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Segmentation Processes and Pattern Recognition in Retina and Brain Imaging

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia Biomédica. Este trabalho foi realizado sob a orientação do Professor Doutor Rui Manuel Dias Cortesão dos Santos Bernardes e do Professor Doutor Miguel de Sá e Sousa de Castelo-branco, na Associação para investigação Biomédica e Inovação em Luz e Imagem e no Instituto Biomédico de Investigação da Luz e Imagem.

Pedro Guimarães Sá Correia
2010/2011

Aos meus pais, irmã e namorada;

Aos amigos, quer feirenses quer ‘conimbricenses’;

Aos meus orientadores;

Às ‘segmentadoras’;

A todos aqueles que directa ou indirectamente

possibilitaram este trabalho;

Obrigado.

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Abbreviations

BOLD - Blood Oxygenation Level-Dependent

CCD – Charge Coupled Device

ELM - External Limiting Membrane

fMRI - Functional Magnetic Resonance Imaging

FOV - Field-of-View

GCL - Ganglion Cell Layer

GM - Grey Matter

ILM - Internal Limiting Membrane

INL - Inner Nuclear Layer

IPL - Inner Plexiform Layer

LGN - Lateral Geniculate Nucleus

mm – Millimeter

MPRAGE - Magnetization Prepared Rapid Acquisition Gradient Echo

MRI - Magnetic Resonance Imaging

ms – Millisecond

MT - Middle Temporal

NFL - Nerve Fiber Layer

OCT - Optical Coherence Tomography

ONL - Outer Nuclear Layer

OPL - Outer Plexiform Layer

PET - Positron Emission Tomography

PL – Photoreceptor Layer

poi – patch-of-interest

RPE - Retinal Pigment Epithelium

TE - Echo-Time

TR - Repetition-Time

VFS - Vector Field Sign

WM - White Matter

μm – Micrometer

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We purposed to establish an automatic correlation between retinal and cortical thickness.

In 2009 Christine C. Boucard et al. [6] showed that acquired long-standing retinal pathologies, such as age-related macular degeneration and glaucoma, appear to be related to grey matter density reduction roughly in the defect projections at the visual cortex. As mentioned in [6], “this indicates that long-term cortical deprivation, due to retinal lesions acquired later in life, is associated with retinotopic-specific neuronal degeneration of visual cortex”.

Also on that year, Gupta et al. [14] confirmed the *in vivo* atrophy of LGN in glaucoma patients, “(...) consistent with *ex vivo* primate and human neuropathological studies (...)”.

Optical coherence tomography (OCT) allows imaging the ocular fundus *in vivo* and is herewith applied as the technique of choice for measuring the retinal thickness. We resort to the high-definition spectral domain system from Zeiss (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA, USA) to compute maps of retinal thickness covering the central 20 degrees of the human macula.

Similarly, we resort to the Siemens Trio 3T (Siemens AG, Healthcare Sector, Erlangen, Germany) to gather MRI (Magnetic Resonance Imaging) from the human cortex and the Brain Voyager QX® (Brain Innovation B.V., Maastricht, The Netherlands) software to derive cortical thickness from the human visual cortex.

While with the former technique a fundus reference is made available, therefore the thickness map of the macula can be easily located within the ocular fundus. The latter does not provide *per se* a reference, thus requiring proper visual stimuli in order to establish to which location within the ocular fundus a given visual cortex location refers to.

Each visual area contains a representation of the visual space and therefore a representation of each retinal point. In order to achieve the intended correlation we need to map the retina in each visual area, thus visual area segmentation is mandatory. An algorithm was developed and validated against manual segmentation, using a total of 6 data sets from healthy subjects.

In order to correlate the retina to the brain it is mandatory to find a continuous transformation function that links each retinal point to the cortex and vice-versa. Interpolation method used is based on Delaunay triangulation.

Introduction

The visual system is a complex system which allows us to describe our world in terms of color, size, texture, shape or even location and movement. It is the ultimate responsible for our ability to see. Light that is reflected by the environment enters the eye through specialized transparent tissue. Its energy is transduced into electrical signals by photoreceptors located at the retina and transmitted to the brain where it still has to be processed... A beautiful and complex process where brain and eye work together, achieving a better understanding of our world.

The eye

The purpose of the eye is to capture and focus light into a thin layer of photoreceptors and convert it to refined electrical signals that are transmitted to the visual cortex through the optic disk. Its anatomy will be shortly described and is represented in figure 1.

Light beams enter the eye through the **cornea**, a transparent avascular structure that provides ideal light transmission. The cornea has two main functions: to transmit light minimizing scattering and distortion and to reflect light that can be harmful to the eye. It is continuous with **sclera** which is an opaque white tissue layer that surrounds the whole eye acting as its limiting “wall”. Sclera is able to maintain globe shape, present resistance to internal and external forces, and serves also as an attachment for the ocular muscles. [3, 30, 31]

Adjacent to sclera lays a second layer of tissue, the **uveal tract** (or simply Uvea), which is formed by three continuous structures: the iris, the ciliary body and, the most posterior one, the choroid:

- Before reaching the retina, the amount of light entering the eye is controlled by the **iris**. It is a round structure positioned anterior to the **lens** and it contains sets of muscles with different functions that can vary the size of its center aperture, the **pupil**; [30,31]
- The **ciliary body** is composed by two components, a muscle component, strong smooth muscles called **ciliary muscles** are connected to the lens and are able to adjust its refractive power and, a vascular component, responsible for the production of fluid present in the anterior chamber (aqueous humor); [30, 39]
- The **choroid** is mainly composed by blood vessels and it plays an important role in nourishing the retina;

Before reaching the retina light beams also pass through two fluid-filled chambers: the **anterior chamber**, located between the cornea and the lens, which contains the **aqueous humor** that supplies nutrients to both these structures, and the **vitreous chamber**, between the lens and the retina, containing the **vitreous humor** that accounts for the maintenance of the eye shape and contains phagocytic cells that are able to eliminate debris that might obstruct light transmission.

Fig. 1 – The anatomy of the human eye.

Retina

The retina is a multilayered structure that, despite its location, is still part of the central nervous system.

The direct pathway of visual information in the retina starts at the light sensitive photoreceptor cells which are located at the outermost layers of the retina. Light beams must first pass through several non-sensitive elements before photons can be absorbed. However, these elements are mainly transparent and since distortion in the final image is minimal, this counterintuitive arrangement allows a better nourishment and maintenance of these essential cells. Photoreceptor cells are able to convert photons into neural signals through a process called phototransduction. [3, 8, 30, 31]

Two types of photoreceptor can be identified: rods and cones. Rods are more sensitive to light but have lower spatial resolution. They are accountable for vision at lower levels of light. Due to their lack of resolution, it is common to have problems discriminating what we see at this conditions. At higher levels of luminance the rod system starts to saturate while cones become more and more dominant. [8]

While rods contain a single photo-pigment, there are three types of cones that contain different photo-pigments sensible to different wavelengths. They are referred as short, medium and long wavelength cones or more commonly as blue, green and red cones, and through their relative levels of activation we perceive color. [8, 30]

Action potentials formed at the photoreceptors are transmitted by the bipolar neurons in the central layers to the ganglion cells of the inner retina. The axons of the ganglion cells exit the eye and form the optic nerve that carries retinal information to the rest of the central nervous system. Ganglion cells are also involved in the detection of luminance changes, i.e. how we perceive brightness variations between adjacent regions. [3, 8, 30]

Other neurons in the retina are the horizontal and amacrine cells which modify and integrate visual information before it leaves the eye. While horizontal cells obtain synaptic input from photoreceptors and project laterally (parallel to retinal surface) to influence neighbor bipolar cells and photoreceptors, amacrine cells receive their input from bipolar cells terminals and project themselves to adjacent bipolar cells and ganglion cells. Resulting from these and other retinal organizations, the visual signals that leave the retina to the brain contain already preprocessed visual information that is able to highlight or discriminate different aspects of the visual space. [30, 39]

Neuroglial cells play an important role in maintaining the normal function of the retina. They are not neurons and therefore do not participate in the transmission or modulation of the visual information. However, they provide support and structure, and act as defense mechanism against injuries and infections. [31, 37]

Three main types of neuroglial cells can be distinguished in the retina: Müller cells, essential in the maintenance of retinal structure, microglial cells, phagocytic cells which number increases in response to tissue inflammation and/or injury, and astrocytes, which provide support and structure to neurons. [5, 31, 37]

Retinal Layers

Based on several imaging technics, retina is considered to be organized in ten layers (Fig. 2) which will be shortly described:

- **RPE** - The most external layer (farther to the center of the eye) is the **retinal pigment epithelium**. It's a layer of pigmented cells (melanin) that is able to reduce light backscattering. It also plays an important role in renewing photoreceptor cells and phagocytosing its debris, and is the outer blood-retinal barrier. [30, 31]
- **PL** - The **photoreceptor layer** contains the outer and inner segments of photoreceptor cells. [12, 31]
- **ELM** - Despite its name, the **external limiting membrane** (also called outer limiting membrane) is not a real membrane. It consists in intercellular junctions between photoreceptor cells and Müller cells. This

