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The Rarebit Fovea Test
- a new measure of visual function

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To my dear family

Abstract

Visual acuity (VA) measurement by the use of letter charts is the most frequently used method for testing foveal function. However, since a decimal visual acuity of 1.0, often used as limit for normality, can be achieved with less than two-thirds of the normal number of optic nerve axons, the letter chart test cannot be regarded as sensitive to low-degree damage. The reason why letter charts are unable to detect small and subtle defects is due to the high receptor density and the proportionally large stimuli used in VA testing.

The Rarebit technique was developed with the explicit aim to improve detection of small defects. The method is built on the principle of detection of very small and bright stimuli, corresponding to half the normal minimum angle of resolution in the tested retinal location. The test principle is easy to understand, the results are easy to interpret, and no expensive equipment is required. The system includes two tests Rarebit Perimetry (RBP) and Rarebit Fovea Test (RFT), the first for evaluation of the 30º visual field (with the possibility to test out to 60º), and the second for evaluation of the foveal function i.e., the most central 4º VF.

Several studies have shown that conventional perimetry and vision function tests are insensitive to minor neuro-visual damage. In Rarebit improved detection of small defects, is intended to be obtained due to two deliberate deviations from standard procedures. Target information content is minimized (i.e., small dots compared to the receptive field at the tested retinal location, briefly exposed) and thresholding is replaced by simple probing of the completeness of the retino-cortical detector matrix.

The aim of the current thesis was twofold. Paper I evaluated the physiologic properties of the test, i.e., the effect of age, binocular summation and different luminance levels in the test stimuli, in normal subjects. In Paper III the fixation stability during the test was studied. In order to determine the potential clinical value, patients with diabetes and cataract were examined. In the patients with diabetes, the results from the RFT were compared with fundus photographs and OCT measurements. The cataract patients were examined with both RBP and RFT prior to and after cataract surgery (Paper II and IV).

The findings from the studies support the idea behind the RFT, i.e., that small stimuli might be useful for detecting small defects in the neuro-retinal architecture and can be expected to give additional information about the visual system, compared to findings from conventional tests, e.g., VA and funduscopic examinations. The RFT can be expected to be of clinical value in patients where early identification of damage is relevant and sensitive methods for follow-up are required.

Key words

Rarebit Fovea Test, Rarebit Perimetry, foveal function, fixation, diabetes, cataract, binocular summation

List of Papers

I. Nilsson M, Wanger P, Martin L. Perception of very small visual stimuli in the fovea: normative data for the Rarebit Foveal Test. Clin Exp Optom. 2006 Mar;89(2):81-5.

II. Nilsson M, von Wendt G, Wanger P, Martin L. Early detection of macular changes in patients with diabetes using Rarebit Fovea Test and optical coherence tomography. Br J Ophthalmol. 2007 Dec;91(12):1596-8.

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IV. Nilsson M, Abdiu O, Laurell CG, Martin L. Rarebit Perimetry and Fovea Test before and after cataract surgery.
(Submitted)

Contents

List of abbreviations

AMD	age-related macular degeneration
ANOVA	analysis of variance
ARM	age-related maculopathy
BCVA	best corrected visual acuity
CAP	computerized automatic perimetry
cd/mm^2	candela per square meter
CSF	contrast sensitivity function
D	dioptre
DLS	differential light sensitivity
DM	diabetes mellitus
DPI	Dual-Purkinje-Image
DRP	diabetic retinopathy
EDTRS	Early Treatment of Diabetic Retinopathy Study
e.g.	for example
ERG	electroretinogram
et al.	and others
exam.	examination
FDT	frequency doubling technology
FOS	frequency-of-seeing
GDx	scanning laser polarimetry
HFA	Humphrey Field Analyzer
HRP	high-pass resolution perimetry
Hz	hertz
i.e.	that is
IOL	intraocular lens
K	konicellular
LCD	liquid crystal display
LGN	lateral geniculate nucleus
M	magnocellular
m	minute or meter
MHR	mean hit rate
Mild	mild DRP (diabetic retinopathy)
Min	minimal DRP (diabetic retinopathy)
mm	millimetre
mm Hg	millimetre of mercury (pressure)
ms	millisecond
MTF	modulation transfer function
n	number
nm	nanometre
OCT	Optical Coherence Tomography
P	Parvocellular
PSF	point spread function
RBP	Rarebit Perimetry
RFT	Rarebit Fovea Test

RPE	retinal pigment epithelium
SAP	standard automatic perimetry
SD	standard deviation
sec	second
SWAP	short wavelength automated perimetry
TM	trade mark
V1	visual area 1
V2	visual area 2
V3	visual area 3
V4	visual area 4
V5	visual area 5
VA	visual acuity
ver.	version
VF	visual field
μm	micrometer

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1. INTRODUCTION

Vision is regarded as the most precious of our senses and also the most complex. When studying vision, several different fields of science will be involved, ranging from physics to brain physiology.

1.1 Physical properties of light

The visual system responds to differences in intensity or wavelengths of light. The field of optics describe the behaviour and properties of light and the interaction between light and matter. Light is a form of radiant energy emitted into space by luminous sources. Light can exhibit properties of both waves and particles, the wave–particle duality. This theory of duality emerged from competing theories of light proposed by Christiaan Huygens and Isaac Newton in the seventeenth century (Freeman & Hull 2003) and has recently been experimentally verified (Jacques et al. 2007). Light is the normal stimulus to vision and to be detected by the human eye the wavelength of an electromagnetic radiation needs to be between ~380–780 nm (Rabetts 2007). Light is composed of elementary particles called photons. The energy carried by a single photon is just enough to excite a single molecule in a photoreceptor cell of an eye and thereby contribute to vision (Baylor et al. 1979).

1.2 Optics of the Eye

The eye is often compared with a camera. Both have imaging lenses, variable apertures and a photosensitive surface. However, the eye is superior on almost every count. The eye has a wider field of view, is sensitive to a much wider range of luminance levels, has a resolving power close to the theoretical limit, and the complex processing within the retina makes it entirely different from a simple photographic film or electronic sensor.

Like any other optical system, the eye suffers from errors of focus and a variety of optical aberrations. Aberrations are usually expressed as Zernike polynomials, which is the standard way to describe the shape of a wave front produced by an optical system (American National Standards Institute 2004). Fortunately the most common aberrations and the ones with largest influence on optical image quality, refractive aberrations or lower-order aberrations, can be corrected with spectacles, contact lenses or refractive surgery. The average of the higher order aberrations is small in comparison with the refractive errors. Thibos et al. (2002a) studied monochromatic aberrations in 200 normal healthy eyes and found the average amount of higher order aberrations present for a 7.5 mm pupil equivalent to the wave-front error produced by less than 0.25 dioptres (D) of defocus. They also found that the population averages of Zernike coefficients were nearly zero for all higher-order modes except spherical aberration, which is systematically biased towards positive values.

Spherical aberration is a defect of an optical system due to a variation in the focusing between peripheral and paraxial rays. The larger the aperture/pupil size, the greater the difference in focusing between two rays. The mean spherical aberration is approximately 0.1μm in an eye with a pupil size of 6.0 mm (Thibos et al. 2002b).

Chromatic aberrations are defects of an optical system due to unequal refraction of different wavelengths which results in an extended image along the optical axis (longitudinal chromatic aberration) and that the size of the image of a point object is extended by coloured fringes (lateral chromatic aberration). However, reduction of contrast sensitivity is moderate and reduction in visual acuity is minor due to chromatic aberrations (Thibos 1991).

Every eye has its own specific pattern formed by individual aberrations, these aberrations always blur the retinal image, but our subjective impression is that the visual world is sharp and clear. Artal et al. (2004) suggested that the brain might compensate for our aberrations and influence on subjective experience. By using adaptive optics they showed that subjects preferred their own aberrations rather than unfamiliar ones and concluded that the neural visual system is adapted to the eye's aberrations, thereby reducing the blurring effect.

