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An Investigation of Microperimetry In Macular Function Monitoring of Retinal Vascular Diseases, Their Treatment and Visual Rehabilitation

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Declaration

Except where acknowledged in the text, I declare that this thesis is my own work and is based on research that was undertaken by me in Academic Ophthalmology, under the supervision of Mr. Winfried M Amoaku and Prof. Martin Rubinstein at the School of Medicine, University of Nottingham, UK.

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Abstract

Age-related macular degeneration (AMD) and diabetic eye disease are two of the main causes for blindness and severe visual loss in developed countries. Late AMD may be in the exudative, or neovascular form, whilst the main cause of visual loss in diabetic eyes is caused by an increase in microvascular endothelial permeability which leads to macular oedema. Treatment outcomes have improved considerably with the introduction of intravitreal therapies that inhibit vascular endothelial growth factor (VEGF). However, the advanced form of macular diseases, can result in a progressive and irreversible loss of the central retina, affecting central vision including the capacity to fixate. In contemporary clinical practice, assessment of retinal pathologies typically includes visual acuity, fundal examination, and optical coherence tomography (OCT), which has been established as the gold standard for the management of retinal pathologies. However, since it is a diagnostic technique based exclusively on the structural image, it does not offer the possibility of evaluating functional data regarding visual function. Microperimetry (MP) technology assess functional characteristics of the retina, however is not commonly used in retinal clinical practices. This research poses two questions related the use of MP: the optimisation and standardisation in the clinical practice. It aims to understand and clarify the benefits of retinal functional analysis performed with the latest generation of MP instruments.

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1. General Introduction.

1.1 Rationale

The macula is the central part of retina, the centre of which is the fovea. The fovea is responsible for detailed vision, colour perception, and fixation capabilities. Some of the major diseases that affect the macula, such as dry age-related macular degeneration (AMD), Stargardt's disease and other similar pathologies, progress slowly and cause mild blurring of central vision in the early or intermediate stages of the pathology. The advance stage of dry AMD may affect large areas of the central retina causing central vision loss commonly resulting in poor vision and unstable fixation. AMD progression may result in significant visual impairment.

The advanced stage of AMD and other similar pathologies affecting the macula have no available proven treatment to date. Individuals with late stage macular pathologies can only improve their residual vision and quality of life through vision rehabilitation services. The typical rehabilitation process for patients with foveal involvement secondary to late stage AMD has included the use of prisms, magnifiers and other non-technological aids as mobility training, counselling and education.

The accurate control of eye movements involves several different structures, from the extraocular muscles to the frontal cortex of the brain. There are three major types of eye movement. Smooth pursuit, or the following of a target accurately; Saccadic eye movements, where a sudden shift of the eyes to a new target occurs; and Sustained gaze, where the eyes are fixed in one direction to a specific visual target.

In healthy eyes, the anatomical area of the retina used to fixate is the fovea, as described previously. In cases of eyes with central vision loss, fixation attempt is performed with an eccentric retinal area known as the Preferred Retinal Locus (PRL). However, retinal loci of fixation in these eyes are not always optimal for the best visual performance and result, in most cases, in constant involuntary eye movement with unstable fixation. In essence, peripheral (eccentric) PRLs are associated with highly unstable fixation and very poor visual acuity, where fixation stability decreases whilst PRL eccentricity increases (Von Noorden and Mackensen, 1962, Sullivan et al., 2008).

Biofeedback is a therapeutic technique used to increase awareness of a physiological function. Biofeedback methods used in rehabilitation are based on biomechanical measurements and measurements of the physiological systems of the body, the neuromuscular system, the respiratory system and the cardiovascular system. Biomechanical feedback can be obtained through different physical measurements, such as movement, postural control and force output. Biofeedback is generally delivered using visual displays, acoustic or haptic signals, however more recently virtual reality (VR) or exergaming

technology have been used as biofeedback signals. Biofeedback has shown to be effective in improving exercise technique in musculoskeletal populations. While a number of studies in this area have been conducted, further large scale studies and reviews investigating different biofeedback applications in different clinical populations are required (Giggins et al., 2013).

Biofeedback rehabilitation in ophthalmology is seldom used, nonetheless few authors have reported its use in patients with unstable fixation secondary to central vision loss. This technique is based on the theory of neural plasticity where areas of residual vision can be identified and repetitively stimulated, improving ocular motor control for better residual visual performance. In recent years a biofeedback therapy has been incorporated in microperimetry devices, with the purpose to improve eccentric visual function in cases of maculopathies affecting the central macula.

Microperimetric biofeedback training is a visual rehabilitative strategy based on fixation stability improvement, reinforcing or creating a new preferential fixation locus. The rationale consists in re-educating the visual system to a new visual condition, promoting retina-brain transmission, and thus cortical plasticity. Its major application in visual diseases is related to macular pathologies affecting central vision, however this therapeutic process is still limited due to poor knowledge of the procedure and inconsistent standards of practice, which implies an incipient scepticism on its efficacy (Vingolo et al., 2018).

The first Microperimeter with Biofeedback rehabilitation software (Nidek-MP1), was introduced in 2003 by Nidek Technologies (Padova, Italy). Unfortunately, the rehabilitation method of the MP1 was not widely used due to the difficulty of operation and the high cost of the instrument. More recently, Centervue (Padova, Italy) developed a 3rd generation of microperimetry systems, the Macular Integrity Assessment (MAIA). The biggest advantage of the MAIA is the ease of operation and the much lower cost than similar instruments of its kind. Although few authors have suggested the effectiveness of biofeedback eccentric visual training with both the MP1 and the MAIA, the methodology of such therapeutic process has never been properly described or correlated in a large cohort of patients. As such there are several open questions about this technique that have limited its use to few international experts.

For years microperimetry (MP) technology has been confined to the use of few specialist and researchers due to the complexity of the previous technology. This research aims to understand and clarify the benefits of retinal functional analysis performed with the latest generation of MP instruments.

1.2 Aims and Objectives

This research poses two questions related the use of MP: the optimisation and standardisation in the clinical practice. It aims to understand and clarify the benefits of retinal functional analysis performed with the latest generation of MP instruments.

The purpose of the present study is to analyse retinal functional characteristics such as light threshold sensitivity, fixation location and fixation stability with MP, in common macular diseases including AMD, and determine its value in monitoring disease progression, treatment and visual rehabilitation of macular diseases.

The study has 2 main sections:

a) MP in the monitoring of macular diseases.

The aim of this section is to assess the benefit of MP functional analysis in clinical practice for patients undergoing surgical or clinical treatments, related to pathologies affecting central vision, such as AMD, diabetes macular oedema (DMO), macular oedema secondary to retinal vein occlusions (RVO), macular holes (MH) and epiretinal membranes (ERM). This study may allow a better understanding of retinal functional characteristics, complementing existing standard eyecare tests, aimed at predicting better prognosis for patients with different macular pathologies, such as the OCT structural analysis, and the well-known visual acuity (VA) test.

b) Eccentric fixation training for visual rehabilitation with MP biofeedback technology.

This section analyses MP biofeedback fixation training (BFT) as a visual rehabilitation process in patients with loss of central vision, with the aim of studying the effectiveness of such technology and proposing guidelines for this

rehabilitation method, allowing the use of this technique to a wider pool of eye specialists with minimal experience in eccentric vision rehabilitation. In this section, the different variables responsible for unstable fixation, and the selection of the best eccentric functional retinal locus will be studied. The definition of the best eccentric functional retinal locus, may lead to better outcomes in other vision therapies commonly performed in low vision centres, increasing possibilities for a better quality of life in patients with loss of central vision secondary to macular pathologies.

1.3 Structure and function of the retina

The Retina

In normal eyes, light rays enter the eye through the cornea, the pupil and through the crystalline lens to converge into a sharp focusing point on the retina. The retina is a delicate sheet of nervous tissue located in the posterior wall inside the eye. It is formed of several layers of nerve cells interconnected by synapses, which sense light (visual information) that is converted into electrical signals that are transmitted along the optic nerve to the brain. The outer surface of the retina is in contact with the choroid; the inner surface is adjacent to the vitreous body. The neural retina forms the inner layer of the retina where neural cells (photoreceptor, bipolar, and ganglion cells) are located. The retinal pigment epithelium (RPE) forms the thin outer retina which supports the retinal photoreceptors. The neural retina is anchored only at the optic disc, where nerve

fibres congregate before passing through the sclera to form the optic nerve (Krause, 2005).

Histologically, the retina can be divided into 10 detailed layers (Kolb et al., 1995, Krause, 2005, Willermain et al., 2014). From the inner to the outer part of the retina, these retinal layers are organized as follows. The inner limiting membrane (ILM) is formed by the conical end of the Müller cells and astrocytes. The nerve fibre layer (NFL) consists of axons of ganglion cells, retinal vessels and glial cells. The ganglion cell layer (GCL) predominantly contains the nucleus of ganglion cells, vessel cells, glial cells, and some displaced amacrine cells. The inner plexiform layer (IPL) where bipolar, amacrine, and ganglion cells interact. The inner nuclear layer (INL) harbours the nuclei from bipolar, horizontal, amacrine, and Muller cells. The outer plexiform layer (OPL) is where photoreceptor cells connect with bipolar cells, and where horizontal cells interact closely with both photoreceptors and bipolar cells. The outer nuclear layer (ONL) contains nuclei from photoreceptor cells. The external limiting membrane (ELM) is created by junctional complexes between adjacent Muller cells as well as between Muller and photoreceptor cells. The photoreceptor layer (PL) contains tightly stacked cones and rods forming a palisading layer of photoreceptors. The tenth layer results from tight junctional complexes between RPE cells forming a continuous RPE monolayer. Moreover, the RPE is separated from the choriocapillaris by the Bruch's membrane composed of 5 layers: the basement membrane of the choriocapillaris, an outer collagenous layer, a central elastic layer, an inner collagenous layer, and the basement membrane of the RPE. The RPE absorbs light after it has passed through the neural retina and prevents

reflection within the eye. It is a storage site for vitamin A, a precursor of rhodopsin, which is recycled to the membranes of the outer rod segments.

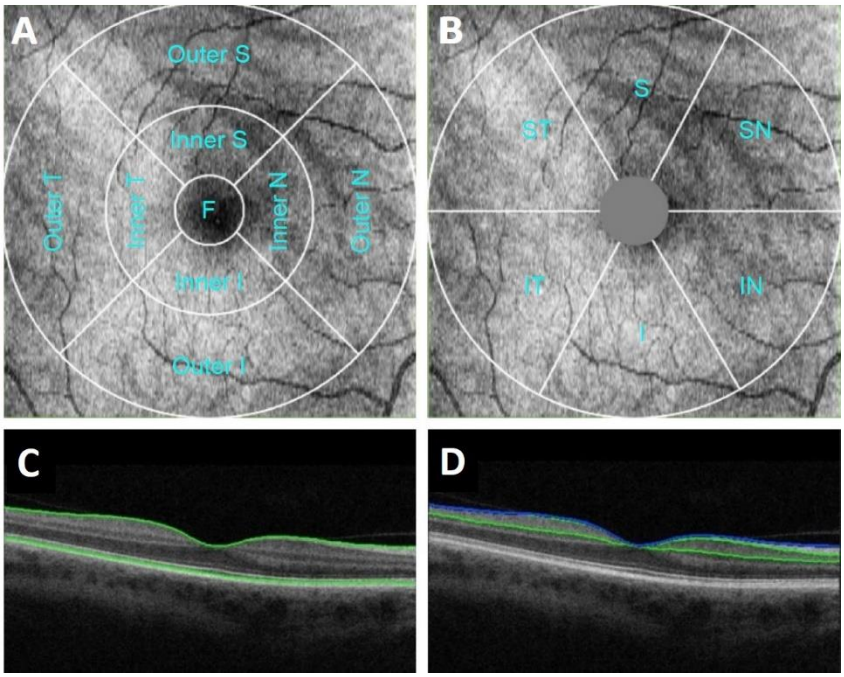

Figure 1.1 OCT image of a normal retina

*Measurement areas for macula Early Treatment Diabetic Retinopathy Study grid (**A**), and macula 6 circle (**B**). Single-frame OCT B-scan images overlaid with boundaries (green and blue lines) demonstrated the retinal layers in various retinal thickness measurements, including full retinal thickness (**C**); ganglion cell + inner plexiform layers (distance between the 2 green boundaries), ganglion cell complex (distance between the blue and green boundaries, vitreal to inner nuclear layer) (**D**).(Chaglasian et al., 2018)*

Vascular Supply of the Retina

The inner layers of the retina are supplied by retinal vessels arising from the central retinal artery, which enters the eye in the optic nerve. Capillary networks from this source lie in the nerve fibre layer and in the inner plexiform layer. The outer nuclear and plexiform layers and the layer of rod and cone inner segments lack blood vessels. These parts of the retina are nourished by diffusion of nutrients from capillaries of the choriocapillaris layer of the choroid. Nutrients

cross the pigmented epithelium to enter the intercellular spaces of the outer neural retina (Krause, 2005).

Photoreceptors

The photoreceptor cells are directly sensitive to light. There are two types of photoreceptors in the human retina, rods and cones. Rods function mainly in dim light (scotopic vision) with low spatial acuity and do not mediate colour vision. Cones are active at higher light levels (photopic vision), mediate colour vision and provide high spatial acuity. The light levels where both are operational are called mesopic (Kawamura and Tachibanaki, 2012). The human cone photoreceptor distribution has a high peak packing density at the foveal centre, which decreases rapidly within the central 2 mm of retina. Cone density decreases as rod density increases longitudinally from the fovea to the retinal periphery (Song et al., 2011). Recent studies confirmed that cone photoreceptor density decreases at 1, 2, 4 and 6 degrees of eccentricity, showing a homogenous drop in each of the four retinal meridians and high agreement between nasal and temporal locations (Song et al., 2011, Lombardo et al., 2013).

The Macula & Fovea

The anatomic macula of the eye is the area of retina extending from the temporal optic disc margin, and enclosed by the supero-tempral and infero-temporal retinal blood vessels. The central part of the retina, known as the macula lutea, is a flattened oval area in the centre of the retina approximately 3 to 4 mm (15°) temporal to and slightly below the optic disc. The macula appears as a yellow, oval area, when examined under green light. Its diameter is roughly equal to that

of the optic disc (1.5–1.9 mm). Located in its centre is the avascular fovea centralis, a funnel-like depression in direct line with the visual axis. The fovea centralis is populated exclusively by cones (no rods), which explains why it is the point at which visual perception is sharpest. Foveal cones are longer and thinner than cones elsewhere in the retina. Light stimuli in this region can directly act on the sensory cells, as retinal layers beyond the outer nuclear layer are displaced laterally, giving light an almost free pathway to the photoreceptors (Lang, 2015). Detailed visual tasks, such as fixation, are only performed with the fovea in healthy subjects.

Retinal Visual Processing

Retinal visual processing starts when light photons pass through the neuroretina triggering a change in the excitatory signalling to bipolar cells and subsequently ganglion cells. Bipolar and ganglion cell responses are modulated by horizontal and amacrine cells responses, respectively. Ganglion cell axons converge to form the optic nerve which carries the signal to the brain (Lang, 2015).

Visual Field

Visual field (VF) can be defined as all the space that one eye can see (visualise) at any given instant. Perimetry is defined as the study of the VF, whilst any instrument design to measure VF is called a perimeter (Tate and Lynn, 1977). Although VF consists in a three-dimensional volume of space, it is represented in perimetry in two dimensions and is described as the “area” rather than the “volume”. The extent of the normal VF, measured in degrees, for a bright stimulus is 60° superior, 75° inferior, 100° temporal and 60° nasal. With both

eyes open the VF has a horizontal extent of approximately 200°. The binocular field is the region where both eyes can see contemporarily a stimulus.

The sensitivity of the eye is not constant across the whole VF, it varies with eccentricity, the nature of the test stimuli and adaptation level, corresponding to photoreceptor density distribution. When the eye is light-adapted, the sensitivity peak is at its centre. When the eye is dark-adapted, sensitivity is more distributed eccentrically with lower values on the fovea due to the cone-rod distribution.

1.4 Clinical features and treatments of common macular pathologies.

Age Related Macular Degeneration (AMD)

Age-related macular degeneration (AMD) is the leading cause of blindness and visual disability in the elderly across the developed world (Klein et al., 1999). There are 2 types of late AMD: dry and neovascular (wet) AMD (Ferris et al., 2013). Vision performance in the late stage of AMD is regularly decreased due to the loss of central vision. Late stage AMD is present in approximately 5% of over 65's and 12% of over 80's (Smith et al., 2001). By 2040, the number of individuals in Europe with early AMD will range between 14.9 and 21.5 million, and for late AMD between 3.9 and 4.8 million (Colijn et al., 2017). The worldwide projected number of people with AMD in 2020 is 196 million, increasing to 288 million in 2040 (Wong et al., 2014).

Risk Factors

AMD is a multifactorial disease involving ocular, systemic and genetic risk factors. The ocular risk factors include iris pigmentation and hyperopic refraction, while systemic risk factors include cigarette smoking, obesity, sunlight exposure and cardiovascular diseases (Chakravarthy et al., 2010, Choudhury et al., 2011). Genes influence several biological pathways related to AMD, including the immune processes, mechanisms involving collagen and glycosaminoglycans synthesis and angiogenesis. All these factors have been associated with the onset, progression and bilateral involvement of early, intermediate, and advanced states of AMD (Lombardo et al., 2012).

Classification of AMD

There are two clinical types of AMD, the "dry" and "wet" form. Early (dry) AMD progresses slowly and causes mild blurring of central vision in the early or intermediate stage of the pathology. In these stages, insoluble extracellular aggregates called drusen accumulate in the retina. The late stage of dry AMD, which is also known as Geographic Atrophy (GA), is characterized by scattered or confluent areas of degeneration of RPE cells and the overlying retinal photoreceptors, which rely on the RPE for trophic support. This late stage may also affect large areas of the central retina, causing the total loss of central vision (scotoma) with important decrease of visual acuity (VA).

AMD may also progress (10–15%) in an exudative (wet) form, termed neovascular AMD (nvAMD) which is typified by development of choroidal neo-

vascularization (CNV), where newly immature blood vessels grow toward the outer retina from the underlying choroid with subsequent progression to the retina, and leak fluid below or within the intra retinal layers. Patients with sight loss due to nvAMD are expected to increase from 145,697 to 189,890 by the end of the decade in the U.K. (Minassian et al., 2011).

AMD assessment

Fundus fluorescein angiography (FFA) is the gold standard to diagnose CNV due to AMD. Leakage of dye (hyper-fluorescence) is noted and classified by location (subfoveal, juxtafoveal, or extrafoveal) and by type (classic, occult, or mixed). Dynamic high-speed indocyanine green angiography (ICG) improves identification and characterisation of neovascular variants of AMD (e.g., the polypoidal choroidal vasculopathy) as it delineates the choroidal circulation more clearly than FFA (Chakravarthy and Williams, 2013). Optical coherence tomography (OCT) is excellent at detecting leaked fluid from abnormal vessels. It enables high-resolution in vivo cross-sectional or volumetric tomographic visualisation of the retinal micro-architecture. Although OCT outlines the neovascular choroidal complex, its components are difficult to distinguish from fibrous components, haemorrhages or dense exudates within the lesion. Similarly, OCT is not dynamic, and cannot detect the origin of leakage from abnormal vasculature. The newer modality of OCT angiography (OCT-A) may provide an advancement of OCT technology, and allow detection of leakage. With the advent of anti-VEGF therapy, OCT imaging is widely used for screening and early diagnosis of CNV, and for the monitoring of treatment outcomes and re-treatment management (Chakravarthy and Williams, 2013,

Ambati and Fowler, 2012). The sensitivity and specificity of OCT in diagnosis of nvAMD is lower than that of FFA (Castillo et al., 2015).

In contemporary clinical practice, assessment of AMD, including re-treatment decisions in nvAMD, typically includes visual acuity, fundal examination, and OCT at monthly follow-up intervals (Chakravarthy and Williams, 2013). However, it is generally accepted that VA change or OCT alone may not be the optimum parameter(s) for measuring visual function in AMD and other macular diseases, as these are dependent on function in limited parts of the central macula. Thus, microperimetry systems may be considered a more reliable mean to assessing the sensitivity of the central retina due to the precise topographic correlations of macular anatomy and threshold light sensitivity, even in cases of highly unstable fixation capabilities (Rohrschneider et al., 1996, Midena et al., 2004).

Management of AMD

Intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF) therapy have become the standard for treatment of CNV or nvAMD, as it can efficiently block the pathophysiological process of CNV, restore retinal morphology and increase/maintain neurosensory function in most patients with neovascular AMD (Rosenfeld et al., 2006). Current treatment options include bevacizumab (Avastin, Genentech, San Francisco, CA), ranibizumab (Lucentis, Genentech), and aflibercept (Eylea, Regeneron, Tarrytown, NY). They can be delivered at monthly intervals until stable, followed by different maintenance

regimen including 1-2 monthly fixed dosing, or pro re nata. However, no treatment has been proven to be effective in the management of dry AMD.

Diabetic Retinopathy (DR) & Macular Oedema (DMO)

Diabetes mellitus, commonly referred to as diabetes, is a metabolic disease in which the patient has a high serum glucose level. An estimated 346 million of people worldwide have diabetes and the number is projected to rise to 360 million by the year 2030 and approximately 4.5% of the UK population is diabetic (Wild et al., 2004). According to the World Health Organization, diabetes mellitus is responsible for about 12% of new cases of blindness between the ages of 45 and 74 years in the developed world. Diabetic retinopathy (DR) is the commonest cause of visual impairment in persons below the age of 65 years (Hendrick et al., 2015). DR is a frequently occurring complication of diabetes, and is a progressive condition with microvascular alterations that lead to retinal ischemia, retinal permeability, retinal neovascularization and macular oedema (Klein et al., 2009, Scully, 2012, RCOphth and Ophthalmologists, 2013).

Risk Factors DR

Both the duration of diabetes and glycaemic control are independent risk factors for severity and progression of DR. Younger-onset diabetics' have twice the prevalence of advance (proliferative) diabetic retinopathy compared with the older-onset group that takes insulin (RCOphth and Ophthalmologists, 2013): The prevalence of DR is 48.4% in the population with type 1 diabetes mellitus

(T1DM) and 28.3% in the population with type 2 diabetes mellitus (T2DM). Among patients with T2DM, severe DR risk increased in South Asian groups and more deprived groups. Relative risk of DR for patients with T1DM varied by age and region, but not by gender, ethnic group or deprivation (Mathur et al., 2017).

Classification of DR

Various DR classification systems have been described in literature. Such classifications consist of 2 different approaches. The first is based on the presence or absence of new vessels (traditional background [non-proliferative] vs proliferative retinopathy, which may be associated with maculopathy. The second is based on the presence or absence of centre-involving/subfoveal macular oedema and/or ischaemia. These 2 systems are not mutually exclusive.

Diabetic macular oedema (DMO) can be divided in focal and diffuse subtypes. Focal DMO is caused by focal leakage of microaneurysms. Diffuse DMO is caused by a generalized breakdown of the inner blood/retinal barrier. The Early Treatment Diabetic Retinopathy Study (ETDRS) used the proportion of microaneurysmsal fluorescein leakage to differentiate focal and diffuse DMO conditions. Eyes with ≥ 67% of leakage originating from microaneurysms were classified as focal DMO. Eyes with microaneurysmsal leakage between 33% and 66% were determined to have intermediate DMO. Eyes with ≤33% microaneurysmal leakage were classified as having diffuse DMO (ETDRS, 1995). ETDRS defined clinically significant macular oedema (CSMO) as "thickening of the retina at or within 500 μm of the centre of the macula; or hard

exudate at or within 500 μm of the centre of the macula associated with thickening of adjacent retina; or a zone of retinal thickening 1-disc area or larger, anywhere in macula (ETDRS, 1995, ETDRS, 1991b).

Regarding the presence of neovascularisation, DR can be classified as non-proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR). NPDR is further graded as mild, moderate and severe according to the ETDRS severity scale (ETDRS, 1991a). The International Clinical Disease Severity Scale for Diabetic Retinopathy defined five severity stages for DR (Wilkinson et al., 2003). The first is "no apparent retinopathy". The second is "mild NPDR". This stage is characterized by the presence of a few microaneurysms. The third stage is "moderate NPDR" which is characterized by the presence of microaneurysms, intraretinal haemorrhages or venous beading that do not reach the severity standard. The fourth is "Severe NPDR", this classification is based on the 4:2:1 rule of the ETDRS, where severe NPDR is present if: haemorrhages are present in all 4 quadrants, 2 quadrants or more have venous beading, 1 or more quadrant has intraretinal microvascular abnormalities (IRMA). The final stage is "proliferative diabetic retinopathy". PDR is characterized by neovascularization of the disc, neovascularization of the retina, neovascularization of the iris, neovascularization of the angle, vitreous haemorrhage or tractional retinal detachment (Murphy, 1995, Wu et al., 2013a). PDR increases risk of vitreous haemorrhage, retinal fibrosis, and tractional retinal detachment with significant risk of vision loss.

DR assessment

DMO can be assessed evaluating retinal thickness through OCT devices in the nine ETDRS subfields. Centre-involving DMO is defined as presence of oedema in zone 1 (fovea). Non-centre-involving DMO is related to oedema located in one or more of the subfields 2–9. Approximately 8% of people with diabetes have centre-involving DMO and a further 8% have non-central DMO. Not all patients with centre-involving DMO have impaired vision, therefore progression must be measured as an increase in retinal thickness in all nine zones as well as total macular volume. Fundus fluorescence angiography (FFA) is of great value to detect microvascular damages and retinal ischemia in the early stage of DR. It is currently the gold standard for evaluating the retinal vasculature. In FFA microaneurysms appear as punctate areas of hyperfluorescence. Patchy areas of hypofluorescence can signify ischemia from nonperfused retinal capillaries. An increase in the foveal avascular zone from macular ischemia can be seen using FA, which may help explain vision loss in some diabetic patients, however macular function can only be presumed from FFA images. FFA can also show abnormal blood vessels in the eye such as intraretinal microvascular abnormalities (IRMA) or retinal neovascularization. The visualization of leakage of fluorescein dye over time is useful in showing the breakdown of the blood-retinal barrier. Retinal neovascularization also can cause fluorescein leakage, and FFA is a useful test to confirm the diagnosis of neovascularization of the disc and elsewhere in proliferative diabetic retinopathy (Salz and Witkin, 2015).

Management of DR

Diabetic patients can be managed in an appropriate screening programme. Patients with PDR and patients with centre-involving macular oedema are referred to the hospital eye service for treatment. Other patients with less severe retinopathy may be observed with close monitoring. Based on severity signs, retinal laser photocoagulation may be considered. Pan-retinal photocoagulation (PRP) laser treatment reduces risk of vision loss in PDR. Focal or grid macular laser photocoagulation was standard in management of DMO which was non-ischaemic. Treatment outcomes have improved considerably with the introduction of intravitreal therapies to inhibit vascular endothelial growth factor (VEGF), which is thought to be the central mediator of neovascularisation in these retinal vascular diseases, and inhibitors of inflammation. Intravitreal anti-VEGF injections can be useful in stabilising PDR although more permanent treatment is still reliant on PRP (Alexander et al., 2012).

Patients with non-centre-involving diabetic maculopathy can be treated with laser photocoagulation according to modified ETDRS criteria or with MicroPulse laser. Patients with centre-involving macular oedema (central macular thickness ≥250 μm and visual acuity in the region of 6/10—6/90) would benefit most from anti-VEGF (with ranibizumab or aflibercept) treatment, which may be combined with laser treatment. Where ranibizumab (Genentech/Novartis) or aflibercept (Regeneron/Bayer) is not available, bevacizumab (Roche/Genentech) may be considered. Intravitreal steroid therapy with dexamethasone implant (Ozurdex, Allergan) or Fluocinolone (Iluvien, Alimera) injections offer other potential options. Where these licensed steroids

are not available, intravitreal injection of triamcinolone can be considered as an alternative option. Patients unsuitable for injections can be offered macular laser treatment. If there is evidence of vitreomacular traction, vitrectomy may be considered with or without adjunctive anti-VEGF/steroid treatment. Patients needing laser treatment require 3-monthly or 4-monthly follow-ups, whereas patients undergoing anti-VEGF injections need monthly follow-up, at least in the first year. Patients with early maculopathy (no CSMO) and background retinopathy can be followed up through colour images and OCT, at 4–6 monthly interval (RCOphth and Ophthalmologists, 2013).

