MEASURING AND MODELLING FORWARD LIGHT SCATTERING IN THE HUMAN EYE

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for the degree of Doctor of Philosophy,
in the Faculty of Life Sciences.

2015

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Optometry
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Figure 3.1: HS wavefront sensor detector. A HS detector is composed of an array of microlenses that analyse an incident wavefront by focusing the light passing through each one of the microlenses on a HS image. In case of an optically perfect eye, a plane wavefront projected into the eye would leave the eye (after a double pass) still being a plane wavefront. However, diffraction, aberrations and intraocular scatter in the eye will modify the pattern of an incident plane wavefront to a distorted wavefront (after the double pass). Incident plane wavefronts create HS images with clear dots while distorted wavefronts create HS images with dots and a distribution of light around those dots as a consequence of diffraction, aberrations and light scatter.

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Equation 1-3: Local parameter (LP) calculation (also called neighbourhoods) (Perez Sanchez, 2009).

Equation 1-4: Global scatter parameter (GSP) calculation (Perez Sanchez, 2009).

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<tr>
<td>BAT</td>
<td>Brightness Acuity Tester</td>
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<tr>
<td>BLS</td>
<td>Backward light scatter</td>
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<tr>
<td>BS</td>
<td>Beam splitter</td>
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<tr>
<td>CIE</td>
<td>Commission Internationale de l’Eclairage</td>
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<tr>
<td>COA</td>
<td>Coefficient of agreement</td>
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<tr>
<td>CS</td>
<td>Contrast sensitivity</td>
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<td>CSF</td>
<td>Contrast sensitivity function</td>
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<td>DP</td>
<td>Double pass</td>
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<td>ESD</td>
<td>C-Quant standard deviation</td>
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<td>FLS</td>
<td>Forward light scatter</td>
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<td>GRIN</td>
<td>Gradient index media</td>
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<tr>
<td>GSP</td>
<td>Global scattering parameter</td>
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<td>HS</td>
<td>Hartmann-Shack Aberrometer</td>
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<tr>
<td>LASEK</td>
<td>Laser assisted Subepithelial keratomileusis</td>
</tr>
<tr>
<td>LASIK</td>
<td>Laser assisted in-situ keratomileusis</td>
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<tr>
<td>LOCS</td>
<td>Lens Opacities Classification System</td>
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<tr>
<td>LP</td>
<td>Local parameter</td>
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<td>Max_SD</td>
<td>Maximum standard deviation</td>
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<tr>
<td>MTF</td>
<td>Modulated Transfer Function</td>
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<tr>
<td>OQAS</td>
<td>Optical Quality Analysis System</td>
</tr>
<tr>
<td>OSI</td>
<td>Objective scatter index</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethyl Methacrylate</td>
</tr>
<tr>
<td>PRK</td>
<td>Photorefractive keratectomy</td>
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<tr>
<td>PSF</td>
<td>Point Spread Function</td>
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<tr>
<td>Q</td>
<td>Quality factor of the psychometric function – C-Quant</td>
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<tr>
<td>RPG</td>
<td>Gas permeable rigid lenses</td>
</tr>
<tr>
<td>RK</td>
<td>Radial keratotomy</td>
</tr>
<tr>
<td>SD/ESD</td>
<td>Standard Deviation</td>
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<tr>
<td>VA</td>
<td>Visual Acuity</td>
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<tr>
<td>VDB</td>
<td>van den Berg Straylight meter</td>
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<tr>
<td>VIF</td>
<td>Variance inflation factor</td>
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ABSTRACT

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DEGREE Doctor of Philosophy – Optometry
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BACKGROUND: Intraocular scatter is an important factor when considering the performance of the human eye as it can negatively affect visual performances (e.g. glare). However, and in contrast to other optical factors that also affect vision such as high order aberrations, there is currently no efficient method to measure accurately and objectively the amount and the angular distribution of forward light scatter in the eye. Various methods and instruments exist to assess forward light scatter (FLS) but the relation between these methods has rarely been quantified. In addition, FLS measurements obtained with existing instruments cannot be related to any physiological factors due to the absence of a valid model.

PURPOSE: To investigate the relations between some of the main methods to measure forward light scatter, and to develop an experimental set-up for the objective measurement of forward light scatter that could be ideally related to physiological parameters.