Beside aberrations, the optical quality through the eye or the resolution ability is limited by diffraction and photoreceptor density. Diffraction refers to various phenomena associated with the bending of waves when they interact with obstacles in their path. In the eye, this occurs when the light passes through the pupil. Diffraction is only a limiting factor for eyes with pupils smaller than 2 mm (Thibos et al. 2002a).

When the eyes optical system is limited by diffraction rather than aberrations the minimum resolvable angle between two images is given by the Rayleigh criterion - invented by Lord Rayleigh (Rabetts 2007). When the separation distance on the retina is calculated for a 2 mm pupil it is found to be proportionate with the retinal receptor spacing at the fovea (Freeman & Hull 2003). There are theoretical calculations arguing that normal photoreceptor density is capable of providing a decimal visual acuity about 2.0-3.0 (Seiler et al. 2000, MacRae & Williams 2001).

The image quality of a point can be described by a point spread function (PSF) and the image quality of a pattern in terms of a modulation transfer function (MTF). The PSF describes the response of an imaging system to a point source or point object. The image will be a non-point image caused by a mixture of aberrations and diffraction (Tunnacliffe 1993). The MTF describes the degradation of contrast when a sinusoidal grating is imaged by an optical system (Tunnacliffe 1993). This loss of quality is also caused by the effect of aberrations and diffraction, and MTF is a useful parameter to describe the eyes sensitivity to luminance contrast.

1.3 Optical aspects of the retina

The Stiles-Crawford effect refers to the directional sensitivity of the cone photoreceptors in the fovea. The cones are more sensitive to light passing though the centre of the pupil and thereby parallel to the photoreceptors, than to light passing near the edge of the pupil (Stiles & Crawford 1933). This phenomenon is caused by the optics of the retina. The inner segment of the cones has a high refractive index, which helps to funnel photons into the outer segment and thereby increase the probability that they will be absorbed (Burns & Lamb 2004). This is advantageous, since light, which enters through the centre of the pupil forms a sharper image because the eye's optics has its highest quality in the centre. Compared to cones, rods are less directionally sensitive (Makous 2004).

1.4 The biochemistry of light detection

A photoreceptor is a specialized type of neuron that is capable of phototransduction, i.e., converting light energy to electrochemical signals. In the human eye there are two types of photoreceptors, cones and rods. The photoreceptor uses rhodopsin to absorb photons and by complex biochemical processes signals are produced that can change the cell membrane potential. The signal leads to transmission of either excitatory or inhibitory signals to the brain.

The sensors in the retina can actually respond to a single photon (Baylor et al. 1979). However, the impulse from a single photon is not enough to trigger a conscious response. Yet, only a few photons are needed for the scotopic (i.e., dark adapted) visual system to react (Hecht et al. 1942, Vimal et al. 1989). Exactly how the retina interprets the rod signals and differentiates between noise and relevant potential changes is unclear (Field et al. 2005) but the process of rod-to-rod bipolar cell signal transmission has strong implications for setting the absolute threshold for seeing (Okawa & Sampath 2007).

1.5 Structure and function of the retina

Several layers of specialized cells and membranes build up the retina and there is a close interaction between the choroid, the retinal pigment epithelium (RPE) and the neural retina.

Proteins used during photo transduction are phagocytosed by the RPE, a layer of "nurse" cells behind the neural retina. The RPE sustains photoreceptor health in numerous ways, including maintaining proper ionic balance and hydration, transporting and filtering nutrients, providing retinoid intermediates to reload photo pigments bleached by light exposure, and absorbing stray photons (Schraermeyer & Heimann 1999).

Nutritients to the RPE and photoreceptors are supplied by the choroidal vascular system, which has the proportionally highest blood flow in the body. Between the RPE and the choriocapillaris, Bruch's membrane is located. All nutrients to and metabolites from the RPE and photoreceptors must cross Bruch's membrane (Jackson et al. 2002).

The retina contains five main classes of cell types (Masland 2001). On the deep surface, furthest from the incoming light, lie the rods and the cones that are responsible for night and daylight vision respectively. They are connected to the bipolar cells, which in turn connect to the ganglion cells. Horizontal cells and amacrine cells makes side-to-side connections between photoreceptors and ganglion cells respectively. Another type of cells, Müller cells, constitute a functional link between neurons and vessels (Bringmann et al. 2006).

Before the signals are sent from the retina to the brain they are processed by a variety of interactions among the retinal neurons. Lateral inhibition is an important part of this interaction and it can modulate the direct pathway for signals both via horizontal cells in the outer retina, where photoreceptors synapse onto bipolar cells, and via amacrine cells in the inner retina, where bipolar cells synapse onto ganglion cells. The purpose of lateral inhibition in the retina is to increase the spatial tuning of ganglion cells and thereby sharpen the image (Cook & McReynolds 1998).

1.5.1 Macula, fovea and foveola

Macula is the specialized region of the retina responsible for high-resolution vision. It is anatomically defined as the "portion of the posterior retina that contains xanthophyll and two or more layers of ganglion cells" (Berger et al. 1999). This roughly circular area is about 5-6 mm in diameter and corresponds to approximately 15-20º of the visual field (VF).

The central part of the macula, called the fovea, is typically more pigmented than the surrounding tissue and has a diameter of about 1.5-2.0 mm, equal to ~5.2º VF. In the parafovea, just outside the fovea, the retina is thickest because of multiple layers of

ganglion cell. Along the foveal slope, the axon of the photoreceptors runs radially and forms the Henle fiber layer. Within the fovea the retinal structure is modified to allow the incoming photons a more direct access to the photoreceptors. In this area, the light does not need to pass through any other retinal cell layers or blood vessels, which otherwise might absorb or scatter the incoming light.

The foveola is the most central part of the fovea, with a diameter of approximately 0.3 mm or 1º VF, and is entirely composed by cones. The cone density in this area provides high spatial resolution. The average peak density is almost 200,000 cones/mm^2 but decreases rapidly with increasing eccentricity (Curcio et al. 1990). There are three types of cones, sensitive for short, middle, and long wavelengths, i.e., to blue, green and red light respectively. The foveal cones are very thin with a diameter of approximately 2.5 μm, the thickness increases to 4-10 μm outside the fovea (Curcio et al. 1990).

1.5.2 Receptive fields

A receptive field is defined by the receptors sending information through the same ganglion cell. With increasing distance from the fovea, the number of receptors per ganglion cell increases, together with the size of the receptive field. The radius of a receptive field is in most cases larger than the distance between the ganglion cells, resulting in overlap between the receptive fields. However, this is not the case in the fovea (Fischer 1973).

The ratio between number of ganglion cells and photoreceptors is close to 3:1 within a 2-3º VF. Outside the fovea, at ~7.5º eccentricity the ratio is reduced to 1:1 and outside 19º the ratio is 1:2 (Sjöstrand et al. 1999). The ganglion cell/photoreceptor ratio is further decreased with increasing eccentricity to a relation of up to 1:125 in the most peripheral parts (Curcio & Allen 1990). This means that when the ganglion cell/photoreceptor ratio is less than 1:1 a receptive field is no longer defined by photoreceptors but by ganglion cells and the resolution is not limited by photoreceptor density but by ganglion cell density.