1.5 Low Vision

Despite recent progress in the treatment of nvAMD, there are currently no proven treatments available for dry AMD. However, fortunately dry AMD progresses slowly, and usually spares the central vision till late in the disease. As such, only a proportion of dry AMD cases progress up to the point to cause legal blindness (Buckle et al., 2015). Instead individuals with moderate to advanced dry AMD develop central or paracentral visual field defects (scotomas) or moderately/severe reduced vision, ending up as Low Vision (LV) patients.

Patients are categorised as having LV when a significant loss of vision cannot be corrected medically, surgically, or with the best possible corrective lenses, but which may be improved with special aids or devices. LV may be the result

of different ocular pathologies of either congenital disease, such as retinitis pigmentosa, or of acquired conditions such as AMD. Subjects with LV secondary to maculopathies can only improve their quality of life through vision rehabilitation services by learning how to use their remaining vision more effectively.

LV patients with central scotoma perform visual tasks with the eccentric parts of the retina, which regularly leads to unstable fixation. The size of central scotoma has already been established as an important determinant of visual acuity, reading capability and reading speed. The rehabilitation process for such LV patients typically consists of the use of prisms, magnifiers and other non-technological aids as mobility training, counselling and education (Klein et al., 1997).

Some of the visual disturbances related to low vision are (Lamba and Ahmed, 2016):

i) *Lower central visual acuity or fluctuating vision*. These patients initially report the inability to read small print, or that the letters of the reading target move constantly.

ii) *Metamorphopsia.* People describe a distortion of vision and may complain of haze all the time. Objects appear to bulge, curve, or "look funny".

iii) *Photophobia.* Patients either complain of abnormal sensitivity to light or do not report the problem but use very dark glasses or avoid high levels of illumination. Recovery from glare is slow, and adaptation to light is difficult.

iv) *Colour Distortions.* Persons with this condition indicate they cannot detect colours, or functional observations show they have trouble identifying colours.

v) *Field Defects.* These patients report they have no vision in specified sectors of the visual field; objects disappear to the right, left, and so forth, and parts of an object being viewed are always missing. Frequently, field loss is detected through functional observations rather than from direct reports from the person experiencing it. Common losses include: (1) general contraction or depression (objects in the periphery are not seen), (2) Hemianopsia (the right half, left half, upper or lower half of the visual field is missing), and (3) Central Scotoma (in which the macula is no longer functioning but all the retina tissue around the scotoma area is intact).

vi) *Night Blindness.* Persons who have night blindness indicate a decreased ability to see at night and difficulty in performing specific tasks at night or are observed to function worse at night. This condition can be confirmed by clinical tests such as electrodiagnostic examinations.

vii) *Entopic Images.* Persons with this condition see floaters or spots before their eyes, including stationary spots that move with the eye; these floaters or spots momentarily interfere with vision. Such symptoms may indicate an active pathology, so appropriate care should be provided by the staff optometrist or ophthalmologist.

viii) *Oscillopsia.* Persons with oscillopsia report that the world seems to be moving or jumping around. This condition may be related to a loss of foveal function or a sign of neurological disorder; therefore, persons suffering from it should be referred to a neurologist or another appropriate physician.

Many of the above described LV conditions provoke involuntary eye movements as a reflex of the oculomotor system aiming a visual target during a redefinition of a retinal locus which may substitute central vision. Manifestations of disordered eye movement include a loss of conjugate movements, broken pursuit movements, inaccurate saccades, gaze palsies and nystagmus (Tarita-Nistor et al., 2008).

Management of LV

Patients with central vision loss can benefit from eccentric vision therapy by using their remaining vision more effectively. Fixation control is the essential part of such therapeutic process. Biofeedback fixation training (BFT) can aid patients to learn how to use their best functional eccentric retinal locus, in a

totally controlled environment, with the scope to convey newly acquired oculomotor skills into daily living improving quality of life. BFT is a technological aid used in the rehabilitation process for low vision patients with eccentric and unstable fixation. This technique is based in the theory of Brain Plasticity where areas of residual vision can be identified and repetitively stimulated, improving the ocular motor control.

BFT rehabilitation consists of asking patients to move their gaze toward a pre-selected direction related to a specific retinal zone with good light sensitivity (Nilsson et al., 1998); the gaze movement is accompanied by an intermittent beep sound which increases its frequency whilst it approaches the selected area. When the eye movement hits the target the beep sound becomes constant whilst visual stimuli are presented. The combination of visual stimuli and audio feedback helps patients develop a better functional residual vision (Tarita-Nistor et al., 2009b).

The incorporation of BFT in MP devices adds the advantage of the anatomico-functional analysis, whereas MP maps out precisely areas of good retinal sensitivity over a fundus image, that may be used for eccentric vision. Unfortunately, to date, there are no standardised methods for eccentric vision training with MP and biofeedback.

2.Materials

2.1 Microperimetry

Standard Automated Perimetry (SAP)

Standard automated perimetry (SAP) is a well-known method of measuring the field of view. This visual field analysis, examines light threshold sensitivity (LTS) of different spatial locations of the retina. The function of perimetry is to indicate the site and the extent of involvement of the visual pathway. It is a standard examination in glaucoma management, where monitoring of LTS progression of the peripheral retina is an integral part of the glaucoma evaluation. However, such tests may not be reliable in macular pathologies due to patients' inability to hold their fixation during the long perimetry testing, particularly in those cases when central vision is affected.

The Humphrey Visual Field Analyzer (HFA) is the most commonly used SAP. Visual field analysers have standardized quantitative results on SAP instruments through practical statistical methods to measure early functional loss in glaucoma. The high sensitivity of the methods has required attention to the multiple psychological and physiologic variables that may affect measured thresholds. Although SAP has reduced variability in the examination technique,

the test still depends on the reliability of the patient's responses, the ability to see the instrument's fixation target, and may be affected by optic, neural, and psychological factors.

Although detailed measurements of the visual field can be obtained, differentiating long-term fluctuation from progressive loss remains one of the greatest clinical challenges in visual field interpretation (Wong and Plant, 2015).

To map the visual field, perimetry measures the differential LTS of a stimulus projected at multiple locations in different retinal loci, with variable luminance against a constant illuminated background. Currently, the standard SAP test is a Goldmann size III stimulus, a white circular projection, whose diameter is 0.43° of visual angle. However, other stimulus sizes can be used (Swanson, 2013). The highest limitation of perimetry testing is subjectivity, as patients are asked to press a response button once the stimulus is seen.

Visual field test in SAP can be either static or dynamic. In static perimetry, sensitivity thresholds are determined at specific locations of the visual field. These thresholds are then compared to those of normal controls. Small changes in LTS can be detected with accuracy; however, this is highly dependent on patient's ability to hold their gaze steady on the fixation target for a long period of time. Probably, the major limitation of static perimetry is that, examination time depends on the number of stimuli projected, as this increases patient's fatigue, reducing the ability to fixate. Therefore, a compromise between stimuli separation and visual field coverage must be achieved, resulting on a low spatial resolution test. For such reason, static perimetry is typically limited to the central

30° of the visual field. Conversely, kinetic perimetry, provides higher spatial resolution and is faster for peripheral testing. However, it involves greater interaction between the patient and the examiner. In kinetic perimetry, sensitivity thresholds are determined by moving stimuli of various sizes and luminosities from a region of non-seeing to a region of seeing.

Perimetry outcomes are a grayscale representation of the visual field map with numeric readouts in decibels which indicate the location and density of visual defects, where the non-seeing regions are defined as scotoma.

The biggest limitation of perimetry testing in patients with macular pathologies, is the assumption that, the point of fixation is the fovea, and that patients are able to hold their fixation steady during the whole examination time, which is not the case of patients with central vision loss secondary to macular pathologies.

Microperimetry (MP)

Microperimetry (MP) is also known as Fundus-related perimetry. Today MP is a well-known instrument in studying retinal functional characteristics, such as light threshold sensitivity (LTS) and patient's fixation capacities. It was firstly reported by Sunnes et al. (Sunness et al., 1995) as a Scanning Laser Ophthalmoscope (SLO) perimetry, a perimetry test that, with the aid of an eye tracker, based on retinal landmarks, compensates for eye movements during testing, therefore, the correct retinal location is tested, even if fixation changes, allowing for accurate testing of patients with central scotomas and for repeating testing longitudinally at the same retinal locations even if central fixation is lost.

Sunnes studied, for the first time, fixation behaviour and reading rates in patients with central scotoma with the SLO perimetry (Sunness et al., 1996). Rohrschneider et al., correlated reading capacities with the SLO fundus perimetry and later proposed that the analysis of functional outcomes with SLO perimetry was a better predictor of visual function after laser photocoagulation treatment (Rohrschneider et al., 1996, Rohrschneider et al., 1997). However, such SLO perimetry was barely used as a consequence of its high cost, and a lack of automatism and standardization of the visual field test.

The automated MP test is a fundus related perimetry with similar functional characteristics as those to the SAP or visual field test, where patients are asked to look at the centre of a fixation target whilst, points of white light are automatically projected several times at different light intensities over different parts of the retina. The stimuli grid distribution and projection strategy are equivalent to those of a basic field analyser. Patients are asked to press a response button when they perceive the light stimulus, thus a LTS map is created.

The disadvantage of MP compared to SAP is that, the retinal area covered by the test is reduced to less than 45 degrees on account of the fundus image size limitations. For that reason, MP examinations have been limited to the analysis of the central retina as initially reported by Midena et al., who described MP as a reliable technique to analyse detailed macular function in AMD patients, exalting the advantage of topographic correlations between macular anatomy and LTS (Midena et al., 2004). In essence, the main difference with SAP is that, MP is a fundus imaging device which controls eye movements through a retinal

landmark eye tracker, such that retinal LTS outcomes are fundus correlated. In addition, microperimetry assesses fixation characteristics such as fixation stability and the area of the retina used during fixation attempts, also known as the preferred retinal locus (Von Noorden and Mackensen, 1962, Fletcher and Schuchard, 1997).

In 2003, Nidek Technologies (Padova, Italy) introduced the first automated MP, the MP-1. It was first reported by Midena et al., in a study that analysed macular function in patients with subfoveal CNV secondary to AMD (Midena et al., 2004). In that study, Midena evoked MP as an instrument to quantify in detail (better than visual acuity), the effects on macular function of subfoveal CNV treatments. The value of MP evaluation in AMD and other macular diseases is that, light threshold assessments are independent of patients' capabilities to hold their fixation during the examination, particularly in patients with foveal involvement. The fundus correlation allows precise follow up for comparisons of sequential examination.

The MP-1 produces a 45° field of view fundus image in real time on a video monitor using an infrared (IR) fundus camera with an image resolution of 768 x 576 pixels and a 25Hz eye tracker that samples the eye movement by following 2 retina landmarks selected at the beginning of the procedure. Fixation target and stimuli are projected on a liquid crystal colour monitor. Adopting the perimetric standard of the Octopus automated perimeter, the MP-1 background illumination was set on 4 apostilb (asb), equivalent to 1.27 candles/square meter. The brightest stimuli luminance, which was limited by the colour monitor, was 400

asb (127 candles/square meter), allowing a stimulus dynamic range from 0 to 20 decibels (dB).

The MAIA MP

The Macular Integrity Assessment (MAIA) system (Centervue S.p.A, Padova, Italy), is the 3rd MP generation. It improved the limitations of the previous technologies with an easy to use instrument and lower price. The MAIA combined the advantage of a high-resolution retinal imaging system using a SLO technology (1024 x 1024 pixels) and a more effective eye tracker which automatically tracks the whole retina image and not only 2 landmarks as in the case of the MP-1. This improved the reliability and usability of the MAIA for operators and patients, as described by Vujosevic et al (Vujosevic et al., 2010a). The eye tracker speed for both technologies is 25Hz, as it is the maximum effective frame rate in synchrony with the imaging technology. The MAIA maximum stimulus luminance is 1000 asb, whilst the stimulus dynamic range is 36 dB compared to the maximum luminosity of 400 asb and 20 dB dynamic range of the MP-1. Vujosevic reported the MAIA average LTS outcomes compared to a normal reference database (Vujosevic et al., 2010a).

The MAIA manufacturer conducted an internal sponsored study with the purpose of establishing a normative, age-related database on LTS, and fixation stability for the MAIA in normal, early-stage AMD, and intermediate-stage AMD eyes. The results served to generate an algorithm that differentiates normal eyes from those with early- and intermediate-stage of AMD.

The study included 494 eyes from 265 subjects with normal visual conditions, 200 eyes with classified early-stage AMD, and 155 eyes classified with intermediate AMD. Both eyes were analysed for normal participants, whilst only one eye was analysed for each patient with AMD. The analysed data was, the mean LTS, fixation stability and fixation location. Age range was from 21 to 86 years of age. Outcomes were analysed in five age groups. Group 1 included patients from 20 to 29 years, group 2 from 30 to 39, group 3 from 40 to 49, group 4 from 50 to 59 and group 5 was composed for participants with more than 60 years of age.

A Goldmann III stimuli grid of 61 stimuli distributed in 5 rings separated at 1° was used (Figure 2.1). The projection threshold algorithm was the standard full 4-2 staircase.

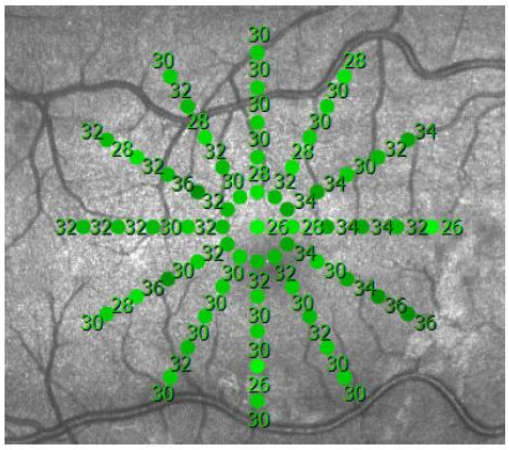

Figure 2.1 MAIA normative grid

Stimuli grid used to establish the normative database for the MAIA microperimeter. Separation between stimuli was 1°.

A linear regression was used to compute mean LTS and participant's age on all 494 normal eyes (Figure 2.2).

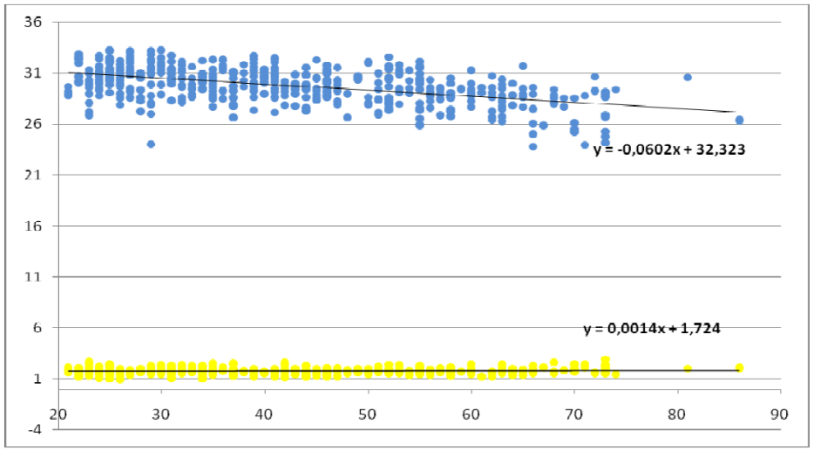

Figure 2.2 MAIA normative data

Means (blue) and standard deviations (yellow) in dB of threshold values for normal eyes vs. age. Linear regression line and equations are shown (N = 494).

A significant decrease in retinal LTS was found in AMD patients compared to normal, while FS showed a reduction between normal and AMD, patients, but the reduction among AMD stages was not significant, as shown on Table 2.1.

Table 2.1 MAIA normative values

	Normal	AMD 2	AMD3
Sensitivity (dB)	29.78±1.71	24.85±3.87	21.76±5.36
P1	88.79±15.18	79.09±24.88	77.46±26.83
P2	97.05±6.18)	91.5±16.92	89.85±20.55

Summary statistics for mean light threshold sensitivity and fixation stability for all groups. Values are expressed in the mean ± SD. P1: percentage of points having distance less than 1° from the centre of mass of all fixation points. P2: percentage of points having distance less than 2° from the centre of mass of all fixation points.

Spatial variability of LTS was assessed as the mean value for all points located at each ring, and showed significant changes between groups 1 (age 20-29), 4 (age 50-59) and 5 (age 60+), as shown in Table 2.2.

Table 2.2 Normative group results

Ring	Group 1 (20Y - 29Y)	Group 4 (50Y – 59Y)	Group 5 (60Y+)
1	31.0 dB	29.5 dB	28.5 dB
2	31.3 dB	29.9 dB	29.0 dB
3	30.9 dB	29.4 dB	28.6 dB
4	30.4 dB	29.2 dB	28.3 dB
5	30.1 dB	28.7 dB	28.0 dB

Mean light threshold sensitivity of each stimuli ring for age groups 1, 4 and 5.

The normative study concluded that macular sensitivity is reduced in patients with early and intermediate AMD when compared to age-matched normals. LTS reduction is more relevant in intermediate than in early AMD. Fixation is altered in AMD patients when compared to normal subjects. Fixation does not differ significantly between AMD 2 and AMD 3 groups. Therefore, the macular MP test may be useful in early diagnosis of AMD, mainly in the screening setting.(Vujosevic et al., 2010b).

Scotopic Microperimetry

Standard microperimetry is considered a mesopic examination. The test is performed under dim light conditions (mesopic) as LTS of the retina is assessed with achromatic (white) stimuli, which stimulate both cones and rods photoreceptors. However clinical studies have shown that in some degenerative

retinal diseases the sensitivity of the rods and cones may change asynchronously (Nebbioso et al., 2014). Therefore, there was an intrinsic need to study independent function of cones and rods. Scotopic microperimetry fulfils this need by performing LTS under dark adaptation. The scotopic MAIA system (S-MAIA) is equipped with two different chromatic stimuli, cyan (505 nm) and red (627 nm), so that cones and rods mediation can be studied independently under scotopic conditions. The cyan testing is intended to be largely derived from rod photoreceptor-mediated function, whilst the scotopic red would be more influenced by cone-mediated function (Pfau et al., 2017a).

More recently, the microperimeter MP-3 (Nidek Co, Japan) became available. The technology is similar to the one in the MP-1, with an infrared imaging system used during testing for motion capturing. In contrast to previous devices, the eye tracking frame rate is 30 Hz, the background luminance is set at 31.4 asb, whilst the maximum luminance is 10,000 asb, with a stimuli dynamic range of 34 dB. However, such device was not available at the time when the present investigation took place.

Preferred Retinal Locus

The anatomical area of the retina used to fixate is known as Preferred Retinal Locus (PRL). The PRL in microperimetry is defined by the cloud of fixation points distribution, superimposed over the fundus image. The PRL in healthy retinal conditions is centrally located on the fovea, whilst in patients with central vision loss it is located eccentrically commonly associated with unstable fixation

(Sullivan et al., 2008). In general, the more peripheral the PRL is located, the more unstable the fixation becomes (Morales et al., 2013).

Fixation Stability

Patients' fixation characteristics in MP are determined by sampling the position of a reference fundus image at the eye tracker speed (25 Hz). The centre of each sampled frame is superimposed on the initial reference image creating a cloud of fixation points. The location and distribution of such points are used to determine fixation stability (FS) and the PRL. MP systems currently use two different approaches to measure FS. The first FS measurement method calculates the percentage of fixation points falling inside a circle of 1° and 2° radii (defined in the MAIA MP as P1 and P2, respectively) centred on the barycenter of the cloud of fixation points (Morales et al., 2013). The main advantage of this method is the clinical classification of FS as suggested by Fujii et al., where eyes with P1 greater than 75% are classified as having stable fixation; if P1 is less than 75% and P2 is more than 75%, fixation is classified as relatively unstable; and if both P1 and P2 are less than 75%, the pattern is described as unstable fixation (Fujii et al., 2002). This methodology has been criticized in the literature because of the arbitrarily selected parameters of the distances of 1° and 2° used to establish such FS index.

The second FS measurement method suggested by Crossland et al., is known as the bivariate contour ellipse area (BCEA) (Crossland et al., 2009). It calculates the area and orientation of an ellipse encompassing a given proportion (ρ) of the fixation points' dataset. This is a two-dimensional elliptical representation that describes the limits of the retinal surface area used during a fixation attempt,

where lower BCEA values define better fixation stability (Crossland et al., 2009, Crossland et al., 2004, Crossland and Rubin, 2002). The advantage of the BCEA calculation over the percentage of fixation points is that it is based on a mathematical model used in statistics to describe movement of variables. However, it is not related to any clinical classification in MP.

Biofeedback Fixation Training

When central vision loss results from macular degeneration or retinal dystrophies affecting the fovea, fixation becomes unstable. In such cases, MP is useful in mapping out, with precision, the vision loss zone and determining LTS on different areas of the eccentric retina. This analysis is useful in determining the best functional eccentric locus that may be used by patients with loss of foveal vision when attempting visual tasks.

Automatic MP includes biofeedback technology which has the scope to improve eccentric vision capabilities by improving fixation stability in patients with central vision loss. Through oculo-motor training, patients are instructed to use their best potential eccentric retina, by moving their gaze in a way that a visual target falls on the healthier eccentric locus. With several sessions, patients may learn to use their residual vision more effectively (Markowitz, 2006).

Biofeedback fixation training is, therefore, a technological aid used in the rehabilitation process for LV patients with eccentric and unstable fixation. This technique is based on the theory of Brain Plasticity (Markowitz, 2006), where areas of residual vision can be identified and stimulated, improving the ocular

motor control for better residual visual performance. Unfortunately, this technique is not well known, and there are no standardised methods for such rehabilitation method.

Clinical Review on Microperimetry

It has been reported that MP may provide insight into the changes in macular function after retinal pigment epithelial (RPE) transplantation, macular thickness changes in diabetic retinopathy, anti-VEGF therapies in AMD, and retinal vein occlusion (Winterhalter et al., 2012). Karacorlu et al found that intravitreal bevacizumab (Genentech, Inc., South San Francisco, CA, USA) therapy in previously untreated patients induced a significant increase in mean retinal LTS and a reduction in mean scotoma size (Karacorlu et al., 2010). Hatef et al., showed that in eyes with diabetic macular oedema, with a retinal thickness value of 280μm or less at the fovea, retinal thinning was associated with decreased retinal sensitivity (Hatef et al., 2011). Landa et al., suggested that retinal sensitivity was correlated with the status of the underlying IS-OS junctional layer in both dry and wet forms of AMD (Landa et al., 2011).

Alexander et al., reported the relationship between macular sensitivity, central retinal thickness and visual acuity in the maintenance phase of ranibizumab therapy using the MAIA microperimetry. Some patients with stable visual acuity and central retina thickness were shown to have deteriorating retinal sensitivity, which may indicate subclinical disease activity (Alexander et al., 2012), while Ozdemir et al., reported reduction in mean scotoma size with intravitreal bevacizumab therapy (Ozdemir et al., 2012).

Recently, in a report from the NEI/FDA workshop on age-related macular degeneration and inherited retinal diseases, MP was acknowledged as an instrument for generating retinal sensitivity maps. Jaffe has suggested that the OCT (anatomical) and microperimetry (functional) correlation may be a suitable functional outcome measure for progression in Stargardt disease. However, Chambers reported that in certain areas of the retina, particularly in the ellipsoid zone at the edge of the lesion, the correlation between microperimetry and OCT data is not perfect; however, if the extent of change is outside the 'questionable area', then this parameter is likely to be acceptable as a surrogate endpoint for such retinal pathologies (Csaky et al., 2017).

2.2 The Topcon Optical Coherence Tomography (OCT)

Optical coherence tomography (OCT) is one of the most successful imaging technologies implemented in the ophthalmology practice. It is based on low-coherence interferometry, used in the diagnose and monitoring of retinal pathologies capturing two- and three-dimensional structural images of the retina. OCT is analogous to ultrasound imaging, where a sound pulse is launched and the reflections (echoes) are measured to create an image of tissue. In OCT light reflections are measured by a Michelson interferometer, using the low coherence properties of a broadband light source. By measuring this interference, the location and strength of the reflections can be determined. There are in essence two types of OCT technology according to their evolution: Time Domain (TD-

OCT) and Fourier Domain (FD-OCT), which can in principle be performed in two ways: either by using a spectrometer, commonly named Spectral Domain OCT (SD-OCT), or by using a fast tuneable laser, called Swept Source OCT (SS-OCT). Both approaches have in common that the reflectivity is measured for a multitude of wavelengths separately. By combining the information of those wavelengths, a depth profile with high resolution can be created (de Boer et al., 2017). OCT has been established as the "Gold Standard" for the diagnosis of ocular pathologies. However, since it is a diagnostic technique based exclusively on the structural image, it does not offer by itself the possibility of evaluating functional data regarding visual function. Nowadays with the emergence of Anti-VEGF treatments, OCT has fulfilled the need for a precise and exhaustive follow-up of patients, in virtually all ophthalmological practices as routine tests. The Topcon SD-OCT (Tokyo, Japan) is able to produce up to 85000 A-Scans for up to 256 parallel B-Scans, creating structural retinal images with resolution that reaches 5 microns, whilst the B-Scan length goes from 6 mm to 12 mm.

2.3 International Reading Speed Test (IReST).

Visual acuity is normally assessed by the Snellen or the ETDRS charts. These are a reliable visual performance assessment in cases of patients with functional foveal. They represent the ability to distinguish one single letter as a visual task. However, they are often poor predictors of everyday visual function such as reading, especially in cases of patients with central scotoma (Ahn et al., 1995).

Different reading texts have been developed to assess visual performance in clinical trials. Reading performance analysis may serve as a useful tool in such situations because the evaluation of reading speed and reading acuity is easy to perform and provides a large amount of information that can easily be interpreted. Texts with single sentences for measuring reading acuity, reading speed, and critical print size are available, such as MN Read or Radner's charts. The Minnesota Low-vision Reading Text (MN- READ) uses simple sentences and common vocabulary to minimize cognitive and linguistic demands. Sentences are presented at high magnification so that subjects with low vision do not need to manipulate magnifiers. The Radner reading charts use sentences, in German language, developed to be comparable in number and length of words, as well as in difficulty and construction, with the purpose to offer a reliable and valid test for measuring reading acuity and speed (Radner et al., 2002).

However, for measuring reading speed, a whole paragraph of text is preferable to single sentences, because the percentage error of reading time measurements in seconds is smaller for longer texts. Furthermore, reading whole paragraphs is closer to the demands of everyday reading. The International Reading Speed Text (IReST) was created with the purpose to standardized reading performance texts through multiple equivalent texts for repeated measurements, and for texts equated across languages for multi-language studies. Ten paragraphs of German text were designed by a linguist from material for sixth grade reading (age 10–12 years) with a mean length of 132 words (SD 6 3.2). They were matched for difficulty and linguistic/syntactic complexity. The texts were translated into 16

languages, and adapted by linguists of the respective language, to be similar in number of words, character counts, difficulty and complexity to the German original (Trauzettel-Klosinski and Dietz, 2012).

A study to established normal reading speed test parameters was performed with 436 normally-sighted subjects, who were native speakers of the respective languages. Participants were 18 to 35 years of age with normal or corrected-to-normal vision. The texts were printed and presented in black on white paper at a viewing distance of 40 cm and a size of 1 M (5 minutes of arc at 1 m), or 10-point Times New Roman font, which corresponds to many newspapers print sizes. The paragraphs had a maximum line length of 8.5 to 10.0 cm. The mean number of lines/text was 14.3. The texts were read aloud. A stopwatch was used to measure reading time. Words read incorrectly or omitted were counted. Reading speeds was assessed in four different ways: texts/min, words/min, syllables/min, and characters (without spaces and punctuation marks)/min. Means and SDs were computed for each language using these four measures of reading speed.

The MN Read and Radner texts use short and simple (second and third grade material) single sentences in different print sizes to assess reading acuity, critical print size, and magnification need. Variability among participants depends on reading habits and skills. However, within one individual reader the variability is relatively low.

The IReST differs from prior text charts that used single sentences by employing linguistically standardized paragraphs. The IReST instead, due to lower variance, measuring reading time of a complete paragraph rather than a single sentence or random words is more reliable. Furthermore, it can provide some information about fluency, fatigue, and mistakes.(Trauzettel-Klosinski and Dietz, 2012) In patients with low vision and central scotoma, it is suggested to read the IReST with their magnifying aids.

3.Methods.

As summarised in the Aims and Objectives section, this study is divided in 2 projects: a) Microperimetry in the monitoring of common macular diseases, and b) Eccentric fixation training for visual rehabilitation with MP biofeedback technology.

The main research question for both projects are:

Project A.

Is Microperimetry (MP) technology able to provide relevant information regarding macular function, to such extent that MP examinations can be recommended as an integral part of the standard of care in the management of macular diseases?