METHODS: After a short review of intraocular light scatter, the three main methods used to assess forward light scattering are compared. In this sense, the C-quant (CQ) straylight meter is compared to the van den Berg (VDB) straylight meter and the Hartmann-Shack spot pattern analysis obtained from the Hartmann-Shack aberrometer. The potential of the new Oculus Pentacam functionalities for providing information on backward light scatter (BLS) are also investigated. Finally, an innovative prototype for objective assessment of intraocular light scattering together with a scatter model of the eye is presented.

RESULTS and DISCUSSION: Although no significant relationship was found between the different instruments considered (VDB straylight meter, CQ, Pentacam), our results allowed us to clarify some possible confusion introduced by previously published results and to illustrate the fact that existing commercial instruments such as aberrometers and the Pentacam cannot be used to measure FLS without at least some major modifications (hardware or software). Preliminary results with the prototype built in this study suggest that it could be used for the objective measurement of intraocular light scatter. Relating this measurement to physiological parameters stays however elusive, a fact that widens the future scope of this research.
DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of The University of Manchester, or any other university or institute of learning.

THESIS FORMAT

This thesis is presented in ‘Alternative Format’. The decision to present the thesis this way was taken as several of the chapters featured here had already been either published, or prepared for submission to peer-reviewed journals. Where manuscripts based on these chapters have been published, or submitted for publication in a refereed journal it is indicated on the first page of the chapter. The author’s contribution to the work presented in each chapter is also identified on the first page of each chapter.
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1. INTRODUCTION

1.1 OVERVIEW

The main optical factors degrading the quality of the retinal image, and hence our vision, are diffraction, aberrations and scatter. Diffraction is an optical phenomenon related to the finite size of the pupil and thus inherent to any optical system. For the human eye, diffraction is a limiting factor when the pupil diameter is approximately less than 2.4 mm (Charman, 1991, Rovamo et al., 1998). Aberrations, usually divided into monochromatic and polychromatic aberrations (Donnelly and Applegate, 2005), can be measured efficiently using different wave front sensing technique (e.g. Hartmann-Shack (Miranda et al., 2009), pyramid (Mierdel et al., 2001), curvature sensor (Diaz-Douton et al., 2006)). Among them, low order aberrations can be easily corrected by various means including spectacles, contact lenses, intraocular lenses or refractive surgery. Light scatter is due to small scale inhomogeneities within the ocular media, whose refractive indices with respect to the surrounding media modify the original trajectory of the light inside the eye. These inhomogeneities will affect light propagation within the eye and degrade the retinal image quality. An example of such inhomogeneities is the activation of keratocytes in laser surgery. When quiescent, their refractive index is similar to the surrounding stroma. However when activated, their composition changes and therefore their refractive index. As a result of this change, light is deviated and does not participate “correctly” (according to the optical ray tracing) to the retinal image’s formation process.

Intraocular scatter levels play an important role next to visual acuity in defining our overall visual performance (Donnelly and Applegate, 2005) and many studies that were published during the last decades presented possible clinical applications, from controlling
the cataract progression to diagnosing different types of ocular pathology (see the following review papers as reference (Pinero et al., 2010, van den Berg et al., 2013)). However, the objective and accurate assessment of intraocular light scatter remains a difficult task when it is compared to the measurement of aberrations and diffraction. In addition, measurements obtained with existing instruments cannot be related to any physiological factors. Obtaining accurate and reliable measurements, and being able to relate them to the source of scatter through a model, would allow for a better understanding and assessing of how various factors (e.g. old age, ocular pathologies) may lead to a decrease of visual performances (e.g. disability glare) through increased scatter. The development of a prototype for objective measurement of forward light scatter (FLS) and the creation of a numerical eye model to relate the experimental data to physiological parameters is therefore the main objective of this PhD research.

This first chapter intends to give a review of the work that has been done in the field, clarify important concepts, summarize the scatter structures within the human eye and main techniques used on the assessment of that intraocular scatter that were necessary for the research conducted during this PhD project.

1.2 WHAT IS INTRAOCULAR LIGHT SCATTER?

As stated above, ocular scatter can be defined as the change in the trajectory that light experiences inside the eye due to the presence of inhomogeneities within the ocular media. Another definition of intraocular light scatter is “the percentage of reflected, diffracted or refracted light along the optical media of the eye due to the differences in
refractive index of particles in the trajectory of the incident beam” (Donnelly and Applegate, 2005).

Intraocular scatter is usually divided in two different categories, depending on the change in trajectory of the incident light. When the angle of deviation is less than 90°, it is called forward light scatter (FLS). FLS reaches the retina and produces a veiling luminance (also called straylight), reducing in this way the contrast of the retinal image and possibly the visual performance (Donnelly and Applegate, 2005). On the contrary, when the deviation angle is more than 90°, light does not reach the retina. In this case, the phenomenon is called backward light scatter (BLS).