1.5.3 Optic nerve

The optic nerve is composed of the axons from retinal ganglion cells. The variation in number of axons is more than two fold in humans, between 750,000 – 1,500 000 (Curcio & Allen 1990). Two main classes of ganglion cells, parasol and midget cells, processes information from the retina to the lateral geniculate nucleus (LGN). Parasol cells are large and receive information from larger receptive fields and are sensitive to motion as well as changes in contrast. The smaller midget cells receive information from smaller receptive fields and respond to light of different wavelengths (colors) with fine spatial discrimination. The midget cells constitute ~80% of the total number of ganglion cells (Nicholls et al. 1992). From the LGN to cortex, the visual system processes visual information through three main types of pathways. Magnocellular (M), parvocellular (P) and koniocellular (K) pathways, each kind processing different aspects of visual information in parallel. M-cells carry information from the parasol ganglion cells, P-cells from midget ganglion cells. Little is known about the K-cells and their role in vision, it has been speculated that blue-yellow retinal ganglion cells provide input to the K-cells (Sincich & Horton 2005). P-cells, by far outnumber both M- and K-cells (Masland 2001).

The highest density of ganglion cells is found in an elliptic ring in the most central part of the retina and 50% of the ganglion cells are located within the 16º VF (Curcio

& Allen, 1990). During the embryogenesis lots of ganglion cells are lost due to apoptosis, i.e., genetically programmed cell death. Only ganglion cells with appropriate synaptic connections in the brain will remain (Quigley et al. 1990). The fetal human optic nerve has 3.0-3.5 times more axons or fibres at 16-17 weeks' gestation than in adulthood (Provis et al. 1985). Sing et al. (2000) calculated expected values of axon loss due to aging based on a review of several post-mortem studies. They argued that normal aging could account for a loss of about 25% (~275,000) by the age of 60-69 years, assuming that one started life with approximately 1.1 million initially. Further they calculated the average loss to about 5% of the axons each decade or 5,500 axons per year.

1.5.4 Lateral geniculate nucleus

The LGN in the thalamus is the major target of the retinal ganglion cell axons. It receives input from both eyes and transmits the information to the primary visual cortex via the optic radiation. On its way to the LGN the optic nerve passes through the optic chiasm where the axons from the nasal part of the retina cross over to the contralateral side. Thereafter, the axons form two optic tracts that combine input from the ipsilateral temporal hemiretina and the contralateral nasal hemiretina.

The LGN has six layers of cells. There is a very strict organization and layer 2, 3 and 5 receives information only from the ipsilateral eye, while layer 1, 4 and 6, receive information from the contralateral eye (Hubel 1988). The retinal parasol and midget ganglion cells project to two ventral magnocellular layers and four dorsal parvocellular layers of the LGN respectively. Some axons pass through the LGN and end in the colliculus superior (Nicholls et al. 1992).

The central VF project onto greater portion of each geniculate layer and there are relatively few cells devoted to the peripheral retina/VF. This neural organization reflects the psychophysical features of high-resolution central acuity and lower spatial discrimination in the VF.

1.5.5 Visual cortex

The over-representation of the fovea in the LGN can also be found in the visual cortex. In 1991 Horton and Hoyt calculated the area apportioned to central vision and suggested that the central 10º occupies at least 50-60% of the striate cortex. A few years later, McFadzean and co-workers (1994) confirmed this proposition.

The primary visual areas in the cortex, localized in the posterior part of the occipital lobe, are called visual area 1 (V1) and 2 (V2), anatomically grouped together as area striata. In V1 signals received from LGN are processed and transmitted to separate domains in V2. Higher, extrastriated visual areas known as visual areas 1-3 (V3, V4 and V5) interact with V1 and V2 and there is a substantial feed-back projection to the LGN and the pulvinar, a part of thalamus (Guillery 2005). Area V1 and V2 contain similar retinotopic maps and occupy comparable brain surfaces.

The input from both eyes are combined in binocularly driven neurons in the cortex, providing stereopsis and slightly better visual performance, usually referred to as binocular summation (Hubel & Wiesel 1962, 1968, Joshua & Bishop 1970).

1.5.6 Secondary visual areas

Neurons in area V3 are specialized for processing information relating to form. In area V4 color information is processed, whereas neurons in area V5 are specialized for detection of movement (Nicholls et al. 1992). There are several hypotheses regarding the organization of visual input or cortical projection (Wandell et al. 2007). In 1983, Mishkin and coworkers presented an idea about two cortical pathways. These two major white matter pathways were thought to carry projection from V1 through a ventral and a dorsal part of extrastriate cortex. The ventral connection enables visual identification of objects and lesions produce visual discrimination deficits, while the dorsal stream allows visual locations of objects and lesions produce deficits in spatial orientation (Mishkin et al. 1983).

1.5.7 Subcortical visual connections

Pulvinar is argued to have an important role in vision and even if its exact function is unknown it is likely to play an important role in visual information processing and visual spatial attention (Leh et al. 2008). The pulvinar is connected to various parts of subcortical and cortical areas, such as amygdala, pons, superior colliculus and the areas V1, V2, V4 and V5 (Leh et al. 2008). The subcortical visual connections allow us to be aware and react in response to visual stimuli in the spatial domain. This system intergrades sensory and motor functions, and initiates eye movements and maintains gaze control (Leigh & Zee, 2006).

1.5.8 Age related processes in the normal eye

With age comes a gradual reduction in the eye's sensitivity due to factors such as changes in pupil size, in the transmission of the ocular media and in the sensitivity of the retina and visual pathways.

Owsley et al. (1983) found reduced best corrected visual acuity (BCVA) and reduced contrast sensitivity function (CSF), especially at mid and high spatial frequencies, in older subjects. The modulation transfer function (MTF) has been used to evaluate the overall optical performance of the eye as a function of age in a normal population (Guirao et al. 1999). The reduction in image quality was thought to reflect an overall degradation in optical performance, partly caused by an increase of optical aberrations in the lens and cornea.

The relative contribution of optical versus neural factors to the deterioration of the visual function due to age is somewhat controversial. Whether the reduction is caused by reduced retinal illumination due to senile pupillary miosis and increased lens absorption, by the greater light scatter in the aged eye, or by retinal and neural cell loss and degeneration was studied by Elliott in 1987 and 1990. The results implied that the sensitivity decline at medium and high spatial frequencies is primarily due to retinal and neural changes, with optical factors having a slight effect at the highest spatial frequency only. Johnson and co-workers (1989) showed that a decline in visual field sensitivity with increasing age was not correlated with the increase of lens density, thus, neural loss rather than preretinal factors were suggested to be the primary cause of age-related visual field changes. Since visual abilities decline during normal aging it may be difficult to distinguish between normal aging and pathological changes. Neural cell loss is one of the characteristics of aging in the human retina. In a review by Brusini (2007) it was argued that if a normal optic disc is composed by ~1.2 million optic fibers, upon reaching a hypothetical age of 200 years a very limited number of retinal ganglion cells and nerve fibers would remain, resulting in a

condition of “physiological blindness”. The loss of neural tissue is also one of the essential pathologic changes in glaucoma.

Loss of photoreceptors also occurs with increasing age. Rods tend to be more affected by age than cones and Curcio et al. (1993) showed that the rod density is decreased by 30% between the age of 34 and 90 years, whereas the number of cones remained stable. Photoreceptor degeneration due to changes in RPE is a fundamental part of age-related maculopathy (ARM) and death of photoreceptors accounts for the vision loss in ARM (Jackson et al. 2002).