Fig. 2 – Representation of the retina and its layers

layer has the ability to block passage of large molecules and thereby acts as a metabolic barrier. [12, 30, 31]

- **ONL** - Cell bodies of rod and cone cells lie in the **outer nuclear layer**. This layer is thicker in fovea region due to cone distribution (much larger in this area). [30, 31]
- **OPL** - In the **outer plexiform layer** occur the synaptic contacts between photoreceptor terminals, bipolar cells and horizontal cells.
- **INL** - The **inner nuclear layer** contain the cell bodies of horizontal, bipolar, Müller and amacrine cells, and some displaced ganglion cells [12]. Horizontal and amacrine cells are located closer to where their synapses occur, i.e. while horizontal cells can be found next to the outer plexiform layer, amacrine cells are positioned nearer to the inner plexiform layer. [31]
- **IPL** - Bipolar cells make synaptic contacts on the dendrites of ganglion cells in the **inner plexiform layer**. Amacrine cells contact with this two set of cells also in this layer. [30]
- **GCL** - **Ganglion cell layer** is mainly constituted by ganglion cell bodies. However, it can also contain some displaced amacrine and astroglial cells. [31]
- **NFL** - In the **nerve fiber layer** ganglion cell axons run parallel to retinal surface. This axons exit the eye at the optic disk to form the optic nerve.
- **ILM** - The most internal layer (closer to the center of the eye) is the **internal limiting membrane**. As its name may indicate, this membrane limits the retina, i.e. it separates the retina from the vitreous.

Glaucoma

Glaucoma is the world second most common cause for blindness and the primary cause for irreversible visual loss. It was estimated that 130 thousand people in the United States of America alone and 7 million worldwide were blind due to primary glaucoma. [22, 29]

Despite technological advances, glaucoma processes are still poorly comprehended. Several factors may contribute to the development of this optic neuropathy but ultimately it leads to damage and loss of ganglion cells and axons at the retinal nerve fiber layer and therefore optic nerve damage that causes progressive and irreversible loss of peripheral vision (Fig. 3). Damage is often linked to increased ocular pressure caused by insufficient drainage of the aqueous humor. As pressure inside the eye begins to rise to unhealthy levels, it starts to damage the retinal nerve fiber layer, either directly by applying mechanical pressure or indirectly by obstructing the normal blood flow. [11, 30, 31, 35, 38]

Two main types of glaucoma can be distinguished: open-angle glaucoma, where drainage channels become gradually clogged through months or even years, and the less common closed-angle glaucoma, where drainage channels block due to narrowing of the angle between the cornea and the iris. In closed-angle glaucoma channels blockage can be instantaneous or gradual. [22, 29]

Fig. 3 – Simulation of glaucoma progression: patient perspective.

Several diagnosing and monitoring methods can be applied when dealing with glaucoma. Imaging technics like scanning laser tomography, scanning laser polarimetry and optical coherence tomography can provide important knowledge on disease progression by measuring the nerve fiber layer thickness and optic disk contour. [17, 38]

Optical Coherence Tomography

Optical Coherence Tomography (OCT) has gained increasingly importance in areas such as biomedical engineering and medicine. It provides high-resolution cross-sectional images that allow us to see tissue microstructure, thereby becoming an important tool in diagnose and research for several optical diseases in ophthalmology. It provides images in vivo and in situ of the ocular fundus with resolutions one or two orders of magnitude higher than conventional ultrasound technics.

OCT imaging method uses the interferometer principle. It can be compared to an echography since it uses echoes to visualize through biological tissue. Rather than sound waves, it relies on light, which provides higher spatial resolution and allows non-contact examination. As mentioned, retinal layers are transparent to a certain range of wavelengths. However, some of the light projected into it is back reflected by the internal microstructure of the retina. [7, 9]

In OCT, light passes through a beam splitter therefore being divided into sample and reference beams. As the light beam is directed onto the eye, OCT machines measure the echo time delay of the back reflected light in the internal structures, which can be used to compute its travel distance. [7, 9, 23]

A reflected sample beam comprises multiple echoes from different structures. Reference beams are reflected in a reference mirror that is positioned at a previously known distance. Reflected reference and sample beams are once again combined at the beam splitter. When the reference beam and the sample beam travel the same distance, a phenomenon called interference occurs. Its detection allows inferring the echo time delay for each reflecting structure.

A single depth scan is called an A-scan and it provides measurements of the backscattering versus depth. Combining several A-scans obtained at different transverse positions along a line, we get a representation of the backscattering along a cross-sectional plane (B-scan). These images can also be combined to form a three-dimensional representation. [2]

Time-Domain OCT

The first generation of commercially available systems was able to envision tissue microstructure in the time domain, i.e. depth information was acquired as a function of distance or time [33]. The most widely in use machine of this type is the Stratus OCT (Carl Zeiss-Meditec, Dublin, California, USA) with an axial resolution of 10 μm and a scan velocity of 400 axial scans per second [21].

A time-domain OCT sequentially measure echo time delays. It uses a movable reference mirror, adjusting echo time delays for the reference beam by changing the mirror position. The interference pattern is measured by a light sensitive detector. The pre-known distance of the mirror and the detection pattern provide the distance of the reflecting structure. [23, 24]

Spectral-Domain OCT

Spectral-domain OCT is also called Fourier-domain or even frequency-domain OCT. In these systems all echo time delays are measured simultaneously by a spectrometer and a high-speed charge coupled device (CCD), leading to increased speed when acquiring data (Fig. 4). [15, 23, 35]

Fig. 4 - B-scan obtained from:

b) a time domain machine;
a) a spectral domain machine.

An interference spectrum is obtained which undergoes Fourier transformation making it possible to acquire A-scan measurements and therefore remove the requirement to adjust the reference mirror. [15, 23, 33]

Two machines are in use at AIBILI: the Cirrus high definition OCT (Carl Zeiss-Meditec, Dublin, California, USA), with speed of 27.000 A-scans per second and 5 μm axial resolution, and the Spectralis Heidelberg Retinal Angiography (HRA)-OCT (Heidelberg Engineering, Heidelberg, Germany), able to get 40.000 A-scans per second with 7 μm optical and 3.5 μm digital axial resolution.

Studies have showed that retinal thickness measurements obtained from Spectral-Domain OCT systems are consistently superior than those obtained from Time-Domain OCT systems. The reason may be that the lower resolution provided by time domain machines, results in different calculations of the reference retinal structures by the system software. [15, 21]

Retinal Thickness

Several retinal pathologies, such as macular edema or glaucoma, may be expressed by changes in retinal thickness. This measurement allows inferring on the integrity of the retina, thus being an important tool in diagnosis and monitoring of pathology progress and treatment response. [10, 36]

Imaging technics such as slit-lamp biomicroscopy and stereo fundus photography were used to evaluate retinal thickness. However, these technics did not provide the necessary accuracy to track slight alterations in the degenerative processes. Since its appearance, OCT technology has been extensively applied in this field and studies have shown the good reproducibility of its measurements. [26]

Measuring retinal thickness by OCT imaging depends on the identification of the internal limiting membrane and the retinal pigment epithelium (Fig. 5). The distance between these two layers is regarded as the retinal thickness.

Fig. 5 – OCT image: RPE represented in red and ILM represented in green.

From Retina to the Cortex

The visual pathway between the retina and the cortex starts with the ganglion cell axons leaving the retina through a circular region in its nasal part, the optic disc, and forming the optic nerve. No photoreceptors can be found in this region, thus giving rise to the blind spot.

Axons from ganglion cells that lie at the nasal region of each retina cross from one side to another at the optic chiasm (Fig. 6), while other axons continue in the same side. Fibers leave the chiasm to form the optic tract that contains information from both eyes

Fig. 6 - Projections of retinal ganglion cells towards the Lateral Geniculate Nucleus: ganglion cells that lie at the nasal region terminate at the LGN of the opposite side.

and extends towards several targets in the diencephalon and the midbrain. [13]

Thalamus receives information from all the sensory systems with the exception of the olfactory. Processing of the visual information in this structure is conveyed to the Lateral Geniculate Nucleus (LGN). It is the major destination of the optic tract, a multilayered structure located on the dorsolateral side of the thalamus, where retinal axons terminate (axons from each eye terminate in different layers). [30, 37]

Two layer types can be distinguished based on cell size: magnocellular layers and parvocellular layers which receive input from different sets of ganglion cells (Fig. 7):

- Two magnocellular layers (layers 1 and 2), located at the ventral side of the LGN, are composed by large cells that receive input from M ganglion cells. These ganglion cells have large dendritic trees, fast conduction speed, large diameter of their axons and aren't able to transmit chromatic information. These properties are important to understand the role of the magnocellular organization at the visual perception. The magnocellular system is therefore accountable for processes that require high temporal resolution such as perceiving fast moving objects. [13, 20, 27, 30, 37]

- Located at the dorsal side of the LGN we can find four parvocellular layers (layers 3, 4, 5 and 6) composed by smaller cells that receive input from P ganglion cells. These neurons have smaller cell bodies and center-surround receptive fields, slower conduction speeds and are present in larger number than M ganglion cells. Because their receptive field centers and surrounds are selective and distinct in the class of cones they receive input from (i.e. while the center receptive field may receive input only from red cones, its surround receptive field receive input from a distinct but specific set of cones like blue or green), they are able to perceive variances in wavelengths, thus conveying color information. Like as in the magnocellular organization, P neurons properties allow us to infer on the role of the parvocellular system: it is important in processes that require high spatial resolution such as the identification of size, shape and color of objects. [13, 20, 27, 30]

Besides these two distinct pathways, a third one, the koniocellular pathway, has been identified in the inter-laminar space of the LGN (Fig. 7). Its contribution to visual perception is not still completely understood.