![Types of scatter produced by different sizes of particles](image)

**Figure 1.1:** Types of scatter produced by different sizes of particles: Scatter produced by small particles whose size is less than 1/10 the size of the incident wavelength) is principally Rayleigh (A) and produces a homogenous scatter pattern around the particle. For larger particles than the incident wavelength Mie scatter takes place (B and C). The Mie scatter pattern produced by large particles is narrower in forward direction when the particle is larger (C).

Physically, intraocular scatter can be complex since it depends on the shape, size and refractive index of the scatter structures with respect of their surrounding media. The shape of these elements is usually considered to be spherical in order to use Mie scatter modelling solutions (Mie, 1908) and the associated approximations. When the size of the particle is close to the wavelength, Mie scatter approximation takes place and the distribution of scattered light is predominantly forward (Figure 1.1-B and Figure 1.1-C).
When the scatter sources are much smaller than the wavelength of light, the Rayleigh approximation can be used. Rayleigh scatter occurs in both forward and backward directions (Figure 1.1-A). Alternatively, if the size of particle is much larger than the wavelength, the light is scattered predominately in backward direction (30 ± 180°) and a geometrical approximation can be used. Mie scatter is precise for spherical particles of any size, while Rayleigh only for very small particles, spherical or not, with a size less than one tenth of the incident light wavelength (Bohren and Huffman, 2007). Intraocular scatter is due to the scatter contribution of different type of particles and so can be described as a combination of Mie and Rayleigh scatter (van den Berg et al., 1991, Whitaker et al., 1993). Rayleigh scatter has been observed in the nuclei of excised human lenses, however, scatter in the eye is dominated by large size particles with less wavelength dependency (van den Berg, 1997). Scatter of light for a fixed particle size depends on wavelength, but only a weak relationship has been found in the human eye (Whitaker et al., 1993). While Rayleigh scatter is wavelength dependent (1/λ^4), Mie scatter is almost independent. Table 1.1 shows the different approximations that can be applied to each type of particle.

<table>
<thead>
<tr>
<th>METHOD TO CALCULATE LIGHT SCATTER</th>
<th>SHAPE OF PARTICLE</th>
<th>SIZE</th>
<th>SCATTER ANGLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mie</td>
<td>Spherical</td>
<td>Any size</td>
<td>Mainly 0 ± 30°</td>
</tr>
<tr>
<td>Rayleigh</td>
<td>Spherical or non-spherical</td>
<td>&lt;1/10 size of incident light wavelength</td>
<td>30 ± 180°</td>
</tr>
<tr>
<td>Geometrical approximation</td>
<td>Non spherical</td>
<td>&gt;1/10 size of incident light wavelength</td>
<td>0 ± 180°</td>
</tr>
</tbody>
</table>

*Table 1.1: Methods to evaluate light scatter (Bohren and Huffman, 2007).* While Mie scatter approximation is only valid for spherical particles of any size, Rayleigh approximation can be used only for particles whose size is less than 1/10 the size of incident wavelength. In case of particles whose size is larger than 1/10 the size of the incident wavelength, a geometrical approximation is applied which consist in a combination of both Mie and Rayleigh approximations. With respect to the scatter angle produced by the particles, Mie approximation considers a scatter pattern which is predominantly in forward direction, while Rayleigh is in backward direction.
When a ray of light passes through a particle inside the eye, that ray can pass the particle without modifying its trajectory or be scattered. The overall light scattered is proportional to the intensity of the incoming light and its angular distribution will depend on the scatter source (size, shape, refractive index and spatial distribution). Light has also an associated energy. This energy can be partially or totally absorbed by a particle the light is passing through. Different ocular structures have been found to absorb determined wavelengths (Miller et al., 2005).

In normal and healthy human eyes, FLS represents 1-2% of incident light (Vos, 1984). Figure 1.2 shows the differences between scatter light produced by large particles (Figure 1.2-A) and small particles (Figure 1.2-B).

