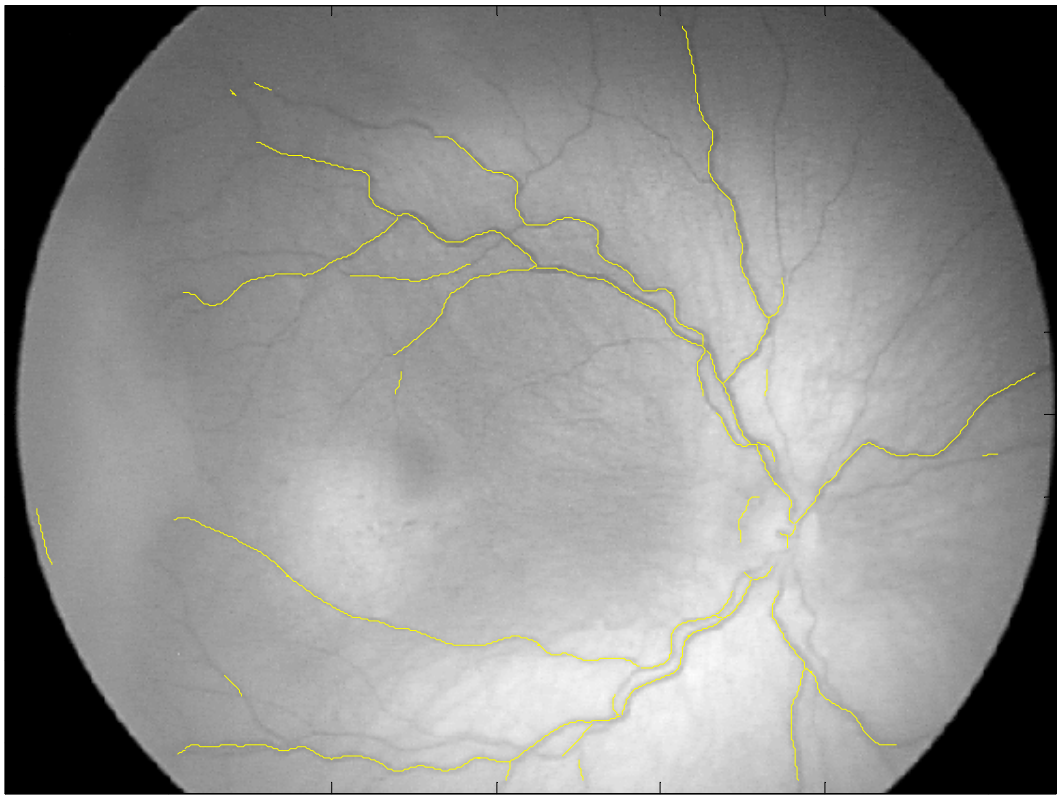
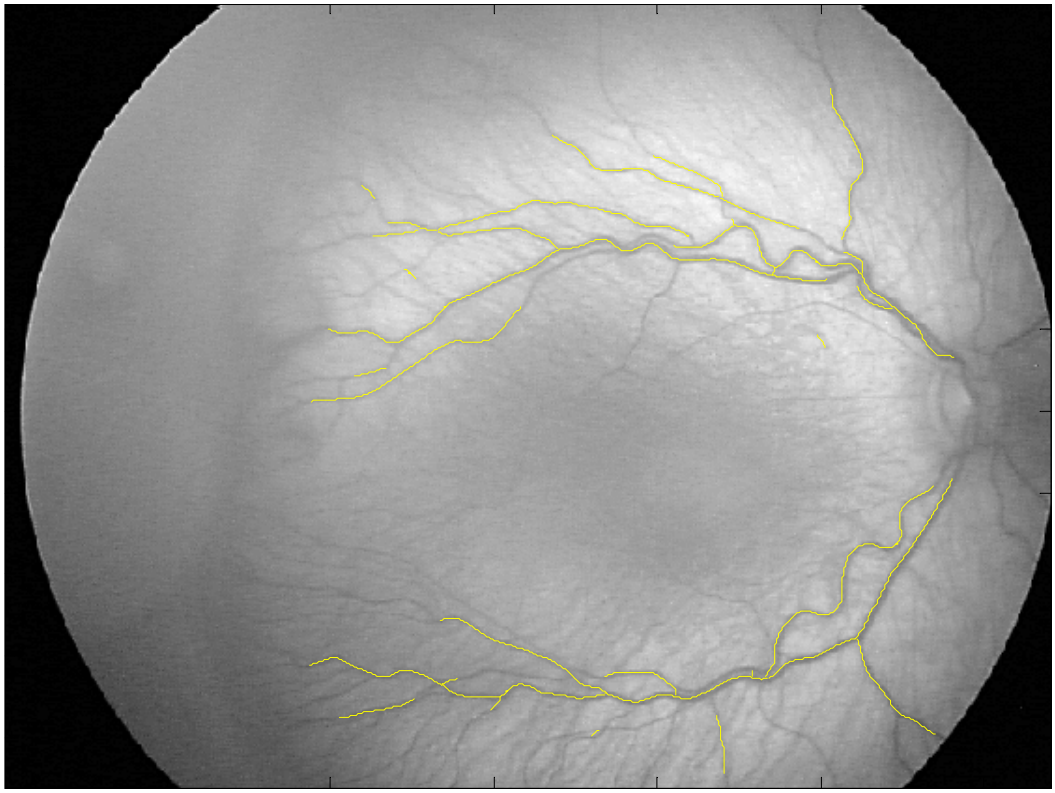