Fibers leaving the LGN are called optic radiations and terminate at the primary visual cortex that also sends feedback to this structure.

The Cerebral Cortex

The cerebral cortex is a convoluted sheet of tissue that surrounds the rest of the brain with an average thickness of 3 mm. Deviations can occur due to physiological or pathological reasons. Physiological variation depends mainly on measurement spot and age. Normal non-pathological range stands between 2 and 6 millimeters. Anatomical observations suggest that it is divided vertically into layers, generally 6, and horizontally into columns. [1, 19, 42]

Primary Visual Cortex

The primary visual cortex, also named Brodmann area 17, striate cortex or simply V1, can be found at the posterior limit of the brain, in the occipital lobe, surrounding the calcarine sulcus. It is the first visual cortical area, the most important structure in the visual system and the most systematically studied one.

Axons from LGN neurons terminate at layer 4 (subdivided into different strata) of the striate cortex, while axons from layer 6 send feedback to this structure. LGN division into magnocellular and parvocellular organization (M and P pathways) continues into this structure (Fig. 8): while the magnocellular system reaches layer 4C α that extends to layer 4B and from there to the extrastriate cortex, axons from the parvocellular system reach to a distinct, deeper layer 4C β , which in time project to the extrastriate cortex. [3, 31, 39]

The koniocellular pathway and also projections of the M and P pathways from layer 4C, send axons to layers 2 and 3 of V1 (Fig. 8), a pathway of conversion where it has been suggested to handle the analysis of object color. [3]

Fig. 8 – Visual information pathway at the striate cortex.

Extrastriate Cortex

Placed next to the striate cortex, the extrastriate cortex is associated with an intermediate level of visual processing. Four distinct areas are distinguishable in this region: V2, V3, V4 and middle temporal (MT).

Areas V2 and V3 constitute two cortical strips that surround V1. Each of these two regions presents a discontinuous hemifield of the visual space divided along the horizontal meridian (i.e. two quarterfields).

Two independent visual information streams (Fig. 9) of the extrastriate cortex visual processing were identified in the macaque monkey brain:

- The dorsal stream starts at V1 and connects V2, V3 and MT visual areas. Its neurons are similar to those in the magnocellular portion of V1, and are thought to be involved in motor coordination and on the analysis of motion. [3, 39]

Fig. 9 – Dorsal and Ventral streams representation.

- On the other hand, the ventral stream is associated with V1, V2 and V3 towards the temporal cortex. It is associated with perception of the visual space and object identification. [3, 39]

Magnetic Resonance Imaging

In 1976 at the University of Nottingham, Sir Peter Mansfield and his team presented for the first time images of human anatomy using magnetic resonance technology (MRI) [18]. Since then MRI has become an essential tool in almost all fields of medicine.

When an object is placed under a strong magnetic field, the nuclei of certain atoms will align with it, spinning at a frequency dependent of that field's strength. A radiofrequency wave adjusted to that frequency is then emitted, so that, when those atoms receive it, they are removed from their alignment, and consequently release oscillatory energy by slowly realigning with that field. [30, 32]

The emitted energy is then detected by the scanner. Different molecules emit energy at different frequencies and the strength of the captured signal is dependent on the nuclei number involved in the process. [30, 32]

Cortical thickness

Cortical thickness is a powerful tool in longitudinal studies and diagnosis of a wide range of neuropathologies since it may provide incisive knowledge about neurodegenerative and neurodevelopmental patterns. *In vivo* cortical thickness measurements can be achieved by using magnetic resonance images and appropriate data processing software. [1, 4, 41]

What is cortical thickness? An anatomical perspective may lie in its communal organization: it is the length of the functional cortical columns. [25, 34]

Two surfaces are defined by finding the boundaries between gray matter (GM), white matter (WM) and cerebrospinal fluid.

The convoluted disposition of the human cortex poses as a difficult challenge to overcome. How to retrieve thickness values from a curved structure? The shortest path or simply perpendicular lines to the surface are useless as shown in Figure 10. To any given point defined at any of the surfaces, a single cortical thickness values should correspond. [1, 19, 25, 43]

One solution that is widely accepted by the scientific community was first presented by Jones et al. [19] based on the Laplace's equation:

$$\Delta^2\psi = \frac{\partial^2\psi}{\partial x^2} + \frac{\partial^2\psi}{\partial y^2} + \frac{\partial^2\psi}{\partial z^2} = 0. \text{ (Eq. 1)}$$

The suggested principle is that one surface is set to the null potential and the other as a potential of one thousand Volts, while Laplace's equation is solved to the potentials in between, similar to the electrostatic field. Streamlines are the lines that connect both surfaces and are defined as orthogonal to equipotential surfaces as defined by the ruling equation. The final outcome is represented in figure 10. Streamlines are independent from initial potential

values and result in smooth transitions between surfaces. Cortical thickness is therefore defined by the length of these streamlines. [19, 43]

Fig. 10 – Diagram illustrating cortical thickness estimation by:
a) surface normals (green) and minimum distance (red);
b) Laplace's equation with equipotential field lines.

Functional MRI

Functional magnetic resonance imaging (fMRI) is a variant of MRI and its major source of contrast is the blood oxygenation level-dependent (BOLD) signal.

When a brain area is activated, the consumption of oxygen in this area increases leading to a rise in blood flow. Local blood oxygenation level at the venous portion of the circulatory system rises, since oxygen delivered exceeds its consumption. Subsequently, the levels of deoxygenated hemoglobin drop. [32]

Unlike oxygenated, deoxygenated hemoglobin (deoxyhemoglobin) has strong paramagnetic properties, and it locally distorts the magnetic field, i.e. it distorts the magnetic resonance properties of hydrogen nuclei in its neighborhood. The decrease in the concentration of deoxyhemoglobin leads to the local smoothing of the uniform magnetic field. Magnetic resonance signal is stronger in this region. [30, 32]

As conditions modify during a functional paradigm, different regions will activate or deactivate.

Retinotopic Mapping

In retinotopic mapping, stimuli are provided to subjects in order to assign the visual space to a pattern of temporal activation, i.e. to link every position in the visual space to a single delay in stimulation. This is achieved by measuring the stimulus position that causes the largest response at each cortical coordinate. [40]

Studies in primates have led to the discovery of several areas that handle the processing of visual information. These areas can be found at the occipital, parietal, and temporal lobes and each comprises a map of the visual space. [30, 40]

In humans, a strong correlation was observed between visual deficit and lesions located in the primary visual cortex (V1). Later, using positron emission tomography (PET), V1 maps were characterized in human living brains. Only with developments in functional imaging and advanced data-analysis methods have allowed the identification of additional visual structures in human brains. [30, 40]

The first fMRI research application involved retinotopic mapping, as the early stages of cortical visual processing were the main target of the involved researchers. The very first human fMRI research study tried to demonstrate that the primary visual cortex located at the calcarine fissure was essential to the early visual processing. [32]

Currently, retinotopic analysis with specific stimuli has made possible to locate on 16 separate maps of the visual space (Fig. 11). The segmentation of these regions it's of extreme help since it may provide useful information about visual system pathologies, a new insight into the organization of the visual cortex and even enlighten different visual responses in cognitive experiences. [40]

Fig. 11 – Visual field maps in the human cortex.

Methods

As aforementioned, each visual area contains a representation of the visual space and therefore a representation of each retinal point. In order to achieve the intended correlation we need to map the retina in each visual area, thus visual area segmentation is mandatory.

Through retinotopic mapping we can link the visual space to the visual cortex. The visual space will serve as a reference that links both ends of the correlation.

Retinal Thickness Measurements

Optical coherence tomography allows imaging the ocular fundus in vivo and is herewith applied as the technique of choice for measuring the retinal thickness. We resort to the high-definition spectral domain system from Zeiss (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA, USA) to compute maps of retinal thickness covering the central 20 degrees of the human macula.

The 200x200x1024 Macular Cube protocol was used for OCT data acquisition, while retinal thickness was obtained using proprietary software.