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size of particle</strong></td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td><strong>Intensity pattern</strong></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td><strong>Size of angle scatter</strong></td>
<td>Narrow</td>
<td>Wide</td>
</tr>
<tr>
<td><strong>Signal intensity</strong></td>
<td>High</td>
<td>Small</td>
</tr>
</tbody>
</table>

*Figure 1.2: Scatter light produced by large (A) and small particles (B). Large particles scatter light giving a narrower pattern than small ones. Signal intensity from small particles is however smaller than for large particles.*
The angular dependence of intraocular scatter and its impact on our vision is usually described using the Stiles-Holladay equation:

\[ L_{eq} = k \cdot E_{glare} \cdot \Theta^{-n} \]  

(Vos, 1984)

**Equation 1-1: Disability glare formula.** \( L_{eq} \) is the equivalent veiling background (cd/m²), \( E_{glare} \) is the illuminance upon the pupil produced by the glare source (lux). \( \Theta \) (degrees) represents the angle between the direction of viewing and the glare source. \( k \) is a constant that varies with age.

Where \( L_{eq} \) is the equivalent veiling background (in cd/m²) and \( E_{glare} \) is the illuminance due to the glare source in the plane of the pupil (in lux). \( \Theta \), in degrees, is the angle between the scatter source and the direction of viewing; \( k \) and \( n \) are constants that vary in different eyes. The constant \( k \) has a value of 10 for a healthy eye according to the Stiles-Holladay approximation (Holladay, 1926, Stiles, 1929). However, more recent work has shown its dependency on age (Vos, 1984, Hennelly et al., 1998, Vos, 2003).

### 1.3 INTRAOCULAR SCATTER, GLARE AND STRAYLIGHT

Glare can be defined as an excessive contrast of light on the scene, or an inappropriate distribution of light that limits the ability to distinguish details and objects (The Commission Internationale de l’Eclairage (CIE) (http://www.cie.co.at, 2014)). Glare is sometimes also defined as the reaction of the eyes to a greater luminance in the visual field than the luminance to which the eyes are normally adapted to. This excess of luminance may cause discomfort (discomfort glare), annoyance, or loss in visibility and visual performance (disability glare) (Van den Berg et al., 2010). Discomfort glare provokes a disagreeable sensation without reducing the performance necessarily (Vos,
Disability glare occurs when the incoming light from the bright source is scattered by the ocular lens, forming a veiling luminance over the retina, reducing the contrast of the final retinal image or even producing almost total blindness when high intensity glare sources are positioned near the eyes (Vos, 1984, van den Berg, 1995). The effect of disability glare is mainly due to intraocular scatter of light (van den Berg et al., 2009b). Thus, the evaluation of disability glare through the assessment of straylight became a CIE standard (Vos, 1984).

1.4 INTRAOCULAR SCATTER AND CONTRAST SENSITIVITY

Contrast sensitivity (CS) is another important factor used to evaluate vision performances. CS determines the lowest level of contrast that can be detected for a patient for a fixed stimulus size. CS is thus different from visual acuity (VA). CS depends on the size and the contrast of the target while VA depends only on the size since the contrast of the target is always fixed (black and white or 98% to 100%). For this reason, a score of 20/20 in Snellen test, which means a normal VA, does not necessarily mean a good quality of vision or visual performance.

CS can be estimated through different methods. A good correlation was found between low contrast sensitivity scores across a range of spatial frequencies and intraocular light scatter (Barbur et al., 1999, Aguirre et al., 2007). This result is not surprising since the main effect of FLS is the reduction of contrast of the retinal image. In this context, glare tests which assess the reduction of contrast of the retinal image could be seen as an indirect way to assess FLS. In this sense, the agreement between a measure of glare and FLS
(results from the van den Berg straylight meter being taken as gold standard) has been investigated for various glare test. The best agreement was obtained when using the Pelli-Robson chart together with the Brightness Acuity Tester (BAT) (glare test) in the presence of a glare source (Elliott and Bullimore, 1993).

1.5 SCATTER AND POINT SPREAD FUNCTION

Point spread function (PSF) is the distribution of light obtained in the retina from an ideal point source of light. The PSF describes the quality of the visual system, and as stated above, is degraded by aberrations, diffraction and light scatter. Two different scenarios illustrating a perfect (Figure 1.3-a) and a degraded (Figure 1.3-b) PSF are depicted in Figure 1.3.