The National Institute for Health and Care Excellence (NICE) recommendations in the managing of age-related macular degeneration include: fundus examination to people presenting changes in vision, slit-lamp fundus biomicroscopy to confirm diagnosis of early or late dry-AMD, OCT in people with suspected late wet-AMD, and FFA to confirm the late wet-AMD diagnosis if OCT does not exclude neovascular disease. During the treatment and

monitoring phase of late wet-AMD, NICE recommends ongoing monitoring with OCT for both eyes, and fundus examination or colour photography if OCT appearances are stable but there is a decline in visual acuity, or the person reports a decline in visual function. If OCT results suggest macular abnormalities but the abnormalities are not responding to treatment, the NICE guidelines suggest to use "alternative imaging" (NICE, 2018). However, up to date functional retinal examinations, such as MP, have not been considered in the management of AMD at any stage.

Project B.

Is the eccentric fixation training performed with MP technology an effective rehabilitation proposal, which can improve vision in people with central vision loss secondary to macular pathologies?

The NICE guidelines suggests referring to low-vision services, people with visual impairment secondary to late AMD, and consideration of eccentric viewing (EV) training for people with central vision loss in both eyes (NICE, 2018). EV is an adaptive strategy used to compensate for central vision loss in which relatively healthy paracentral areas of the retina are used to fixate objects. People are taught to use a specific paracentral area of retina during EV training, known as the trained retinal locus (TRL). However, there is insufficient evidence to conclude that a particular model of EV training is effective, and there are no robust studies aimed to optimise or standardised EV training techniques (Gaffney et al., 2014).

3.1 Study design and protocol Project A

This was a longitudinal observational, non-randomized, prospective cohort study in patients with treatable macular diseases, to determine the utility of the MAIA MP in monitoring intravitreal treatment outcomes.

Patients from the Macular Clinic of the Nottingham's Queen Medical Centre, scheduled for treatment with anti-VGEF intravitreal injections of one or both eyes for wet AMD or DMO secondary to retinal vein occlusions (RVO) were invited to participate in the study. All patients had a routine clinical evaluation including, best corrected visual acuity, according to the ETDRS standard, slit lamp biomicroscopy of the anterior and posterior segments, and optical coherence tomography (OCT). In addition, a fully automatic MAIA microperimeter (MP) examination was performed to study macular function outputs of retinal light threshold sensitivity and fixation stability. The appropriate intravitreal therapy with ranibizumab or bevacizumab for wet AMD, or DMO, and ranibizumab or dexamethasone implant for RVO was administered as clinically necessary, as recommended by the Royal College of Ophthalmologists guidelines.

All examinations were performed at base-line, before any clinical or surgical intervention, and repeated on follow-up visits at 3 months, 6 months, and 12 months from baseline.

Study treatment and regimen

All participants were involved for 12 months after enrolment, and performed the following tests at baseline, 3, 6, and 12 months

-Best corrected Visual Acuity measure (EDTRS),

-OCT at baseline,

-MAIA MP with the standard macular grid automatically centred on the patient's PRL.

-Fluorescein angiography when clinically indicated.

The MAIA examination was performed over the 10° diameter central retinal area, using the standard macular grid (37 measuring points) with automatic grid centring on the patient's preferred retinal locus (PRL). The MP follow-up test was automatically performed over the same anatomical area tested during the baseline examination.

Primary and secondary endpoints

The primary endpoint was the quantitative analysis of the MP functional outputs of retina sensitivity, fixation stability and fixation location during the assessment of macular therapy correlated to OCT and visual acuity.

The secondary endpoint was a qualitative evaluation regarding the capacity to understand better patient's visual conditions from the MP outputs correlated to OCT outputs and visual acuity.

Recruitment

Participants were recruited from the Medical Retina and Macular clinic of the Nottingham's Queen's Medical Centre. The initial approach was from a member of the patient's usual care team which may include the investigator. The investigator or a member of the research team, informed the participant all aspects pertaining to participation in the study.

Inclusion and exclusion criteria

Inclusion Criteria:

• Signed Informed consent.

• No limitation to race, sex or ethnicity.

• Adult age above 21 years old.

• Visual acuity LogMAR 0.2 to 1.2.

Newly diagnosed patients with:

• Choroidal neovascularization – AMD and non-AMD.

• Retinal vein occlusion (central [CRVO] or branch [BRVO]) with or without MO.

• Diabetic macular oedema (DMO).

• Patients with other macular pathology (Central serous retinopathy [CSR], Macular hole [MH], epiretinal membrane [ERM]), included as untreated controls.

• OCT presence of macular oedema.

• Patients willing to attend for follow-up microperimetry examinations.

• Planned treatment with intravitreal dexamethasone (Ozurdex), ranibizumab, bevacizumab, and/or laser photocoagulation.

• Concomitant treatment, such as appropriate intravitreal therapy with ranibizumab or bevacizumab for AMD, or DMO, and ranibizumab or dexamethasone implant for RVO.

Exclusion criteria:

• Moderate to severe cataract (over grade 2) or other cause of opaque optical media.

• Previous treatments in the particular eye with intravitreal therapies of anti-VEGFs or steroids within the previous 6 months, or macular laser photocoagulation

• Uncooperative patient.

• Previous vascular event in the eye of interest other than current diagnosis.

3.2 Study design and protocol Project B

This was a pilot, longitudinal, observational, prospective cohort, non-comparative, non-randomized, interventional study, on low vision patients with central vision loss secondary to late stage maculopathies, to study visual improvement after eccentric fixation training with MP biofeedback technology.

Patients from the Macula and Low Vision Clinics of the Nottingham's Queen Medical Centre, diagnosed with low vision secondary to late maculopathies, such as, geographic atrophy secondary to late-stage dry AMD, vitelliform dystrophy, or central serous retinopathy, scheduled for regular control visit were invited to participate in the study.

All patients had a routine clinical evaluation including, best corrected visual acuity, according to the ETDRS standard; slit lamp biomicroscopy of the anterior and posterior segments, and optical coherence tomography (OCT). In addition, a fully automatic MAIA microperimeter examination was performed to study retinal light threshold sensitivity and fixation stability with the purpose to perform eccentric fixation training with the MAIA biofeedback technology. The biofeedback fixation training sessions were scheduled on a weekly base during 12 weeks. After 3 months of resting time, a new set 12 weeks of BFT sessions was scheduled.

Study treatment and regimen

All participants had 2 periods of weekly BFT sessions. Each period lasted 12 weeks and each session lasted 10 minutes. A 3 months resting time between periods was scheduled.

At baseline and at the end of the BFT sessions the following tests were performed.

-Best corrected Visual Acuity (EDTRS).

-OCT.

-Reading speed with the International Reading Speed Text (IReST). Different text paragraphs were chosen for baseline and for the end of BFT sessions.

-MAIA MP with the standard macular grid automatically centred on the patient's PRL.

In addition, BFT was performed at baseline with the purpose of identifying the eccentric retinal location to be used to perform BFT. The following MAIA examinations were performed in this regard: A) the "Low-Vision-Assessment" grid-test (30°, 83 stimuli) with the 4-levels-fixed projection strategy, which scores retinal sensitivity as "good" (25dB), "relatively good" (15dB), "relatively poor" (5dB), "poor" (0dB) and "scotoma" (<0 dB); and B) the "Fixation-Training-Target" grid-test (7°x5°, 35 stimuli) with the 4-2 projection strategy. The first test, (A), was centred on the estimated foveal location, or on the patient's PRL in cases of Geographic Atrophy larger than 3 times the optic nerve head size. The Low-Vision-Assessment grid output was used to identify retinal loci with at least 2 consecutive stimuli, distributed horizontally, showing "good" or "relatively-good" light sensitivity, and served as a reference to centre the Fixation-Training-Target grid, prioritizing the superior retina (inferior visual field) and the smaller distance from the anatomical fovea. The second custom test, (B), was used to select the target locus for BFT. This locus was set in the centre of the two adjacent stimuli with highest light sensitivity, and lowest distance from both the anatomical fovea and the patient's baseline PRL.

BFT consisted of asking patients to slightly move their gaze towards the training locus. An auditory signal increased frequency as the desired eye movement reached the target. The microperimeter operator helps the patient with voice commands suggesting the direction of eye movements.

Primary and secondary endpoints

The primary endpoint was classification of FS, the MAIA fixation stability indices, visual acuity, and reading speed (IReST). The secondary endpoint was mean light sensitivity, the anatomical location and the visual field correspondence of the PRL.

Recruitment

The initial approach was from a member of the patient's usual care team which included the investigator.

The investigator or their nominee, e.g. from the research team or a member of the participant's usual care team, informed the participant or their nominated representative (other individual or other body with appropriate jurisdiction), of all aspects pertaining to the participation in the study.

Inclusion and exclusion criteria

Inclusion criteria:

• Signed Informed consent.

• No limitation to race, sex or ethnicity.

• Visual acuity LogMAR 0.4, to LogMAR 2.0.

• Adult age above 21 and below 80 years old.

• All patients categorized as eccentric viewers, geographic atrophy area with scotoma larger than 1 disk size and smaller than 5 disk sizes.

• Stable pathology.

• At least 6 months from last anti-VEGF injection if any.

• Patients willing to improve reading abilities (based on questionnaire).

Exclusion Criteria

• Mild to severe cataract (over grade 2) or other opaque optical media.

• Macular Oedema.

• Uncooperative patient.

• Non-English speakers.

• Patients undergoing any other visual rehabilitation therapy.

3.3 Statistics

Spearman's rank-order coefficient (rs) and a one-tailed Mann-Whitney test was applied to compare baseline and final outcomes, with a significant difference of $P < 0.05$. Linear modelling techniques, paired t-tests (two-tailed) and multivariate analysis were also conducted to assess improvement in reading speed and VA.

As there are no previous sizeable studies of this kind, it was estimated that an appropriate minimum sample size of 100 participants for each project was needed to obtain statistical significance results. Data from participants withdrawn from the trial prematurely was included in the final analysis up to the point of withdrawal.

3.4 Ethical and regulatory aspects

Ethics committee

The trial initiated only after the protocol, informed consent forms and participant information sheets received approval of favourable opinion from the Nottingham Research Ethics Committee (REC), and the University of Nottingham's Research & Development (R&D) department. The trial was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996, the principles of Good Clinical Practice (GCP), and the Department of Health Research Governance Framework for Health and Social care, 2005. All participants provided written informed consent.

Informed consent

The decision regarding participation in the study was entirely voluntary. Both, the investigator and the participant signed the informed consent form before undergoing any interventions (including physical examination and history taking) related to the study. The investigator emphasized that consent regarding study participation could be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. The Investigator explained the details of the trial and provided a Participant Information Sheet, ensuring that the participant had sufficient time to consider participating or not.

4.Clinical Reference Database for Fixation Stability Metrics in MAIA

4.1 Introduction

As described earlier, MP allows an automated analysis of macular function even with unstable fixation, as in the case of central geographic atrophy secondary to dry AMD. Such assessments are independent of the quality of fixation and eye movements. Fixation stability (FS) in microperimetry can be quantified with different indices. One of them is the Bivariate Contour ellipse Area (BCEA). This chapter is related to a published study that we have conducted reporting a reference clinical database for fixation stability metrics of the fixation index BCEA in normal subjects measured with the MAIA MP (Morales et al., 2016).

FS is an objective test performed in MP by means of retinal landmark tracking which plots the scatter of a cloud of fixation points (CFP) over a retinal image reference map. The resulting scatter pattern can then be analysed mathematically.

Two different approaches have been used in MP to measure FS. The first one is related to the quantification of fixation points falling inside a circle of 1° and 2° radii, defined in the MAIA as P1 and P2 respectively (Morales et al., 2013). This method is used to classify FS in 'Stable', 'Relatively Unstable' and 'Unstable', as described in chapter 2 (Fujii et al., 2002). However, such classification has been largely criticized in the literature due to the arbitrarily selected distance of 1° and 2° used to establish such stability index.

The second FS measurement method is known as the bivariate contour ellipse area (BCEA). It calculates the area and orientation of an ellipse encompassing a given proportion (ρ) of the fixation points dataset. This is a two-dimensional elliptical representation that describes the limits of the retinal surface area used during a fixation attempt, and where lower BCEA values define better fixation stability (Crossland et al., 2009, Crossland et al., 2004, Crossland and Rubin, 2002). The advantage of the BCEA calculation over the Fujii's classification is that it is based on a mathematical model used in statistics to describe movement of variables; however, it is not related to any clinical classification in MP.

The common formula used to calculate BCEA (Steinman, 1965) is:

$$BCEA = 2k\pi\sigma_H\sigma_V(1-\rho^2)^{1/2}$$

where $BCEA$ is the bivariate contour ellipse area, σ_H is the standard deviation of point location over the horizontal meridian, σ_V the standard deviation of point location over the vertical meridian, and ρ the product-moment correlation of

these two position components. The value k defines the limit for the ellipse and is dependent upon the probability area chosen given by:

$$P = 1 - e^{-k}$$

where e is the base of the natural logarithm, from where,

$$k = -\ln(1 - P)$$

This method assumes that fixation points are normally distributed. Different authors have used different probability (*P*) values, such as 0.63 (Steinman, 1965, Kosnik et al., 1986), 0.68 (Crossland et al., 2009, Crossland et al., 2004, Crossland and Rubin, 2002), or 0.95 (Schuchard and Raasch, 1992). The clinical and statistical significance of such *P* values have never been reported for MAIA microperimetry.

The MAIA reports two BCEA indices with proportional values of 63% and 95% converted into degree units. Reference databases of macular sensitivity values have been reported, allowing the correlation of location-specific data from the average threshold sensitivity in normal eyes to those data acquired from eyes with different stages of AMD, however there are no studies reporting similar normative reference for the BCEA fixation indices, for such reason, we conducted a study to establish a reference database for fixation stability measured with the BCEA metric in a group of normal subjects tested with the MAIA microperimeter (Morales et al., 2016).

4.2 Methods

Participants

This was a multisite, cross-sectional, observational, prospective study which included normal healthy subjects without signs of ocular pathology, aged 19 to 86 years from June 2012 to June 2016. The clinical study sites involved were located at Nottingham (U.K.), Crete (Greece), Slidell, Louisiana (U.S.A.), and Milan (Italy).

A total of 358 participants with normal visual acuity or corrected to normal (20/20 or better), mean spherical equivalent refractive error of -3.06±1.36 (range, -9.50, 5.28) and with no history of any visual abnormalities, were tested for fixation stability and macular light threshold sensitivity using the MAIA microperimeter device. The MAIA automatically corrects for refraction errors in the spherical equivalent of -15 to +10 diopters; therefore, such range was part of the inclusion criteria. Only one eye of each patient was tested for inclusion within the reference database. The eye with the better visual acuity was selected, and the right eye was chosen if visual acuity was identical in both eyes. Thirty-one patients were excluded from the analysis due to their low reliability index in the MAIA testing, established by CenterVue as a fixation loss value indicated by the percentage of stimuli reported as seen when a stimulus is projected onto the optic nerve head (blind spot). Microperimetry, through the retinal landmark tracker, compensates for eye movements in the location of stimuli projection,

hence gaze deviation will not interfere while testing the blind spot. In essence, the fixation loss value is a validation measure of the test subject's compliance with reporting only those stimuli that are potentially visible. The test is considered reliable when such index is above 70% (*MAIA Operator's Manual*). A total of 326 eyes were included in the analysis after the exclusions.

Patient examination data was anonymised prior to inclusion within the database in order to protect patient privacy. Written informed consent was obtained from all participants, and local Bioethics Board approval was obtained as required for all sites involved in the study. The study was performed in accordance with the tenets of the Declaration of Helsinki.

Equipment and procedures

A fully automated MAIA examination was performed to assess LTS and FS using the MAIA standard macular grid pattern (37 stimuli points) over 10 retinal degrees (±5° around the macula). In the first 10 seconds of the examination, the MAIA microperimeter performs a fixation test in which a central point of reference of the average retinal position is calculated and labelled as PRL_initial (PRL*i*); subsequently the projection of the pre-selected stimuli grid map begins with the grid centred on such PRL*i*. On completion of the test, the centre of the mass (barycenter) of the total fixation points is calculated and defined as PRL_final (PRL*f*), which corresponds to the foveal area in normal subjects.(Morales et al., 2013) The projection strategy was the standard 4-2 and the examination was performed without dilation, in a darkened room (mesopic conditions). Patients were instructed to stare at the centre of the fixation target

during the examination. The fixation target consists of a 1° diameter red circle with light intensity of 10 ± 3 apostilb (asb).

The MAIA can project stimuli targets with a maximum luminance of 1000 asb and with a stimulus dynamic range of 36 decibels (dB). The MAIA microperimeter utilizes a scanning laser ophthalmoscope (SLO) technology to image the retina to a field of 36°; it has an automatic compensation of refractive error between -15 and +10 diopters (D). The automated retinal eye-tracking system samples and corrects for eye movements at 25 Hz with respect to the positioning of stimulus targets. The fixation location is calculated from the eye-tracking output, and plotted 25 times per second as a cloud of fixation points (CFP) describing the fixation pattern overlaid onto a reference SLO retinal image acquired at the start of the test. The average examination time is 5 minutes, 50 seconds; therefore, the CFP may contain more than 8700 points (Fig. 4.1).

Primary outcomes of the study were the four MAIA fixation indices: P1, P2 and the two BCEA proportional values of 63% and 95%. Secondary outcomes were the average of sensitivity, distance between the PRL*i* and PRL*f* (ΔPRL) (Morales et al., 2013), the MAIA examination time and the BCEA orientation, which corresponds to the angle between the ellipse major axis (EMA) and the horizontal axis (HA) of the visual meridian, where values between 0° and +90° correspond to an angle measured counter clockwise between the HA and the EMA; values between 0° and -90° are measured clockwise between the HA and the EMA; 0° corresponds to a horizontal orientation, and 90° to a vertical one.

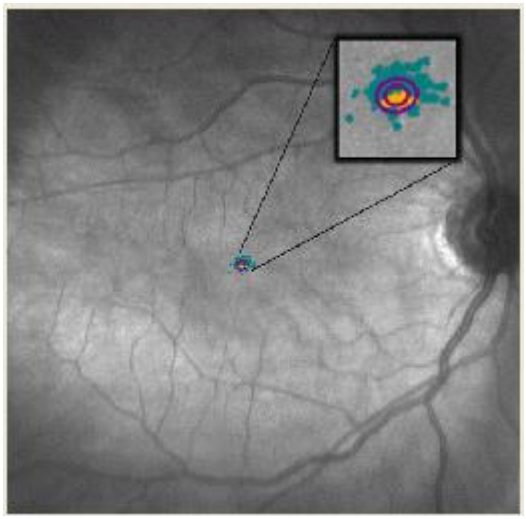

Figure 4.1 MAIA image with CFP

Image of the MAIA microperimeter showing the cloud of fixation points (CFP) and both BCEAs (63% and 95%), which lie at approximately the same foveal location on a normal subject.

Statistical Analysis

All data were exported from the MAIA system as raw data (MAIA software version 1.7.0) and collected into a Microsoft Excel spreadsheet (Microsoft, Bellevue, WA). The statistical analysis was performed with linear regression and Pearson's product-moment correlation coefficient. A *p*-value less than 0.05 was considered statistically significant. Linear regression, best-fit values, 95% confidence and prediction intervals, correlation coefficient of determination, and analysis of variance (ANOVA) with 95% CI were calculated and plotted using GraphPad Prism (version 6; GraphPad Software, Inc., La Jolla, CA).

4.3 Results

When data from all included eyes (n = 326) were analysed (Table 4.1), we found average areas of 0.80 deg^2 (min = 0.03, max = 3.90, SD = 0.68) for the parameter BCEA@63%, and 2.40 deg^2 (min = 0.20, max = 11.70, SD = 2.04) for BCEA@95% (Figure 4.2). The average values of P1 and P2 were 95% (min = 76, max = 100, SD = 5.31) and 99% (min = 91, max = 100, SD = 1.42), respectively. The maximum fixation value (100%) was recorded in 65 (20%) cases of P1 and 199 (61%) cases of P2. MAIA P1 & P2 linear regression analysis showed a more sensitive P1 index than P2 (Figure 4.3). The average of LTS was 29 dB (min = 18, max = 34, SD = 2.60). The mean ΔPRL was 0.2° (min = 0.0, max = 1.0 SD = 0.16) (Fig. 4.4). The angular orientation of the BCEA was variable with a mean angle of 3° (min = -90, max = 90, SD = 53.83). The mean examination time for each eye was 5 minutes, 50 seconds (min = 4 minutes, 2seconds, max = 10 minutes, 40seconds, SD = 59 seconds).

Table 4.1 Linear regression and summary statistics

	BCEA@63% (deg^2)	BCEA@95% (deg^2)	P1 (%)	P2 (%)	LTS (dB)	ΔPRL‡ (deg)	BCEA θ§ (deg)	Exam. time
Mean	0.80	2.40	96	99	30	0.2	3	5'50''
SD	0.68	2.04	5.76	1.37	1.61	0.15	53.83	59''
Min	0.03	0.20	76	91	24	0.0	-90	4'2''
Max	3.90	11.70	100	100	33	0.8	90	10'40''
R^2*	0.068	0.070	0.074	0.048	0.378	0.039	0.001	0.001
***p*-value†**	< 0.001	< 0.001	< 0.001	<0.001	< 0.001	<0.00	0.49	0.001

Linear regression and summary statistics for all parameters of fixation stability (BCEAs, P1 and P2), light threshold sensitivity (LTS), distance between the initial and final PRL (‡), angle of inclination for the BCEA (§), and examination time. (= Coefficient of determination. † = Shapiro–Wilk test for normality).*

The ANOVA comparisons of the FS means within the age groups were highly significant for the indices P1, BCEA@63% and BCEA@95%, but less significant for P2 (Table 4.2).

Table 4.2 Fixation stability in age groups.

	BCEA@63% (deg^2)		BCEA@95% (deg^2)		P1 (%)		P2 (%)	
AGE	Mean	SD	Mean	SD	Mean	SD	Mean	SD
20s (n= 59)	0.56	0.61	1.73	1.80	97.19	4.10	99.60	0.94
30s (n= 57)	0.56	0.57	1.69	1.72	97.08	4.58	99.62	1.15
40s (n= 45)	0.84	0.76	2.52	2.26	94.74	6.17	99.19	1.42
50s (n= 28)	0.85	0.66	2.56	1.94	95.20	4.00	99.44	1.14
60s (n=57)	0.98	0.73	2.93	2.18	92.79	6.33	99.05	1.56
70s (n=60)	0.96	0.68	2.89	2.04	94.37	4.82	98.88	1.86
80s (n=20)	1.07	0.56	3.20	1.67	92.80	4.53	98.70	1.34
ANOVA (P)	0.0003		0.0002		<0.0001		0.0069	

Mean and standard deviation of all fixation stability parameters for different age groups.

The Pearson's product moment test showed an almost perfect correlation index (r = 0.999) between BCEA@63% and BCEA@95%. The P1 index showed a very strong correlation with BCEA@63% (r = -0.924), as well as with BCEA@95% (r = -0.925). P2 demonstrated a slightly lower correlation with both BCEA@63% and BCEA@95% with *r* values of -0.874 and -0.875 respectively. The correlation between P1 & P2 ($r = 0.792$) was lower than that found with the BCEA indices. Finally, low correlations ($r < 0.28$) between any of the fixation indices and the patient's age were found.

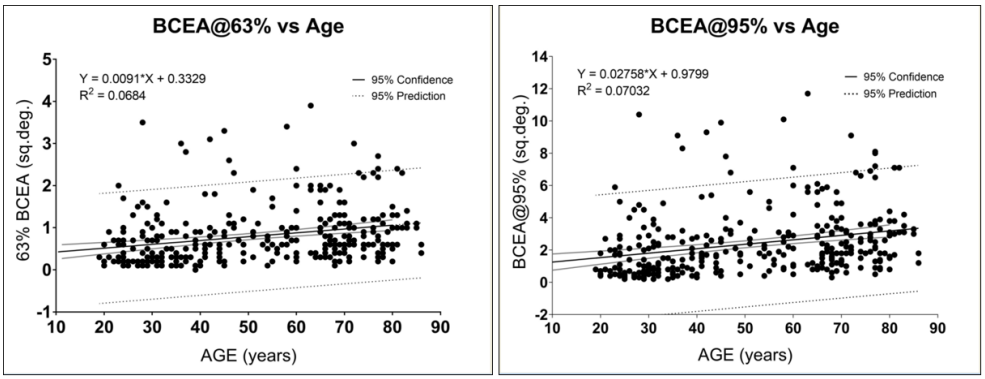

Figure 4.2 BCEA in normals

The BCEA proportional values of 63% shows a mean of 0.80 ± 0.68 deg^2, whilst the BCEA proportional values of 95% shows a mean of 2.40 ± 2.04 deg^2. Note the distribution similarity between both proportional values datasets.

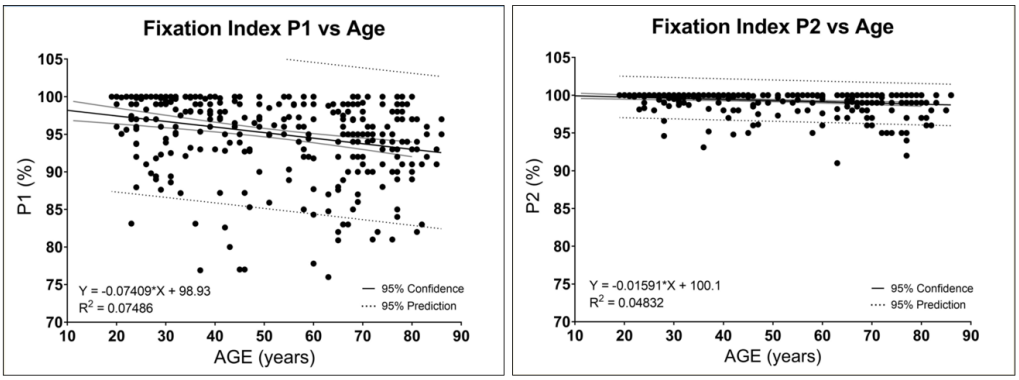

Figure 4.3 P1 in normals

P1 shows mean values of 95% ± 5.31, whilst P2 shows mean values of 99% ± 1.42. Note that the R-squared value of P1 (0.07), fits better the regression model than P2 (0.04), which demonstrates higher ceiling effect than P1.

4.4 Discussion

Currently available MP instruments can deliver four or more different indices of fixation stability, making the comparison to a reference database overly complicated and somewhat arbitrary in terms of a standard. As such, the MAIA

MP delivers two PFP indices (P1 and P2) as proposed by Fujii (Fujii et al., 2002), as well as two BCEA indices (63% and 95%) as proposed by Crossland (Crossland and Rubin, 2002). The PFP indices allow the classification of fixation as stable or not stable, but the BCEA indices are not related to any clinical stability standard at the present. This study reports a normative reference database for BCEA fixation stability indices for the MAIA instrument.

In addition, we explored the strongest correlations among all fixation indices, highlighting the near-perfect correlation between BCEA@95% and BCEA@63%, as well as the very strong Pearson's power product between BCEA@95% and P1. Moreover, the P1 and P2 linear regression analysis showed a more sensitive P1 index than P2, with P2 having a higher ceiling effect than P1 (100% maximum percentage).

Various geometric and statistical models using multi-variable datasets have been used to simplify and describe the complexity and dynamic nature of movement sampling. The Bivariate Contour Ellipse Area (BCEA) model describes the horizontal and vertical displacements of the retinal position over the course of time during fixation attempts. By describing fixation stability in this manner, the BCEA has a graphical advantage in its ability to represent a specific proportion of the total CFP dataset points collected. The dimensions and orientation of the BCEA illustrate the total extent of the retinal area used by subjects while attempting to maintain constant fixation.

Crossland et al. (Crossland and Rubin, 2002) suggested the analysis of fixation stability by means of BCEA instead of PFP after demonstrating a strong relationship between fixation stability measured with BCEA and many parameters of reading ability, as well as a very poor correlation between PFP indices with any of the standard parameters of reading ability in Low Vision patients.

Tarita-Nistor et al. (Tarita-Nistor et al., 2008) have stated that the BCEA description of fixation is scientifically more acceptable than the Fuji classification due to the analysis being based on a well-known mathematical model versus the quantification of fixation points falling into an arbitrarily selected fixed circular area of 1° and 2° in radius. However, the main advantage of the Fuji metrics is that it has established a differentiation between normal and non-normal fixation with cut-off values of 75% within 1° radius from the centroid of the fixation dataset.