MRI Data Acquisition

The acquisition of structural data was performed using a Siemens Trio 3T (Siemens AG, Healthcare Sector, Erlangen, Germany) MRI scanner. Data was acquired using two MPAGE (magnetization prepared rapid acquisition gradient echo) T1-weighted sequences with voxel size of $1 \times 1 \times 1 \text{ mm}^3$.

The two nine minutes-long sessions of optimized MPAGE sequences were averaged to obtain a single high-resolution anatomical image therefore improving signal-to-noise ratio.

We resort to Brain Voyager QX® (Brain Innovation B.V., Maastricht, The Netherlands) software to derive cortical thickness from the human visual cortex. Cortical thickness measurements are computed based on the Laplace method following white and grey matter (WM and GM) segmentation.

In order to segment WM and GM, Brain Voyager uses a region growing approach based on voxel intensity values and therefore intensities across voxels of the same tissue should be uniform. White matter voxels at one position might have similar intensities as grey matter voxels at other locations, which poses a problem in this type of segmentation. Performing inhomogeneity correction is thus mandatory.

Some of Brain Voyager automatic segmentation tools exploit anatomical knowledge in their processes. Transforming anatomical data to Talairach coordinate system is therefore required. Talairach coordinate system of the human brain is a unified framework that allows localizing of brain structures in an independent manner from overall brain size and proportions: structures of the brain that cannot be identified only by the visual interpretation of anatomical images are defined relatively to other anatomic cerebral structures which we can be pinpointed.

Surface Model

The construction of a surface model allows for a better visualization of functional data mainly inside the sulci. Two main types of models can be used: the inflation and the flattened models. In both cases the process introduces distortion to the final model. We chose to work with the flattened one because it is two dimensional thus reducing processing time in subsequent stages.

Flattening is accomplished with Brain Voyager software following guidelines provided by the manufacturer. Due to its strong curvature, the surface requires to be sectioned. Despite the automatic tools provided by the software for this requirement, automatic sectioning at the calcarine fissure is not accurate enough, thus being required to identify 5 reference points along it. An automatic distortion correction tool provided by the software is applied in order to minimize this problem.

Functional MRI data

Functional data was also obtained using Siemens Trio 3T scanner. Technical features were:

- TR (repetition-time) = 2000 ms
- TE (echo-time) = 39ms
- Interslice time = 76 ms
- Voxel size = $2 \times 2 \times 2 \text{ mm}^3$
- FOV (field-of-view)= 256 mm x 256 mm
- Slice thickness = 2 mm
- Number of slices = 26
- Imaging matrix = 128 x 128

Brain Voyager QX software was used to gather functional maps from the gathered raw data.

Head movements in the scanner at acquisition time, diminishes fMRI data quality, leading to hampered activation patterns.

Motion correction through Brain Voyager software allows rectification of small movements. Three translation parameters as well as three rotation parameters are able to describe head motion. These are iteratively assessed by evaluating in what way the volume has to be translated or rotated so that a better alignment with a reference volume is achieved.

Another important task in preprocessing functional data is to correct slice scan time. The problem is that the entire volume is normally not covered at once. Instead, a series of two dimensional scans are sequentially acquired and assembled to form 3-D data. In the current case, with a TR of 2000 ms, the last scan is acquired virtually 2 seconds after the first one.

One solution to this problem is to perform the statistical analysis at each of the whole 2-D scans and then shift the time point to the reference scan, thereby considering that the volume was acquired simultaneously in time, i.e. at the beginning of TR. This method requires temporal interpolation of each scan.

In order to display the final functional data, anatomical and functional volumes need to be aligned with each other. Brain Voyager software initially performs a scaling transformation so that anatomical and functional data sets have the same resolution. Finally, rotation and translation transformations allow the required alignment.

Retinotopic Mapping

Visual stimuli are provided to subjects in real time by computerized imaging through a mirror, which was used to reflect the image from a screen while the subject is undergoing the fMRI imaging. In this process, subjects should focus on a fixation target (a yellow dot) in the center of the image. Magnetic resonance imaging sequences are obtained simultaneously.

Stimuli

One of the most applied techniques is the travelling-wave method or phase-encoded retinotopic mapping. In this technique two functional paradigms must be presented to subjects (Fig. 12):

- Polar angle paradigm: consists on a checkerboard wedge that rotates around the fixation target;
- Eccentricity paradigm: consists on a checkerboard ring that expands from the fixation target;

Fig. 12: Polar Angle (a) and Eccentricity (b) stimuli.

Together, these two measurements specify visual field positioning in polar coordinates, providing angle and eccentricity values. Three polar angle data sets are combined into a single volume for improved data quality.

The checkerboard panel presented on the stimuli intends to ensure good neural response.

Segmentation of Visual Areas

Manual segmentation of the visual cortex is time consuming, labor intensive and presents undesired variability. Automated software that maps the visual field is thus desirable. An algorithm (Fig. 18) was developed and implemented in Matlab® (The MathWorks Inc., Natick, Massachusetts, United States).

Segmentation Algorithm

Data are imported into Matlab using the BVQXtools v0.8, a Matlab toolbox available at the BrainVoyager site. It allows reliable data exporting to Matlab, for most of the major BrainVoyager QX's file formats.

Brain Voyager analysis produces a large amount of files, most of them not required at the present stage, and each of these files still contains unnecessary information. We retrieve vertex coordinates using the surface file and polar angle and eccentricity stimulus position for each vertex from 'smp' files.

Additionally using BVQXtools v0.8, a threshold map of brain activation is created. Retinotopic data is then filtered using this map so that only relevant vertices are displayed, which significantly improves visualization and facilitates subsequent algorithm processing phases.

The representation is casted into an image for future analysis so that more efficient methods could be applied, hence reducing processing time and enhancing results. The limitations imposed by the triangular mesh are thus avoided.

Because of its local computation gradients are sensitive to noise leading to the need low pass filtering. A low-pass filter of $[30 \times 30]$ kernel was used.

The Kernel size was decided on a try-and-error basis. In figure 13 it is possible to visualize filter size effect in the image and in the corresponding gradient. As kernel size increases, the boundaries become more visible.

A previous study combined Polar Angle and Eccentricity information to compute a Vector Field Sign map (VFS) [42]. In this way, the gradient of the polar angle ($\nabla\theta$) and eccentricity ($\nabla\rho$) maps are required to be computed on the cortical surface:

$$VFS = \text{sign}(\det(\partial\psi)), \text{ (Eq. 2)}$$

where ψ is the function that maps each point on the cortical surface position in the visual field. Therefore:

$$VFS = \text{sign}(\det(\nabla\theta, \nabla\rho, \hat{n})), \text{ (Eq. 3)}$$

where $\hat{n}$ is normal to the surface.

This method was implemented using Matlab in a first attempt. However, it didn't prove itself as robust as we expected, i.e. the method was too much sensitive to data artifacts. The achieved results presented artifacts that turn into a challenge for the delineation of the visual areas.

Several attempts to improve achieved results were made. By using a flattened surface model we removed the requirement of including $\hat{n}$ (Eq. 3), therefore:

$$VFS = \text{sign}(\det(\nabla\theta, \nabla\rho)). \text{ (Eq. 4)}$$

Higher order filters were also used in order to get smoother data. However, it didn't seem the results were enhanced to a sufficient degree which ultimately led us to look for alternatives.

Longer acquisition time and/or using the same combination method for eccentricity paradigms as the use of polar angle paradigms might have improved results (three polar angle data sets were combined).

Segmentation of the visual areas V1, V2 and V3, using retinotopic data, is only possible since the representation of the visual space between contiguous areas changes its orientation, i.e. adjacent areas have mirror or non-mirror visual representations. Therefore, using a flattened surface model, polar angle is enough to determine boundaries between retinotopic visual areas.

Attending to this information we propose that the reversals on the orientation of polar angle representations can be easily perceived in a directional gradient map. In this way:

$$Orientation = sign(\partial F_{\theta} / \partial x), \text{ (Eq. 5)}$$

where F_{θ} represents polar angle function.

A similar process is applied to eccentricity data:

$$Orientation = sign(\partial F_{\rho} / \partial y), \text{ (Eq. 6)}$$

where F_{ρ} represents eccentricity function. The inversion in its gradient direction allows us to identify the boundary between dorsal and ventral regions of the visual cortex.

Two binary images are created, one containing pixels with positive orientation and the other with negative orientation. We perform morphological opening, erosion followed by dilation, in order to break weak links between clusters of pixels with the same orientation. The resulting clusters become candidate visual areas (Fig. 15).

Information from each of the candidate visual areas, such as gradient orientation (mirror or non-mirror visual representation), centroid, area, neighbor regions and pixel position in the image, is calculated/retrieved and stored for future application.

Neighbor regions are defined as the regions that directly contact with the region of reference but only in the direction of the visual pathway (posterior to anterior), i.e. V2 would be neighbor of V1, but V1 would not be neighbor of V2.