![Figure 1.3: Retinal PSF from a point light source. A shows the image from that point in ideal conditions while B shows the image from that point considering aberrations, diffraction and scatter.](image)

The PSF can be related mathematically to intraocular light scatter (van den Berg et al., 2009a). In these calculations, the area within 1±90° around the centre of the PSF is affected by intraocular scatter and receives around 10% of the light reaching the retina (Vos, 1984, Franssen et al., 2007). Equation 1-2 relates straylight and PSF between 1-90° (van den Berg, 1995).
\[ s(\Theta) = (\Theta)^2 \times \text{PSF}(\Theta) \] (van den Berg, 1995)

*Equation 1-2: Relationship between straylight and PSF.*

Where “s” is the straylight parameter (in degrees\(^2/sr\)) and \( \Theta \) is the angle subtended between the glare source and the direction of viewing in degrees. The size of the pupil only affects the parameter “s” if the ocular media is not homogeneous (van den Berg, 1995).

Figure 1.4 shows a graph corresponding to the relationship obtained between PSF and scatter angle for a subject (Ginis et al., 2012).

*Figure 1.4: Relationship between PSF (Log (PSF)) and the scatter angle \( \Theta \) (in degrees) obtained for a subject using a double pass system (Ginis et al., 2012). As it can be seen on the figure, Log(PSF) becomes smaller as the measured scatter angle increases.*
1.6 SOURCES OF SCATTER

As previously stated, the amount of light forwardly scattered by the human eye (Figure 1.5) correspond to approximately 1-2% of incoming light (Vos, 1984). For a healthy eye, the contribution of FLS by the cornea is up to 30% (Vos and Boogaard, 1963) and 5% more for iris and scleras from lightly pigmented eyes (van den Berg, 1995). The contribution of the crystalline lens is around 40% though this value can be much higher in case of advancing age or cataract (Bettelheim and Ali, 1985). The retina contributes up to 20% (Delori, 2004). Floating particles in the vitreous increases the amount of light scatter in case of symptomatic eyes (Castilla-Marti et al., 2015). Since the intraocular scatter is mainly due to the cornea, the iris, the lens and the retina, we review their respective scatter structures in the following subsections as it will be useful to develop a numerical model of intraocular scatter.

![Figure 1.5: Light scatter structures of the human eye. Major light scatter structures are the cornea (30% of the incident light), crystalline lens (40%) and the retina (20%). Light scatter through the iris in light pigmented eyes and through the sclera can be up to 5% of the incident light.](image-url)
1.6.1 CORNEA

The cornea can be divided in 6 different layers (Figure 1.6). The layer situated next to the tear film is the epithelium, with a thickness of approximately 40µm. The more internal cells of this layer are attached to the basement membrane (0.05µm thickness), which is over the Bowman’s layer (10µm). Bowman’s layer is formed by a collagen matrix with no cells. The stroma (500µm thick) occupies 90% of the corneal thickness and is situated between the Bowman’s layer and the Descemet’s membrane. Behind the Descemet’s membrane is the endothelium, a single layer of cells. Both, the Descemet’s membrane and the endothelium have a thickness of 10µm (Freund et al., 1986). The stromal cornea is composed of about 250 lamellae with 2µm thickness each (Hogan et al., 1971). Lamellae are made of parallel collagen fibrils with a thickness of 0.025 µm each and a refractive index of 1.47. Lamellae have regular shape and size and they are floating in a matrix of refractive index 1.354 (Maurice, 1969). Fibrils extend across the cornea and those from lamellae make large angles with others from adjacent lamellae (Freund et al., 1986). The transparency of the cornea is assumed to be due to the regularity of separation of fibrils within the stroma of the cornea (Maurice, 1957). However, the array of fibrils is not infinite, and because the separation width is not insignificant compared to the wavelength, some scatter is wavelength dependent (Hart and Farrell, 1969).
Figure 1.6: Structure of the cornea depicted from the epithelial to the endothelial layer (epithelial layer, Bowman’s layer, stoma, Descemet’s membrane and endothelial layer). Stroma is composed of regularly separated fibrils. Anatomically, both fibrils and keratocytes are parallel to the different layers of the cornea, but have been depicted perpendicularly for illustration purposes.