Functional and structural changes also occur in the aging human lens. Yellowing and increased thickening result in reduced transparency. The human lens continuous to grow throughout life and with Scheimpflug photography the lens thickness and light scattering have been shown to increase linearly with increasing age (Sasaki et al. 1999). When these changes cause visual impairment they are defined as cataract. The changes in the optical properties due to the cataract are not uniformly distributed. Posterior polar cataracts can involve a very small volume of the lens but if they are located close to or at the visual axis, their effect on vision can be significant. Cortical cataracts are generally less disturbing until late in its progression (Duncan et al. 1997). However, all types of cataract may be associated with reduced visual acuity and contrast sensitivity, as well as disability due to glare (Chua et al. 2004). The presence of lens opacities may also influence the outcome of visual field testing and complicate the interpretation of examination results. The results from conventional (Hayashi et al. 2001) as well as Frequency Doubling Technology™ (FDT) (Carl Zeiss Meditec Inc., USA), High-pass Resolution Perimetry™ (HRP) (Visumetrics and HighTech Vision, Sweden) and Rarebit Perimetry (RBP) are all influenced by lens opacities (Martin 1997, Siddiqui et al. 2005, Salvetat et al. 2007).

Cataract, glaucoma, age-related macular degeneration (AMD), and diabetic retinopathy are the most common sight-threatening conditions in the industrialized world (Jackson & Owsley 2003). Diabetes mellitus (DM) affects the eye in numerous ways, e.g., alterations in the refractive power of the lens and increased risk of devolvement of cataract are related to the disease (Rowe et al. 2000). The most important DM induced complication in the eye is diabetic retinopathy (DRP) and it is one of the main causes of visual impairment and blindness in people at working age (Fong et al. 2004). Diabetic retinopathy has features of both micro vascular occlusion and leakage due to breakdown in the blood-retinal barrier (Fong et al. 2004). The micro vascular occlusion leads to retinal hypoxia with arteriovenous shunts and neovascularization, which causes hemorrhage and edema. Severe retinopathy can occur without patient noticing it but when the edema engages the macula it affects the visual acuity (VA) (Ferris et al. 1984).

The hyperglycemia associated with diabetes, not only leads to functional and structural microvascular lesions but also induces retinal neuro-degeneration. During the last years several studies have reported retinal functional and structural changes in DM patients without advanced retinopathy, supporting the hypothesis that chronic neuro-degeneration is a component of DRP (Lieth 2000, Barber 2003).

1.6 Visual function tests

A perfect visual function test should define the degree, cause and location of visual dysfunction, something that no test alone is capable of. Visual function can be evaluated in aspects of detection, resolution, contrast sensitivity, color vision, visual field, stereopsis and adaptation to light. The results of psychophysical measurements

are influenced by cognitive factors including, e.g., subject attention, motivation, fatigue, and response bias. Characteristic for all psychophysical tests is that the frequency of correct responses is a function of the difficulty of the task. When a task gets more difficult, the test variability increases and the uncertainty of the result increases. This phenomenon occurs both in normal and abnormal vision, and can be demonstrated by frequency-of-seeing (FOS) curves (Frisén 1990). When measuring visual function there are also a number of variables that influence the test result such as involuntary eye movements and oscillations of the pupil and accommodation (Kutzko et al. 2000). To reduce the influence of fatigue and reduce testing time different screening strategies are often used. However, by simplifying a test the sensitivity and specificity may decrease. In screening methods, balance between the specificity and sensitivity has to be adjusted according to the severity of the medical condition.

1.6.1 Visual acuity

Visual acuity (VA) or the ability to resolve fine details of high contrast is probably the most studied of all sensory functions and measures the ability to discriminate two stimuli separated in space at high contrast relative to the background. The most commonly used test chart in ophthalmological and optometric settings is the letter chart. In research, the ETDRS chart has become standard (Ferris et al. 1982). Originally, the chart was used in the Early Treatment Diabetic Retinopathy Study. The ETDRS chart has high-contrast letters, displayed in a box with internal illumination. Each letter line contains five Sloan letters of equal difficulty and there is a geometric progression in letter size from line to line. Charts with different letter sequences are used for testing right and left eyes to avoid the effect of learning. However, since a decimal visual acuity of 1.0, often used as limit for normality, can be achieved with less than two-thirds of the normal number of optic nerve axons (Frisén & Quigley 1984), the letter chart test cannot be regarded as sensitive to low-degree damage. Due to the high receptor density and the proportionally large stimuli used in VA testing using letter charts are unable to detect small and subtle defects.

1.6.2 Perimetry

The visual field is the total area in which objects can be perceived while the eye is focused on a central point. The VF is evaluated using perimetry which may be kinetic, where stimuli are moved inwards the central field until the subject sees them, or static, where stimuli are projected onto a defined background and the observer is asked to respond if he or she sees it. The purpose of the VF examination is to measure the ability to resolve or detect stimuli (depending on test principle) in a number of different locations in the visual field. Perimetry has an important role in screening, diagnosing, and follow-up of various disorders in the visual system.

The Goldmann perimeter was long considered to be the gold standard. It relies on differential light sensitivity (DLS) technique, which measures the ability to detect a light stimulus against a neutral background (also called white-on-white). The Goldmann perimeter is a manual method, highly dependent on the skill and experience of the examiner and permits testing of both the central and the peripheral VF.

Computerized automated perimetry (CAP) is now the standard procedure in most ophthalmological practices. The technique relies on threshold strategies with repeated testing in each location. There are a number of automated perimeters and one of the

most commonly used is the Humphrey Field Analyzer™ (HFA) (Carl Zeiss Meditec Inc., USA). HFA uses white-on-white stimuli, but the apparatus also includes blue-on-yellow perimetry or Short Wavelength Automated Perimetry (SWAP) (Heijl & Patella 2002). The test targets used in DLS CAP, equivalent to Goldmann III-V, can be assumed to stimulate several receptive fields at a time (Garway-Heath et al. 2000) and may therefore fail to reveal small and subtle defects in the neural pathways.

Full threshold testing can be a very demanding task for subjects/patients and the test-retest variability in DLS perimetry is high (Artes et al. 2002). When white-on-white stimuli become smaller and less luminous in purpose of making the test more sensitive, they are easily confused with the internal noise in the visual system (Faisal et al. 2008). Thresholding is also a time consuming method, which makes the test less preferable in a psychophysical perspective. Supra-threshold perimetry involves presentation of stimuli above the average normal age-corrected threshold, which gives lower variability (Henson & Artes 2002), but also lower sensitivity for minor damage.

Another computerized perimetry method is the FDT, relying on contrast sensitivity and motion detection (White et al. 2000, Anderson & Johnson 2002). The FDT and the SWAP technique were both designed to be more sensitive to early glaucomatous changes than standard automated perimetry (SAP) (Ferreras et al. 2007). The HRP is another computerized method, measuring resolution thresholds. This method is unique in the sense that there is a direct correlation between resolution thresholds and retinal ganglion cell separation (Frisén 1993, Popovic & Sjöstrand 2005).

The ideal perimetric test should be accurate, repeatable, stimulate accurate fixation and have a stable relationship between the test results and the extent of underlying neural damage. Stable fixation is essential for all visual field testing and has been found to have a large influence on test reliability during perimetry (Katz & Sommer 1988, Birt et al. 1998). White-on-white is the most common form of perimetry, although histological studies demonstrate that retinal ganglion cell loss can be very high (25 to 50%) before any deficits can be revealed by this technique (Quigley et al. 1988, Harwerth et al. 1999, 2004). Therefore, new techniques have been developed with the aim to improve detection of small defects.

One of these is the Rarebit Perimetry, built on the principle of detection of very small and bright stimuli, corresponding to half the normal minimum angle of resolution in the tested retinal location (Frisén 2002).

1.7 Retina structure measurements

1.7.1 Fundus photography

The advent of fundus cameras has improved documentation and diagnosis of diseases in the retina and the optic nerve head. Digital technology has greatly facilitated fundus photography. The use of high-resolution digital fundus photographs in diagnosing and follow-up is widely accepted and considered as an accurate and reproducible method. The technique also produces objective documentation of the retinal state for future comparisons. In screening for detection and classification of diabetic retinopathy the use of fundus photographs is thought to be the most sensitive method (Hutchinson et al. 2000). However, in glaucoma diagnosis and follow-up, more advanced techniques, e.g., the Heidelberg Retina Tomograph™ (Heidelberg Engineering Gmbh, Germany) and the GDx™ (Carl Zeiss Meditec Inc., USA), for

measuring the retinal nerve fiber layer has in some settings replaced fundus photography.