The candidate regions are labeled and a mask is created (Fig. 14) using a structuring element such as:

$$\begin{bmatrix} 1 & 1 & 0 & 0 & 0 \end{bmatrix}.$$

The mask is then applied to the labeled image as the neighbor regions are the ones labeled with the values of the resulting non-zero pixels. This process turned out to be helpful since, as we will see, the classification is performed sequentially: it limits the number of regions which will go under consideration, eliminating lateral and posterior regions.

Fig. 14 – a) Labelled image; b) Mask.

Neighbor
Information

As shown in figure 15 the pixel clusters obtained don't possess a clear boundary between them. Instead we clearly distinguish the regions and an interregional space. The use of the boundaries of each singular region would go against anatomy, since it would

Fig. 15 – Candidate Visual Areas.

mean the presence of two different boundaries between two visual areas. To obtain the final boundaries we achieve the inter-regional space skeleton.

Fig. 16 – Inter-regional space skeleton and loose ends removal.

The resulting skeleton is shown in figure 16. It presents the wanted boundaries in addition to loose ends needing to be pruned. The boundaries are continuous, with no endpoints (Fig. 17). Pruning is performed by sequentially calculating and removing endpoints until the boundaries become clean and no endpoints can be found.

Fig. 17 – The central pixel is:

- a) not an endpoint;
- b) an endpoint.

Skeleton
Gathering

Pruning

The next step is the classification of the candidate visual areas, and it starts by dividing regions into dorsal or ventral. We then find ventral V1 since it is the more robust one. First we need to know that visual areas have typical orientation and relative positions, so candidate regions are matched against for these features.

As it has been said before, V1 is the most posterior visual area, and by using this anatomical feature we are able to locate this region. When tracing a line between dorsal and ventral V1 it would not be a vertical line (Fig. 15). This natural orientation makes ventral V1 classification less affected by lateral artifact resulting regions. Dorsal V1 is found by a similar “neighbor finding” process but instead of using a horizontal structuring element, a vertical one is used.

Sequentially the algorithm finds V2 that must be a neighbor of V1 and V3 that must be a neighbor of V2. Please note that this process is divided in dorsal and ventral streams. Some discrepancies might be found in this process, such as small clusters that are the result from fMRI artifacts, but are overcome by the ranking system that is able to exclude them.

A Brain Voyager ‘poi’ (patch-of-interest) file is then created. This file contains information about each visual area such as name or vertices that belong to that area.

Fig. 18
Algorithm schematics

Algorithm Validation

The segmentation algorithm was validated against manual segmentation using a total of 6 data sets from healthy subjects. Inter and intra-subject boundaries variation of manual segmentation was compared to variation values obtained between manual and automatic segmentation.

Manual segmentation was performed by two researchers in order to gather inter-subject variation. One of the researchers repeated the segmentation process two weeks after to achieve intra-subject variation.

For each boundary point we calculated relative distance (r) and angle (θ) to a fixed center of each region, therefore computing their polar coordinates (Fig. 19 a). In order to obtain a continuous line between the points, we performed linear interpolation of r between $-\pi < \theta \leq \pi$. The first and last points are repeated at the end and at the beginning respectively, to achieve an accurate interpolation in the full length of the interval (Fig. 19 b).

Fig. 19 – a) Region Boundary and schematics of r and θ ; b) Boundary points (blue) and interpolation (black). In red we can see the repeated points.

The average ($\bar{x}$) and the standard deviation (sd) of the variation of the two segmentations was calculated using:

$$\bar{x} = \frac{1}{N} \sum_{i=1}^N x_i; \quad sd = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}; \quad (\text{Eq. 7 and 8})$$

where n is the number of elements in the sample and x the data.

Visual Areas Cortical Thickness

A Matlab function was developed to retrieve cortical thickness values and other statistic measurements, such as average, standard deviation, median or standard error, from visual areas.

It receives as input: 'poi' Brain Voyager files containing the visual areas, and 'smp' files containing cortical thickness surface map. The output is an excel file as well as a scatter plot graphic.

Correlating the Retina to the Visual Cortex

Retinotopic mapping links each vertex in the visual cortex to a polar angle and eccentricity stimulus position. A total of 24x12 patches are created mapping the visual space, therefore it is a poor resolution technique at least when compared to data obtained by OCT (Fig. 21).

In order to correlate the retina to the brain it is mandatory to find a continuous transformation function that links each retinal point to a cortical point and vice-versa. The problem of attributing a value for new points within a given set of scattered points is called interpolation [28].

Fig. 20 – Polar angle and eccentricity mapping.

Interpolation

Using Delaunay triangulation, interpolation of a data set which is defined by its location (x, y) produces a surface:

$$surf = F(x, y), \text{ (Eq. 9)}$$

therefore the value v of any given location (x', y') within the smallest convex set comprising the original set of points (the convex hull) can be obtained by:

$$v = F(x', y'). \text{ (Eq. 10)}$$

As the word suggests, a triangulation of a given set of points is the organization of those points in triangles. In order to achieve a 'good' triangulation two models can be distinguished. One that minimizes the maximal angle, or alternatively, that maximizes the minimal angle of all the angles of the triangles in the triangulation. [16]

Delaunay triangulation is defined at the convex hull of the given set of points and uses the MaxMin angle criterion. It is built so that no point of the set is contained in any triangle circumcircle (Fig. 22). [16]

Fig. 21 - Delaunay triangulation with its circumcircles represented.

Retrieving a scattered data set from the retinotopic data implicates finding and crossing the representative lines obtained from each of the polar angle and eccentricity regions. They are obtained by performing object reduction, i.e. sequential boundary pixels removal while maintaining the Euler number. The result is a line equidistant to the region boundaries.

Fig. 22 – Polar Angle (a) and Eccentricity (b) before and after filtering in V2 region of the visual cortex.

Data is filtered through a low pass filter (Fig. 23). By obtaining a smoother set of representative lines we reduce pruning and therefore processing time. The improvement achieved can be easily perceived in figure 24.

Fig. 23 – Polar angle and eccentricity representative lines before (a and c) and after filtering (b and d).

Pruning

In the process of retrieving the representative lines, redundant branches are often created that may corrupt the final results (Fig. 24). Crossing these lines often creates duplicated points for the same polar angle and eccentricity values.

Fig. 24 – The pixel is:
a) a branchpoint;
b) not a branchpoint.

Trying to overcome this limitation a pruning method was developed. The first step involves labeling each branch. Starting at the line endpoints, each branch is tracked until it reaches a branchpoint (Fig. 25). Tracking is processed by scanning the current point neighborhood (Fig. 26) for the following one.

Representative lines that don't possess any branchpoints are considered final and don't take part in this process.

Having labeled each branch, we scan the branchpoints finding conflicts where we are able to apply our algorithm, i.e. the branchpoint has at least two branches connected to it that possess an endpoint (end-branches). If at a certain branchpoint this is not the case, it means that other branchpoints need to be solved first.

$(i+1,j-1)$	$(i+1,j)$	$(i+1,j+1)$
$(i,j-1)$	(i,j)	$(i,j+1)$
$(i-1,j-1)$	$(i-1,j)$	$(i-1,j+1)$

Fig. 25 – Neighborhood.

In each conflict, identifying a reference branch is mandatory:

- If one of the branches is connected to another branchpoint it is considered as the reference;
- If not, the longest branch is chosen.

In order to solve each conflict three criteria are used:

- If a branch is shorter than five pixels it is automatically deleted;
- If one of the branches is much smaller than the other, the longer branch is chosen;
- Finally the branch is chosen based on a continuity criterion, i.e. the branch which vector forms the smallest angle with the reference branch vector (Fig. 27).

Fig. 26 – Continuity criterion.

End-branches vectors are created between its branchpoint and endpoint. In the reference branch the vector is created between the branchpoint and the point located at the same distance as the one between endpoint and branchpoint of the smallest end-branch.

Instead of calculating the angle and since:

$$\cos(\theta) = \widehat{v1} \cdot \widehat{v2}, \text{ (Eq. 11)}$$

where $\widehat{v1}$ and $\widehat{v2}$ are two unit vectors, we retrieve the branch whose unit vector generates the maximum dot product with the reference branch.

Finally if there still are branchpoints present, the process is repeated.

Results

In this study, we were able to export Brain Voyager data, develop and implement automated software that maps the visual field, retrieve and cross representative lines, to employ an effective pruning method and to interpolate polar angle and eccentricity data.

In this section, we show MRI resulting data and demonstrate our approach for segmenting the visual areas V1, V2 and V3. We also present the results of the comparison between our automated method and the manual segmentation one.

In addition the reader may visualize the processing stages in order to achieve the interpolation of retinotopic data and the final results.

Magnetic Resonance Imaging

With magnetic resonance imaging we are able to obtain cortical thickness maps (Fig. 28). Cortical thickness measurements allow us to evaluate cortical integrity.

Fig. 27 – Cortical thickness map on a flattened surface model. Blue color represents thinner regions while green represents thicker ones (range: 0.5 – 5 mm).

Two functional paradigms: polar angle and eccentricity, allow us to map the visual space. Functional data in figure 29 is presented in the flattened surface model and was obtained on the right hemisphere of two healthy subjects.