Fig.15 Detection of the seed points in an image.

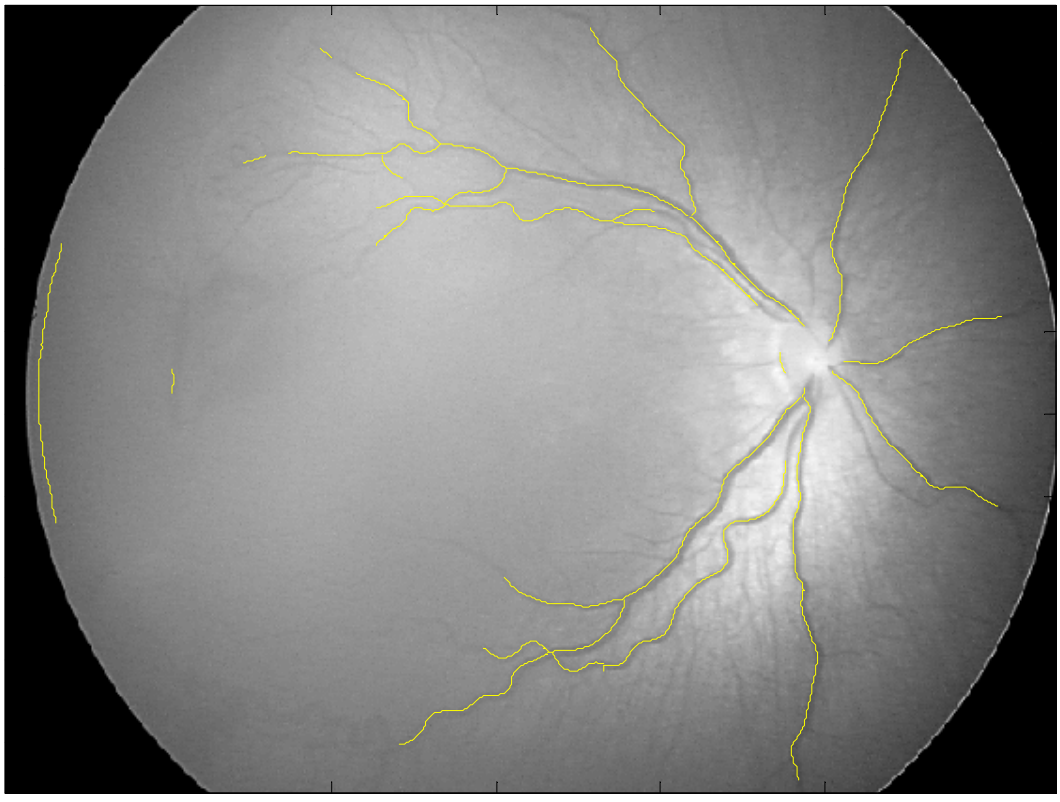
After the seed points extraction the exploration begins from every starting node in every directions. This phase consists of a modified version of the Dijkstra's algorithm. When two trees meet, they provide the shortest path connecting their roots but the algorithm does not stop. Instead, the two trees merge in a single new tree, from which the search goes on successive iterations.|6|
This stage represents the heart of the algorithm and includes parameters, which have major effects on the retinal vascular network. In case of ROP the vessels are often at a reduced distance from each other and tortuous. That is why it is important to make a precise exploration of the areas interested by the disease and at the same time avoid to slow down the process too much.
These parameters have been carefully analyzed in order to allow the algorithm to trace as far as possible the peripheral areas of the retina, without leading to paths which can easily be confused between each other.
This phase is followed by several methods aimed to track the whole vessel network in an even more detailed way. Actually, the final results can be considered as definitive, only after using such methods are run. Among them, it is noteworthy to mention the *merging* method: this operation allows the resulting merged tree to coherently interact with the others in succeeding iterations. These methods have not been modified or optimized, because they are considered suitable for the images to analyse.