According to the above, the discrepancy between our MAIA-based findings and the cut-off values of stable fixation proposed by Fujii (Fujii et al., 2002) is worthy of comment. Our studied population demonstrated higher P1 values (fixation points within 1° radius) than those proposed by Fuji (95±5% vs 75%) while the P2 index (fixation points within 2° radius) reached a celling effect in a high number (61%) of the studied subjects. In contrast, our BCEA values showed only 1 participant who reached the floor effect in the BCEA@63% while none of the subjects reached the lowest limiting value in the BCEA@95%.

A literature search reveals reports of different BCEA ellipse values for normal control groups evaluated with microperimetry systems other than the MAIA (Tarita-Nistor et al., 2008, Dunbar et al., 2010, Cutini et al., 2014). It is noteworthy that some of these previously reported results are based on a fixation-only test lasting between 10 and 30 seconds, and the amount of data collected is less than 800 points. In contrast, the MAIA-based BCEA ellipse is derived from a CFP with more than 8,000 data points, a greater than 10-fold difference in data sampling. Furthermore, because the complete MAIA microperimetry examination has a median duration of 5.5 minutes, fixation fatigue plays a major role in creating a larger BCEA ellipse.

In particular, the majority of the participants in our study were naïve clinical patients with no previous experience in maintaining fixation for long periods of time. Other possible explanations for BCEA outcome discrepancy include differences in the *N* values of the study groups, the motivation and inclusion criteria for selecting study participants, differences in the fixation target dimensions and appearance, and other protocol differences that are beyond the scope of this report.

Tarita-Nistor et al. (Tarita-Nistor et al., 2008) reported in a study with the Nidek MP1 (Nidek, Gamagori, Japan), 10 experienced healthy controls (age 41 ± 18.1 years) with very small mean BCEA_68% values (0.053 ± 0.022 deg2).This is different from the results obtained by Dunbar et al., (Dunbar et al., 2010) who reported the BCEA@68% values of 16 normally sighted volunteers with the Rodenstock SLO and the Nidek MP-1, showing different values among both of

their studied instruments which are even larger than our findings (3.3 deg^2 with the Rodenstock and 5.0 deg^2 with Nidek).

Liu et al. (Liu et al., 2015) reported BCEA data in subjects with and without maculopathies with two different systems, the MP-1 and optical coherence tomography (OCT)/SLO. The technologies behind the two instruments are different. The MP-1 uses an infrared camera to image the retina controlled by a 25-Hz eye tracker, which is the same eye-tracker frequency found in the MAIA. The second is an SLO imaging device with a much slower eye tracker (8 Hz). Such a study was based on a fixation-only test of 20 seconds, which is largely different from the examination time (mean 350 seconds) studied in this article. Their results suggest that the patient's pattern of FS may be the same, although calculated with different technologies. Furthermore, the authors normalized their data with a log transformation of the BCEA units (minutes of arc2) to undertake statistical comparison of the two technologies. In our study, the whole dataset was analysed using the MAIA output layout with the purpose of establishing reference values for the MAIA that is useful to clinicians.

Similar to the findings reported by Cutini et al (Cutini et al., 2014), who evaluated BCEA indices in the MP-1 microperimeter (Nidek), our study demonstrates a weak, but statistically significant correlation between the fixation indices and the patient's age.

Our study data, which was collected from multiple clinical sites, represents true characteristics of the clinical data that will be encountered by eye care

professionals using the MAIA system. Because of the aforementioned protocol and technical differences among various systems, the quality and accuracy of fixation results reported for other microperimetry devices are questionable and therefore not directly relatable to the MAIA system.

In light of these correlations, we conclude that in clinical practice, the single parameter of the BCEA@95% should be considered as accurately reporting fixation stability and serves as an age-dependent reference database of normal subjects with a cut off area of 2.4 ± 2.0 deg^2 in MAIA microperimetry. The study established an important age-related normative standard for future fixation stability studies of diseased and disease-suspect eyes analysed with BCEA in the MAIA microperimetry (Figure 4.4).

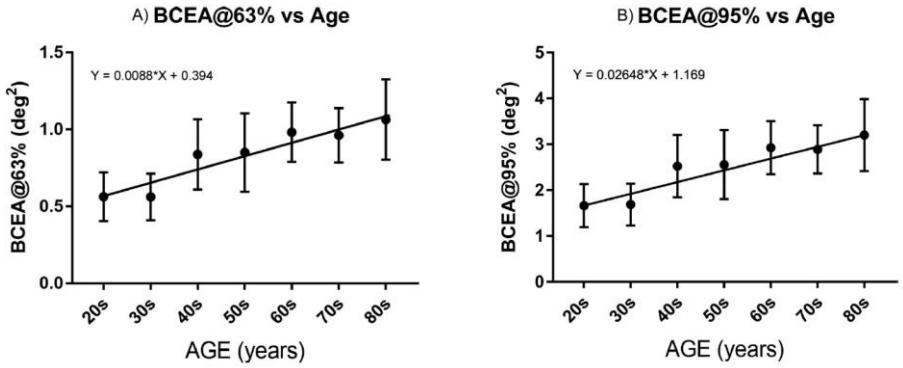

Figure 4.4 BCEA vs age

Mean BCEA@63% and BCEA@95% values ± 95% confidence interval (CI) of the mean in the different age groups.

5.The MAIA PRL initial and final

5.1 Introduction

The retinal area used during any fixation attempt, in cases of central vision loss, defines the preferred retinal locus (PRL). It is presumed that during prolonged fixation attempts there may be various representative reference points within the PRL area. The MAIA microperimeter defines two main PRL reference points during any light threshold sensitivity (LTS) examination. However, the understanding of its clinical validity is incomplete (Morales et al., 2013).

Several different retinal diseases affecting the macula will interfere with the fixation of the eye. These include retinal dystrophies, in particular: Stargardts' disease, age-related macular degeneration AMD, retinal vascular occlusions (RVO), diabetic macular oedema (DME), and intraocular inflammations. Patients with visual loss from the foveal involvement of these diseases tend to adopt eccentric viewing by developing an eccentric PRL. Previous studies have identified eccentric PRLs. However, the characteristics of these PRLs are still not fully understood, or clearly defined. Current knowledge suggests that PRLs may change depending on the type of macular disease, levels of illumination, and the tasks being performed (Sunness et al., 1996, Lei and Schuchard, 1997,

Safran et al., 1999, Schuchard et al., 1999, Deruaz et al., 2002, Midena et al., 2004, Crossland et al., 2011a).

During MP examinations, patients are asked to fixate on a target displayed inside the instrument. While patients attempt fixation, eye-tracking algorithms compensate retinal movement. Image sampling is carried out in real time. The center of all retinal positions is plotted as a cloud of fixation points (CFP) describing the fixation pattern overlaid onto a reference retinal image acquired at the start of the test. The graphical result of the CFP is nominated in MP instruments as the PRL. Hence, the resulting PRL describes patient's fixation characteristics in terms of location (central or eccentric) and stability (stable, relatively stable or unstable) (Fujii et al., 2002). In addition, the PRL graphic representation provides information on size of PRL span density, as well as PRL span orientation.

The MAIA is the only MP system that offers automatic estimates on PRL characteristics during early and late fixation. It utilizes a Scanning Laser Ophthalmoscope (SLO) to image the retina while projecting Goldman III size stimuli directly on the retina by means of a white LED. The eye tracker speed is 25Hz, which is the maximum effective tracking speed to synchronise the SLO imaging system. In the first 10 seconds of the examination, the MAIA performs an initial fixation test in which a central point of reference of the average retinal position is calculated and labeled as PRL_initial (PRL*i*); subsequently the projection of the pre-selected stimuli grid map begins using the PRL*i* as the center of the stimuli grid array. On completion of the test, the center of the mass

(barycenter) of the total plotted CFP is calculated and nominated as PRL_final (PRL*f*) which is at the same time the reference point of fixation stability calculations, including the parameters used to classify fixation in stable, relatively unstable and unstable as described in previous chapters (Fujii et al., 2002).

The aim of this study was to improve understanding of the clinical validity of the MAIA PRLs. The location of both PRLs (initial and final), and their correlation with fixation stability and visual acuity in a group of patients with different pathologies affecting the central macula were investigated.

5.2 Methods

Participants

Sixty-five eyes from forty-one (41) patients attending the Retina and Low Vision Clinics of the Queens Medical Centre, Nottingham, were investigated in this study. They included 26 (40%) male and 39 (60%) female patients (mean age 70, SD = 13.82), with varying retinal pathologies affecting the central vision such as dry AMD, neovascular AMD (nvAMD), non-AMD choroidal neovascularization, anterior uveitis with macular oedema, chorioretinitis, diabetic macular oedema, and branch retinal vein occlusion (BRVO) with macular oedema. All the participants signed an informed consent form and were assessed for visual function with the MAIA microperimetry. The inclusion criteria for the study were: adults over 21 years old with macular diseases as

listed above, and with a visual acuity (VA) from 0.0 to 1.6 LogMAR. There was no limitation in terms of race, sex or ethnicity. Excluded from the study were subjects with moderate to severe cataract (over grade 2 LOCS) or with other causes of opaque optical media and patients who were uncooperative or unable to use the MAIA after instruction.

Equipment and Procedures

All patients underwent the MAIA microperimetry standard exam grid consisting of the projection of 37 Goldman III size stimuli points with a presentation time of 200 ms covering the central 10° degrees of vision. The test contains one central stimulus and three concentric rings with 12 evenly distributed stimuli separated at 1°, 3° and 5° from the centre. During test, patients are asked to gaze steadily at the centre of the 1° red circle fixation target, which is concentric to the instrument's optical path. The MAIA examination starts with 10 seconds of fixation test with no stimuli projection in order to define the initial PRL (PRL*i*). Once the PRL*i* is estimated, the projected stimuli grid map is automatically centred in such PRL*i* and the randomized stimuli projection starts. At the end of the examination, the final PRL (PRL*f*) is calculated as the barycenter of all the plotted fixation points (Figure 5.1). MP outcomes are presented as the LTS of individual stimulus, their average value, the CFP, the reference point of both PRLs (initial and final) and the estimates of fixation stability (stable, relatively-unstable, or unstable).

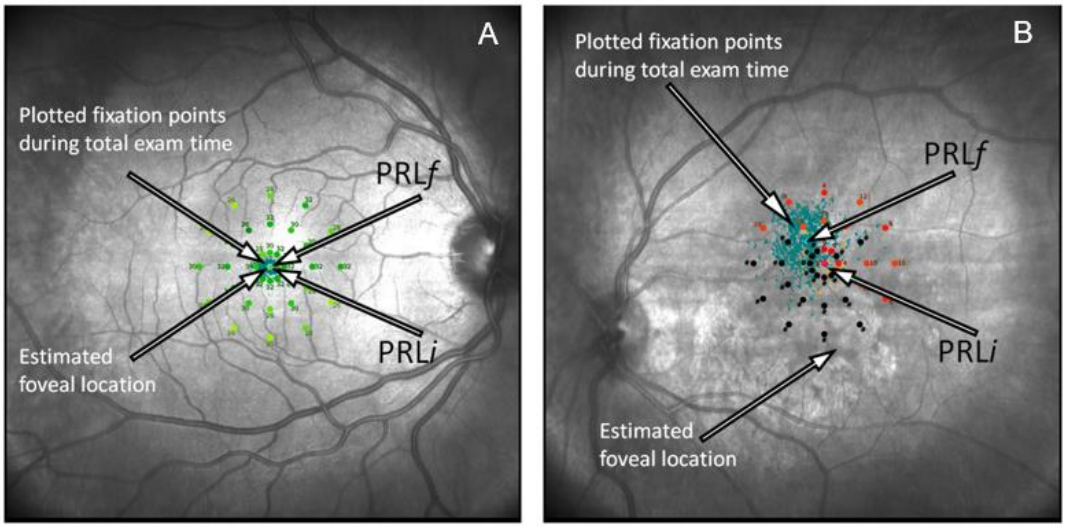

Figure 5.1 PRL initial and final

Microperimetry examination outcomes: A) Normal patient showing PRL_initial (PRLi) and PRL_final (PRLf) in the same anatomical position with a mean light threshold sensitivity (LTS) = 29.8 dB and stable fixation with fixation points in P1 and P2 more than 95%. B) Patient with GA and eccentric preferred retinal locus (PRL); PRLi and PRLf in different loci, mean LTS = 2.9 dB and unstable fixation with P1 = 23%, P2 = 70% of fixation points. In both cases, the microperimetry MAIAstd grid is centred on the PRLi.

Statistical Analysis

Patient characteristics are summarized with percentages, means, and standard deviations. Association of the FS and VA are evaluated using linear regression. All statistical analyses were performed using SPSS16 (IBM), and the significance level was set at 5% (2 sided).

5.3 Results

A total of 41 patients (n = 65 eyes) completed the microperimetry test within an average time of 6 minutes. PRL*i* and PRL*f* values were calculated by the MAIA

instrument after the first 10 seconds, and at the end of the test respectively. Forty-two eyes (65%) of the studied population had diagnosis of dry AMD or nvAMD; 75% of these subjects showed a different locus of PRL*i* and PRL*f*.

Forty-four eyes (68%) with mean age = 66yrs (SD = 14), different visual acuities (mean = 0.24, min = 0.0, max = 1.60 LogMAR) and different average LTS (mean = 22.5, min = 9.4, max = 29.9 dB), demonstrated almost identical locations for PRL*i* and PRL*f*, and were classified as having stable fixation (mean P1 = 95.1, P2 = 99.2) (Figure 5.2).

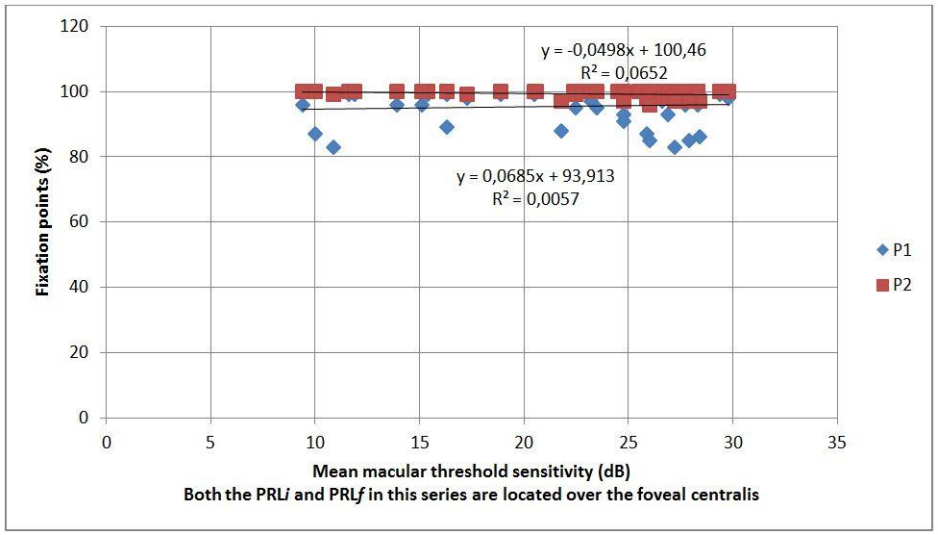

Figure 5.2 Fixation vs sensitivity

Eyes (44) with both PRL_initial (PRLi) and PRL_final (PRLf) located over the central macula demonstrated good fixation stability (P1 and P2 < 75%) despite the wide range of threshold sensitivity values (min 9.4 dB, max 29.8 dB).

Forty eyes (62%) were found to locate both PRLs in the same anatomical position corresponding to the fovea centralis (VA mean = 0.19 LogMAR, SD = 0.26, LTS mean = 23.46 dB, SD = 5.28) (Figure 5.3A). Another four eyes (6%) with stable fixation had central geographic atrophy (cGA) with both PRLs located in the same extrafoveal loci (VA mean = 0.79 LogMAR, SD = 0.55, LTS

mean = 13dB, SD = 3.36) (Figure. 5.3B). Two eyes with stable fixation, both with VA = 0.1 LogMAR (LTS 19.5 and 25.3 dB respectively), had the PRL*i* located over the foveal area, whilst the PRL*f* was in a different locus.

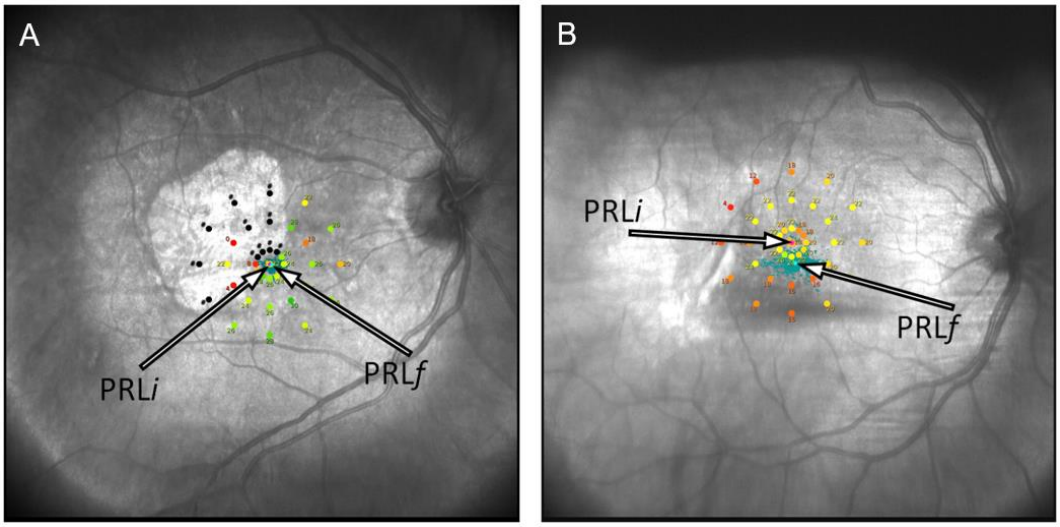

Figure 5.3 PRLi-PRLf near and far

Two patients classified as having stable fixation with different PRL characteristics. A) Patient with GA with both PRL_initial (PRLi) and PRL_final (PRLf) in the same eccentric (inferior) loci with very stable fixation (P1 = 99%, P2 = 100%), low mean light threshold sensitivity (LTS) and visual acuity (VA) due to the geographic atrophy, LTS = 15.4 dB, VA = 0.7 logMAR. B) Patient with nvAMD having PRLi located over the foveal area, whereas the PRLf is located inferiorly about 1° with P1 = 86%, P2 = 99%, LTS = 19.5 dB, and VA = 0.1 logMAR.

From the total studied population (n = 65), 20 (31%) eyes demonstrated different locations of PRL*i* and PRL*f* (Fig. 5.4), including 11 eyes (55%) with nvAMD, 4 (20%) with dry AMD, 3 (15%) with disciform degeneration (end stage nvAMD) and 2 (10%) with DME. The mean distance from PRL*i* to PRL*f* was 2.27° (SD = 1.43), with a mean VA = 0.75 LogMAR (min = 0.1, max = 1.9) and a mean LTS = 12.06 dB (SD = 6.46). Eighteen of these 20 eyes (90%) were classified as having unstable or relatively-unstable fixation and only 2 eyes (10%) had a

stable fixation, both with VA = 0.1 LogMAR (mean LTS 19.5 and 25.3 dB respectively) (Figure 5.4).

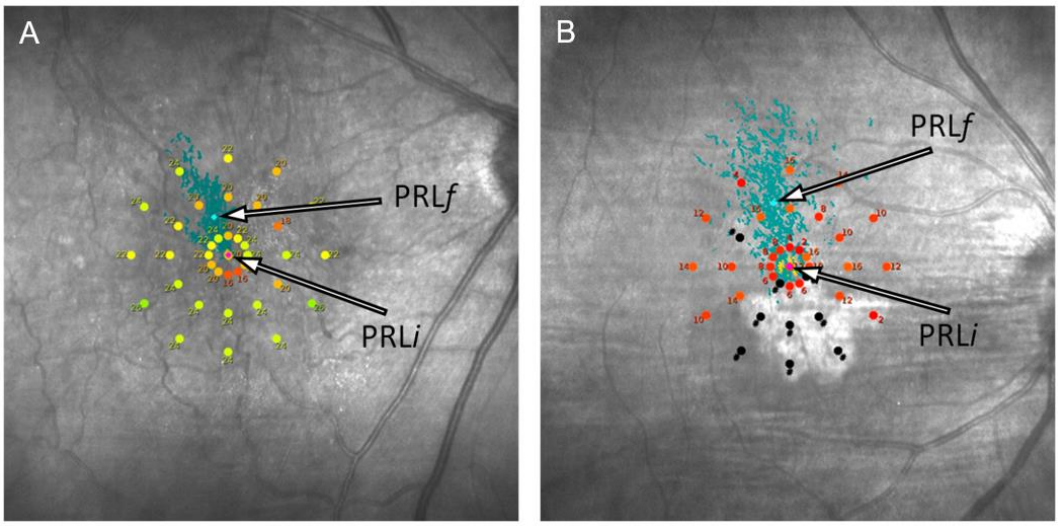

Figure 5.4 PRL eccentric

Patients with different location of PRL_initial (PRLi) and PRL_final (PRLf). A) Patient diagnosed with nvAMD and ERM with separation distance of 2° between the preferred retinal loci (PRLs), visual acuity (VA) = 0.3 logMAR, mean LTS = 21.9 dB, and a relatively unstable fixation (P1 = 40%, P2 = 84%). B) Patient diagnosed with dry cGA AMD; PRLi located adjacent to the geographic atrophy and PRLf separated 3° from PRLi, VA = 0.66 logMAR, mean LTS = 7.4 dB, and an unstable fixation (P1 = 22%, P2 = 51%).

Fourteen (21.5%) eyes of the studied population had cGA (mean VA = 0.89 LogMAR, SD = 0.36). All 14 (100%) presented the PRL*i* immediately adjacent to the dense scotoma. 10 (71%) of these cases showed different PRL*i* and PRL*f* loci (PRL*i* to PRL*f* distance min = 0.5°, max = 5°) with an unstable or relatively unstable fixation. The other 4 (29%) shared the same eccentric PRL*i* & PRL*f* loci with a stable fixation. 5 (36%) cases showed the PRL*f* in a more eccentric locus away from the cGA limit (Fig. 5.4B). Those patients with cGA, demonstrated a visual acuity reduction as the distance between the PRL*i* from the fovea centralis increases.

Six cases with non-central GA (4 of whom had been diagnosed with nVAMD secondary to angioid streaks), demonstrated a stable fixation (P1 > 86%, P2 > 96%) with both PRLs over the foveal centralis.

Patients classified as having an unstable and relatively-unstable fixation demonstrated that as the distance among PRL*i* and PRL*f* increases, VA (Figure 5.5A), LTS (Fig. 5.5B), and FS (Fig. 5.5C) tend to decrease. However, the mean LTS and VA may have a wide range of values when both PRLs are located in the same position showing a stable fixation.

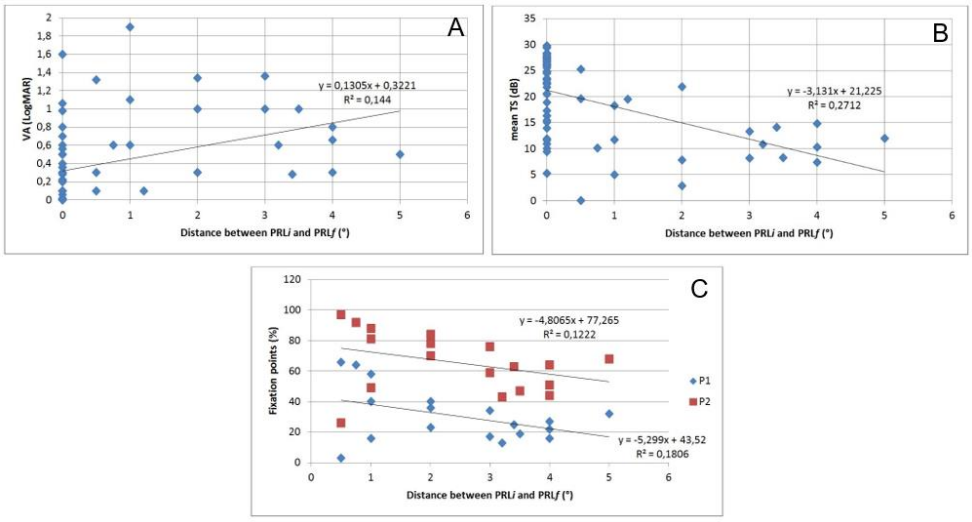

Figure 5.5 Distance PRLi & PRLf

Graphics show that when the distance between PRL_initial (PRLi) and PRL_final (PRLf) is minimal, visual acuity (A) and threshold sensitivity (B) may have a wide range of values, whereas they tend to decrease, as well as fixation stability (C), when the distance between both preferred retinal loci (PRLs) increases.

VA showed a tendency to decrease when the distance between the PRLi and the fovea centralis increases Figure 5.6A), as well as when FS (Fig. 5.6B) and LTS (Fig. 5.6C) decrease. Normally, the greater the distance between PRLi and PRLf, the lower the fixation stability. However, there may be cases where the fixation stability is low, even when the distance between both PRLs is not large, particularly in cases where the PRL is located away from the fovea centralis (Figure 5.7).

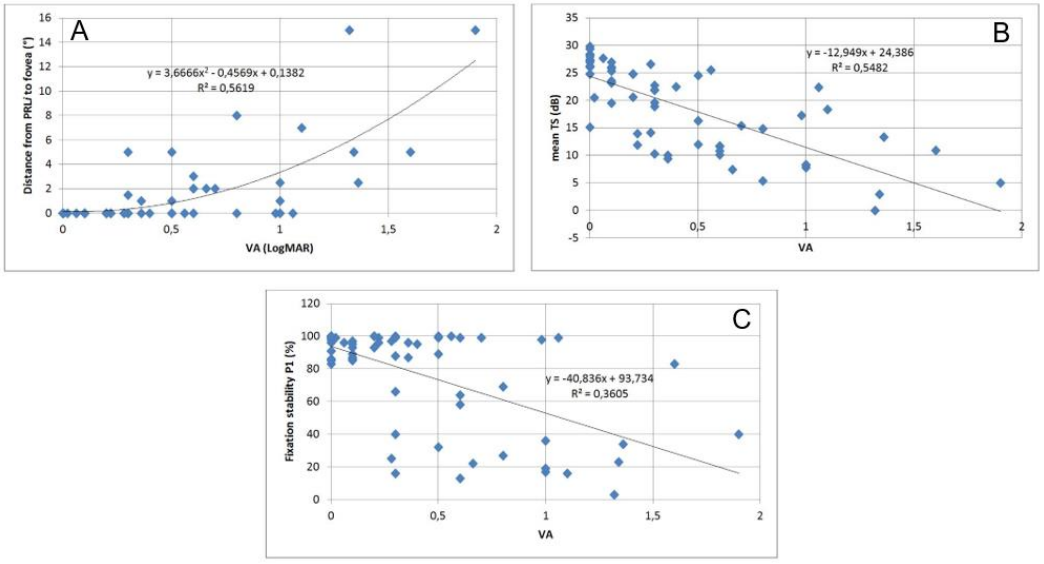

Figure 5.6 VA linear regresion

Visual acuity shows a tendency to decrease when the distance between the PRL_initial (PRLi) and the fovea centralis is increased (A), and also when fixation stability (B) and threshold sensitivity (LTS) (C) decrease.

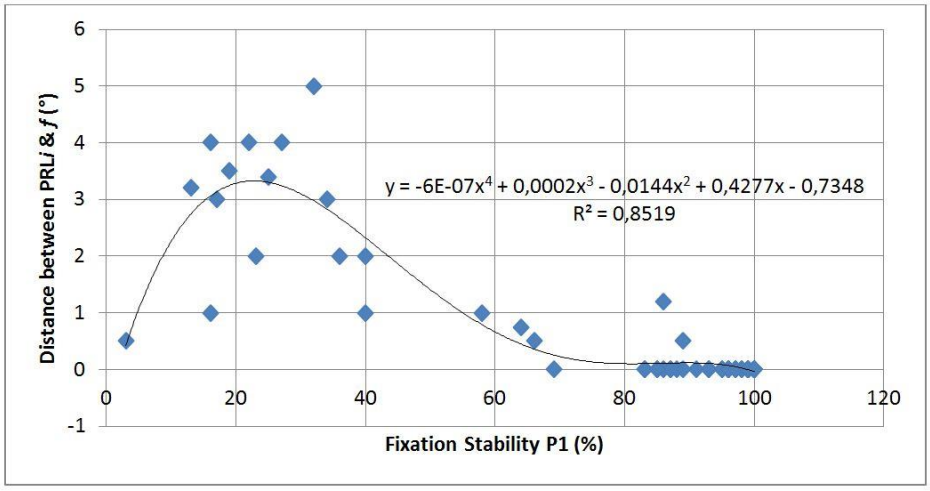

Figure 5.7 Fixation stability vs PRL distance

Graph shows that when there is no distance between the PRL_initial (PRLi) and PRL_final (PRLf), the fixation is stable (P1 = 75%), and normally, the greater the distance between PRLi and PRLf, the lower the fixation stability, although our study showed 1 case with small preferred retinal loci (PRLs) distance and unstable fixation (P1 = 10%).