Fig. 28 – Polar angle and eccentricity fMRI results for two different subjects;
Subject one: a) and b); Subject two: c) and d)

Data Importing

The first step in order to achieve the intended correlation was to make data fully accessible. Cortical thickness and retinotopic data was successfully exported from Brain Voyager QX® into Matlab.

By removing vertices whose activation is not related to the provided stimulus and casting the covered triangular mesh into an image, we enhance subsequent algorithm processes. Figure 30 shows unprocessed polar angle and eccentricity maps within Matlab.

Segmentation

Our approach was based on the computation of directional gradient maps which allows identifying inversions of polar angle representations. Our algorithm is capable of the segmentation and classification of dorsal and ventral V1, V2 and V3 visual areas. The segmentation results presented in this section were obtained on the data presented above and followed the procedure already described.

Data Filtering

As mentioned, in order to get an accurate gradient representation, it is essential to have smooth data transitions. Figure 30 shows polar angle data after filtering (as an example arrows represent gradient direction).

Fig. 30 - Polar Angle representations of the two subjects after filtering.
Arrows in white indicate local gradient direction.

Gradient Gathering and Visual Areas Delineation

Figure 31 shows the obtained directional gradient maps. These maps allow the direct delineation of the visual areas: positive and negative directional gradients indicate opposite directions in which the visual space is represented in the visual cortex. The reversal lines in the gradient map of the polar angle correspond to visual areas boundaries (Fig. 32).

Fig. 31 - Polar angle directional gradient maps.

Fig. 32 - Polar Angle representations of the two subjects.
In white visual areas boundaries obtained

Visual Areas Classification and Final Results

Figure 33 shows segmentation result after being imported back into Brain Voyager software.

Fig. 33 – Final segmentation results for both subjects.

Algorithm Validation

To test the validity of the herewith proposed algorithm, we performed automated segmentation on retinotopic data from six healthy subjects. The same data was also manually segmented by two researchers. One of the researchers repeated the analysis two weeks after. Reference boundaries for inter- and intra-subject validation were obtained by averaging the two manual boundaries.

Pre-processing stages for all the subjects were performed following the same method.

Error was computed at each boundary point and averaged to the entire boundary. Two types of error measures were used:

- Each boundary point variation value was computed and normalized with the standard deviation (sd) of the manual segmentations (standard score). Since $N=2$ (two graders) the boundaries may cross, therefore it is possible that $sd=0$. To avoid this normalization was performed with $(sd+1)$:

$$z = \frac{p - \bar{x}}{sd+1}, \text{ (Eq. 12)}$$

where z is the 'standard score' and $\bar{x}$ the average of the two manual segmentations.

- Standard deviation of the computed boundary points.

Data was excluded when the graders or the algorithm were not able to produce valid boundaries.

Due to the similarity and to the large amount of data produced by this process, only particular cases will be further discussed.

Inter-Subject Validation

For each individual region and for each hemisphere, table 1 presents inter-subject variation values normalized with (sd+1), while table 2 presents standard deviation values in mm. Two researchers provided manual segmentations (N=2).

Tab. 1 - Normalized Inter-subject Variation (N=2).

Subject	Hemisphere	Dorsal			Ventral			Average
		V1	V2	V3	V1	V2	V3	
S1	Left	0.08	0.37	0.38	0.29	0.35	0.37	0.31
	Right	0.62	0.52	0.11	0.25	0.46	0.22	0.36
S2	Left	0.32	0.19	0.47	0.09	0.19	0.22	0.25
	Right	-	-	-	-	-	-	- *1
S3	Left	0.16	1.68	0.37	0.53	0.04	0.38	0.53
	Right	0.70	0.29	1.13	0.13	0.25	0.79	0.55
S4	Left	0.14	0.63	0.57	0.93	0.36	0.13	0.44 *2
	Right	0.59	0.35	0.28	0.36	0.60	0.22	0.40
S5	Left	0.57	0.68	0.68	0.10	0.38	0.21	0.42
	Right	-	-	-	-	-	-	- *3
S6	Left	0.04	0.35	0.53	-	0.57	-	0.37 *4
	Right	0.57	0.68	0.68	0.10	0.38	0.21	0.42

Tab. 2 - Inter-subject Standard Deviation values (mm).

Subject	Hemisphere	Dorsal			Ventral			Average
		V1	V2	V3	V1	V2	V3	
S1	Left	1.47	1.17	1.10	0.74	0.64	0.55	0.95
	Right	1.38	1.24	1.19	0.86	0.95	0.75	1.06
S2	Left	0.67	1.21	1.33	1.07	0.85	1.10	1.04
	Right	-	-	-	-	-	-	-
S3	Left	1.14	3.17	2.11	0.92	0.53	0.84	1.45
	Right	1.75	1.00	1.81	0.85	0.77	1.60	1.30
S4	Left	0.83	1.16	1.51	2.69	0.79	0.92	1.32
	Right	1.29	0.82	0.86	0.65	0.98	1.06	0.94
S5	Left	1.14	1.28	1.30	0.65	0.93	0.67	0.99
	Right	-	-	-	-	-	-	-
S6	Left	1.32	0.84	0.87	-	1.64	-	1.17
	Right	1.14	1.28	1.30	0.65	0.93	0.67	0.99

*1

*2

*3

*4

- * 1. The algorithm was incapable of producing valid data;
- * 2. Despite Brain Voyager being able to read the file containing data from the first segmentation, when imported into Matlab, it appeared to be corrupted. Data from the second segmentation was used instead;
- * 3. One of the researchers was not able to provide a valid segmentation, due to poor data quality;
- * 4. The manual segmentations contained completely different boundaries for ventral regions. This case will be further explained in a subsequent section.

Intra-Subject Validation

For each individual region and for each hemisphere, Table 1 presents intra-subject variation values normalized with (sd+1), while Table 2 presents standard deviation values in mm. Manual segmentations were provided by the same researcher two weeks apart (N=2).

Tab. 3 - Intra-subject Variation (N=2) normalized with (sd+1).

Subject	Hemisphere	Dorsal			Ventral			Average
		V1	V2	V3	V1	V2	V3	
S1	Left	0.16	0.31	0.87	0.41	0.73	0.52	0.50
	Right	0.64	0.42	0.12	0.10	0.39	0.45	0.35
S2	Left	-	0.27	0.24	0.39	0.22	0.32	0.29 * ¹
	Right	-	-	-	-	-	-	- * ²
S3	Left	0.71	1.95	0.54	0.41	0.42	0.26	0.71
	Right	1.12	0.42	1.19	0.37	0.32	1.26	0.78
S4	Left	-	-	-	-	-	-	- * ³
	Right	0.83	0.48	0.40	0.25	0.30	0.35	0.43
S5	Left	0.58	0.30	0.53	0.39	0.17	1.26	0.54
	Right	-	-	-	-	-	-	- * ⁴
S6	Left	0.13	0.52	0.76	0.75	0.40	0.43	0.50
	Right	0.62	0.86	0.58	0.11	0.49	0.22	0.48

Tab. 4 - Intra-subject Standard Deviation values in mm.

Subject	Hemisphere	Dorsal			Ventral			Average
		V1	V2	V3	V1	V2	V3	
S1	Left	1.42	1.23	1.61	0.92	1.10	0.91	1.20
	Right	1.39	1.28	1.09	0.89	0.92	0.87	1.07
S2	Left	-	1.36	1.47	1.11	1.09	1.42	1.29 * ¹
	Right	-	-	-	-	-	-	- * ²
S3	Left	1.09	3.38	2.10	0.86	0.75	0.67	1.47
	Right	1.56	1.01	1.86	0.98	0.84	1.75	1.33
S4	Left	-	-	-	-	-	-	- * ³
	Right	1.34	1.02	0.84	0.80	0.91	1.01	0.99
S5	Left	1.01	0.77	1.06	0.81	0.74	2.70	1.18
	Right	-	-	-	-	-	-	- * ⁴
S6	Left	1.31	0.77	1.05	1.14	1.02	0.93	1.04
	Right	1.17	1.27	1.49	0.70	1.05	0.65	1.06

* 1. When imported into Matlab, dorsal V1 from the second segmentation is not correctly read;

* 2. The algorithm was incapable of producing valid boundaries;

* 3. This analysis was not possible, since when imported into Matlab data from the first segmentation appeared to be corrupted;

* 4. One of the researchers was not able to provide a valid segmentation, due to poor data quality.

Validation Analysis

In this section we will analyze overall results and present segmentation results for the best and worst performances achieved, i.e. the highest and lowest standard deviation. Table 5 summarizes the validation data.

Tab. 5 – Validation Data Summary.