1.7.2 Optical Coherence Tomography

Optical Coherence Tomography (OCT) (Carl Zeiss Meditec Inc., USA) is considered to be the most precise method for measurement of retinal thickness (Goebel et al. 2002; Massin et al. 2006). The technique, first described by Huang et al. (1991), is non-invasive and provides high resolution, cross-sectional imaging of internal structures in biological tissues by measuring their optical reflections. The technique has been described as "…an indispensable tool in clinical practice for the diagnosis and management of ocular diseases involving the macula, optic nerve and anterior segment" (Chen & Lee 2007) and "a key to the future management of patients with diabetic macular oedema" (Massin et al. 2006). Since the OCT can provide information about the internal structure of the retina it has the potential to detect pathological changes before clinical signs or visual symptoms occur (Noval et al. 2006, Subbiah et al. 2007).

1.8 The Rarebit test principle

Rarebit Perimetry is a computerized visual function test, described as "…fun, fast, robust and user-friendly" (Frisén 2002). Bright dots are presented against a dark background on a computer screen and the patient's task is to identify the number of dots seen by clicking a mouse button. The test principle is easy to understand, the results are easy to interpret, and no expensive equipment is required. The system includes two tests, Rarebit Perimetry (RBP) and Rarebit Fovea Test (RFT). The first for evaluation of the 30º visual field (with the possibility to test out to 60º), and the second for evaluation of the foveal function or the most central 4º VF. The RBP has turned out to be well tolerated among patients, from 7 years of age (Frisén 2002, Martin & Wanger 2004, Martin et al. 2008) and the test results correlate well with findings from other perimetry techniques like, HRP, HFA and FDT (Frisén 2002, Martin & Wanger 2004, Brusini 2005).

Fig.1. *Rarebit equipment*

The Rarebit technique was developed to detect small subtle defects in the neural detector system. In a first study describing and evaluating the technique, the RBP identified more defects in patients with minor chiasmal lesions compared with the HRP perimetry (Frisén 2002). In a later study, impaired RBP result was found in patients suffering a first neurological episode, suggestive of multiple sclerosis, without any visual symptoms (Frisén 2003). Further, Frisén (2004) reported that the

Rarebit technique was better suited to identify the relationship between dose and VF damage in patients treated with Vigabatrin compared to Goldmann perimetry.

As mentioned earlier, several studies have shown that conventional perimetry and vision function tests are insensitive to minor neuro-visual damage. Frisén (2002) discuss this issue in his article "New, sensitive window on abnormal spatial vision: rarebit probing" and presents explanations of the poor sensitivity of clinically available tests. These were proposed to include the reliance on empirical references for normality and the redundant amount of information contained in test targets, i.e., targets that are large in relation to the receptive fields. In Rarebit, improved detection of small defects was intended to be obtained due to two deliberate deviations from standard procedures. Target information content was minimized (i.e., small dots compared to the receptive field at the tested retinal location) and thresholding was replaced by simple probing of the completeness of the retino-cortical detector matrix.

1.8.1 Test stimuli and stimulus presentation

The size of the Rarebit stimuli is adapted to the size of the receptive fields in the tested retinal location and range from 0.5 to 6 min of arc. For comparison, the smallest spots (Goldmann I stimulus) used in clinical perimetry subtend ~6.6 min of arc (0.11°). In theory, a Goldmann I object covers some 150 cones in the center of the fovea of an average retina (Curcio et al. 1990) while the image of a Rarebit stimulus will be about as small as a single photoreceptor. The most commonly used stimulus in conventional perimetry is the Goldmann III. This must be considered as a large stimulus in relation to receptive field size, since it subtends a visual angle of approximately 25.8 minutes of arc (0.43°). Regarding RBP the stimuli used are small compared with the receptive fields, rather than with a single photoreceptor, since the RBP aims at evaluating peripheral parts of the retina.

The Rarebit test principle is to probe the integrity of the retinal architecture, which in a normal eye will be complete without overlaps or gaps. This means that individual variation in the number of photoreceptors or ganglion cells do not influence the test

Test stimulus in RBP and RFT consists of pair of dots and presentations with one dot only or none at all serves as controls (false positives) and constitutes ten percent of the total number of presentations. The use of pairs as stimulus intends to increase effectiveness in testing without making the test task to complex for patients. The distance between dots that are presented two at a time is adjusted to receptive field size in the tested area. In RBP the dots are separated by 4 degrees and in RFT by 1 degree. The stimuli presentations are divided into passes or runs. During each run, one pair of dots is presented in each location. Five runs are recommended, giving a number of presented stimulus of 240 in RBP and 100 in RFT, and thought to be a useful compromise between patients' attention span and what could be accepted as a proper evaluation of the visual function. The stimulus presentation time is short (< 200 ms) in order to prevent eye movements from sweeping receptive fields across the dot image. A presentation time of 200 ms makes re-fixation difficult (Leigh & Zee 2006). Rarebit uses high-contrast targets, i.e., supra-threshold stimuli, and tests the integrity of the retino-cortical detector matrix. This detector matrix, in fovea defined by the photoreceptor density, and peripherally by the retinal ganglion cell density, is presumed to be complete in a healthy eye and therefore normal results are expected to be very close to 100%.

1.8.2 Test design

The RBP evaluates a 30x20º (horizontal x vertical) VF with the possibility to also test the flanking regions between 30 and 60º. No stimuli are presented within the central 4º VF and instead the Rarebit Fovea Test (RFT) tests this area. The RFT covers the most central 4x3º VF. During the RBP and RFT tests, stimuli are presented in 24 and 10 areas respectively. Stimuli are randomly distributed within these locations and since they are small relative to receptive fields and displayed in ever-new position it is not likely that they would hit the same receptor twice. In RBP the rectangular areas increase in size with eccentricity from 8x6° to 14x6°. In RFT the areas are squares and about 1.5x1.5°. The average RBP test duration is about 5 minutes (Martin & Wanger 2004, Salvetat et al. 2007) and for RFT slightly more than one minute (Malmer & Martin 2005). The above given examination time corresponds to test sessions including five runs.

The test is performed on a personal computer with a high quality LCD screen with a resolution of 1280 x 1024 pixels is required, see Fig 1. The test can be calibrated for different screen sizes through a simple procedure. The standard viewing distance is set to 0.5 and 1.0 m for testing of outer and inner RBP VF, and 2m for the RFT examination.

1.8.3 Fixation target

The fixation target is animated and gives auditory and visual feedback to enhance and keep the tested subjects´ attention. In the RBP test the fixation mark flickers before each presentation to enhance attention, and moves to different locations on the screen during examination in order to test a larger area than covered by the screen. This fixation target movement also encourages fixation. The subject is instructed to maintain fixation on and follow the target. In RFT the fixation target stays in the centre of the screen during the test, but constantly flickers and makes a sound before stimulus presentation. The fixation target provides feedback to the subject. A false positive response causes an annoying sound and a red circle replaces the fixation target. A correct response generates an encouraging sound and a green circle replaces the fixation target. In order to save time and spare the examiner the difficulty of evaluating fixation data, fixation is not monitored during tests.

1.8.4 Results – presentation and interpretation

The results are expressed as mean hit rate (MHR), i.e., the number of dots seen relative to dots presented, given in a percentage format. A typical printout is shown in Figure 2.