The integrity of the endothelial layer has also been found to be crucial in the maintenance of the stromal transparency, acting as a shield (Steele, 1999). Between fibrils, there are flat cells of 15 µm each called Keratocytes, occupying 3-5% of corneal volume and 9-17% of corneal stromal volume (Hahnel et al., 2000). There are around 2.0-3.5 million keratocytes in the corneal stroma (Moller-Pedersen, 1997) with a thickness of 1 µm of nuclei and several cell-processes of up to 50 µm long, giving the keratocyte a stellated shape that covers an area of about 1000 µm² in the frontal plane (Hahnel et al., 2000). However, some authors have found a keratocyte size covering an area of 78-211 µm² (Prydal et al., 1998). The density of keratocytes is about 20522 ± 2981 cells/mm³ (mean ± standard deviation) (Patel et al., 2001). Light travelling through the cornea might have to pass about 100 layers of keratocytes with their scatter properties (density, volume and size estimates), and therefore, the development of an optical model eye including the scatter properties of the keratocytes seems to be reasonable (Moller-Pedersen, 2004).
Another study found that for scatter angles larger than 30° with respect to the visual axis, the main corneal layer contributing to scatter is the stroma (70%), and the remaining 30% might be probably related to scatter by the epithelial layer, the endothelial layer, Bowman’s layer and stromal keratocytes (Freund et al., 1986). Other studies have shown that major sources of light scatter within the cornea are the endothelium and the epithelial cell layer, with scatter from the stroma limited to the keratocytes nuclei and not to the broad cell bodies (Jester, 2008). A study analysing corneas from postnatal rabbits found a good relationship between decreased density of keratocytes and reduction of light scatter (Jester et al., 2007).

Several studies investigated the wavelength dependency of corneal transparency. As previously stated, it is related to the space between structures of different refractive index (McCally and Farrell, 1988) and the magnitude of fluctuation of refractive index of structures. In fact, if the space separating scatter structures such as fibrils is small or less than half of visible light wavelength (400-700nm), hence the environment is transparent (Benedek, 1971).

1.6.2 CRYSTALLINE LENS

Previous studies have found that scatter in the crystalline lens is due to the change of refractive index between the surrounding cytoplasm and cell membranes, the increase in the separation of fibres (Kerker, 1969), or the aggregation of protein molecules (Bettelheim and Ali, 1985, Hemenger, 1992, Whitaker et al., 1993).

The crystalline lens is composed of a biconvex lens capable of modifying its shape to perform its main objective, the accommodation mechanism. The crystalline lens can be
defined as a matrix of fibres (Figure 1.7) placed very closely to each other (Trokel, 1962). Recent investigations of crystalline lens fibres and their refractive index have shown that fibres produce significant scatter and are homogenously spaced in the lens cortex (i.e. the peripheral part of the lens). This order of the fibers makes the crystalline lens more transparent. However, the fibers can act as a diffraction grating that is responsible in some circumstances to the lenticular halo (Charman, 1991b).

![Figure 1.7: Structure of the crystalline lens. The crystalline lens is internally composed of a matrix of fibers which are equally distributed and surrounded by the cytoplasm. Cells from the cytoplasm can migrate to different areas of the crystalline lens and create large molecules that produce light scatter.](image)

The refractive index of the crystalline lens is not constant and presents a gradient index profile (GRIN) that improves the focusing properties of the lens. The refractive index of the fibres’ membrane and cytoplasm decreases from the centre (1.409) towards the periphery (n = 1.380 (Kasthurirangan et al., 2008)). This difference in refractive indices is attributed to proteins from the fibres cytoplasm (Lovicu and Robinson, 2004). The transparency of the crystalline lens is attributed to the high concentration of proteins and their short-range-order interactions between them, that produce destructive interferences eliminating “virtually” the light scatter (Delaye and Tardieu, 1983).
It was also noted that the concentration of large particles need to be high to explain the amount of light scattered forwardly by the lens (Bettelheim and Ali, 1985). Continuing with this investigation, many studies have tried to identify the effect on scatter produced by proteins packed in the cytoplasm of the crystalline fibre cells (Gilliland et al., 2004). In this sense, the Rayleigh-Gans approximation allows describing light scatter when a scatter source has a refractive index similar to the one of the surrounding media. Given this situation, if the size of the scatter source increases, FLS becomes greater and BLS decreases (Kerker, 1969). This confirms that the aggregation of proteins molecules in the crystalline lens produces an increase of FLS. In the same way, scatter of light through cataracts is probably due to: the differences in the refractive index of the lens fibres (Hemenger, 1992); the aggregation of protein molecules in the crystalline’s nucleus (Bettelheim and Ali, 1985) and the posterior migration of cells from the epithelia, creating large organelles and eventually, posterior subcapsular cataracts (George, 2005).