(a)



(b)



(c)

Fig.16 (a), (b), (c) Tracking of retinal vessels

Chapter 5

Presentation of the results

5.1 Mask comparison

The proposed system for the automatic tracking of the retinal vessel network was evaluated on the basis of 20 images, that belong to our own clinical dataset.
These images were manually segmented in order to be compared with those that were tracked through the algorithm. While the background is black, vessels was labelled in white. Manual tracking focused on recognizing the vessels that definitely belong to retina, and on determining their width for a correct comparison.

Fig.17 Example of manual tracking of a ROP image.

5.2 Results

In order to assess the performance of the method, two parameters are considered: *sensitivity* and *false positive rate*. For a correct definition of them, it was necessary to generate the skeleton of the mask image. Sensitivity has been evaluated as the percent fraction of the length of correctly tracked vessels over the total length of the ground-truth vessels (skeletonization of the ground-truth vessel mask). Practically, *sensitivity* corresponds to the ratio between true positives (in other words, the result of the intersection between manual and automatic tracking) and those that belong to the skeleton. On the other hand, *false positive rate* has been evaluated as the percent fraction of the length of the false vessels over the length of all estimated vessels.

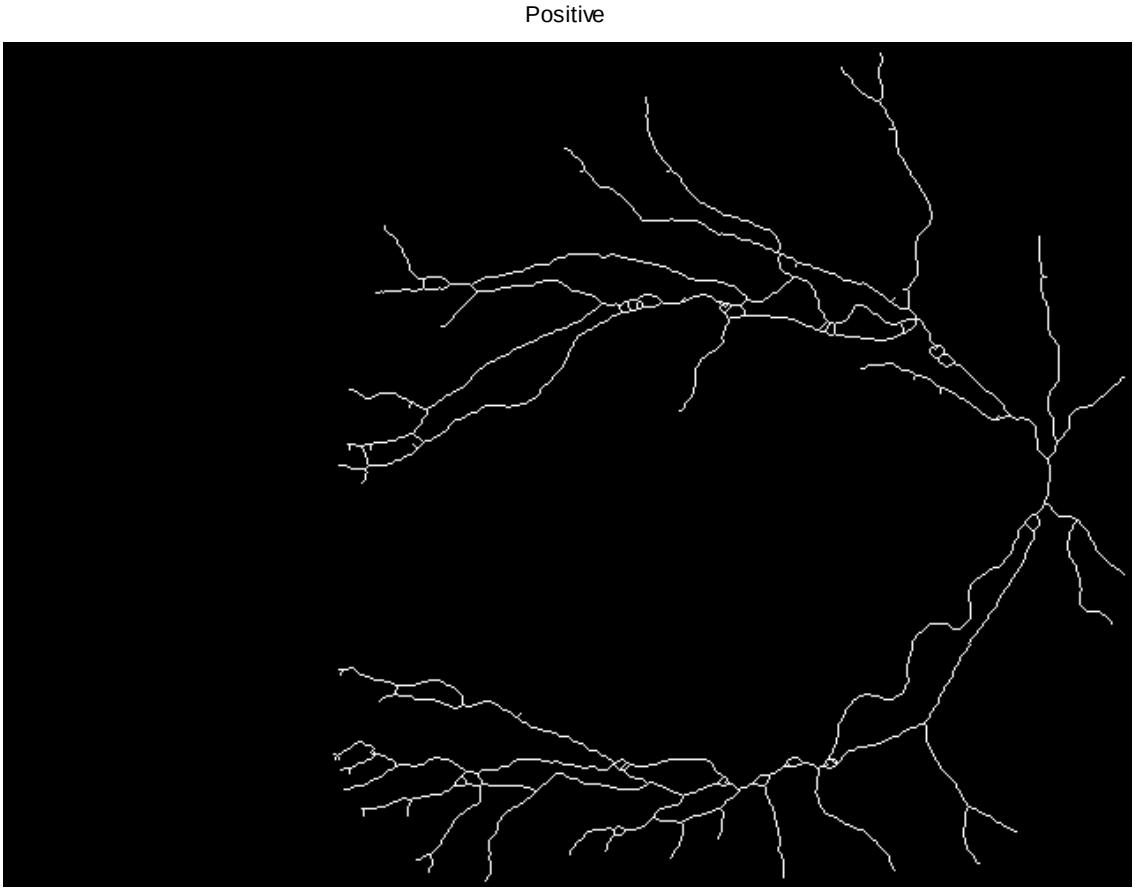


Fig. 18 Skeleton of the image

Results are summarized in Table 1.

	Sensitivity	False positive rate
Figure 1	0.8082	0.1596
Figure 2	0.9515	0.3897

Figure 3	0.9470	0.3814
Figure 4	0.6275	0.0293
Figure 5	0.9551	0.3395
Figure 6	0.9541	0.2751
Figure 7	0.7747	0.1102
Figure 8	0.6907	0.3593
Figure 9	0.7918	0.1440
Figure 10	0.7375	0.4202
Figure 11	0.5052	0.2840
Figure 12	0.8582	0.3118
Figure 13	0.4182	0.6129
Figure 14	0.6049	0.2337
Figure 15	0.7677	0.3673
Figure 16	0.7329	0.4208
Figure 17	0.6652	0.4169
Figure 18	0.8006	0.3580
Figure 19	0.7032	0.4677
Figure 20	0.6705	0.4358

Table 1

The importance of this comparison is that, analysing the skeleton of the image issued from manual tracking, it stands out that wider vessels were more considered. To be more precise, the skeleton of the wider vessels is wider and is not shown as a simple line representing the vessel axis. Moreover, the analysis of the emerging results shows that low values of false positive rate demonstrate the presence of a low rate of false vessels which were instead considered as such.

5.3 Conclusions