5.4 Discussion

Previous studies have characterized PRLs in eyes with subnormal vision. Of special interest are the temporal characteristics of PRLs during same or consecutive fixation attempts. Different authors have stated the importance of the analysis of FS and fixation location in patients with eccentric PRL as essential predictors during the evaluation of the effects of treatment (Tarita-Nistor et al., 2009a, Greenstein et al., 2008). The analysis of PRL*i* and PRL*f* combines both FS and location, and may be useful information to complement prognosis data for visual outcomes in the treatment of pathologies affecting the central vision. In the assessment of retinal function, the anatomical location of PRL*i* and PRL*f* may be important because these fixation characteristics may

reflect the possible association with oculomotor functions that ultimately dictate the span and direction of the PRL and FS.

The MAIA instrument by design allows identification of locations for the PRL at different stages of fixation (PRL*i* = 10 seconds, PRL*f* > 5 minutes) under same testing circumstances. To the best of our knowledge, this is the first study to characterize changes in PRLs during the course of a prolonged fixation attempt using microperimetry.

Eyes with both PRL*i* and PRL*f* close to each other are noted to have stable fixation located mostly over the fovea centralis. However, patients with eccentric PRL may also have both the PRL*i* and PRL*f* in the same anatomical location showing eccentric but stable fixation. Eyes with PRL*i* and PRL*f* far from each other are noted to have, as a rule, unstable fixation mostly located on the peripheral retina. Interestingly, LTS may not be related to fixation characteristics particularly in cases of stable fixation. These characteristics may be important in eccentric fixation training.

The location of PRL being immediately adjacent to the geographic atrophy (GA) had been previously reported (Timberlake et al., 1987). This is supported by our finding that at least 1 of the PRLs in all cases of cGA (except 1) was located immediately adjacent to the GA. However, a large number (71%) of the eyes with cGA presented unstable or relatively unstable fixation with different PRL*i* and PRL*f* loci, although some (29%) shared the same eccentric PRL*i* and PRL*f* loci showing stable fixation.

Previously, PRLs had been reported as "multiple" because they changed characteristics according to the type of macular disease, levels of illumination, and activity in which the patient is engaged (Sunness et al., 1996, Lei and Schuchard, 1997, Safran et al., 1999, Schuchard et al., 1999, Deruaz et al., 2002, Crossland et al., 2011a, Markowitz et al., 2011). Lei and Schuchard found cases with 2 different PRLs depending on the brightness of objects used in visual tasks (Lei and Schuchard, 1997). Similarly, Safran et al., and Deruaz et al., found multiple PRLs during the reading process; in both studies, 2 well-defined PRLs were located in different retinal zones (Safran et al., 1999, Deruaz et al., 2002). Conversely, our study has demonstrated cases with 2 different loci of high density of fixation points, within the same PRL span, in 2 different times of the same examination.

Previous studies have demonstrated that VA can vary depending on the FS and location (Tarita-Nistor et al., 2009a). Our results show how VA tends to decrease as the distance between both PRL*i* and PRL*f* increase. However, further studies are needed to fully correlate the behaviour of both of the PRLs and VA during different stages of the pathology, treatment, and rehabilitation process.

It is important to note that the studies of the PRL during the fixation attempt with microperimetry have been monocular; thus, the fixation characteristics in stereoscopic conditions remain to be explored in the future. The PRL is the graphic representation of all the retinal area used during a fixation attempt by

patients with compromised central vision. Characteristics of fixation, stability, and location appear to be dependent on the pathology and affect visual acuity.

The importance of defining more than 1 reference point within the whole PRL area (PRL*i* and PRL*f*) is to give eye care practitioners a better understanding of the dynamic of the visual function. The representative points, PRL*i* and PRL*f*, may vary at different stages of fixation during prolonged fixation attempts. The PRLs are important and need to be considered during retinal functional assessment.

This study suggests that when both PRL*i* and PRL*f* are located in the same position, fixation is stable. Conversely, a greater distance between the PRL*i* and PRL*f* may be a defining parameter of unstable fixation. The superimposition of clinical features and microperimetric characteristics such as LTS, FS, and fixation location may facilitate the determination of the differential impact of macular disorders and their corresponding VA. This could be relevant as prognostic factors for treatment and rehabilitation purposes.

6. Role of microperimetry in monitoring functional changes in eyes receiving intravitreal therapies.

6.1 Introduction

Age-related macular degeneration has, for many years, been the leading cause of blindness and visual disability in the western world (Bourne et al., 2014, Klein et al., 1999). Late AMD may be exudative, or neovascular form (nvAMD), which is typified by development of choroidal neo-vascularization (CNV), or geographic atrophy (GA), the advanced stage of dry AMD. The number of people with late AMD in Europe will increase from 2.7 million in 2013 to 3.9 million by 2040 (Colijn et al., 2017), whilst patients with sight loss secondary to nvAMD are expected to increase from 145 697 to 189 890 by the end of the decade in the UK (Minassian et al., 2011). Similarly, diabetic retinopathy (DR) is the commonest cause of visual impairment in persons below the age of 65 years; retinal vein occlusions (RVO) comes second. Treatment outcomes have improved considerably with the introduction of intravitreal therapies that inhibit vascular endothelial growth factor (VEGF) [thought to be the central mediator of vascular permeability and neovascularisation in these retinal vascular diseases], and inhibitors of inflammation e.g. intravitreal dexamethasone

implant (Ozurdex, Allergan). Other macular pathologies, including choroidal neovascularization secondary to pathologic myopia and other CNV with pathophysiology similar to nvAMD receive anti-VEGF therapy similar that in nvAMD. Epiretinal membranes are treated with intravitreal injections (IVT) of Ocriplasmin, whilst diabetic macular oedema (DMO) and macular oedema (MO) secondary to RVO may be treated with either IVT anti-VEGF or intravitreal dexamethasone implant. Non-infectious intraocular inflammation (uveitis) with macular oedema or vitritis is treated with dexamethasone implant.

In contemporary clinical practice, assessment of macular pathologies typically includes visual acuity (VA), fundal examination (biomicroscopy), and optical coherence tomography (OCT) at monthly follow up intervals (The Royal College of Ophthalmologists, 2009), following confirmation of diagnosis and initial treatment. It is generally accepted, however, that changes in either VA or OCT alone may not be the optimum parameter(s) for measuring visual function in AMD and other macular diseases as these parameters (VA and OCT measurements including central retinal thickness [CRT]) are dependent on function in limited parts of the macula.

As described in earlier chapters MP assesses the sensitivity of the central retina and allows precise topographic correlations of macular anatomy and light sensitivity. In addition, retinal sensitivity, as measured by MP, may be more sensitive to changes in macular function than the conventional distance visual acuity because it assesses function in a larger retinal area. As such, retinal sensitivity could be a better indicator of practical visual capabilities for patients

with macular dysfunction as in AMD, retinal vein occlusions, diabetic maculopathy etc. Retinal images in MP are correlated with retinal functional characteristics such as retinal sensitivity, PRL and fixation stability (FS), even in cases when central vision (VA) is reduced as a consequence of macular pathologies (Schneider et al., 1996, Midena et al., 2004).

A few investigators have reported the usefulness of MP in the management and follow-up of pathologies affecting the macula, including complementary changes in retinal sensitivity during intravitreal therapies (Winterhalter et al., 2012, Karacorlu et al., 2010, Hatef et al., 2011, Landa et al., 2011, Ozdemir et al., 2012). However, until now, retinal morphology assessment with OCT is used as the main criterion, in addition to VA, in determining treatment outcomes by ophthalmologist during the initiation and continuation of therapy, or temporary cessation, and treatment termination. This practice has continued in spite of a pilot study reported by Alexander et al., who reported a relationship between macular sensitivity measured with the MAIA MP, central retinal thickness and VA in the maintenance phase of ranibizumab therapy, and hypothesized that deteriorating retinal sensitivity in patients with stable VA and central retina thickness, may be an indicator of subclinical disease activity (Alexander et al., 2012). The MAIA identifies two PRLs (initial and final) in two different examination times. Our team has reported that eyes with both PRLi and PRLf close to each other are noted to have stable fixation located over the fovea centralis, whilst eyes with PRLi and PRLf far from each other (higher than 0.8°) are noted to have unstable fixation located eccentrically from the fovea (Morales et al., 2013).

6.2 Methods

This was an observational, cross-sectional, descriptive, study of retinal function changes monitored with an MP system in a group of patients from the Macular Clinic of the Queen's Medical Centre, Nottingham (U.K.), with treatable macular diseases, undergoing IVT for different retinal pathologies affecting the central vision. Local ethical board approval and written informed consent was obtained, and the study met the tenets of the Declaration of Helsinki.

Participants

One hundred and three patients, mean age 70.7 years (min = 37, max = 97) attending the Macular Clinic for intravitreal therapy of one or both eyes for specific pathologies (including nAMD, DMO, MO secondary to RVO, and VMT) were recruited. Inclusion criteria were as follows: no limitation to race, sex or ethnicity, adult age above 21 and below 99 years, OCT presence of macular oedema, patients willing to attend for follow-up MP examinations, patients undergoing treatment with IVT of anti-VEGF therapies, dexamethasone implants, and ocriplasmin.

The exclusion criteria were: moderate to severe cataract (over grade 2) or other cause of opaque optical media, previous treatments in the particular eye with IVT of anti-VEGFs or steroids within the previous 6 months, or macular laser photocoagulation, and uncooperative patient.

Equipment and Procedures

Before commencing the IVT injection therapy, all patients had a routine clinical evaluation including best corrected visual acuity (VA) (LogMAR at 4 metres), slit lamp biomicroscopy of the anterior and posterior segments, MAIA microperimetry and OCT examinations. Only one eye for each patient was evaluated. If both eyes were treated, only the right eye was selected for inclusion. Appropriate IVT was administered as clinically necessary, as recommended by the Royal College of Ophthalmologists guidelines.

The selected MP test was the standard MAIA grid, which covers a diameter of 10° on the retina, with 37 stimuli points distributed in 3 concentric rings of 12 stimuli located at 1°, 3° and 5° from the centre (equivalent to 2°, 6° and 10° in diameter respectively) plus a central stimulus. The grid was automatically centred by the MAIA on the patient's PRL_initial. (Morales et al., 2013). Distance between PRL_initial and PRL_final (PRL*i-f*) on each MP test was annotated and used to determine if patents had foveal PRL (PRL*i-f* $\leq$ 0.8°), or eccentric PRL (PRL*i-f* > 0.8°) (Morales et al., 2013).

MP assessments consist in asking the patient to look steady at a fixation target inside of the instrument, while white stimuli of Goldmann III size (0.46°) are projected at different light intensities, from 4asb to 1000asb, over the selected grid pattern. Patients are asked to press a response button whenever they perceive light stimuli. The result of the minimum perceived light stimuli in every point is defined as the retinal threshold sensitivity map measured in a decibel (dB) scale

with a 32dB dynamic range. If no light is perceived, the retinal sensitivity in that point is defined as scotoma (<0 dB). Threshold of normal vision is known to be 29.78 ± 1.71dB when assessed with the MAIA (Smolek et al., 2010). Examinations were performed with the full threshold 4-2 projection strategy. During the whole process the eye movement is registered by the MAIA eye tracker defining FS and the PRL area. Two of the MAIA FS indices were annotated, the P1 and the BCEA@63% (Morales et al., 2016). Classification of FS is defined in the MAIA as stable, relatively stable, or unstable ((Fujii et al., 2002). The follow-up test is an automatic reproduction of the stimuli grid over the same anatomical points projected during base line examinations.

Assessments were schedule on base-line, and repeated as follow-up after 3 months, 6 months, and 12 months. The studied variables were, MP light threshold sensitivity (LTS), FS indices of P1 and BCEA@63%, FS classification, distance between PRL initial and PRL final to evaluate foveal or eccentric fixation, central retinal thickness (CRT) on OCT (the average retinal thickness in the central 1mm of the ETDRS circle), and VA.

Primary outcomes were variable comparisons between MP-LTS, CRT and VA from base line and month 3 after therapy. Secondary outcomes were comparisons of the same variables between months 3, 6 and 12 after therapy. Final outcomes were correlations between base line values of FS, CRT, with VA in months 3 and 12. Foveal or eccentric PRL and fixation stability was analysed.

Statistical Analysis

Statistical analysis (GraphPadPrism_8.20) included standard errors, 95% confidence level of intervals with significance differences set as $P < 0.05$. Ordinary one-way ANOVA test was applied to evaluate significant difference among means of each evaluated variable. Paired, parametric, one-tailed t-test was performed to compare the means. Finally, correlations were analysed through Spearman's rank-order coefficient (r_s). The correlation strength was described for the absolute value of *r* as, very weak (0.00 to 0.19), weak (0.20 to 0.39), moderate (0.40 to 0.59), strong (0.60 to 0.79), and very strong (0.8 to 1.0) (Evans, 1996).

6.3 Results

Patients with poor MP reliability test, or who missed one or more study visits were excluded from the analysis. A total of 74 patients were analysed and divided in 4 groups according to the intravitreal therapy:

Group 1 - IVT anti-VEGF drugs for nAMD.

Group 2 - IVT dexamethasone implant for the treatment of DMO.

Group 3 - IVT dexamethasone implant for the treatment of MO secondary to RVO.

Group 4 - IVT ocriplasmin for the treatment of VMT and macular hole (MH).

Group 1 (Anti-VEGF for nAMD)

Forty patients, mean age 72.8 ± 11.2 years, were included. The measured variables at baseline (BL) and 3 months (3M) after therapy were as follows: LTS changed from 16.83 ± 5.96 to 16.82 ± 6.30 dB, CRT from 235 ± 70.3 to 237 ± 91.5 μm, and VA from 0.36 ± 0.34 to 0.34 ± 0.32 LogMAR. The t-test showed no significant difference for any of the variables at 3M compared to BL, and include for LTS, $t(39) = 0.01$ $p = 0.493$; for CRT $t(39) = 0.260$ $p = 0.398$ and for VA $t(39) = 0.84$ $p = 0.202$ (Table 6.1).

The one-way ANOVA test conducted to compare the effect of the studied variables at BL, 3, 6 and 12 months after treatment showed no significant effects between the same variable at different time points but with a significant interaction (correlation) within groups. For LTS, $F(2.79, 109) = 0.124$, $p = 0.936$, and $F(39, 117) = 17.70$, $p < 0.0001$. For CRT, $F(2.06, 80.4) = 1.91$, $p = 0.153$ and $F(39, 117) = 17.10$, $p < 0.0001$. For VA, $F(1.60, 62.5) = 2.27$, $p = 0.122$ and $F(39, 117) = 14.12$, $p < 0.0001$).(Figure 6.1).

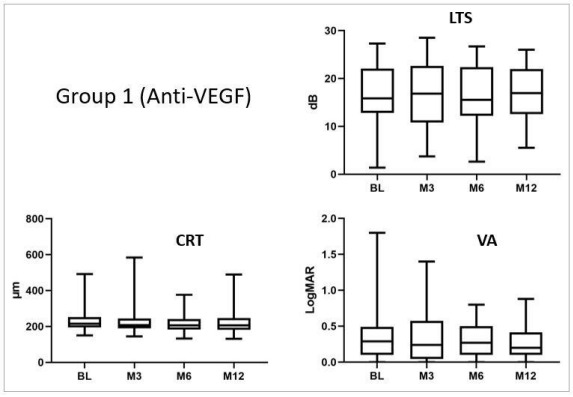

Figure 6.1 ANOVA results group 1

Fig 6.1 *Box & whisker ANOVA outputs from Group 1, where significance between groups is: no significant for LTS (p = 0.936), no significant for CRT (p = 0.153) and no significant for VA (p = 0.122).*

A Pearson's product-moment correlation coefficient was computed to assess the relationship between the two fixation stability indices (P1 and BCEA), LTS, CRT and VA in base line (BL), with VA at 3 months (VA-3M), and 12 months (VA-12M) after initial treatment. P1-BL showed a strong correlation (r = -0.65) with VA-3M, and moderate correlation (r = -0.56) with VA-12M. Similarly, BCEA-BL showed moderate correlations with VA-3M (r = 0.50) and VA-12M (r = 0.53). LTS-BL showed moderate correlation with VA-BL (r = -0.42). Whilst CRT-BL showed very weak (r = -0.10) and weak (r = -0.20) negative correlations with VA-3M and VA-12M respectively. Overall, VA-BL had the strongest correlation with VA after treatment (r = 0.90). A heatmap of the correlation matrix summarizes the results (Figure. 6.2).

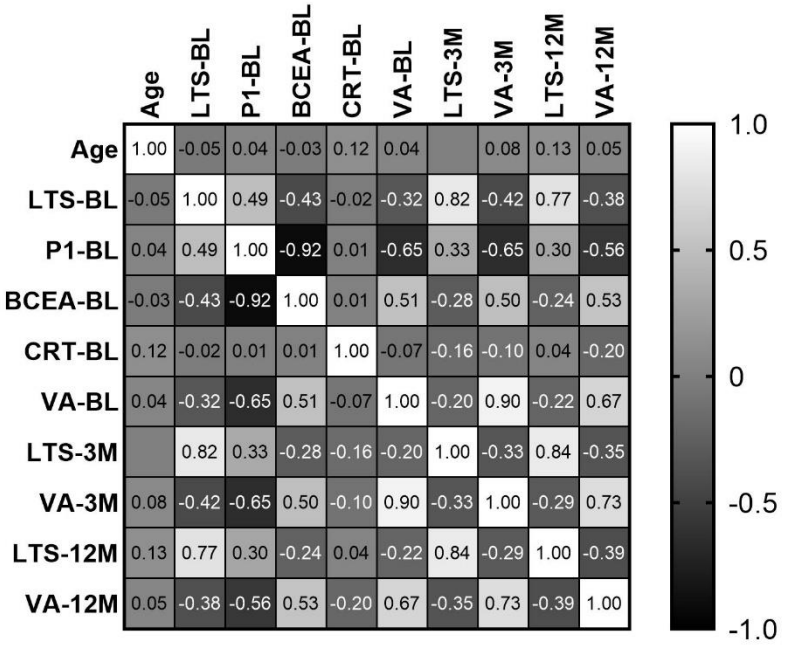

Figure 6.2 Correlations Group 1

Correlation heatmap graphic in Group 1 showing Pearson's coefficient between age, base line fixation indices (P1-BL and BCEA-BL), base line visual acuity (VA-BL), and visual acuity after 3 (VA-3M) and 12 months (BL-12M).

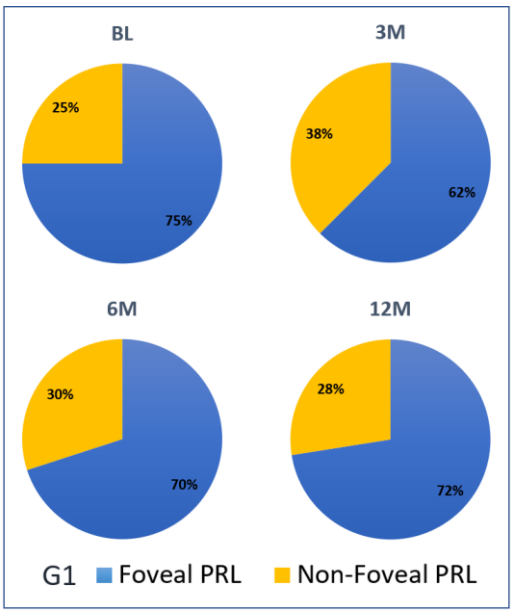

Figure 6.3 Patients with foveal PRL in Group1

Number of patients (%) in Group 1 with foveal PRL (blue) and non-foveal PRL (yellow) as demonstrated with microperimetry examinations during the studied time.

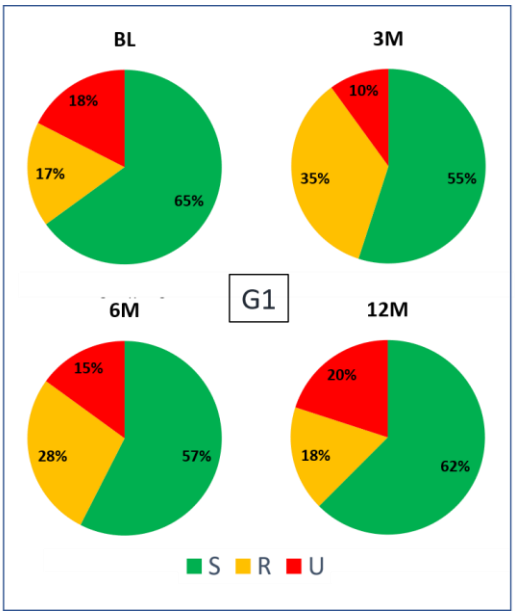

Figure 6.4 Fixation classification in Group 1

Number of patients (%) in Group 1 with fixation stability classified as stable (green), relatively stable (yellow) and unstable (red) as demonstrated with microperimetry examinations during the studied time.

Group 2 (Dexamethasone for DMO)

Fifteen patients, mean age 72.0 ± 9.6 years, showed a difference in studied variables between BL and 3M after therapy as follows: LTS changed from 18.75 ± 3.84 to 19.56 ± 2.97 dB, CRT from 490 ± 188 to 327 ± 119 μm, and VA from 0.54 ± 0.23 to 0.48 ± 0.25 LogMAR. The t-test showed, no significant difference for LTS, $t(14) = 1.25$ $p = 0.116$, but was significant for CRT with the effect $t(14) = 3.72$ $p = 0.001$, and not significant for VA $t(14) = 1.24$ $p = 0.117$ (Table 6.1).

The one-way ANOVA test conducted to compare the effect of the studied variables on base line, 3, 6 and 12 months after treatment showed no significant effects between the same variable at different time points but with a significant interaction (correlation) within groups for LTS, $F(2.10, 29.3) = 2.31$, $p = 0.115$, and $F(14, 42) = 13.40$, $p < 0.0001$. However, it was significant for CRT and VA in both, between and within groups, with the effects $F(1.64, 23.0) = 13.2$, $p = 0.0003$ and $F(14, 42) = 8.22$, $p < 0.0001$ for CRT, and for VA, $F(1.59, 22.2) = 4.16$, $p = 0.037$ and $F(14, 42) = 15.8$, $p < 0.0001$ (Figure. 6.5).

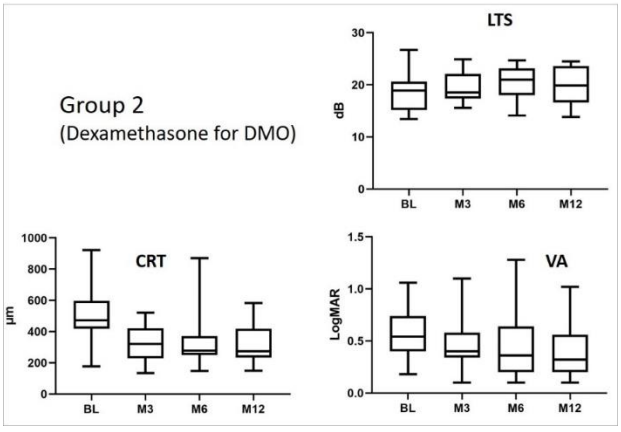

Figure 6.5 ANOVA results group 2

Box & whisker ANOVA outputs from Group 2, where significance between groups is: no significant for LTS (p = 0.115), significant for CRT (p = 0.0003) and significant for VA (p = 0.037).

A Pearson product-moment correlation coefficient was computed to assess the relationship between the two fixation stability indices (P1 and BCEA), CRT and VA in base line (BL), with visual acuity three months (VA-3M), and 12 months (VA-12M) after treatment. P1-BL showed a strong correlation (r = -0.61) with VA-3M, and moderate correlation (r = -0.53) with VA-12M. Similarly, BCEA-BL showed moderate correlations with VA-3M (r = 0.50) and VA-12M (r = 0.45). CRT-BL instead showed moderate (r = 0.58) and strong (r = 0.70) correlations with VA-3M and VA-12M respectively. A heatmap of the correlation matrix summarizes the results (Figure. 6.6).

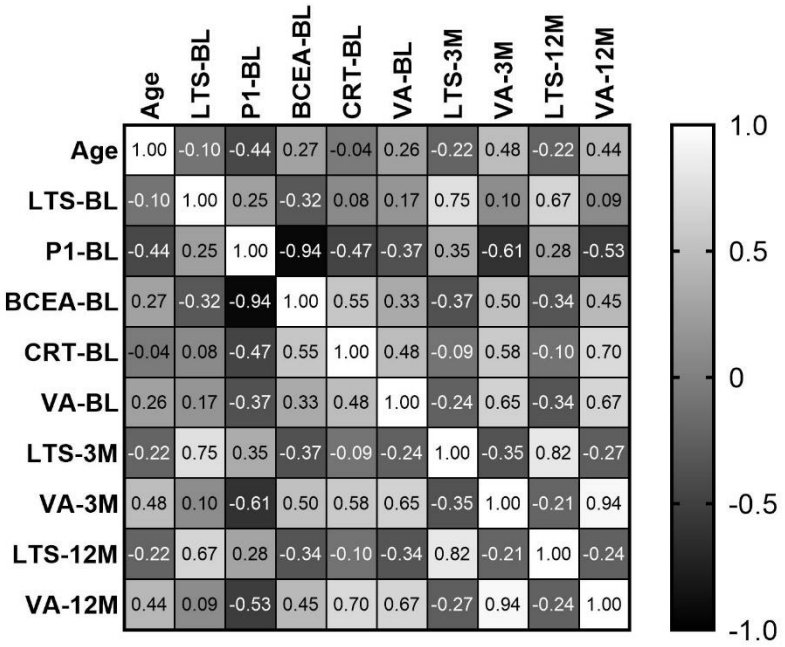

Figure 6.6 Correlations Group 2

Correlation heatmap graphic in the Group 2 showing Pearson's coefficient between age, base line fixation indices (P1-BL and BCEA-BL), base line visual acuity (VA-BL), and visual acuity after 3 (VA-3M) and 12 months (BL-12M).

From the studied population (n = 15), microperimetry outcomes showed, 11 (73%) patients with foveal PRL at BL examination, 9 (60%) at 3M after therapy, 8 (53%) at 6M, and again 8 (53%) at 12M (Fig. 6.7). Fixation stability (FS) was classified as stable in 9 (60%) patients at BL, 9 (60%) at 3M, 6 (40%) at 6M, and 5 (33%)12M (Figure. 6.8).

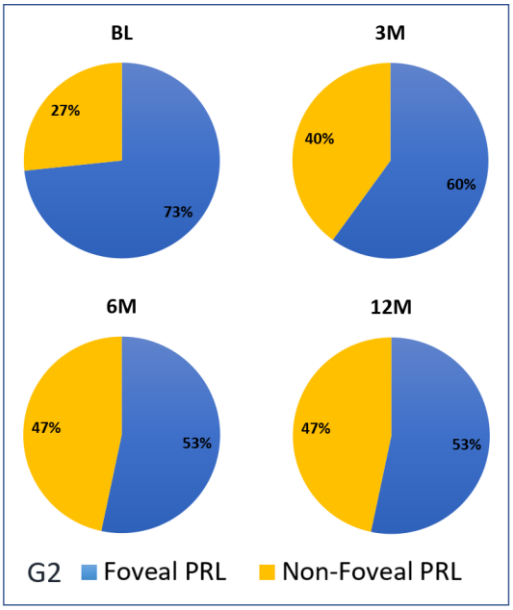

Figure 6.7 Patients with foveal PRL in Group 2

Number of patients (%) in Group 2 with foveal PRL (blue) and non-foveal PRL (yellow) as demonstrated with microperimetry examinations during the studied time.

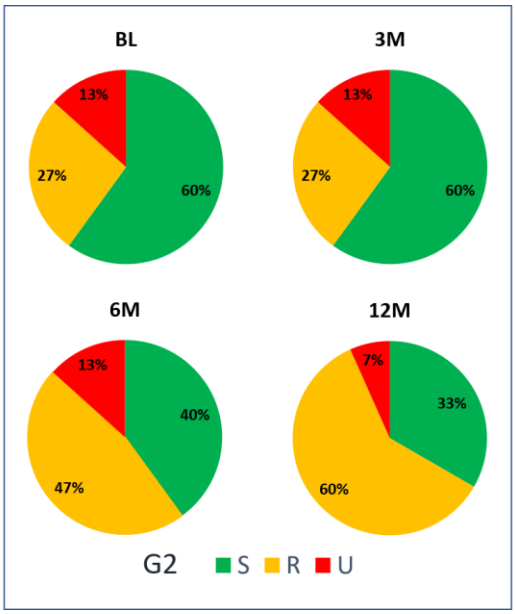

Figure 6.8 Fixation classification in Group 2

Number of patients (%) in Group 2 with fixation stability classified as stable (green), relatively stable (yellow) and unstable (red) as demonstrated with microperimetry examinations during the studied time.