These proteins, once they are produced, stay in the fibers and may suffer from some modifications such as cross-linking, creating proteins aggregates and changing their refractive indices, leading to an opacification and an increase in FLS (Hanson et al., 2000). With ageing, these proteins can suffer from oxidation as well, damaging the fiber cell membranes, and resulting in larger particles than the aggregates produced by protein cross-linking (Gilliland et al., 2004). These large particles which are called Multillamellar bodies (MLBs) (Gilliland et al., 2001), have a spherical shape and occupy a volume lower than 0.005% of the membrane. With an average diameter 2.4µm, they are located in the equatorial axis of the nucleus of the lens. MLBs are 7.5 times more frequent in cataracts than transparent crystalline lens (Gilliland et al., 2001), and they show Mie scatter (van den Berg, 1997, van den Berg and Spekreijse, 1999).
Other studies have found that MLBs have an average diameter of 2.7µm and are coated by 3-10 thin bilayers with a thickness of 5µm each. The nucleus of the MLBs has an index of refraction of 1.49, while the coating might be 1.40 (Costello et al., 2007).

1.6.3 IRIS, SCLERA AND UVEAL TRACT

A small fraction of the total FLS (1% or less depending on the colour of the eye (van den Berg et al., 1991)) can be attributed to illumination through the ocular wall. Greater scatter levels have been found in lightly pigmented eyes than in dark brown irises of non-Caucasians (Van den Berg et al., 1990, Elliott et al., 1991, van den Berg et al., 1991, de Waard et al., 1992). In fact, blue eyes from Caucasians produce about 18% more light scatter than brown Caucasian eyes (van den Berg et al., 1991).

In the next table (Table 1.2), some results from a study are presented in which a strong relationship between FLS and the type of iris pigmentation was found (van den Berg, 1995).

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Mean Age</th>
<th>Log(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Θ=3.5°</td>
</tr>
<tr>
<td>Caucasians blue eyed</td>
<td>33</td>
<td>33.5</td>
<td>0.88</td>
</tr>
<tr>
<td>Caucasians blue-green eyed</td>
<td>6</td>
<td>39.5</td>
<td>0.87</td>
</tr>
<tr>
<td>Caucasians brown eyed</td>
<td>19</td>
<td>37.7</td>
<td>0.82</td>
</tr>
<tr>
<td>Pigmented non-Caucasians</td>
<td>20</td>
<td>34.2</td>
<td>0.74</td>
</tr>
<tr>
<td>All Caucasians</td>
<td>109</td>
<td>20-82</td>
<td></td>
</tr>
</tbody>
</table>

*Table 1.2: Relationship between FLS (Log(s)) and the type of iris pigmentation measured with C-Quant straylight meter for different angle eccentricities (Θ) (Van den Berg, 1995).*
With reference to the uveal tract, transmittance of light through this media was found reduced by 0.2-1% due to the different absorption properties of the melanine in visible spectrum (van den Berg et al., 1991).

1.6.4 RETINA

The retina is made of several layers that partially reflect the light received. Hence, some of the incident light will be scattered backward or laterally. This scatter depends on the level of pigmentation of the pigmented retinal epithelium and of the choroid (Vos and Boogaard, 1963, Vos, 1984, Franssen et al., 2007) and is thus wavelength dependent (mainly due to the presence of melanin and oxyhaemoglobin (Hodgkinson et al., 1994). Other studies found that retinal straylight increases with ageing and also with the axial length of the eye (Rozema et al., 2010).

The retina produces a Rayleigh type scatter on light, which is wavelength dependent (Stiles, 1929) and proportional to $\lambda^{-4}$ (McCally and Farrell, 1988).

1.7 FACTORS AFFECTING INTRAOCULAR LIGHT SCATTER

In the previous section, the physical structure of the main sources of intraocular scatter was described, i.e. the cornea, transillumination through the iris, the crystalline lens and the retina. However, intraocular scatter can also be affected by several additional factors such as physiological changes related to age, pathologies or refractive corrections. The purpose of this section is to review these different factors.
1.7.1 PHYSIOLOGICAL

Numerous studies investigating the relation between scatter and age have reported an increase of intraocular scatter with age (Van Den Berg et al., 2007, Rozema et al., 2010). This increase is assumed to be due to changes in the crystalline fibers and the aggregation of protein molecules, especially in the cortex and nucleus (Allen and Vos, 1967).

FLS in a 70 years old person with normal ocular media has been found to be about 2.0 to 2.57 times higher than in a 20 years old person (Whitaker et al., 1993). Major increments of FLS start after 40 years old (Wolf, 1960, Elliott et al., 1991, Hennelly et al., 1998), coinciding with a study in which it was found that the transmittance through the ocular media did not change significantly between the first and third decade (Coren and Girgus, 1972, Norren and Vos, 1974).