	Hemisphere	Dorsal			Ventral			By	
		V1	V2	V3	V1	V2	V3	Hemisphere	
Inter-subject analysis	z (sd+1)	Left	0.22	0.65	0.50	0.38	0.33	0.26	0.39
		Right	0.62	0.46	0.55	0.21	0.42	0.36	0.44
		By Region	0.42	0.55	0.52	0.29	0.38	0.31	Total Average
								0.41	
	sd (mm)	Left	1.10	1.47	1.37	1.22	0.89	0.82	1.15
		Right	1.39	1.08	1.29	0.75	0.91	1.02	1.07
By Region		1.24	1.28	1.33	0.98	0.90	0.92	Total Average	
							1.11 mm		
Intra-subject analysis	z (sd+1)	Left	0.40	0.67	0.59	0.47	0.39	0.56	0.51
		Right	0.83	0.52	0.57	0.21	0.38	0.57	0.51
		By Region	0.61	0.60	0.58	0.34	0.38	0.56	Total Average
								0.51	
	sd (mm)	Left	1.21	1.50	1.46	0.97	0.94	1.33	1.24
		Right	1.37	1.15	1.32	0.84	0.93	1.07	1.11
By Region		1.29	1.32	1.39	0.90	0.94	1.20	Total Average	
							1.17 mm		

Differences between the algorithm and manual segmentations are consistently higher in dorsal regions, which supports the theory that ventral V1 is less affected by artifacts. No major differences are present between left and right hemisphere error measures.

Both error measures are higher in intra- than inter-subject validation. However, the difference in 'z' values ($\approx 19.3\%$) are higher when compared to sd ($\approx 5.4\%$).

One of the reasons for the higher difference verified in 'z' values, may lay in the fact that sd values of the manual segmentations for the intra-subject validation are consistently lower than the ones from inter-subject validation (Tab. 6).

Tab. 6 – Variation values for the Manual Segmentations (mm).

Subject	Hemisphere	Inter-Subject	Intra-Subject
S1	Left	1.63	0.85
	Right	1.60	0.71
S2	Left	2.45	1.24
	Right	-	-
S3	Left	1.53	1.52
	Right	1.97	1.38
S4	Left	-	-
	Right	1.88	0.76
S5	Left	2.10	1.92
	Right	-	-
S6	Left	1.55	0.91
	Right	1.39	1.02

Subject S4; Right Hemisphere

The right hemisphere of subject S4 provided the lowest average standard deviation values for the inter- and intra-subject validation. Figure 34 shows reference (green) and obtained (blue) boundary for ventral V1 (inter-subject validation), which is the region with the lowest standard deviation. Red and black curves represent the standard deviation of the obtained boundary and from the manual segmentations respectively.

Fig. 34 – Validation data for ventral V1. Reference (green) and obtained (blue) boundary representation. In red and black, sd values for the obtained and manual segmentation, respectively.

Notice that standard deviation values for our algorithm are lower than the ones for the graders.

The major differences are located in the superior and inferior boundaries. As we will see, this is constant throughout the results, even when comparing manual segmentations of different researchers.

Fig. 35 – Inter- (a) and intra-subject (b) validation data for dorsal region V2.

Figure 35 presents inter- and intra-subject validation results for dorsal region V2. Despite the reference boundaries being similar for both analyses, manual boundaries standard deviation values are lower for the intra-subject validation, which may explain the higher normalized variation values.

This hemisphere is demonstrative that the developed algorithm usually performs worse on dorsal regions than on ventral regions.

Subject S3; Left Hemisphere

The worst performance from inter- and intra-subject validation was found on the left hemisphere of subject S3, mainly due to dorsal regions V2 and V3.

Fig. 36 – Inter- (a) and intra-subject (b) validation data for dorsal region V2.

Figure 36 shows inter- and intra-subject validation results for dorsal region V2, which is the region with the highest standard deviation values in both analyses.

In contrast, the algorithm performs considerably better in ventral regions. Figure 37 shows validation results for ventral region V2. In both analysis, this region presents low standard deviation and normalized variation values.

Fig. 37 – Inter- (a) and intra-subject (b) validation data for ventral region V2.

Subject S6; Left Hemisphere

Fig. 38 – Manual segmentation of V1, V2 and V3 performed by two researchers.
IV – Segmentation in red; OA – Segmentation in black.

In figure 38, IV (black) and OA (red) boundaries represent two different manual segmentations. Notice that in the ventral region:

- V1 and V2 from OA segmentation are completely inside V1 from IV;
- V2 from IV corresponds to V3 provided by OA;
- Finally IV segmentation of V3 has no correspondence at all.

To avoid losing all the data from this hemisphere, and since retinotopic data seems to support OA segmentation, we assumed V2 provided by IV as V3 and excluded other ventral regions. The algorithm resulting regions are similar to those from the OA segmentation.

Correlating the Retina to the Visual Cortex

To correlate the retina to the visual cortex we need to compute a continuous transformation function that maps each retinal point in the cortex and vice-versa.

By retrieving and crossing representative lines from polar angle and eccentricity data we are able to create a scattered set of points with polar angle and eccentricity values well defined. In order to get a continuous representation, data in between that set of points, needs to be interpolated through the Delaunay triangulation approach.

Before being intersected, representative lines need to be pruned. In this section it will be presented pruning and crossing results.

Pruning

Representative lines are attained by object reduction, i.e. sequential boundary pixels removal while maintaining the Euler number. As a result we get line equidistant to the region boundaries.

Since smoother transitions reduce pruning necessity, data is filtered with a low pass filter. Figure 39 shows visual areas dorsal and ventral V2 representative lines before and after pruning.

Fig. 39 – Polar angle (a) and eccentricity (b) representative lines before and after pruning.

Pruning is the process in which redundant branches are removed. Our method was based on the three aforementioned criteria. The proposed method was employed with success, and a consistent small increase in points with singular representations was verified (Fig. 40).

Fig. 40 – Resulting scattered points. Represented in red are points removed by pruning.

Interpolation

After intersecting the representative lines, the outcome is a collection of scattered data points that can be visualized in a polar angle or eccentricity scale (Fig. 41).

Fig. 41 – Eccentricity (a) and Polar angle scale (b) on resulting scattered points.

Figure 42 and 43 shows initial data and interpolation results for two subjects. The number of points that we find by intersecting the lines is the major limitation to this process. The higher the number of points, the more accurate is the interpolation. In these particular cases almost 40% of the points weren't located.

Fig. 42 – Initial maps and interpolation results. Interpolation data in b) represents an augmented region of a).

Notice that the interpolation is only achieved in the visual areas V1, V2 and V3. Data in figure 42 and 43 b) represents an augmented region of a).

a)

b)

Fig. 43 – Initial maps and interpolation results. Interpolation data in b) represents an augmented region of a).

Discussion and Conclusion

In order to reduce consumed time, labor necessity and variability, we implemented an automatic method that segments visual areas V1, V2 and V3.

The number of experienced graders available for the validation process is limited, thus the small number of cases presented (N=6).

Inter- and intra-subject validation presented a total average standard deviation of 1.11 and 1.17 mm respectively. One of the limitations for this technique is the imaging method itself. On the validation set, the average distance to the closest point in the visual cortex for the surface model, ranges between 0.75 to 0.85 mm. As a curiosity, we computed standard deviation for our segmentation before and after being casted to the original vertices, the average was of 0.27 mm.

The algorithm performed worst with the left hemisphere of subject S3. Figure 44 shows its polar angle map. Notice that white and blue lines represent automatic and manual boundaries respectively.

As shown in figure 45, our algorithm is able of interpolating 'holes', i.e. vertices whose activation values were lower than the threshold due to acquisition artifacts. However, it does not overcome overall image boundaries. In contrast, manual segmentations may disregard these boundaries, which can contribute to higher standard deviation values.

Fig. 45 – Polar angle map of subject S3 (left hemisphere) and the manual segmentations provided by different researchers.

Between dorsal V1 and V2, a depression in polar angle values (Fig. 44 b) creates distortions in gradient directions, which directly influence dorsal V1 and V2 boundaries and subsequently V3. Manual segmentations may perceive it as an artifact and overcome it. However, it will always be a region of divergence that depends on the applied criteria. Figure 45 shows for the same subject the two researchers' manual segmentations.

Fig. 46 – Polar angle map of subject S1 (right hemisphere) and the manual segmentations provided by different researchers.

Figure 46 shows the manual segmentations provided by different researchers. As mentioned, the major differences are located in the superior and inferior boundaries. Lateral boundaries have been the main target of study in the literature, therefore superior and inferior boundaries are not so well defined and the criteria might vary.

Interpolation quality depends on the number of located scattered points. Due to data quality not all points are found. In several cases, retinotopic data is not distributed in the full scale or, only a very small region becomes activated to a specific stimulus position (Fig. 47).

Fig. 47
Polar angle
map.

In conclusion, the algorithm is viable. However, due to the large variability in the data, supervision from the user may be required. The inclusion of a semi-automatic mode that can be applied to problematic data may be beneficial (inclusion of changeable parameters).

Increasing acquisition time of the functional data may improve interpolation but also segmentation results.