Group 3 (Dexamethasone for MO related to RVO)

Thirteen patients, mean age 58.8 ± 14.3 years, showed a difference in studied variables between BL and 3M after therapy as follows: LTS changed from 21.35 ± 4.88 to 23.05 ± 4.11 dB, CRT from 407 ± 147 to 317 ± 116 µm, and VA from 0.62 ± 0.40 to 0.48 ± 0.36 LogMAR. The t-test showed, no significant difference for LTS, $t(12) = 1.66$ $p = 0.061$, but significant differences for both CRT and VA, $t(12) = 2.00$ $p = 0.034$, and $t(12) = 1.88$ $p = 0.042$. (Table 6.1).

The one-way ANOVA test conducted to compare the effect of the studied variables at BL, 3M, 6M and 12M after treatment showed no significant effects

between groups (therapy time) with a significant interaction within groups for LTS, $F(2.21, 26.5) = 2.38$, $p = 0.107$, and $F(12, 36) = 8.71$, $p < 0.0001$. CRT instead showed significance in both effects, $F(2.11, 25.4) = 4.50$, $p = 0.019$ and $F(12, 36) = 2.84$, $p = 0.007$. VA showed no significance between groups, however it was significant within groups, $F(1.65, 19.9) = 3.55$, $p = 0.055$ and $F(12, 36) = 7.79$, $p < 0.0001$ (Figure. 6.9).

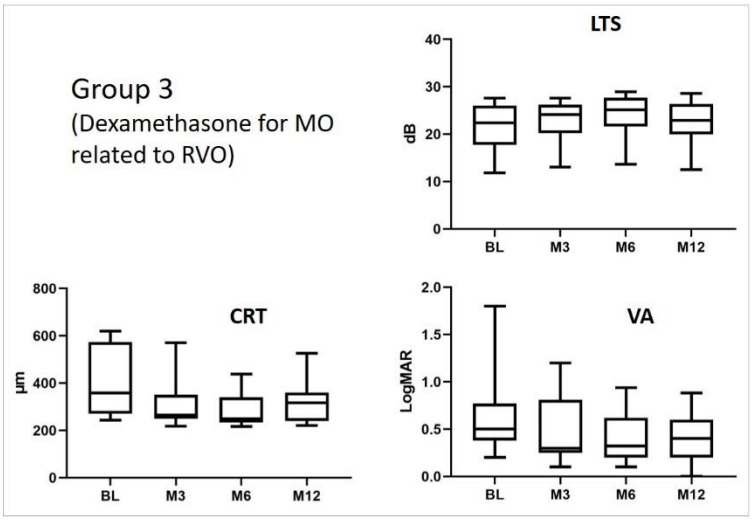

Figure 6.9 ANOVA results Group 3

Box & whisker ANOVA outputs from Group 3, where significance between groups is: no significant for LTS (p = 0.107), significant for CRT (p = 0.019) and no significant for VA (p = 0.055).

A Pearson product-moment correlation coefficient was computed to assess the relationship between the two fixation stability indices (P1 and BCEA), CRT and VA in BL, with VA-3M, and VA-12M after treatment. P1-BL showed very weak correlation ($r = -0.19$) with VA-3M, and null correlation ($r = 0.00$) with VA-12M. Similarly, BCEA-BL showed weak correlations with VA-3M ($r = 0.27$) and VA-12M ($r = 0.04$). Likewise, CRT-BL showed very weak ($r = 0.02$) and

null correlation (r = 0.00) correlations with VA-3M and VA-12M respectively. A heatmap of the correlation matrix summarizes the results (Figure. 6.10).

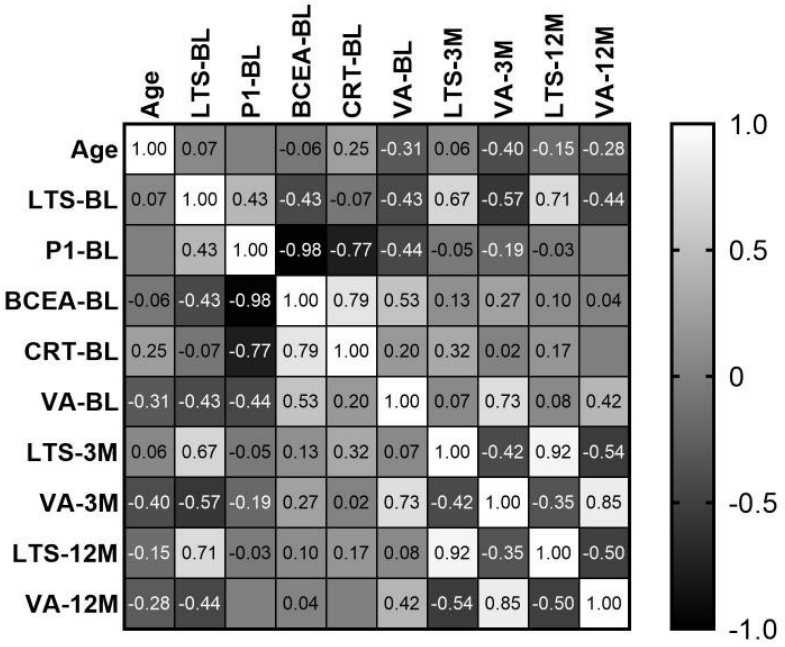

Figure 6.10 Correlations Group 10

Correlation heatmap graphic in the Group 3 showing Pearson's coefficient between age, base line fixation indices (P1-BL and BCEA-BL), base line visual acuity (VA-BL), and visual acuity after 3 (VA-3M) and 12 months (BL-12M).

From the studied population (n = 13), MP outcomes showed, 8 (62%) patients with foveal PRL during BL examination, 11 (85%) 3M after therapy, 10 (77%) at 6M, and 10 (77 %) at 12M (Fig. 6.11). Fixation stability (FS) was classified as stable in 7 (54%) patients at BL, 10 (77%) at 3M, 10 (77%) at 6M, and 8 (61%) at 12M (Figure. 6.12).

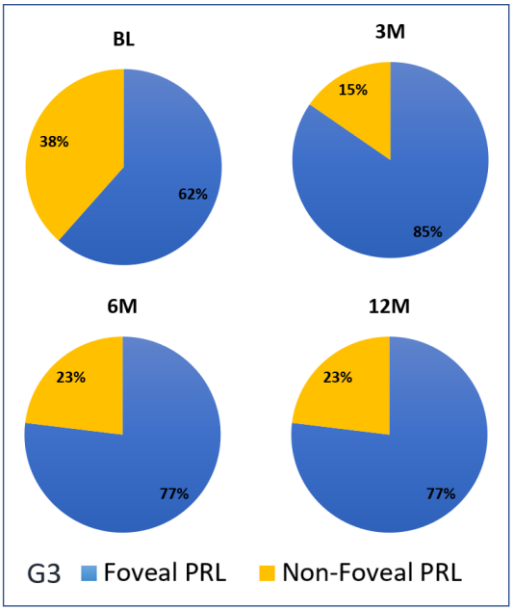

Figure 6.11 Patients with foveal PRL in Group 3

Number of patients (%) in Group 3 with foveal PRL (blue) and non-foveal PRL (yellow) as demonstrated with microperimetry examinations during the studied time.

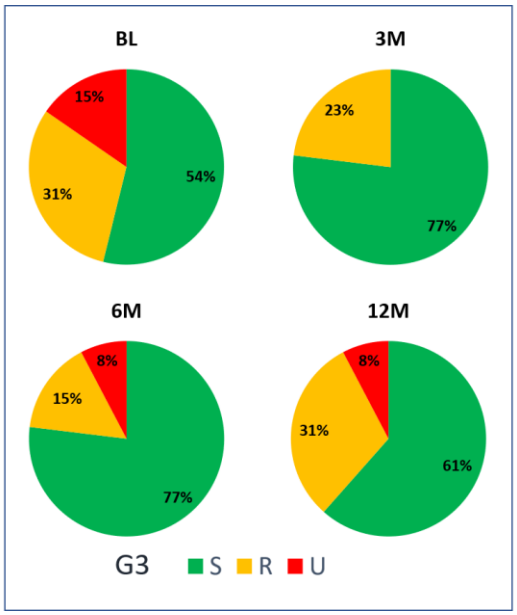

Figure 6.12 Fixation classification in Group 3

Number of patients (%) in Group 3 with fixation stability classified as stable (green), relatively stable (yellow) and unstable (red) as demonstrated with microperimetry examinations during the studied time.

Group 4 (Ocriplasmin for VMT and MH)

Six patients, mean age 79.8 ± 10.8 years, showed a difference in studied variables between BL and 3M after therapy as follows: LTS changed from 17.77 ± 3.83 to 21.45 ± 2.26 dB, CRT from 295 ± 50.7 to 217 ± 27.9 μm, and VA from 0.54 ± 0.14 to 0.32 ± 0.15 LogMAR. The t-test showed no significant difference with the following effects for LTS, $t(5) = 1.62$ $p = 0.083$; significant differences were observed at 3M for CRT $t(5) = 3.23$ $p = 0.011$ as well as for VA $t(5) = 5.34$ $p = 0.001$ (Table 6.1).

The one-way ANOVA test conducted to compare the effect of the studied variables at BL, 3M, 6M and 12M after treatment showed for LTS no significant effects between the same variable at different time points but a significant interaction within groups (correlation) as follows, $F(1.20, 6.0) = 2.63$, $p = 0.155$, and $F(5, 15) = 11.90$, $p < 0.0001$. The opposite way was for CRT with a significant effect between the same variable at different time points groups but no effect within groups, $F(1.37, 6.87) = 7.62$, $p = 0.023$ and $F(5, 15) = 2.47$, $p = 0.079$. Whereas VA showed significant difference in both effects, $F(2.43, 12.2) = 19.6$, $p < 0.0001$ and $F(5, 15) = 8.93$, $p = 0.0004$ (Figure. 6.13).

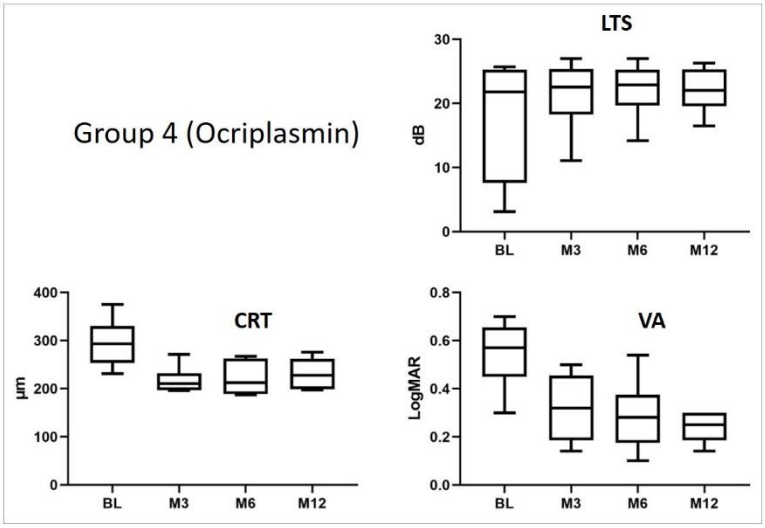

Figure 6.13 ANOVA results in Group 4

Box & whisker ANOVA outputs from Group 4, where significance between groups is: no significant for LTS ($p = 0.155$), significant for CRT ($p = 0.023$) and significant for VA ($p < 0.0001$).

A Pearson product-moment correlation coefficient was computed to assess the relationship between the two fixation stability indices (P1 and BCEA), CRT and VA at BL, with VA-3M, and VA-12M after treatment. P1-BL showed very weak correlation (r = -0.05) with VA-3M and VA-12M (r = 0.10). Similarly, there were no significant correlations for BCEA-BL with VA-3M (r = 0.09) and VA-12M (r = 0.08), as well as the case for CRT-BL (r = -0.09) and (r = 0.08) with VA-3M and VA-12M respectively. Overall, VA outcomes did not show any correlation with any other measured variable, with the exception of base line VA. A heatmap of the correlation matrix summarizes the results (Figure. 6.14).

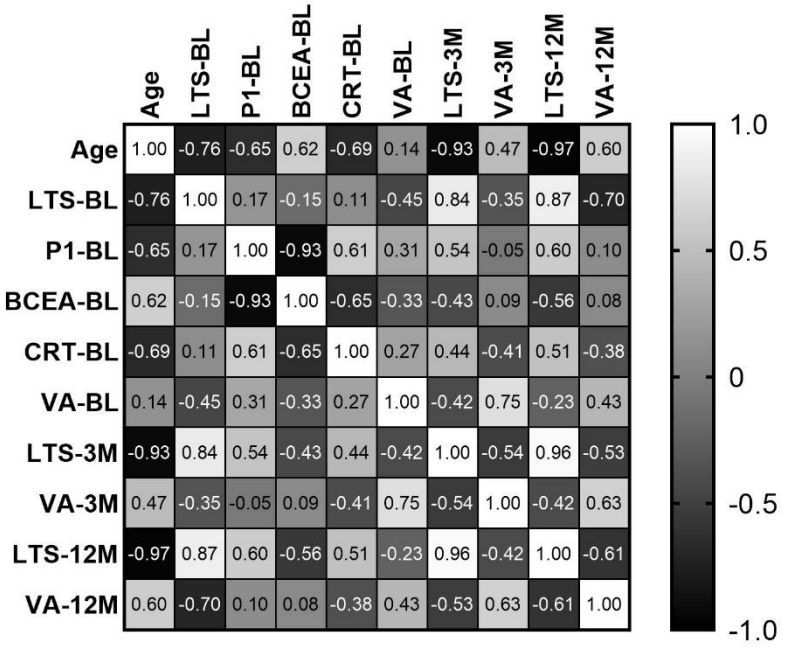

Figure 6.14 Correlations in Group 4

Correlation heatmap graphic in the Ocriplasmin group showing Pearson's coefficient between age, base line fixation indices (P1-BL and BCEA-BL), base line visual acuity (VA-BL), and visual acuity after 3 (VA-3M) and 12 months (BL-12M).

From the studied population (n = 6), MP outcomes showed, 2 (33%) patients with foveal PRL at BL examination, 15 (83%) at3M after therapy, 3 (50%) at 6M, and 2 (33 %) at 12M (Fig. 6.15). Fixation stability (FS) was classified as stable in 1 (16%) patients at BL, 2 (33%) at 3M, 1 (16%) at 6M, and 1 (16%) at 12M (Figure. 6.16).

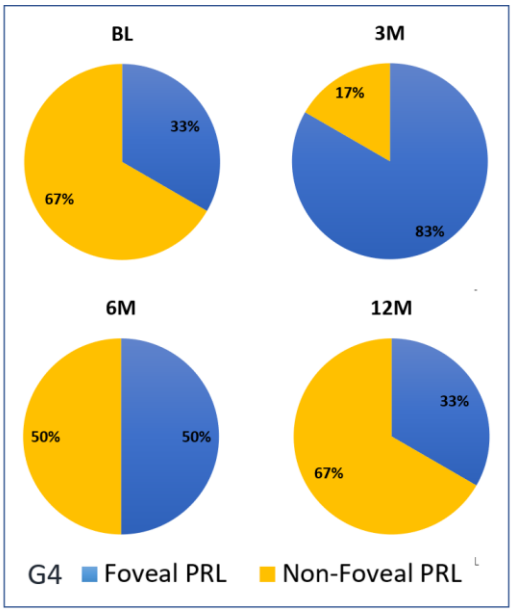

Figure 6.15 Patients with foveal PRL in Group 4

Number of patients (%) in Group 4 with foveal PRL (blue) and non-foveal PRL (yellow) as demonstrated with microperimetry examinations during the studied time.

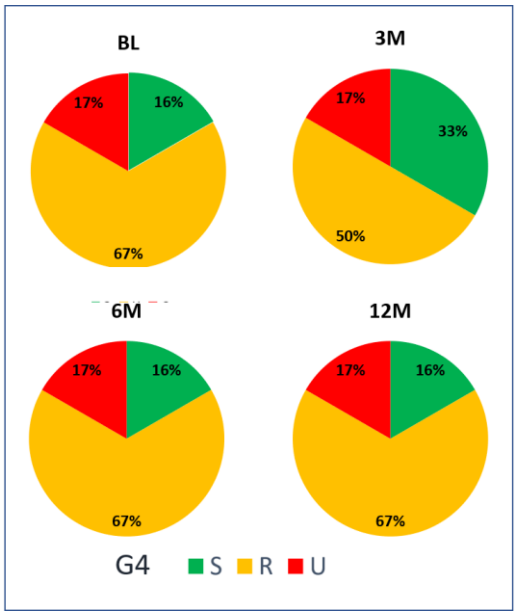

Figure 6.16 Fixation classification in Group 4

Number of patients (%) in Group 4 with fixation stability classified as stable (green), relatively stable (yellow) and unstable (red) as demonstrated with microperimetry examinations during the studied time.

Table 6.1 Descriptive statistics

Anti-VGEF	Base line		3 months		One tailed *t*-test		One-way ANOVA	
n = 40	Mean	SD	Mean	SD	P value	Significance	P value	Significance
LTS (dB)	16.83	5.96	16.82	6.3	0.493	No	0.936	No
CRT (μm)	235	70.3	237	91.5	0.398	No	0.153	No
VA (LogMAR)	0.36	0.34	0.34	0.32	0.202	No	0.122	No

Triamcinolone	Base line		3 months		One tailed *t*-test		One-way ANOVA	
n = 15	Mean	SD	Mean	SD	P value	Significance	P value	Significance
LTS (dB)	18.75	3.84	19.56	2.97	0.116	No	0.115	No
CRT (μm)	490	188	327	119	0.001	Yes	0.0003	Yes
VA (LogMAR)	0.54	0.23	0.48	0.25	0.117	No	0.037	Yes

Dexamethasone	Base line		3 months		One tailed *t*-test		One-way ANOVA	
n = 13	Mean	SD	Mean	SD	P value	Significance	P value	Significance
LTS (dB)	21.35	4.88	23.05	4.11	0.061	No	0.107	No
CRT (μm)	407	147	317	116	0.034	Yes	0.019	Yes
VA (LogMAR)	0.62	0.4	0.48	0.36	0.042	Yes	0.055	No

Ocriplasmin	Base line		3 months		One tailed *t*-test		One-way ANOVA	
n = 6	Mean	SD	Mean	SD	P value	Significance	P value	Significance
LTS (dB)	17.77	3.83	21.45	2.26	0.083	No	0.155	No
CRT (μm)	295	50.7	217	27.9	0.011	Yes	0.023	Yes
VA (LogMAR)	0.54	0.14	0.32	0.15	0.001	Yes	< 0.0001	Yes

Descriptive statistics and one tailed t-test outcomes comparing the studied variables between base line and 3 months after therapy with their ANOVA significant difference between groups.

6.4 Discussion

There has been an incremental use of MP testing in clinical trials recently, as several authors have reported that test re-test capabilities of the same anatomical locus may be more efficient in MP than conventional standard automated perimetry. This is on account of the introduction of the retinal tracker system, an essential component of the MP design, which aids to compensate for eye movements during examinations.

MP outcomes describe retinal function in terms of light threshold sensitivity and patient's fixation capabilities. However, the majority of clinical studies which include MP testing, have predominantly reported outcomes of mean sensitivity in the selected grid, and correlations with VA and OCT macular morphology.

The ETDRS Study Group defined 9 regions (sectors) of the macula for evaluation of changes in macular thickness in DR. The regions are located in three rings with diameters of 1, 3, and 6 mm. The 1 mm ring contains a 1 mm perifovea ring called the Central Retinal Subfield. In this study we have analysed the CRT which represents the average thickness within the central ETDRS 1 mm ring. Different methods have been described to calculate foveal size dimension, in essence studies using the contrast adjustment, or non-contrast adjustment (Fingler et al., 2009) have reported foveal diameter of 0.7mm and 1.02 mm respectively. Recently the size and shape of the foveal avascular zone (FAZ) determined by OCT angiography (OCTA) in normal subjects has reported the foveal size to be 0.749 ± 0.129 mm.. (Shiihara et al., 2018). According to the laboratory calculations performed with the MAIA designers (M. Morales unpublished observation), 0.7 mm correspond to 2.5° of the MAIA on the focal plane. The standard MAIA macular grid used in this study and in the majority of similar studies, consists of three concentric rings of 12 stimuli each, where the first ring has a diameter of 2°, corresponding to 0.56 mm on the focal plane. Therefore, to measure sensitivity in the central retinal thickness more accurately, a dedicated quantification of the 12 stimuli of the first MAIA inner grid may be advisable.

This assumption may be meaningful when the MP grid is centrally located on the anatomical fovea. In this study, it is observed the distance between both of the PRLs (initial and final) reported in the MAIA, where eyes with both PRLs far from each other are noted to have unstable fixation associated with non-foveal vision. The results indicate that from 25% and up to 67% of our studied cases were associated with non-foveal vision in the different groups. These observations do not imply that the results from correlation in this study, as well as other reports in the literature which include MP are wrong. Rather, it is postulated that clinical studies analysing correlations between MP and central macular thickness, must ascertain that the MAIA grid is effectively centred on the fovea, and consider quantifying an eccentricity margin of error in patients with non-foveal function.

Likewise, when analysing central retinal thickness from OCT data special care must be taken, specifically in cases where the PRL is slightly eccentric. In the present study we identified cases of patients with slightly eccentric fixation, where the OCT imaging system is wrongly centred over a non-foveal location, resulting in erroneous quantification of the central retinal thickness. (Figure 6.17).

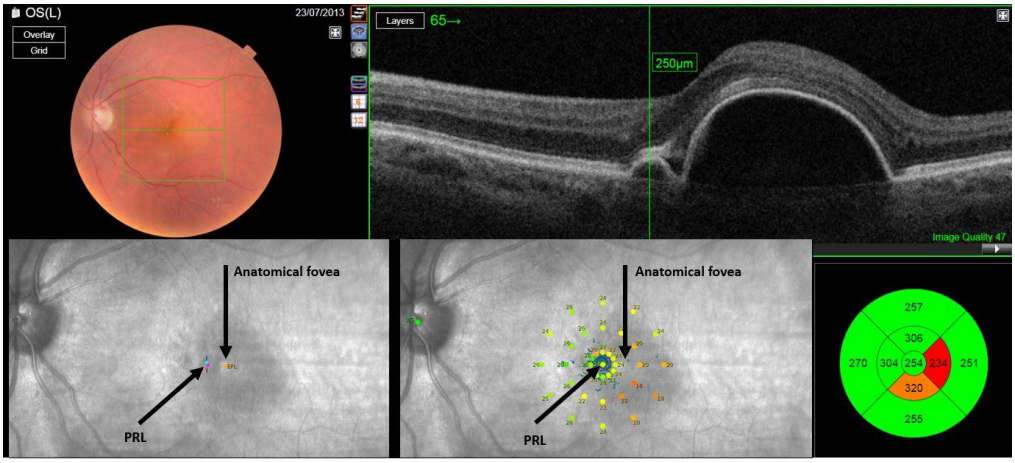

Figure 6.17 OCT with eccentric fixation

Patient from Anti-VGEF group showing the MAIA grid centred on the PRL located approximately 1° from the fovea. The OCT shows normal values on the central fovea, where in reality, the central fovea should've been reported with the abnormal values shown on the adjacent quadrants from the fovea.

A significant finding in this study is that fixation stability may be better correlated to post-treatment VA, and that retinal sensitivity and macular thickness are not always correlated. This reinforces the importance of including retinal function variables in macular treatment studies, as such variables may represent subclinical indicators, not anatomically perceived with OCT examinations.

Although fixation indices showed a better correlation with VA than retinal sensitivity and thickness, it was observed that fixation stability, according to the Fujii classification (Fujii et al., 2002), have variable changes during follow-up visits. The interpretation of these findings is that, on the one hand, when pathologies affect the integrity of foveal photoreceptors, intravitreal injections therapy may not recover such cellular function. As such, patients who have

developed unstable fixation in the course of their pathology, may remain with their unstable fixation even after intravitreal therapies. On the other hand, the Fujii classification of fixation stability, which states 3 fixation levels, may require revision, as important changes on the raw data related to both fixation indices (P1 and BCEA) within the relatively-stable and unstable classification were noted in this study. It is suggested that improving the classification of fixation stability from 3 levels to 5 levels, may provide better correlation of new fixation indices with VA, which may serve as better predictors of therapy outcomes. Such new classification requires further studies.

7. Impact of dark adaptation time in scotopic microperimetry testing

7.1 Introduction

Patients with age-related macular degeneration have particularly decreased vision in dim light conditions and require high ambient light in some settings (e.g. when reading). Such changes occur even at the early stages of AMD. Multiple retinal diseases are associated with rod photoreceptor functional impairment including rod-cone dystrophies (Lorenz et al., 2000), macular telangiectasia type 2 (Schmitz-Valckenberg et al., 2009), and retinitis pigmentosa (Arden et al., 1983). In earlier studies, some investigators have demonstrated that parafoveal rods, but not cones, decrease during the course of normal adulthood (Curcio et al., 1993). Moreover, they have hypothesised that early parafoveal loss of rods may be a subclinical sign in AMD, not yet visible in the fundus, whilst rod loss precedes cone loss in both exudative and nonexudative AMD (Curcio et al., 1996). Furthermore, psychophysical data in patients with early and late AMD have suggested that rod dysfunction exceeds cone dysfunction (Owsley et al., 2000). This observation of scotopic preceding/exceeding photopic sensitivity loss in individuals with AMD is

supported by histopathologic data that demonstrated a preferential vulnerability of rods (Curcio et al., 1993).

The first commercially available device for automated dark-adapted perimetry was a modified first generation Humphrey Field Analyzer (Jacobson et al., 1986) (HFA, Carl Zeiss Meditec Inc., Dublin, CA, USA). Later, a few reports were generated on scotopic fundus controlled perimeter with a modified version of the Nidek MP-1 microperimeter, the MP-1S (Nidek Technologies, Padua, Italy) (Steinberg et al., 2015, Steinberg et al., 2016, Nebbioso et al., 2014, Crossland et al., 2011c, Salvatore et al., 2014). However, the dynamic range of the MP-1 light stimuli (20 dB) is considered to be low and prone to ceiling and floor effects in both mesopic and scotopic examinations (Steinberg et al., 2015, Crossland et al., 2011c, Midena et al., 2007).

Recently a modified version of the MAIA microperimeter, the Scotopic-MAIA (S-MAIA), became available allowing for mesopic light threshold sensitivity (LTS) testing with achromatic stimuli projection, as well as scotopic testing with two chromatic stimuli, cyan (505 nm) and/or red (627 nm). While the scotopic cyan (blue) testing is intended to be largely derived from rod photoreceptor-mediated function, also excluding S-cone activity, the scotopic red would be more influenced by cone-mediated function, rather reflecting a mixture of both rod- and cone-mediated responses (Pfau et al., 2017a). Scotopic examinations must be performed on patient's dark-adapted conditions. One challenge for the practical application of scotopic microperimetry in clinical settings is the time taken for the patient to dark adapt, which is in addition to the testing time. It has

been reported that fatigue during long psychophysical examinations may reduce patent's capacity to fixate (Morales et al., 2013). As such, the aggregation time of dark adaptation and scotopic microperimetry testing should be kept as low as possible.

We conducted a study to investigate the potential impact of different dark adaptation times on scotopic retinal LTS as well as assessing the test-retest repeatability of the S-MAIA for each dark adaptation time and explored the extent of a practice effect.

7.2 Methods

This was an observational, descriptive, study on individuals with no known ocular pathologies from the University of Belfast campus. The study had institutional board approval from the research ethics committee of The Queens University Belfast, School of Medicine Dentistry & Biomedical Sciences, Belfast, U.K. Informed consent was obtained from all participants, and the study met the tenets of the Declaration of Helsinki.

Participants

Twenty-four adult, medical students and staff colleagues (mean age = 32.3 years), with best corrected visual acuity (VA) of LogMAR 0.1 (ETDRS) or better, were recruited. Inclusion criteria for this study were as follows: adult age above 21 and below 80 years, participants with normal vision, no limitation to

race, ethnicity, or sex; and willingness to rest in absolute darkness for an approximately time of one hour.

The exclusion criteria included any diagnosis of ocular disease, opaque ocular media, diagnosis of squint, or any other condition that would cause any kind of fixation instability, refractive error higher than 6 or lower than -6 diopters, and people with known nyctophobia symptoms.