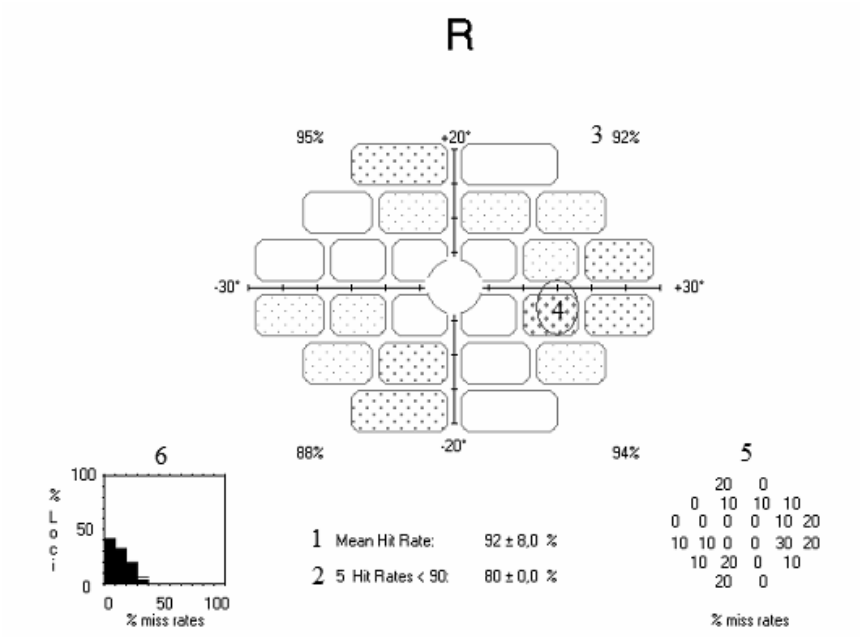

Fig. 2. *The RBP field map, divided in areas that represent all tested locations, is used to present the result. Both MHR for all locations and hit rate for each single test location are presented in a graphic format. In addition, the number of locations with a hit rate below 90% is presented. The proportion of non-perceived dots is represented by various degrees of shading. Further explanations are given for the inserted numbers 1-6 below.*

1. Mean hit rate = the mean ± SD of perceived dots i.e. the number of dots seen relative to dots presented, given in a percentage format. An asterix (*) indicates results outside normal range

2. Number of localisations with a hit rate < 90% and the mean hit rate ± SD in these locations. An asterix (*) indicates results outside normal range

3. Mean hit rate in the quadrant. An asterix (*) indicates results outside normal range

4. The blinda spot area

5. Proportion of missed stimuli in ever location

6. Statistic distribution of the result

Reliability is assessed by counting number of errors, i.e., false positive responses during the examination. In RBP one of the test areas is placed so as to at least partially overlap the blind spot and good fixation is expected to cause reduced MHR in this area. In previous studies of healthy subjects the modal numbers of erroneous responses in RBP were 0 (Frisén 2002) and 1 (Martin 2005). In a study of RFT in healthy subjects below and above 65 years of age, the numbers of errors did not exceed tree and five errors respectively (Malmer & Martin 2005).

1.8.5 Reference RBP values from normal subjects

Reference data for RBP has been presented in at least three studies and normal MHR ranges from 78-100 % (Frisén 2002, Martin & Wanger 2004, Salvetat et al. 2007). For further details see Table 1. The results from all these reports give a significant negative correlation between RBP MHR and age. Frisén (2002) reports a decline of 1% per decade while Salvetat et al. found a decline of 0.21% per year (2007).

Table 1.

	Frisén 2002 n=27	**Martin & Wanger 2004 n=54**	**Salvetat et al. 2007 n=71**
Age range	20-70	17-88	24-79
MHR (range)	Median 96% (88-100%)	Median 97% (78-100%)	Mean 91% (78-99%)

1.8.6 Reference RFT values from normal subjects

Table 2 summarizes the published reference values for the RFT (Malmer & Martin 2005).

Table 2.

	Malmer & Martin 2005 n=66	
N	47	19
Age range	22-64	65-87
Median MHR (range)	100% (97-100%)	88% (34-98%)

1.8.7 Repeatability

Salvetat et al. (2007) evaluated the RBP results from 29 normal subjects at four different occasions during a three months period. Mean variation was found to be 2.9% and no significant improvement was identified. In a study of 24 children aged 7-18 years, examined twice with an interval of two hours, the mean difference between MHR was less than 1 percentage unit (Martin et al. 2008). Since there are no published data regarding repeatability in adults examined with RFT a pilot study was performed (Nilsson et al. unpublished data) by examining eight healthy subjects (aged between 26-42 years). Initially the subjects were tested four times in a row. They were then tested twice after one hour, another two times after another hour, and finally two times after one week, see Table 3. The coefficient of variation was 1.1% and no significant difference was found between examinations (p=0.3).

Table 3.

	Baseline				**1 hour**		**2 hour**		**1 week**	
	1:st exam.	2:nd exam	3:rd exam	4:th exam	1:st exam.	2:nd exam	1:st exam.	2:nd exam	1:st exam.	2:nd exam
MHR(%) ± SD	98.9±1.4	99.5±0.5	98.3±2.8	98.5±2.5	98.8±1.0	99.4±0.9	98.5±1.2	99.5±0.5	99.5±0.5	99.6±0.5

2. AIMS OF THE PROJECT

All studies aimed to evaluate the Rarebit technique from both experimental and clinical points of views. Experimental studies were performed to study stimulus parameters and fixation behavior during the RFT. In addition two clinical studies were carried out to evaluate the possible implementation of the test in a clinical perspective.

Paper I

To establish reference data for the Rarebit Fovea Test and to evaluate the influence of luminance level, age and binocular summation on the Rarebit Fovea Test stimulus.

Paper II

To evaluate foveal function using the Rarebit Fovea Test in comparison with central retinal thickness measured by optical coherence tomography in diabetic patients without previously known retinopathy or maculopathy.

Paper III

To evaluate fixation behaviour and stability during Rarebit Fovea Test examinations.

Paper IV

To study the influence of cataract on Rarebit Perimetry and Rarebit Fovea Test by examining subjects before and after cataract extraction.

2.1 Material and methods

2.1.1 Subjects, patients and data collection

All subjects used as controls in the studies were recruited among staff and students from the Unit of Optometry, Karolinska Institutet and among staff at St Erik Eye Hospital, Stockholm, Sweden (Paper I and II). For Paper III students and staff at the University of Houston, Texas Eye Research and technology Center in Houston, Texas, USA were recruited. Inclusion criteria for all were otherwise healthy and a decimal BCVA of at least 1.0 (except the two subjects with amblyopia in Paper III). The limit for refractive errors was in Paper I set to within ±3D and in Paper II and III to within ±6D.

The patients in Paper II and IV were all referred to the Department of Vitreoretinal Diseases for diabetes screening photography and to the Department of anterior segment surgery for cataract surgery at St Erik Eye Hospital, Stockholm, Sweden, respectively. Inclusion criteria in Paper II were no known maculopathy or retinopathy and a decimal BCVA of least 1.0, measured at their last visit. In Paper IV the inclusion criteria were age $\leq$ 75 years, no previous known ocular disease other than senile cataract. Exclusion criteria were intraocular pressure > 21 mm Hg, medication known to elicit any sort of VF defects and systemic disease that could be presumed to affect VF examination over the time span for participation in the study (~2 months).

In Paper II, both eyes from all patients and subjects were examined but only the results from the right eyes were included in the statistical analysis.

2.1.2 The RFT and RBP setups used in the study

Two different versions of the Rarebit test were used, ver. 3 (Paper I) and version 4 (Paper II-IV). RFT ver. 3 evaluates a 3x3º VF by using both vertically, horizontally and oblique pairs of microdots. Later the test was modified by the inventor and RFT ver. 4 evaluates a 4º horizontal and a 3º vertical VF using only vertically and horizontally presented pairs of test stimuli. These modifications were done to avoid the so-called oblique effect (see program handbook for more information).