The central focus of this work was a reassessment of the tracking algorithm that had already been developed, in order to adapt and optimize it for RetCam images of newborn babies affected by ROP.

The results shown in Sec. 5.2 allows to conclude that the proposed method can be reliably used to assess the retinal vessel network in these patients. In fact, algorithms that are presented in this paper promote the development of a diagnostic instrument that could be a valid support in clinical

praxis, both in case of large screening programs to detect ROP, as well as during all the phases of monitoring of the disease course. In the first phase of the work, the images are processed and the vessels are traced automatically, whereas in the second phase the automatic imaging is directly compared with the manual imaging. The emerging information can be assembled and possibly used by the experts in order to determine the stage of the disease. Nevethless, the reliability of the final results depends on the quality of the blood vessel extraction algorithm starting from the retinal images

The main drawbacks of this method are the difficulty of detecting small capillaries as well as the different behaviors within different datasets. The main difficulty in the optimization of the algorithm resides in dealing with heterogeneous images. In fact, they are strictly related to the stage of ROP and to the conformation of the eye of each patient. But if the image is correctly focused, with retinal blood vessels that are clearly distinguished from choroidal vessels, it is possible to detect a set of seed points, that allow a right and careful segmentation of the retinal vessel tree.

An important advantage of this method, is that it is completely unsupervised, so there is no need for manually labeled images segmented by a human observer, which is time-consuming and subjective, since it depends on the observer. Another advantage is the extremely low number of applied parameters, as well as its simplicity and easy implementation. |6|

5.3.1 Future works

The algorithm adaptation and optimization lead to significant results as far as the images are concerned. Nevertheless, this work paves the way to further studies, aimed to improve its availability.

It is possible to improve the method of acquisition of the image: for example, finding new ways to improve the focusing and the contrast.

Another important aspect is represented by the problems of comparing image results with manual ground truth: new methods for a better skeletonization of the image will be found.

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