Equipment and Procedures

The basic design of the MAIA device has been described in previous chapters. In brief, it is a mesopic test which utilizes a confocal SLO system with a wavelength of 850 nm to image the retina. Retinal sensitivity is assessed with Goldmann size 3 class achromatic (white) stimuli, projected through an LED system within the central visual field. The retinal land mark eye tracker aids test-retest stimuli projection over the same base-line retinal area. The visual target used for fixation during testing is a 645 nm red ring with 1° diameter superimposed over 4 asb (127 cd/m2) background display. The mesopic MAIA examination is a 4–2 full threshold staircase projection strategy with maximum stimuli luminance of 1,000 asb and 36 dB dynamic range. The S-MAIA is an adaptation of the standard (mesopic) MAIA which allows for scotopic LTS testing on patients under dark adaption visual conditions. For that purpose, all internal light sources were eliminated from the device, two colour LED systems were added and the SLO power was significantly reduced during testing with absent background luminosity. The scotopic stimuli projections are available in two different wavelengths, 505nm (cyan) and 627nm (red). The maximum

stimuli luminance is 0.8 asb, with a dynamic range of 20 dB (S-MAIA software version 2.2.0-2016). In this study, a testing grid with 45 stimuli was used. The stimuli grid distribution (Figure 7.1) contained 1 central stimulus, and 5 stimuli concentric rings separated from the centre at 1° (4 stimuli), 2.5° (8 stimuli), 4° (12 stimuli), 6° (12 stimuli) and 10° (8 stimuli). Examinations were performed without pupil dilation.

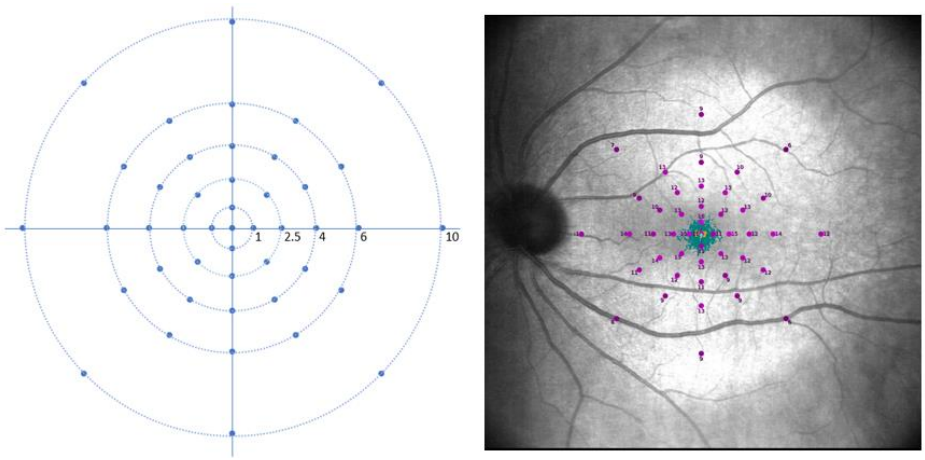

Figure 7.1 Scotopic MAIA grid

S-MAIA stimuli distribution diagram used in this study (left) with the fundus related light threshold sensitivity (LTS) output.

In both examination modes (mesopic and scotopic), the MAIA start testing with 4 paracentral stimuli, corresponding to the central stimulus of each quadrant. These 4 threshold values are then used to adjust the initial brightness levels for measuring the remaining test loci in each of the corresponding quadrants. For mesopic mode, the testing sensitivity started with a level of 2 dB brighter of the respective initial threshold value using a 4–2 dB full-threshold strategy. For

scotopic testing, the testing sensitivity started with a level of 2 dB dimmer than the respective initial threshold, particularly to avoid bleaching, and using a 2–1 dB full threshold strategy. These testing settings were not modifiable in the device (software version 2.2.0-2016). It is noteworthy that after our study report, the company Centervue modified the S-MAIA dynamic range from 20 dB to 36 dB in subsequent software versions.

Two scotopic microperimetry sessions were performed within a maximum separation of one week (7 days). In the first session, measurement of refractive error was carried out with an auto-refractometer (ACCUREF K-900, Shin-Nippon, Japan); the eye with the least refractive error in terms of spherical equivalent was chosen as the study eye. As a microperimetry training test, the participant performed a mesopic supra-threshold examination, known as the fast exam in the MAIA, with the sole purpose to familiarise each participant with the examination task. After that, scotopic microperimetry examinations were performed in 3 different dark-adapted time conditions: 10, 20 and 30 minutes. To reverse the dark adaptation between tests, a 10-minute break with room lights on (luminance 0,10lux) was done. During the dark adaptation period, participants remained in the dark testing room (luminance ≤ 001 lux). This luminance level has been used in other studies as well (Pfau et al., 2017a). Study participants were instructed to keep their eyes open during the dark adaptation period. Two consecutive scotopic examinations were performed on each session, first with the cyan (C) and immediately after with the red (R) stimuli. Test reliability was assessed by measuring the frequency of false-positive responses (measured by presentation of a suprathreshold stimuli to the optic nerve head).

Participants with a false positive rate greater than 33.3% in any of the tests were excluded from the analysis.

Statistical Analysis

Statistical analysis was carried out with SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). All variables were assessed for normality using the Shapiro-Wilk test and transformed if necessary. For comparison between thresholds of the three dark adaptation times the results from the first session only were used in the analysis. The means produced from each dark adaptation interval, for each stimulus (C and R) and their difference (C-R), were compared with one-way ANOVA. Repeatability was assessed producing Bland-Altman plots, whilst reliability using intra-class correlation coefficient (ICC). The categorization and interpretation of ICC was based on published guidelines. These suggest that ICC values <0.5 indicate poor reliability, values between 0.5 to 0.75 indicate moderate, values between 0.75 to 0.9 good and finally values >0.9 indicate excellent reliability (Koo and Li, 2016). The extent of a learning effect was assessed using repeated measures ANOVA.

7.3 Results

Twenty-four persons agreed to participate; two were unable to attend the second session and were excluded from the final analysis. Furthermore, two other participants could not finish one of the tests and were also excluded. This

resulted in a sample of 20 individuals with a total of six scotopic sessions each. Of these, 5 were males and 15 females. Mean age in the studied sample was 32.30 years old ± 10.50 SD. The mean refractive error in terms of spherical equivalent was -1.22 diopters ± 1.86 SD.

Effect of Dark Adaptation time on Scotopic Sensitivity

For the cyan stimuli, we found no significant difference between 20 minutes of dark adaptation when compared to the 10 minutes ($p=0.08$) or the 30 minutes intervals ($p=0.18$). Instead, a significant difference was found when the 30 minutes period of dark adaptation was compared to the 10 minutes interval ($p=<0.01$). For the red stimulus on the other hand, there was no significant difference between any of the adaptation times. Finally, when assessing the differential test (C-R), both 20 and 30 minutes were significantly different when compared to the 10 minutes adaptation time ($p=0.02$, 20' and $p=<0.01$, 30'). When the 20- and 30-minutes intervals were compared with each other, significance was approached ($p=0.07$) (Table 7.1).

Table 7.1 Dark adaptation time

Visit 1 Cyan						
Dark Adaptation Time	Time Compared	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower	Upper
10'	20'	-1.25	0.56	0.08	-2.59	0.1
	30'	-2.24*	0.56	<0.01	-3.59	-0.9
20'	10'	1.25	0.56	0.08	-0.1	2.59
	30'	-1	0.56	0.18	-2.35	0.35
30'	10'	2.24*	0.56	<0.01	0.9	3.59
	20'	1	0.56	0.18	-0.35	2.35
Visit 1 Red						
Dark Adaptation Time	Time Compared	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower	Upper
10'	20'	-0.13	0.36	0.93	-0.99	0.73
	30'	-0.13	0.36	0.93	-0.99	0.73
20'	10'	0.13	0.36	0.93	-0.73	0.99
	30'	0	0.36	1	-0.86	0.86
30'	10'	0.13	0.36	0.93	-0.73	0.99
	20'	0	0.36	1	-0.86	0.86
Visit 1 Cyan – Red						
Dark Adaptation Time	Time Compared	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower	Upper
10'	20'	-1.14*	0.42	0.02	-2.15	-0.13
	30'	-2.10*	0.42	<0.01	-3.11	-1.09
20'	10'	1.14*	0.42	0.02	0.13	2.15
	30'	-0.96	0.42	0.07	-1.97	0.05
30'	10'	2.10*	0.42	<0.01	1.09	3.11
	20'	0.96	0.42	0.07	-0.05	1.97
* The mean difference is significant at the 0.05 level.						

Assessment of the impact of dark adaptation time on scotopic retinal threshold assessed with one-way ANOVA.

Repeatability

The Bland-Altman test, plots the difference of the two sessions (Visit 1 – Visit 2) against the mean (Visit 1 + Visit 2 / 2). The 95% levels of agreement for the C stimuli ranged from -2.70 to 2.19 with a mean difference of -0.25. For the R stimuli -2.27 to 1.75 with a mean difference of -0.26, whilst for the difference (C-R), the 95% levels of agreement ranged from -2.14 to 2.16 with a mean of

0.01 (Fig. 7.2). The widest limits of agreement were found in the scotopic C test. On the other hand, most outliers were found on the scotopic R test as compared to the C, or C-R average threshold values. However, in general, most of the values lie within the 95% confidence limits despite the presence of a few outliers. The finding of outliers though, which indicate a degree of variability, is one which exists in perimetry testing (Wong et al., 2017b, Wong et al., 2017a, Wu et al., 2013b).

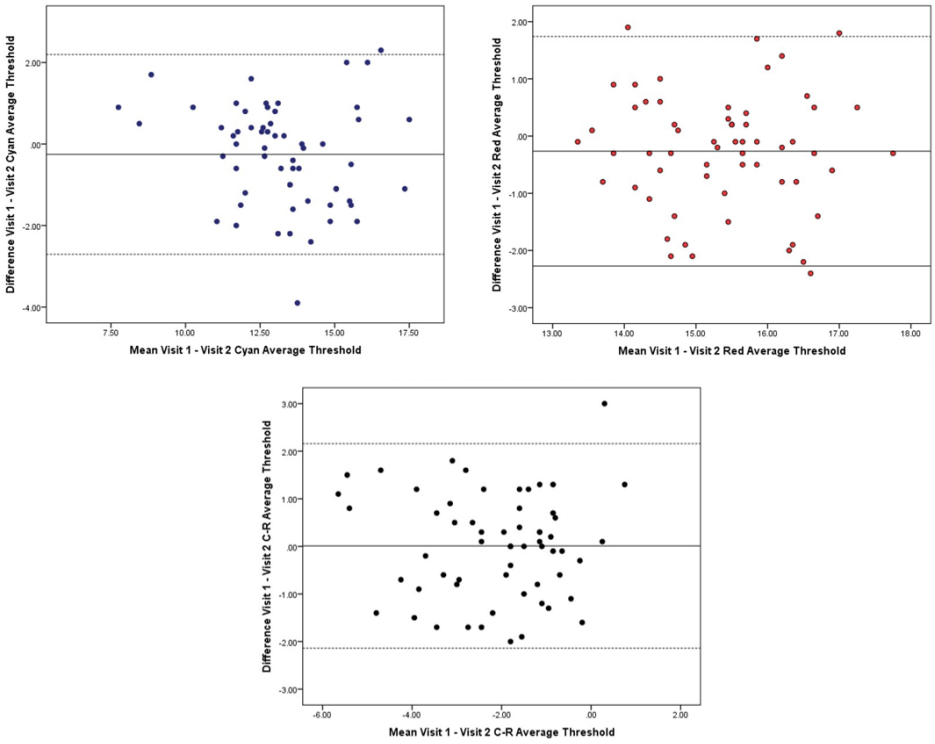

Figure 7.2 Bland-Altman Plots for Scotopic testing

Bland-Altman Plots for Cyan (top), Red (middle) and Cyan – Red average threshold(bottom). The solid lines represent the mean for each measurement while the dashed lines correspond to the 95% upper and lower levels of agreement. On the x-axis the mean average threshold for each test pair is illustrated while on the y-axis the difference (Visit1 – Visit2) between the two tests is illustrated.

In order to further assess the reliability of the device, we calculated the intraclass correlation coefficient (ICC) analysis (Table 7.2). The highest ICC was found

for the cyan stimulus (0.90, p<0.01) approaching an excellent test-retest reliability. The red stimulus on the other hand, showed moderate ICC (0.74, p<0.01). The difference of both stimuli, C-R was also found good with an ICC 0.86 and a p<0.01. The categorization of ICC interpretation was based on published guidelines as described earlier (Koo and Li, 2016).

Table 7.2 Intraclass correlation scotopic test

Intraclass Correlation Coefficient					
	Intraclass Correlation Coefficient (ICC)	95% Confidence Interval		Sig.	ICC Classification
		Lower	Upper		
Cyan*	0.9	0.83	0.94	<0.01	Good
Red*	0.74	0.56	0.84	<0.01	Moderate
Cyan - Red*	0.86	0.77	0.92	<0.01	Good
*Based on average measures					

Intraclass Correlation test (ICC) between examination types. Values <0.5 indicate poor reliability, between 0.5 to 0.75 moderate, between 0.75 to 0.9 good and >0.9 excellent reliability

Learning effect

The paired samples t-tests gave no statistically significant differences (Table 7.3) between study visits. However, the only tests that appeared to show a learning effect were the differences between the two 10' sessions with the C (p=0.03) and R (p=0.03) stimuli. Repeated measures ANOVA, after Bonferroni adjustment, showed no significant learning effect present for the C stimulus (p=0.12) or the C-R (p=0.94), with a borderline suggestion from the R results (p=0.05).

Table 7.3 T-test for dark-adaptation time

Dark Adaptation Time	Cyan				Red				Cyan-Red			
	Visit 1	Visit 2	paired difference	*p*	Visit 1	Visit 2	paired difference	*p*	Visit 1	Visit 2	paired difference	*p*
10'	11.97	12.62	-0.65	0.03*	15.16	15.67	-0.51	0.03*	-3.2	-3.06	-0.14	0.61
20'	13.22	13.55	-0.34	0.11	15.29	15.48	-0.19	0.34	-2.06	-1.93	-0.13	0.58
30'	14.22	13.99	0.23	0.49	15.29	15.38	-0.9	0.74	-1.1	-1.39	0.3	0.25

*T-test evaluation for the three different dark adaptation times on the scotopic-cyan and scotopic-red microperimetry examinations and their difference. (*Statistically significant results on the 0.05 level).*

7.4 Discussion

This study provides data on the impact of dark adaptation time on scotopic retinal sensitivity as obtained with the S-MAIA, the repeatability and reliability of the measurements, and the potential presence of a learning effect.

The results indicated that careful thought was required when deciding on the correct dark adaption period in order to obtain stable threshold results. The data suggest that for the C stimulus, a 30 minutes interval in the dark, produced significant differences when compared with the 10 minutes time, but only a trend to significance when is compared to the 20 minutes adaptation time. For the R stimulus on the other hand, dark adaptation interval did not appear to impact sensitivity thresholds. However, when the results are taken in their entirety, we observed that for the majority of cases there was no difference between the 20' and 30' threshold values. It is thus suggested that a time selection of a minimum

of 30’ of dark adaptation would favour the C-R results a significant difference. Confirmation of this finding would require a larger sample size.

The duplex theory of vision states that for luminance levels above 0.03 cd/m^2, the cone system mediates vision. For luminance levels below that point, rods take over, while the range in which these two mechanisms are working together, is the mesopic vision range (Weale, 1961). Rod peak spectral sensitivity is around 500nm (Kelber et al., 2017), with the C stimulus having a wavelength close to that (λ=505nm) and the topography of rod photoreceptors having been reported (Curcio et al., 1990), our findings come in agreement with Pfau (Pfau et al., 2017a), who reported that the C well reflects rod-mediated visual function. On their point-wise mean sensitivity analysis, these authors observed that the highest mean was at an eccentricity of 7^o from the fovea. This pattern of sensitivity reaching peak values with the C stimulus at a diameter 5^o to 7^o was observed in our study as well. Future work could explore if the impact of dark adaption time differed with retinal eccentricity.

The repeatability test results showed good agreement between measurements. In both scotopic tests, C and R, most points fell within the 95% levels of agreement. The same occurred for their difference (C-R). An interpretation of the few observed outliers is that a degree of variance occurred. Most outliers were found in the R test (3 in total) when compared to C (2 in total).

The results for ICC suggest that the device is reliable to use as it provides high ICC values 0.90 for C, 0.74 for R and 0.86 for C-R respectively. Pfau et al also suggested that the scotopic device is repeatable and reliable to use in both,

healthy and persons with macular diseases (Pfau et al., 2017a, Pfau et al., 2017b). Results for repeatability and reliability of the standard MAIA, have been reported for individuals in normal retinal health, in patients with different ocular diseases and in children (Molina-Martin et al., 2015, Jones et al., 2016, Wu et al., 2015, Wong et al., 2017b).

In agreement with other studies, data from the current study suggests that there is no significant learning effect on the basis of repeated measures ANOVA with the Bonferroni adjustment applied. The absence of a learning effect for the S-MAIA has been reported previously by other authors (Pfau et al., 2017a). However, this is contrary to the results obtained by Wu et al., who reported a learning effect in the standard MAIA device and, therefore, mesopic testing conditions, and recommended discarding the results from the first session (Wu et al., 2013b). It is hypothesized that the use of a training test (fast examination) may have improved repeatability. However, results from the current study may be limited as no randomization was applied in the investigations.

A limitation of this study is the small sample size included, and that analysis could have been extended to measure the effect of time of dark adaptation in different retinal eccentricities. On the other hand, our main focus was to use three different times of dark adaptation to obtain our results, compared to previous studies that used only one-time interval. The previous study also reported short term repeatability with both testing sessions occurring on the same day, whereas this study had a one week window (Pfau et al., 2017a).

In summary, this study is the first to evaluate the impact of dark adaptation time on scotopic retinal sensitivity measured using scotopic microperimetry. The results suggest that the time of dark adaptation did have an impact on the outcome measure. If this is to be estimated from the cyan stimulus, which well reflects rod function, a period of 30 minutes dark adaptation is preferable. If scotopic sensitivity is to be estimated from the difference C-R, the minimum dark adaptation time required, could be slightly reduced to 20 minutes. Additionally, this study provides data which suggests that the MAIA produces repeatable and reliable measurements with no evidence of a significant learning effect, specifically when a pre-test for training purposes is performed.

8. Rehabilitation of eccentric vision with biofeedback technique in MP

8.1 Introduction

Motor neuro-rehabilitation aims to improve patient's functional abilities, replacing skills that have been lost fully or partially. A general neuro-rehabilitation mechanism of action is the potentiation of a group of latent neuronal connections that are utilized repeatedly during challenging behavioural practice. The repeated and persistent practice, over several weeks, of a challenging movement facilitates neural synapsis, which may result in lasting physiological changes in motor neural networks (Lang et al., 2015, Nudo, 2013).

The motor skills acquisition process may be described in distinct phases (Nudo, 2013, Doyon and Benali, 2005) from the early to consolidation stages, when the newly acquired skill is performed with minimal cognitive resources. The final stage is defined as when the performance can be executed after long delays between training sessions (Doyon and Benali, 2005). Although the literature on neurological rehabilitation is vast, there are numerous and inconsistent

parameters of intensity, frequency and therapy duration related to induced movement studies (Lang et al., 2015, Gee et al., 2018).

The fovea is responsible for detailed vision and fixation. Patients with central vision loss attempt fixation with an eccentric retinal zone known as the Preferred Retinal Locus (PRL) (Crossland et al., 2011b). As mentioned in previous chapters macular function can be assessed with microperimetry (MP) by means of light threshold sensitivity (LTS), fixation stability (FS) and the PRL which can be plotted as a cloud of fixation points over a reference retinal image (Morales et al., 2013). FS in microperimetry is classified as stable, relatively unstable or unstable (Fujii et al., 2002). The MAIA microperimeter scores FS with different indexes; the most representative are P1 and the bivariate contour ellipse area (BCEA) with proportional values of 95% (Morales et al., 2016). P1 describes the amount of retinal displacement occurring within 1° from an initial reference point, whilst BCEA describes 95 % of retinal loci used during fixation attempt.

Eyes with eccentric fixation regularly demonstrate unstable FS with associated low vision. However, it has been reported that FS can be improved with oculomotor exercises known as biofeedback fixation training (BFT) (Markowitz, 2006, Vingolo et al., 2007, Tarita-Nistor et al., 2009b, Amore et al., 2013, Morales et al., 2015, Morales et al., 2013), a task-oriented behavioural therapy, which according to some authors may drive neural plasticity changes in the visual system (Tarita-Nistor et al., 2009b, Chung, 2011). BFT consists of asking patients to perform ocular movements towards a specific direction,

attempting to align a selected retinal locus with a visual target. This locus is known as the fixation training target. Biofeedback audio signals (beep sounds) aid patients during the oculomotor task by increasing the auditory frequency as the training target approaches the desired alignment. To be of value, the training target should have better functional characteristics than the PRL previously used by the eye with unstable fixation. However, the selection of this training target has not been described previously.

The index case for this study was published as 'Bilateral eccentric vision training on pseudovitelliform dystrophy with microperimetry biofeedback' in 2015 (Morales et al., 2015), which allowed a methodology for eccentric biofeedback to be developed.

In this study we describe a methodology for selecting the best fixation training target, with the aim of improving eccentric fixation through BFT with MP in patients with unstable fixation.

8.2 Methods

This was a prospective, consecutive, case series study of a cohort of patients with irreversible bilateral central vision loss, poor FS and best corrected visual acuity (VA) of 0.3 LogMAR or worse, who performed and completed BFT sessions with the MAIA MP. The study included participants from the Low Vision and the Macular Clinics of the Queen's Medical Centre, Nottingham (U.K.) and were

recruited from January 2013 to June 2017. Local ethical approval and written informed consent was obtained from all participants, and the study met the tenets of the Declaration of Helsinki.

Participants

Ninety patients (mean age = 67.5 years), with bilateral central vision loss secondary to different pathologies affecting the macula, with unstable fixation and best corrected visual acuity (VA) of LogMAR 0.3 (ETDRS) or worse, were recruited.

Inclusion criteria for this study were as follows: adult age above 21 and below 80 years, patients with bilateral central vision loss, geographic atrophy area with scotoma bigger than 1 disk size and smaller than 5 disk sizes, stable pathology with at least 6 months from last anti-VEGF injection if any, patients willing to improve reading abilities. There was no limitation to race, ethnicity, or sex. The exclusion criteria were: mild to severe cataract (over grade 2) or other opaque optical media, active macular oedema, uncooperative patient, or non-English speaker.

Equipment and Procedures

Patients were scheduled for BFT using the MAIA MP. The eye with the better VA for each participant was selected for this study. If both eyes had equal VA the eye with better FS was selected. Patients were, alternately divided into 2 different groups. In group A (mean age 64.7 ± 22 years), the retinal locus for BFT was set on the baseline patient's spontaneous PRL, assessed with the MAIA

Standard-Macula-Test (10°, 37 stimuli). In group B (mean age 70.4 ± 14 years), BFT was performed on the locus with the best functional characteristics. To determine this locus, two custom MAIA exams were performed: A) the "Low-Vision-Assessment" grid-test (30°, 83 stimuli) with the 4-levels-fixed projection strategy, which scores retinal sensitivity as "good" (25dB), "relatively good" (15dB), "relatively poor" (5dB), "poor" (0dB) and "scotoma" (<0 dB); and B) the "Fixation-Training-Target" grid-test (7°x5°, 35 stimuli) with the 4-2 projection strategy. The first one, (A), was centred on the estimated foveal location, or on the patient's PRL in cases of GA larger than 3 times the optic nerve head size (ONH). The Low-Vision-Assessment grid output was used to identify retinal loci with at least 2 consecutive stimuli, distributed horizontally, showing "good" or "relatively-good" light sensitivity, and served as a reference to centre the Fixation-Training-Target grid, prioritizing the superior retina (inferior visual field) and the smaller distance from the anatomical fovea. The second custom test, (B), was used to select the target locus for BFT. This locus was set in the centre of the two adjacent stimuli with highest light sensitivity, and lowest distance from both the anatomical fovea and the baseline PRL (Figure 8.1).

Adapting the training frequency reported in the literature (Deruaz et al., 2006, Vingolo et al., 2007, Tarita-Nistor et al., 2009b, Amore et al., 2013, Verboschi et al., 2013), and with the scope to reach consolidation and retention therapeutic stages, we performed 2 sets of 12 weekly training sessions separated by a 3 month period of no training. Each session lasted 10 minutes. BFT consisted of asking patients to move their gaze slightly towards the training locus. The

auditory signal increased in frequency as the desired eye movement reached the target. Patients were advised to remember the gaze movement performed during the training sessions, and to try to reproduce the same movement in their daily life when attempting to steadily see a visual target.

The final results were assessed at 2 weeks after completing BFT sessions. Primary outcomes were classification of FS, fixation indices P1 and BCEA@95%, VA, and reading speed (IReST) (Trauzettel-Klosinski and Dietz, 2012). FS values after the first training session, and after 6 months from baseline, were also recorded. Secondary outcomes were mean light sensitivity. The anatomical location and the visual field correspondence of the PRL were also annotated.

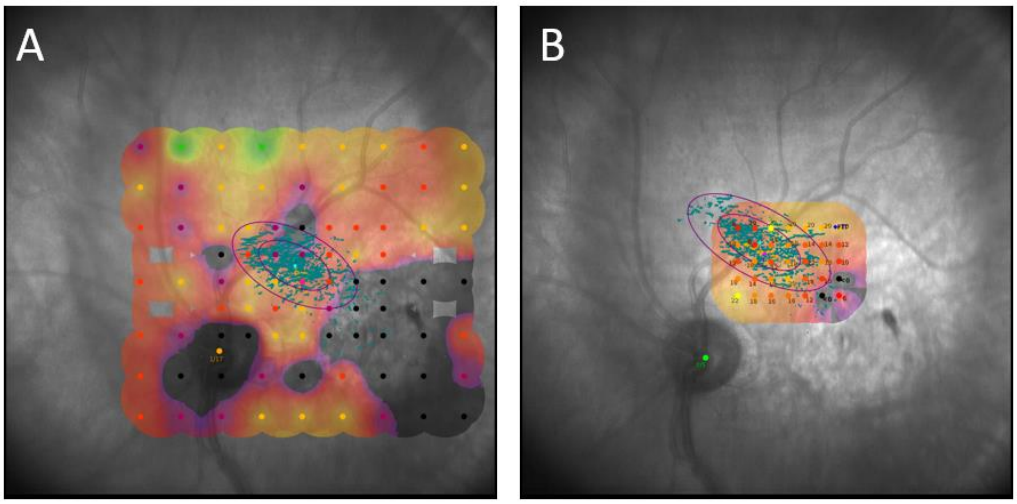

Figure 8.1 Low vision MAIA grids

Microperimetry exam grids: A) "Low-Vision-Assessment" grid centred on patient's PRL with the 4-Levels-Fixed projection strategy, showing five different dB levels of retinal sensitivity: green = 25dB, yellow = 15dB, red = 5 dB, purple = 0dB, black = <0 dB (scotoma). B) "Fixation-Training-Target" grid with the 4-2 projection strategy, used to select the locus with highest retinal sensitivity for subsequent BFT sessions.

Statistical Analysis

Statistical analysis (GraphPadPrism_7.04) included standard errors, 95% confidence, interquartile intervals and robust regression outlier removal. Assuming a non-parametric distribution, a one-tailed Mann-Whitney test was applied to compare outcomes between baseline and the last training sessions for each group, and between groups with a significant difference of $P<0.05$. Outcome correlations were analysed through Spearman's rank-order coefficient (r).

8.3 Results

Twenty-three patients withdrew from the study before completing all BFT sessions either because they did not notice improvement in vision or could not attend regularly for training/assessment. These withdrawals were excluded from analysis. A total of 67 patients completed the study; 30 had Geographic Atrophy (GA), 19 moderate dry AMD, 9 Best's Disease, 6 Myopic Macular Degeneration and 3 chronic Central Serous Retinopathy (CSR). Group A included 20 females and 8 males, whilst group B had 27 females and 12 males. Mean central scotoma sizes were 5.4° ± 3.8° and 5.7° ± 4.5° for groups A and B respectively.

At baseline, the FS classification in group A was unstable in 18 subjects (64%), relatively unstable in 9 (32%), and stable in 1 (4%). At the end of therapy, 16 subjects (57%) were classified as unstable, 11 (39%) as relatively unstable, and 1 (4%) as stable. In group B, the baseline classification was unstable in 19 (49%)

subjects, relatively unstable in 19 (49%), and stable in 1 (2%). At therapy end, 11 subjects (28%) were classified as unstable, 16 (41%) as relatively unstable, and 12 (31%) as stable (Figure 8.2).