Two different LCD screens were used for stimulus presentation, a 15" ViewSonic™ (Paper I) and a 17" Eizo FlexScan™ (Paper II and IV). The first screen used was replaced by a screen with better performances, regarding contrast settings and a more even distributed luminance. Since the experiments in Paper III were performed in another setting (Houston US), an additional screen, a 17" Dell Ultrasharp Genesis™ had to be used. Screen parameters are presented in Table 4.

Table 4.

	ViewSonic VX500	**Eizo FlexScan L568**	**Dell 1707FP**
Display Area	15" (H304mm x V228mm)	17" (H270 x V338mm)	17" (H270 x V337mm)
Pixel Pitch	0.297 mm	0.264 mm	0.264 mm
Contrast Ratio	400:1	1500:1	600:1
Brightness	300 cd/m^2	250 cd/m^2	300 cd/m^2

In Paper I the RGB settings were adjusted to create four new test versions of RFT (ver. 3) in addition to the original version, each with different brightness in the test stimuli. The original settings, given in the program manual correspond to a stimulus luminance of 150 cd/m^2 and a background luminance of ≤ 1.0 cd/m^2. The modified levels used in Paper I were set to 64, 53, 41 and 33 cd/m^2 respectively and the background was set to slightly less than 1 cd/m^2. In Paper II, contrast settings on the screen were modified to give a stimulus luminance of 64 cd/m^2, this setting gave a background luminance < 0.1 cd/m^2. The same settings were used in Paper III. In Paper IV, using both RBP and RFT, the luminance was set close to the original levels.

2.1.3 Additional examinations in the study

2.1.3.1 Photographic methods (Paper II)

Fundus photography

In Paper II fundus photographs were obtained through a dilated pupil using a Zeiss FF450^{plus} IRu ™ (Jena, DE, Germany) camera. Four 50° photographic fields per eye were taken, two centered on the fovea and two on the optic disc. The images were captured in both colour and in red-free black-and-white. Visupac 3.5 Software™ (Pirmasens, DE, Germany) and a high-resolution screen was used for the diagnostic evaluation of the digital images.

2.1.3.2 Grading of diabetic lesions (Paper II)

Four images per eye were analyzed and classified by one of the authors (GvW) of Paper II, based on the ETDRS protocol criteria (von Wendt et al. 1999). All images were classified (for further description see, von Wendt et al. 1999) in 14 stages/levels for diabetic retinopathy and 9 stages/levels for diabetic maculopathy and other

macular lesions. For comparison with the RFT findings, the following categorizes were used: minimal and mild retinopathy, mild maculopathy and other macular lesions or drusen. The last category included drusen and age-related pigment epithelial defects.

2.1.3.3 Optical Coherence Tomography (Paper II)

The macular thickness was measured with optical coherence tomography using the Stratus OCT™, model 3000 (Carl Zeiss Meditec Inc., USA) through a dilated pupil. For each eye, six radial lines (6 mm in diameter) were obtained in a pattern, centered on the fovea. A Retinal Map Analysis Report™ was performed, dividing the macula image into nine regions according to ETDRS (Early Treatment Diabetic Retinopathy Study Research Group 1991). The map contains a central circular area with a diameter of 1000 μm surrounded by two concentric ring-shaped areas, each divided into four quadrants.

2.1.3.4 Scheimpflug photography (Paper II)

The lens thickness and light scatter were evaluated by Scheimpflug photography. The examinations were performed using the Nidek, EAS–1000 ™ (Nidek Inc, USA), after pupil dilation. The lens images were analyzed using the incorporated program with regard to thickness and light scatter. It was not possible to see the posterior lens capsule in all subjects, usually due to insufficient dilation of the pupil. Therefore, the distance from the anterior lens capsule to the central clear zone (zone A-E) was used as a surrogate measure of lens thickness (Sasaki et al. 1999). The lens light scatter was measured in the anterior adult nuclear area, including a part of the deep cortical layer. In a healthy lens this part generates most light scatter and it was assumed that this layer should have the largest influence on the overall optical quality of the lens. The light scatter was assessed from the 8 bit grey scale in the Scheimpflug images, i.e., values from 0 to 255. Higher values implies increased scatter.

2.1.3.5 Recording of eye movements (Paper III)

To evaluate the fixation stability in the third study a three-dimensional eye tracker, also called Dual-Purkinje-Image (DPI) eye tracker, was used (Crane & Steele 1985). The system has a resolution slightly better than 1 arcmin and a sampling frequency of 120 Hz was used under the experiments in Paper III. Eye movements were recorded during two different sessions. Subjects were initiated to fixate a target, just like the fixation stimulus used in RFT, except it was not animated, during approximately 90 sec. In addition, all subjects were instructed to perform the RFT test while eye movements were tracked. During this session, target was animated, stimuli were presented and audio-visual feedback was given as under a “true” test session.

Before each tracking session the DPI system was calibrated with a successive two point fixation task and focus and vergence angle were adjusted to a 2m distance for each subject. An incorporated Badal-optometer was used to correct spherical refractive errors. Before the data was statistically analyzed all eye movement recordings were checked by one of the authors (SBS) and measurements judged as artifacts were discarded from further analysis.

2.1.3.6 Cataract surgery and intraocular lenses (Paper IV)

The cataract surgery in Paper IV was performed by one of the authors (CGL), using a corneal incision of less than 3 mm, capsulorhexis, phacoemulsification of the lens nucleus, and implantation of an acrylic foldable intraocular lens (IOL) in the capsular bag. There were no peri- or post operative complications.

2.1.4 Calculations and statistical analysis

The GraphPad InStat™ version 3.00, SPSS™ statistical programme and Excel™ were used for calculations in Paper I-IV. For comparisons between normally distributed data, Student paired t-test (Paper I, III and IV), un-paired t-test (Paper II and III) and one-way ANOVA (Paper II) were used and for non normally distributed data the Friedman Test (Nonparametric Repeated Measures ANOVA) (Paper I) and the Wilcoxon matched pairs signed ranks test (Paper IV). Spearman Rank Correlation Test was used in Paper I, II and IV and the Fisher's exact test in Paper II.

A p-value of ≤ 0.05 was regarded as significant in Paper I-III, in Paper IV Bonferroni correction was applied to compensate for multiple tests and a p-value of ≤ 0.004 was regarded as significant.

Binocular summation was presented as the increase ratio relative to best monocular result.

Visual acuity values in Paper IV were transformed into LogMar-values when used in statistical calculations.

2.1.5 Ethics

Informed consent was obtained from all patients and subjects before enrolment. The study was approved by the local ethical committee and performed according to the Helsinki declaration.

2.2 Results

2.2.1 Paper I

In this study two experiments were performed in order to investigate the physiological properties of the RFT test stimulus. The influence of changes in target luminance on the RFT result and the effect of age and binocular summation on the RFT result were evaluated. The target luminance could be reduced with more than 50% without getting a reduced mean MHR in normal controls. Reducing the stimulus luminance from 158 cd/m^2 to 53 cd/m^2 resulted in a reduced mean MHR. However, a reduction to 64 cd/m^2 and below revealed an age effect. A significant negative correlation between MHR and age at luminance level 64 cd/m^2 was found ($r=0.44$ $p=0.008$). Normal values for each luminance level is presented in Table 5 (data are not presented in the article).

The effect of binocular stimulation compared with monocular was studied on the lowest luminance setting, i.e., 33 cd/m^2, in order to avoid the ceiling effect. MHR improved with factor 1.54 when subjects were allowed to use both eyes. Mean MHR obtained monocularly was 62.5% in right eyes, 61.7% in left eyes, and 92.5% with both eyes. To calculate the effect of binocular summation, highest value from monocular stimulation was compared with the binocular value in each subject.