References

- 1 Acosta, O., Bourgeat, P., Zuluaga, M.A., Fripp, J., Salvado, O., Ourselin, S. (2009). Automated voxel-based 3D cortical thickness measurement in a combined Lagrangian–Eulerian PDE approach using partial volume maps. *Medical Image Analysis*. 13(5):730-743.
- 2 Baroni, M., Fortunato, P., La Torre, A. (2006). Towards quantitative analysis of retinal features in optical coherence tomography. *Medical Engineering & Physics*. 29(4):432-441
- 3 Bear, M.F., Connors, B.W., Paradiso, M.A. *Neuroscience Exploring the Brain*. (2007). 3rd edition, Lippincots Williams & Wilkins.
- 4 Bernal-Rusiel, J.L., Atienza, M., Cantero, J.L. (2010). Determining the optimal level of smoothing in cortical thickness analysis: A hierarchical approach based on sequential statistical thresholding. *NeuroImage*. 52(1):158-171.
- 5 Bernardos, R.L., Barthel, L.K., Meyers, J.R., Raymond, P.A. (2007). Late-Stage Neuronal Progenitors in the Retina Are Radial Müller Glia That Function as Retinal Stem Cells. *The Journal of Neuroscience*. 27(26):7028-7040.
- 6 Boucard, C.C., Hernowo, A.T., Maguire, R.P., Jansonius N.M., Roerdink, J.B.T.M., Hooymans J.M.M., Cornelissen, F.W. (2009). *Changes in cortical grey matter density associated with long-standing retinal visual field defects*. *Brain*. 132(7):1898-1906.
- 7 Brezinski, M.E. *Optical Coherence Tomography: Principles and Applications*. (2006). Academic Press.

- 8 Dowling, J.E. *The Retina: an Approachable Part of the Brain*. (1987). Belknap Press of Harvard University Press.
- 9 Drexle, W., & Fujimoto, J.G. *Optical Coherence Tomography: Technology and Applications*. (2008). Springer.
- 10 Fabritius, T., Makita, S., Miura, M., Myllylä, R., Yasuno, Y. (2009). *Automated segmentation of the macula by optical coherence tomography*. Optical Society of America. 17(18):15659-15669.
- 11 Fernández, D.C., Salinas, H.M., Puliafito, C.A. (2005). *Automated detection of retinal layer structures on optical coherence tomography images*. Optical Society of America. 13(25):10200-10216.
- 12 Garvin, M.K. *Automated 3-D Segmentation and Analysis of Retinal Optical Coherence Tomography Images*. (2008). The University of Iowa, Iowa City, Iowa.
- 13 Gazzaniga, M.S. *The New Cognitive Neurosciences*. (2000). 2nd edition, The MIT Press.
- 14 Gupta, N., Greenberg, G., Noël de Tilly, L., Gray, B., Polemidiotis, M., Yücel, Y.H. (2009). *Atrophy of the lateral geniculate nucleus in human glaucoma detected by magnetic resonance imaging*. British Journal of Ophthalmology. 93(1):56-60.
- 15 Han, I.C., Jaffe, G.J. (2008). *Comparison of Spectral- and Time-Domain Optical Coherence Tomography for Retinal Thickness Measurements in Healthy and Diseased Eyes*. American Journal of Ophthalmology. 147(5):847-858.
- 16 Hjelle, Ø. & Dæhlen, M. *Triangulations and Applications (Mathematics and Visualization)*. (2006). Springer
- 17 Ishikawa, H., Stein, D.M., Wollstein, G., Beaton, S., Fujimoto, J.G., Schuman, J.S. (2005). *Macular Segmentation with Optical Coherence Tomography*. Investigative Ophthalmology & Visual Science. 46(6):2012-2017.

- 18 Johnson, M.C., Policeni, B., Lee, A.G., Smoker, W.R.K. *Neuroimaging in Ophthalmology*. (2011). 2nd edition, Oxford University Press in cooperation with the American Academy of Ophthalmology.
- 19 Jones, S.E., Buchbinder, B.R., Aharon, I. (2000). *Three-Dimensional Mapping of Cortical Thickness Using Laplace's Equation*. Human Brain Mapping. 11(1):12-32.
- 20 Kandel, E.R., Schwartz, J.H., Jessel, T.M. *Principles of Neural Science*. (2000). 4th edition, McGraw-Hill Medical.
- 21 Kiernan, D.F., Hariprasad, S.M., Chin, E.K., Kiernan, C.L., Rago, J., Mieler, W.F. (2008). *Prospective Comparison of Cirrus and Stratus Optical Coherence Tomography for Quantifying Retinal Thickness*. American Journal of Ophthalmology. 147(2):267-275.e2.
- 22 Krieglstein, G.K. & Weinreb, R.N., . *Essentials in Ophthalmology: Glaucoma*. (2009). Springer.
- 23 Krieglstein, G.K. & Weinreb, R.N., . *Essentials in Ophthalmology: Medical retina*. (2010). Springer.
- 24 Kyung, M.L. *Segmentations Of The Intraretinal Surfaces, Optic Disc And Retinal Blood Vessels In 3D-OCT Scans*.(2009). The University of Iowa, Iowa City, Iowa.
- 25 Lerch, J. *Measuring Cortical Thickness*. (2011). McGill University, Montreal, Canada.
- 26 Liew, S.H., Gilbert, C.E., Spector, T.D., Mellerio, J., Van Kuijk, F.J., Beatty, S., Fitzke, F., Marshall, J., Hammond, C.J. (2006). Central retinal thickness is positively correlated with macular pigment optical density. Experimental Eye Research. 82(5):915-920.
- 27 Livingstone, M., Hubel, D. (1988). *Segregation of form, color, movement, and depth: anatomy, physiology, and perception*. Science. 240(4853):740-749.
- 28 Mastroianni, G. & Milovanovic, G.V. *Interpolation Processes - Basic Theory and Applications*. (2008). Springer

- 29 Morrison, J.C. & Pollack, I.P. *Glaucoma: Science and Practice*. (2002). 1st edition, Thieme.
- 30 Purves, D., Augustine, G.J., Fitzpatrick, D., Hall, W.C., LaMantia, A.S., McNamara, J.O., Williams, S.M. *Neuroscience*. (2004). 3rd edition, Sinauer Associates, Inc.
- 31 Remington, L.A. *Clinical Anatomy of the Visual System*. (2005). 2nd edition, Elsevier Inc.
- 32 Savoy, R.L. *Functional Magnetic Resonance Imaging (fMRI)*. (2007). Encyclopedia of the Brain, Functional Magnetic Resonance Imaging.
- 33 Schmidt-Erfurth, U., Leitgeb, R.A., Michels, S., Považay, B., Sacu, S., Hermann, B., Ahlers, C., Sattmann, H., Scholda, C., Fercher, A.F., Drexler, W. (2005). Three-Dimensional Ultrahigh-Resolution Optical Coherence Tomography of Macular Diseases. *Investigative Ophthalmology & Visual Science*. 46(9):3393-3402.
- 34 Scott, M.L.J., & Thacker, N.A. *Cerebral Cortical Thickness Measurements*. (2005). Medical Image Analysis.
- 35 Sehi, M., Grewal, D.S., Sheets, C.W., Greenfield, D.S. (2009). *Diagnostic Ability of Fourier-Domain vs Time-Domain Optical Coherence Tomography for Glaucoma Detection*. *American Journal of Ophthalmology*. 148(4):597-605.
- 36 Shahidi, M., Wang, Z., Zelkha, R. (2005). *Quantitative Thickness Measurement of Retinal Layers Imaged by Optical Coherence Tomography*. *American Journal of Ophthalmology*. 139(6):1056-1061.
- 37 Squire, L., Berg, D., Bloom, F.E., du Lac, S., Ghosh, A., Spitzer, N.C. *Fundamental Neuroscience*. (2008). 3rd edition, Elsevier Inc.
- 38 Tan, O., Li, G., Lu, A.T., Varma, R., Huang, D. (2008). *Mapping of Macular Substructures with Optical Coherence Tomography for Glaucoma Diagnosis*. *American Academy of Ophthalmology*. 115(6):949-956.
- 39 Tovée, M.J. *An Introduction to the Visual System*. (2008), 2nd edition, Cambridge

University Press.

- 40 Wandell, B.A., Dumoulin, S.O., Brewer, A.A. (2007). *Visual Field Maps in Human Cortex*. Neuron. 56(2):366-383.
- 41 Wang, X., Bauer, W., Chiaia, N., Dennis, M., Gerken, M., Hummel, J., Kane, J., Kenmuir, C., Khuder, S., Lane, R., Mooney, R., Bazeley, P., Apkarian, V., Wall, J. (2008). Longitudinal MRI evaluations of human global cortical thickness over minutes to weeks. Neuroscience Letters. 441(2):145-148.
- 42 Wotawa, N., Thirion, B. Castet, E., Anton, J.L., Faugeras, O. (2005). *Human Retinotopic Mapping Using fMRI*. Rapport de Recherche. Nr 5472.
- 43 Yezzi, A.J. & Prince, J.L (2003). *An Eulerian PDE Approach for Computing Tissue Thickness*. Medical Imaging, IEEE Transactions on. 22(10):1332.1339.