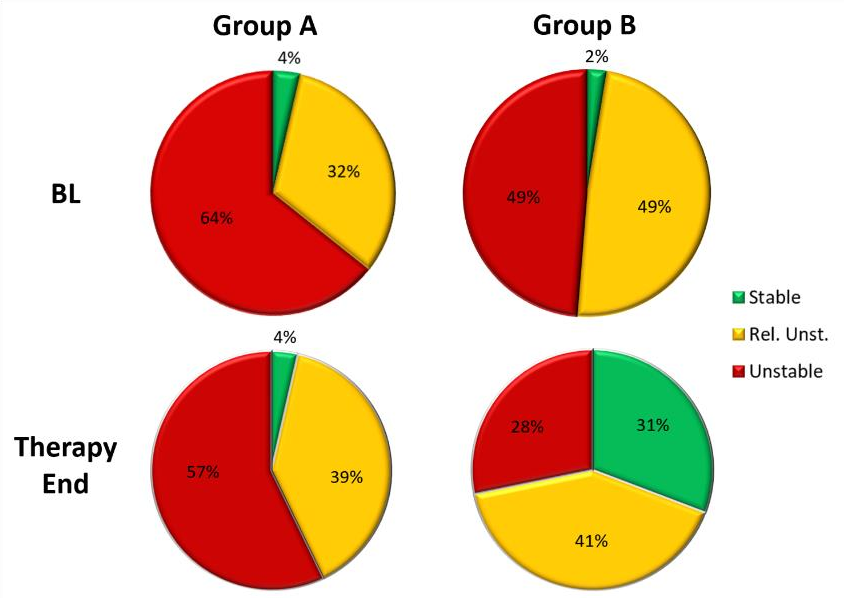

Figure 8.2 Classification of FS in LV group

Classification of fixation stability on baseline (BL) and end of biofeedback training for both groups A & B.

In group A, the mean FS index P1(%) was 32 ± 19 at baseline, 26 ± 18 after the first BFT session, 34 ± 22 at six months, and 35 ± 23 at the end of therapy. In group B, the mean P1 was 40 ± 24 at base-line, 27 ± 20 after the first BFT, 48 ± 29 after six months, and 55 ± 29 at therapy end (Figure. 8.3A).

The mean area (deg^2) of BCEA@95 in group A was 38 ± 23 at baseline, 51 ± 47 after the first BFT, 33 ± 22 at six months, and 32 ± 25 at study end. In group B, the mean area was 39 ± 40 at baseline, 64 ± 70 at first BFT, 30 ± 31 at six months, and 19 ± 18 at therapy end (Figure. 8.3B).

The FS index P1 did not improve in 50% of subjects in group A, and 18% in group B. Similarly, 35% of group A subjects did not improve in BCEA@95%, compared to 10% of those from group B.

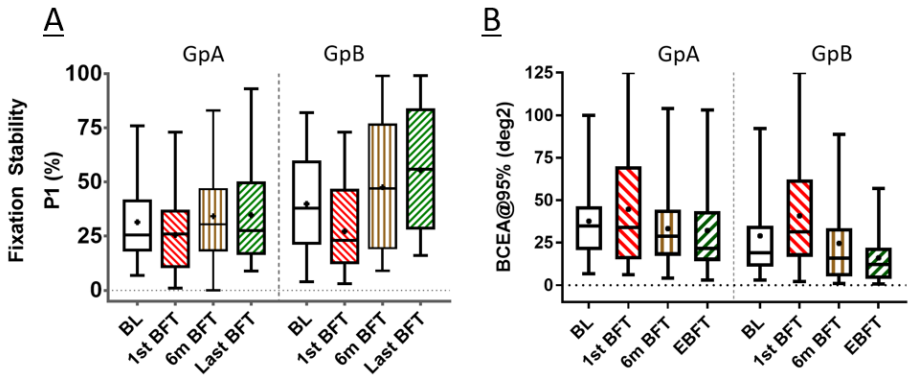

Figure 8.3 FS analysis over time

Box-and-whisker plot showing quartile distribution and mean data of fixation index P1 (A) and BCEA@95% (B) at baseline (BL), after the first BFT session, 6 months after first treatment and at the end of all BFT sessions (EBFT).

Visual acuity (LogMAR) in group A ranged from 0.4 to 1.9 at baseline and 0.3 to 2.0 at the end of therapy, whilst for group B the range was from 0.3 to 2.0 at baseline and 0.1 to 2.0 at the end of therapy. The mean VA in group A improved from 1.0 ± 0.48 to 0.86 ± 0.53 at therapy, whilst for group B the improvement was from 1.0 ± 0.51 to 0.84 ± 0.49. In group A the improvement was observed in 16 (57%) subjects, whilst for group B the improvement was found in 26 (67%) participants. The VA was unchanged in 4 (14%) subjects in group A and 10 (25%) from group B, whilst a decrease in VA was seen in 8 (29%) participants from group A and 3 (8%) from group B. The mean reading speed (wpm) improved from 56 ± 30 to 58 ± 32 in group A, and from 63 ± 36 to 89 ± 46 in group B (Figure 8.4).

Treatment efficacy (baseline vs therapy end) showed no significant difference in any of the studied variables in group A, as demonstrated with the one-tailed Mann-Whitney test (Table 1A). In contrast, differences were found in all group B variables except on light threshold sensitivity (LTS) (Table 1B). When comparing final outcomes between groups, a significant difference in all parameters was found, except for VA (Table 1C).

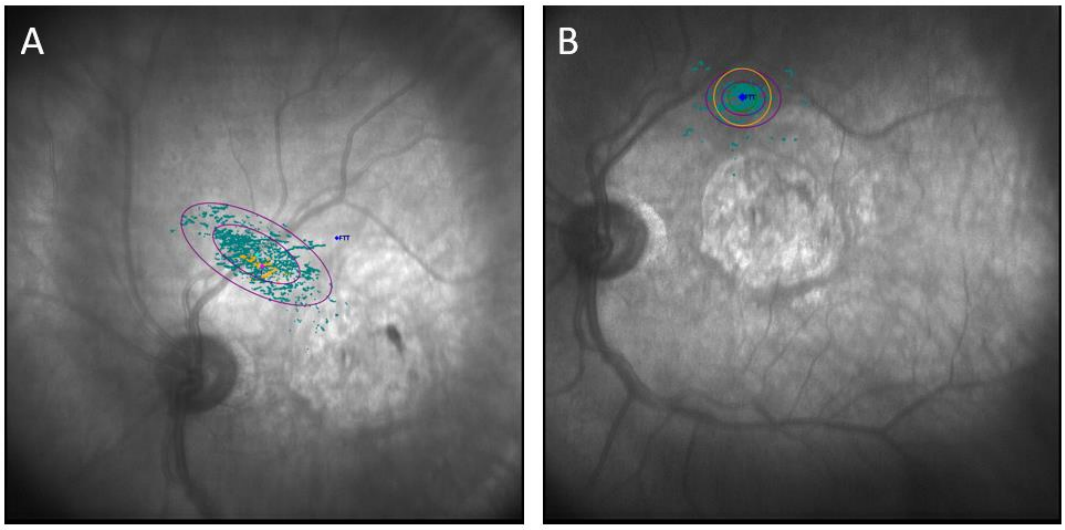

Figure 8.4 PRL after fixation training

Example of a group B patient, showing the cloud of fixation points with different PRL location and improvement of fixation stability from baseline (A) to end of biofeedback training (B). Fixation improved from unstable to relatively unstable, and visual acuity from 1.03 to 0.8 LogMAR.

Baseline FS indexes in group A showed moderate correlation ($0.50 < r < 0.70$) with their final FS values and scotoma extension. However, high correlation ($r > 0.7$) was found with final reading speed. In group B, base-line FS indexes were highly correlated ($r > 0.7$) with final FS values, whilst moderately correlated with final VA and reading speed. FS outcomes demonstrated negligible

correlation with scotoma size (r = 0.2), whilst low dependence with the trained location (r = 0.3) was found. Final VA showed better correlation with baseline FS in group B (r = 0.4) than group A (r = 0.2). Final reading speed was better correlated with base line FS (P1) in group A (r = 0.7) than group B (r = 0.5). Low correlation was found (r < 0.3) in both groups between functional outcomes and patient's age. A week correlation was found (r < 0.3) after performing a subgroup analysis to study the PRL behaviour in the different pathologies investigated.

Table 8.1 Final intergroup comparisons

	P1	*BCEA_95%*	*VA*	*Read-Speed*	*LTS*
A) GpA (n=28): Base-line vs Therapy-end					
P value	0.3895	0.0734	0.1002	0.4306	0.4115
Significantly different (P<0.05)?	No	No	No	No	No
Mann-Whitney U	374.5	303	314	381	378
Median of Base-line	25.5	34.7	1	49	13.25
Median of Therapy-end	27.5	21.65	0.7	46.5	13
B) GpB (n=39): Base-line vs Therapy-end					
P value	0.0098	0.0038	0.04	0.0078	0.1471
Significantly different (P<0.05)?	Yes	Yes	Yes	Yes	No
Mann-Whitney U	528	495	586	519	655
Median of Base-line	38	24.7	0.92	61	17.4
Median of Therapy-end	56	13	0.7	85	18
C) GpA vs GpB Therapy-end					
P value	0.0008	0.0035	0.4582	0.0029	0.04
Significantly different (P<0.05)?	Yes	Yes	No	Yes	Yes
Mann-Whitney U	303	336	537.5	331	408.5
Median of GpA, n=28	27.5	21.65	0.7	46.5	13
Median of GpB, n=39	56	13	0.7	85	18

Mann-Whitney Test of a) Baseline vs Therapy-end for group A. b) Baseline vs Therapy-end for group B. c) Therapy-end comparison for group A vs group B.

8.4 Discussion

Although task-specific training to enhance motor representations has been reported for several decades (Lang et al., 2015), only a few authors have demonstrated FS improvement in patients with foveal impairment using biofeedback and microperimetry (Morales et al., 2015, Verdina et al., 2013, Verboschi et al., 2013, Amore et al., 2013, Tarita-Nistor et al., 2009b, Tarita-Nistor et al., 2009a, Vingolo et al., 2007, Markowitz, 2006, Deruaz et al., 2006, Chung, 2011). Furthermore, detailed methodologies adopted to define the best functional retinal locus for such training have not been fully described.

Ramirez et al. (Ramirez Estudillo et al., 2017). following our suggestions, demonstrated the effectiveness of BFT one week after completion of therapy, whilst Ratra et al. (Ratra et al., 2018), recently demonstrated in a small number of patients that the BFT effect can be maintained for up to six months with slight reduction in fixation stability. Our study demonstrated a similar reduction, suggesting that such visual training should be attempted over longer periods in order to achieve maximum results.

Our study adds additional credence to the notion that fixation in patients with eccentric vision can be improved through biofeedback therapy. Nudo (Nudo, 2013) suggested that without behavioural training, plasticity in spared motor areas, which occurs spontaneously, largely reflects the development of compensatory motor patterns rather than patterns of true recovery. Our findings highlight the concept that localized fixation training may enhance plasticity more

efficiently than when training is performed on the PRL which was spontaneously developed by the individual after the loss of foveal function.

BFT is reported to be dependent on the location, with the highest retinal sensitivity in small central scotomata (Ueda-Consolvo et al., 2015). However, we explored the possibility of standardised BFT in cases with any central scotoma size. The BFT theory is based on neuro-plasticity, where healthy neural sensors are frequently stimulated. When retina photoreceptors and ganglion cells are healthy, microperimetry outcomes demonstrate high light threshold sensitivity values. For this reason, our first selection criteria to define the best locus for BFT is a retinal location with good light sensitivity.

Detailed vision is performed with high packing density of cone photoreceptors. Its density peak, located at the foveal centre, decreases rapidly within the central 2mm, with a gradual decrease further away from the fovea (Song et al., 2011). Subjects with healthy vision perform fixation within the central 2°, as demonstrated with the MAIA (Morales et al., 2016). Consequently, the assumed correlation between density of cones and fixation abilities is valid. Recent studies confirmed photoreceptor's density decreases at 1°, 2°, 4 and 6° of eccentricity, showing a homogenous drop in each of the four retinal meridians, and high agreement between nasal and temporal locations (Song et al., 2011, Lombardo et al., 2013). These results suggest that patients may have similar anatomical visual capabilities at any retinal meridian with eccentric equidistance from the fovea. In light of these associations, the second selection criterion for

the BFT locus corresponds to an area located closer to the anatomical fovea without discrimination of the retinal meridian.

Previous studies suggest that reading with eccentric viewing may be more efficient if the PRL is located on the left hemisphere and the lower visual field.(Fletcher and Schuchard, 1997, Nilsson et al., 2003, Frennesson and Nilsson, 2007) These observations reinforced our third BFT locus selection criterion, suggesting the predilection on the left and/or superior side of the central scotoma whenever good light sensitivity is present.

Finally, it is well known that PRL positions may also depend on the visual task. In the western world, reading is performed from left to right. Reading tasks involve eye fixation and saccadic movements following a horizontal path. For that reason, our methodology locates the training location in the middle of a horizontal line, with at least 2 adjacent stimuli with good sensitivity.

Our investigation contributes to the literature with a thorough BFT analysis, and scope to understand the rationale behind the selection process of an effective retinal locus useful during eccentric fixation training in patients with foveal function loss. This suggested BFT methodology is summarised as follows:

1. Perform the “Low-Vision-Assessment” grid-test with the 4-levels-fixed projection strategy centred on the anatomical foveal, or on the patient’s baseline PRL in eyes with Geographic Atrophy larger than 3 times the optic nerve head (ONH).

2. Identify loci with at least 2 consecutive stimuli, distributed horizontally, of good or relatively-good threshold sensitivity (GTS).

3. Perform the "Fixation-Training-Target" grid-test with the 4-2 projection strategy. Centre the grid on the GTS loci. If there are more than 1 GTS options, prioritize the smaller distance to the fovea on either the superior retina or the left visual field with lower distance from the base-line PRLs.

4. Use the "Fixation-Training-Target" grid outcomes to select the final trained retinal locus to perform BFT. This locus should be set in the centre of the 2 highest horizontal adjacent threshold stimuli.

5. Perform 10-minute BFT sessions over the selected training target on a weekly basis for 12 weeks. After a resting period of 3 months, perform a new set of 12 weekly BFT sessions to aid visual plasticity consolidation.

To conclude, in this study we have described a methodology for BFT with microperimetry, with the scope to improve eccentric vision through better fixation control. Further studies are needed to validate the effectiveness of this methodology in everyday visual tasks, such as reading and other visuomotor activities. Of paramount importance is an investigation of the different motor-sequence adaptation stages during BFT, in particular, the recognition of the consolidation and automatic stages, as these may be the key to optimising frequency and duration for individual therapeutic strategies, as well as to understanding whether long-term plasticity changes are achievable, and retainable.

9. Summary and recommendations.

9.1 General Discussion

The main objective of this research study was to analyse retinal functional characteristics with a MP in common macular diseases, including AMD, and determine its usefulness in the monitoring of disease progression, treatment outcomes, and visual rehabilitation, with the purpose of evaluating its benefits in the everyday clinical practice and propose a standardisation of use. In principle, the studies (with their findings) included in this thesis should allow better understanding of retinal function, as described by MP systems, and complement existing standard eyecare tests of VA (functional) and OCT (structural) analysis, aiming to predict better prognosis for patients with different macular pathologies.

The study was divided in 2 main sections: monitoring of macular diseases and fixation training for eccentric visual rehabilitation.

a) MP in the monitoring of macular diseases. This section included observation of pathologies commonly affecting the central vision and its related intravitreal therapies such as anti-VEGF for nvAMD, DMO and MO secondary to RVO

treated with anti-VEGF or dexamethasone implants and ocriplasmin for symptomatic VMT with or without early macular hole.

b) MP fixation training for eccentric visual rehabilitation. This section included observation of functional visual outcomes in low vision patients with bilateral central vision loss secondary to macular pathologies such as, GA in AMD, Stargardts disease and vitelliform dystrophies, who attended several sessions of eccentric fixation training over a functional preferred retinal locus methodically selected with MP.

In chapter 2, “Materials”, a general review of the differences between standard automated perimetry (SAP) and microperimetry (MP) was included, with a detailed explanation of the MAIA MP. The concepts of preferred retinal locus, fixation stability and biofeedback fixation training, were carefully described. An introduction of Scotopic MP and a general MP clinical review was also part of this section. In addition, a review of the Topcon OCT and the international reading speed test (IReST) were included. This chapter, as well as the preceding one (General Introduction and Rationale), provided useful background information that served as a starting point for the present research project. In particular, the anatomical, and clinical features, as well as the investigative instruments of the retina and macular pathologies affecting central vision are reviewed.

Clinical studies including MP normally describe outcomes related to retinal light threshold sensitivity, whilst only a minority of MP scientific publications refer to fixation stability outcomes, which are mainly related to visual rehabilitation

training where fixation is a descriptive parameter of visual improvement. MP systems currently use different approaches to measure FS, namely P1, P2 and the bivariate contour ellipse area (BCEA), which describe an elliptical area encompassing a given proportion of the fixation points dataset (Crossland et al., 2004). The P1 and P2 indices are used to classify fixation as stable, relatively-unstable, and unstable. However, there was no a reference database available for any of the BCEA indices. For that reason, chapter 4 is dedicated to a study designed to determine normative data for such indices. This study included observations on 358 participants aged from 19 to 86 years, and described normal values of BCEA indices and their correlations with subject's age and P1 and P2 indices.

The preferred retinal locus in microperimetry is described as the graphic representation of a cloud of fixation points plotted as a result of the retina movement during threshold examination. Chapter 5 reviewed the concept of the PRL_initial (*i*) and PRL_final (*f*) unique in the MAIA.

These two PRLs are the barycenter reference point of such cloud of fixation points, but calculated in two different examination times, in the first 10 seconds of the test, before the projection of light stimuli, and at the time when all light stimuli were tested (around 6 minutes for the MAIA standard macula grid). The PRL*i* is used by the MAIA to center the grid map to be tested. The study of 65 eyes from forty-one patients, correlated the distance between both PRLs with fixation stability and visual acuity, demonstrating that both VA and FS tend to decrease as the distance between PRL*i* and PRL*f* increases. The results suggested that eyes with both PRLi and PRLf close to each other are noted to have stable

fixation located over the fovea centralis, whilst eyes with PRLi and PRLf far from each other (higher than 0.8°) are noted to have unstable fixation located eccentrically from the fovea. This information was used on chapter 6 to objectively identify foveal or non-foveal fixation on patients with macular pathologies. This investigation was accepted for publication as the first manuscript describing such characteristics of PRL_initial (*i*) and PRL_final (*f*).

In Chapter 6 a thorough analysis of MP outcomes was conducted and correlated with OCT central retinal thickness and visual acuity. A total of 74 patients attending the macula clinic for intravitreal therapy were included in the analysis. Patients were divided in 4 groups according to the intravitreal treatment. Group 1, Anti-vascular endothelial growth factor (anti-VEGF) drugs for wet (exudative) age-related macular degeneration (AMD). Group 2, Intravitreal dexamethasone implant for the treatment of diabetic macular oedema (DMO). Group 3, Intravitreal dexamethasone implant for the treatment of macular oedema (MO) related to retinal vein occlusion (RVO). Group 4, Intravitreal ocriplasmin for the treatment of vitreomacular traction (VMT) and macular hole (MH). Data from base-line, and follow-up after 3, 6, and 12 months was used in the analysis. Primary outcomes were MP light threshold sensitivity, CRT and VA. T-test and ANOVA computations did not show significant difference with LTS and the other studied variables. However, Pearson's coefficient correlations showed higher levels of agreement between microperimetry FS indices and VA outcomes. This reinforce the importance to include retinal function variables in macular treatment studies, as such variables may represent subclinical indicators, not anatomically perceived with OCT examinations. Although FS

showed a better correlation to VA than retinal sensitivity and thickness, we have observed that the variety of changes in FS classification, according to the Fujii scale (Fujii et al., 2002), may not correspond to the level of change in FS indices P1 and BCEA, suggesting a re-definition of a new fixation stability classification. Of particular importance was the analysis of data on central retinal values with both OCT and MP. This analysis showed that in patients with non-foveal fixation, the lack of perception of eccentric fixation by operators, may lead to examination outcomes with wrong estimates, where eccentric values wrongly deemed to represent central information.

In recent years, a modified version of the MAIA, the S-MAIA, has become available allowing for light threshold sensitivity measurements in scotopic conditions. This is in response to the perceived need of investigating independent threshold sensitivities of cone and rod photoreceptors, as it has been reported by some authors that multiple retinal diseases are associated with rod photoreceptor functional impairment (Pfau et al., 2017a), along with other reports that have described histological cone photoreceptor degeneration in macular pathologies like AMD (Shelley et al., 2009). One challenge for the practical application of scotopic microperimetry in clinical settings is the time taken for the patient to dark adapt, which in addition to the examination time, may increase patient's fatigue, which has been reported to reduce patient's capacity to fixate (Morales et al., 2013). For such reason, chapter 7 described the investigation of three different dark adaptation times, 10, 20 and 30 minutes, on scotopic microperimetry as well as assessing the test-retest repeatability of the S-MAIA for each dark adaptation time, exploring in addition, the extent of a practice

effect. The results suggest that the time of dark adaptation did have an impact on the outcome measure with preference for a period of 30 minutes dark adaptation. Furthermore, this study provides data which suggests that the scotopic MAIA produces repeatable and reliable measurements with no evidence of a significant learning effect, specifically when a pre-test for training purposes is performed.

Finally, chapter 8 is dedicated to the investigation of the use of MP in the fixation training for eccentric visual rehabilitation with biofeedback technology in patients with loss of central vision. Biofeedback fixation training had been reported as a method to improve FS trough oculomotor exercises. However, the selection of the retinal locus target to perform such training had not been described previously. This study had the aim of studying the effectiveness of such technology and proposing guidelines for this rehabilitation method. In this section, the different variables responsible for unstable fixation, and the selection of the best eccentric functional retinal locus were studied. The definition of the best eccentric functional retinal locus, was of paramount importance in this chapter as it may lead to better outcomes also in other vision therapies commonly performed in low vision centres, whilst increasing possibilities for a better quality of life in patients with loss of central vision secondary to macular pathologies. The analysis included 67 patients with various irreversible pathologies affecting the foveal area. Outcomes included FS indices, classification of fixation, VA, and reading speed. At the end of the chapter, step by step instructions were proposed to standardised a methodology to perform this fixation training, with emphasis in the selection of the best retinal locus aiming for effective therapeutic results. At the time of this thesis writing period,

the publication of this investigation had been accepted with minor amendments of the submitted manuscript.

9.2 Limitations and Potential Future Directions

Although this project has contributed to a better understanding of the assessment of retinal function and therapeutic capabilities of microperimetry technology, it is still possible to carry out further investigations. This section is dedicated to the identification of the limitations of this research work, followed by the suggested recommendations for future research:

For project a) MP in the monitoring of macular diseases:

- The number of participants for each study group was small and unbalanced. Therefore, it is recommended to proceed with further research with a higher number of participants for each study group with the purpose to increment the statistical power of the results.
- The MP stimuli grid was the standard macular assessment grid available in the MAIA instrument, however such grid was designed to differentiate normal vs abnormal macular function, which may not be the ideal grid to monitor the progression of different pathologies affecting the macula and cannot be correlated with any standard macular test as those found in OCT instruments. For such reason, it is suggested to design a dedicated MP stimuli grid which can be correlated with all 9 sections of the ETDRS distribution.

- An important limitation of this work comes with the use of the automatic operation mode of the MAIA, which may misplace the centre of the stimuli grid over a non-foveal location. Therefore, it is suggested to create a methodology to assure that both OCT and MP instruments perform examinations accurately centred on the fovea centralis.
- If central retinal thickness is intended to be correlated with MP outcomes, then a mean quantification of stimuli located over the foveal area should be performed. In case of the MAIA standard grid, this corresponds to the inner ring of 12 stimuli located 1° from the central stimuli, in addition to the central stimuli itself.

For project b) MP fixation training for eccentric visual rehabilitation.

- An important limitation of this study was the number and stratification of patients. Bilateral macular atrophy may be present in different dimensions and at different ages, therefore a large number of study subjects divided in several subgroups is recommended.
- This was an interventional unmask trial which may performed by a single researcher which may lead to important bias during the study process. For such reason a multi-site study design performed by naïve operators with no, or minor, experience in visual rehabilitation must be perform to reduce bias.
- A crucial limitation of this study is the lack of a control group. It is well known that patients with low vision conditions in the same visual acuity group may have remarkably different psychophysical characteristics, however it is suggested the inclusion of a control group with the purpose

to compare MP fixation training outcomes with other eccentric vision techniques available today in low vision practices.

Other meaningful recommendations for future research are the following:

- A randomization methodology should be applied to select patients on each group.
- A study with different subgroups according to pathology should be included.
- Different therapy time settings as well as resting time should be tested.
- Results should be presented in different timelines, i.e. 3, 6, 12 and 24 months.
- A longer follow up examination must be included to evaluate permanence of therapy outcomes and compared them with controls.
- A precise quantification of scotomata area should be included in the methodology.
- The study must be registered in any recognised site for clinical trials to increase monitoring and proof of principles reliability.

9.3 General Conclusions

The first aim of this study was to assess the benefit of MP functional analysis in clinical practice for patients undergoing clinical treatments, related to pathologies affecting central vision. This was achieved in full. A better understanding of MP technology and its clinical applications and limitations has been reached. Furthermore, a series of recommendations have been set to improve the use of microperimetry devices, which will increase substantially accuracy in further clinical trials where MP is included.

The second aim was related to the therapeutic capabilities of MP in the rehabilitation of eccentric vision, studying the effectiveness of such technology and proposing guidelines for this rehabilitation method, with the aim of allowing the use of this technique to a wider pool of eye specialists with minimal experience in eccentric vision rehabilitation. This was also achieved in full as the study has created guidelines, which certainly after its publication, will be of significant benefit for MP users worldwide, who may be looking forward to offer this therapeutic technique to patients with irreversible central vision loss. Despite the fact that this technique has proven to be effective in a clinical environment, it leaves open queries regarding the effective benefit in regular daily activities. Furthermore, a couple of additional questions remain open, e.g. the majority of patients clinically diagnosed with low vision have still some degrees of remaining binocular vision, whilst the MP instrument, in contrast, is a monocular system. Therefore, the significant lack of understanding concerning

binocular visual function after monocular fixation training remains open. On the other hand, this therapeutic technique is possible only in a clinical environment, whilst the majority of low vision patients may have mobility difficulties, limiting its benefit only to those patients able to assist constantly and for long period of times a clinical setting. This leaves open opportunities for developments of similar equipment/techniques to be use in a home environment.

People with macular degeneration, and other retinal pathologies affecting the central vision, will derive significant benefit from our research. This research will allow patients and clinical practitioners to better understand visual function in cases of central vision loss, and improve quality of life through better scotoma awareness.

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Abbreviations

AMD: Age related macular degeneration 11

asb: Apostilb 29

BCEA: Bivariate contour ellipse area 125

BFT: Biofeedback fixation training 5

Bivariate Contour ellipse Area : BCEA 54

CFP: Cloud of fixation points 54

CNV: Choroidal NeoVascularization 13

CRT: Central retinal thickness 88

dB: Decibels 30

DMO: Diabetic Macular Oedema 16

DR: Diabetic retinopathy 15

ETDRS: Early Treatment Diabetic Retinopathy Study 16

FFA: Fundus fluorescein angiography 13

FS: Fixation stability 35

GA: Geographic Atrophy 12

GTS: Good light threshold sensitivity 138

ICC: Intra-class correlation coefficient 115

IVT: Intravitreal injections 84

LTS: Light threshold sensitivity 25, 69, 110, 125

LV: Low Vision 20

MO: macular oedema 84

MP: microperimeter 45

MP: Microperimetry 84, 125

nvAMD: Neovascular age related macular degeneration 83

OCT: Optical coherence tomography 13

PDR: proliferative diabetic retinopathy 17

PRL: Preferred retinal locus 69, 88

PRL*f:* PRL final 71

PRLi: PRL initial 70

RVO: Retinal vein occlusions 83

SAP: Standard automated perimetry 25

SLO: Scanning Laser Ophthalmoscope 27

S-MAIA: Scotopic MAIA 110

TS: Threshold sensitivity 25

VA: Visual acuity 72, 87, 111, 127

VEGF: Vascular endothelial growth factor iii, 83

VF: Visual field 10

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