Table 5.

	Luminance level				
	158 cd/m^2	64 cd/m^2	53 cd/m^2	41 cd/m^2	33 cd/m^2
Median MHR (%) (range)	100 (97-100)	99 (80-100)	96 (71-100)	88 (47-100)	66 (17-97)

2.2.2 Paper II

In this study RFT results were compared with fundus photography, optical coherence tomography and Scheimpflug photographs. This was done in order to evaluate the ability of the RFT to detect subtle defects of the foveal function in patients with DM without previously known maculopathy or retinopathy and BCVA ≥1.0. DM subjects had reduced mean MHR (96%) compared to controls (99%). A cut off for normal RFT value was set to MHR > 96% and significantly (p= 0.007) more DM subjects (12/42) showed subnormal or abnormal MHR compared to controls (2/42). RFT results did not correlate with any of the other parameters but the DM group with MHR < 97% had reduced retinal thickness measured in the inner OCT zone compared with controls ($p<0.05$).

2.2.3 Paper III

Fixational behaviour was evaluated both during a RFT test examination and during fixation of a fixation target, without stimulus presentation in a group of 12 normal subjects and two amblyopes. Five out of 14 subjects had abnormal RFT results, i.e., MHR between 57-86 % and two subjects had borderline results, 95% and 96% MHR. There were no significant difference in fixation stability between the subjects with abnormal (n=5) and normal/borderline MHR (n=11, p=0.55). The group of subjects with abnormal results included one of the amblyopic subjects, while the other amblyopic subject had a normal result. Mean SD in fixation stability during the RFT examination was 0.16º±0.09º SD. The fixation stability during the session without stimulus presentations did not differ from the stability values obtained during the RFT examination (p=0.79).

2.2.4 Paper IV

Twenty-five patients were examined before and after cataract extraction with RBP and RFT. The RBP mean MHR result improved from 69.8% (SD ±25.6%) prior to surgery to 90.9% (SD ±8.3%) ($p<0.001$) after surgery. The RFT mean MHR increased from 26.2% (SD ±31.7%) to 89.8% (SD ±11.5%) post surgery. Seven patients had good decimal BCVA, i.e., ≥ 0.8 and RBP (83-99%) but low RFT (0-66%) before surgery.

2.3 Discussion

The findings in the first study (Paper I) were in line with the results from Malmer & Martin (2005), both groups of subjects gave a median MHR of 100% and a range of 97-100% when they were examined with a stimulus luminance of ~150 cd/m^2. However, since reduced luminance to a level of 64 cd/m^2 could identify an age effect without reducing the mean result of the group it was assumed that this might be a more suitable setting to use when looking for small defects in the retino-cortical detector matrix in younger patients. For demonstration of the binocular summation an even lower stimulus luminance had to be used in order to avoid a ceiling effect, which would reduce the expected increase in hit rate from binocular stimulation. The summation factor has been reported to be larger in detection than in resolution tasks (Frisén and Lindblom 1988). The summation factor identified in this study (1.54) agrees with the hypothesis that visual input is transmitted in two channels with uncorrelated noise, i.e., the optic pathways, and ends at a common target in the primary visual cortex (Campbell & Green 1965), where a large proportion of the neurons receives input from both eyes (Read 2005).

In the second study (Paper II) subnormal hit rate was observed in a subgroup of diabetic patients without visible diabetic changes. This finding supports the idea that neural degeneration may occur independently of the vascular changes in diabetes. Traditionally, most of the scientific attention has been focused on retinal vascular changes in DM patients. However, also retinal neurodegeneration has been considered to be an important component of DRP. Review articles by Leith et al. (2002) and Barber (2003), highlights the neurodegenerative aspects and reports on studies showing early neurophysiological changes soon after the onset of diabetes. Further they provide evidence for that vascular permeability and neuronal apoptosis are closely linked components. Both structural and functional changes have been demonstrated in DM patients without or with minor DRP. Chihara et al. (1993) detected nerve fibre layer defects by red-free photography in 6 out of 30 patients without microaneurysms and in 8 out of 40 patients with microaneurysms as the only sign of vascular disturbance. Biallosterski et al. (2007) identified decreased retinal thickness in the pericentral part of the retina in patients with minimal DRP. In addition multifocal ERG can quantify changes in retinal function in the absence of clinical features (Palmowski et al. 1997).

Since both normal ageing and DM is associated with neurodegeneration (Spear 1993, Barber 2003), neural loss could be part of the explanation for the reduced RFT hit rate in older subjects as well as in a subgroup of DM patients.

In Paper III, fixation was found to be very stable during RFT test sessions in young healthy subjects and also in subjects with reduced MHR and/or amblyopia. This indicates that the animated fixation target, giving audio- visual feedback used in RFT manage to stimulate good fixation. Accurate fixation is essential in perimetric methods, which rely on thresholding, where target of different size (HRP) luminance (DLS) or contrast (FDT) are repeatedly presented at the same retinal location. In RFT the design is to present the stimuli at ever new locations. The brief presentations at randomly selected test locations imply that minor changes in gaze direction are unlikely to improve the test results (Leigh & Zee 2006). The subjects with poor hit rate also showed stable fixation. Hence it can be assumed that inability to perceive all stimuli presented does not influence the ability to maintain steady fixation.

The fourth study (Paper IV) showed that cataract had a proportionally larger influence on RFT findings than on VA. Seven patients, had good decimal VA, i.e., ≥ 0.8 and RBP (83-99%) but low RFT (0-66%) before surgery. Thus, RFT seems to be more

sensitive to optic blur caused by cataract than RBP and VA. After surgery 19 out of 24 subjects showed normal results on RBP and RFT, indicating that an IOL is no hindrance for the use of the Rarebit tests. Of the five subjects with abnormal findings after surgery, two had minor macular lesions, two had borderline performance and one was referred to further investigation because of a quadrant VF defect. In one subject no reason was found for the reduced MHR. In the study no attempt was made to compare the patients´ subjective visual problems with RFT or RBP findings. However, the subjects with good VA, in whom cataract surgery was judged to be motivated, the RFT results were very abnormal (hit rate <66%). In one case only, the reverse finding was observed; hit rate 94% and VA 0.4.

The hitherto performed studies indicate that the RFT may provide clinically useful information about visual function, different from findings from other tests. The test is simple and fast and may easily be integrated in clinical practice. The test principle, using very small stimuli similar to the test targets used in RFT, has been used in another study. Makous et al. (2006) used adaptive optic to control the size of the image of the stimulus on the retina. Their stimulus size was 0.75´, somewhat larger than the RFT stimulus (0.45´). They studied one subject with congenital dichromacy, lacking approximately one third of the cones. The technique, with brief flashes of the small stimulus, revealed a ~30% reduction of function. Interestingly, despite the loss of one third of the cone mosaic, ophthalmic examination revealed no abnormalities beside the dichromacy. VA was 20/16, standard visual fields normal and funduscopy revealed no pathologic changes. These results agree with the findings in the current studies, and support the idea behind the RFT, i.e., that small stimuli might be useful for detecting small defects in the neuro-retinal architecture and can be expected to give additional information about the visual system, compared to findings from conventional tests, e.g., VA and funduscopic examinations.

In conclusion, the RFT apparently reveals early or minor disturbances of visual function in common disorders, such as diabetes and cataract, and can be expected to be of value in the selection of patients for current or upcoming treatments, e.g., for age-related macular degeneration, where early identification of damage is relevant and sensitive methods for follow-up are required. Corroborative studies are obviously needed and can be performed quite easily, since the equipment is inexpensive and the test method very straight-forward. Thus, the integration of the Rarebit technique in optometric practice would be an easy task.

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