Aging also produces the yellowing of the crystalline lens with its consequent reduction in transmission (Said and Weale, 1959), a loss of pigment from the iris and a reduction of corneal transparency (Olsen, 1982, Smith et al., 1990).

1.7.2 PATHOLOGICAL

The increase of intraocular light scatter has been associated with various medical conditions. Some of these conditions, including the most common ones, are listed hereafter.

• **Ocular pigment disorders:** As stated before, the level of pigmentation of the ocular wall and iris have an influence on the level of intraocular light scatter. In this sense,
the transillumination through the iris wall was found to decrease from around 1% in a light-blue eye to two orders of magnitude lower in a dark brown eye (van den Berg et al., 1993). In this context, it is not surprising that some conditions such as albinism or pigment dispersion syndrome might produce an increase in the transmittance through the ocular wall and iris, leading to an increase of FLS (Van den Berg et al., 1990).

- **Dystrophies, opacities and corneal oedema**: An increase of both FLS and BLS has been found on corneal scars that produce lower density opacities. These low density opacities were found to produce greater levels of FLS than high density ones (van den Berg, 1986). As a consequence, Woodward, in 1996 said that tattooing the scars may improve the visual performance (Woodward, 1996). In addition, there are some corneal dystrophies such as granular dystrophy that produce changes in the transparency of the cornea and therefore, an increase of the intraocular scatter (van den Berg, 1986). Keratoconus is a type of corneal dystrophy that is related with the increase of ocular light scatter and produces a change of the collagen fibrils in the corneal stroma, making it thinner (Meek et al., 2005). The increase of scatter could be related to the structural degradation of the cornea, in which, alterations of epithelia and stromal changes of keratocytes and lamellae may occur (Jinabhai et al., 2012).

- **Cataracts.** Cataracts are the result of the opacification of the crystalline lens. Age related cataracts are the most common, but a combination of nuclear or posterior subcapsular cataracts may be seen. The increase of the intraocular light scatter due to cataracts is a consequence of the nuclear sclerosis, which may be defined as “the alteration of the crystalline lens metabolism to allow changes in the concentrations of insoluble protein” (George, 2005). In the crystalline nucleus, oxidation of soluble and insoluble lens proteins might be a result of long-wavelength radiation. The sclerosis of the nucleus
produces a cross-linking of proteins that increases the optical density and decreases the nucleus transparency (Gilliland et al., 2004).

The amount of light scatter produced by different types of cataracts has been related to the LOCS III cataract classification (Chylack et al., 1993, Donnelly et al., 2004). In Table 1.3, straylight values for different types of cataracts are represented.

<table>
<thead>
<tr>
<th>Cataract type</th>
<th>Straylight (log(s))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear</td>
<td>1.56</td>
</tr>
<tr>
<td>Cortical</td>
<td>1.52</td>
</tr>
<tr>
<td>Nuclear-cortical</td>
<td>1.72</td>
</tr>
<tr>
<td>Posterior subcapsular</td>
<td>1.83</td>
</tr>
</tbody>
</table>

*Table 1.3: Types of cataracts and mean C-Quant straylight value (Bal et al., 2011).*

Sometimes, there is an increase of FLS after the extraction of the cataract and the implantation of the intraocular lens. This increase is usually related to the apparition of opacities on the posterior surface of the capsular bag (also called posterior capsule opacification) due to the proliferation of epithelial cells. This problem can be easily addressed with a Neodymium-Yag laser capsulotomy, which is used to clean the posterior capsule of the crystalline lens and clear the optical axis.

Regarding the type of cataract, the effects of a posterior cataract on vision are much worse than those from a cataract of same size situated anteriorly. This phenomenon has often been explained by the nearness of nodal points of the eye and the scatter centre. However, the reasons why this has different consequences on the vision of the patient are not well understood (George, 2005).

- **Aphakia and pseudophakia.** An aphakic, or pseudophakic eye, is an eye from which the crystalline lens has been removed surgically. After that process, an intraocular
lens is then usually inserted in replacement. This implant can be itself a source of scatter. The scatter is then related to the physical and optical properties of the lens (e.g. multifocality, diffraction by edges, etc.) (Martin, 1999). As a consequence of the intraocular scatter produced by the intraocular lens, some aphakes and pseudoaphakes patients have a very low contrast sensitivity at mid and high spatial frequencies compared to age-matched patients with normal eyes (Hess et al., 1985, Weatherill and Yap, 1986).

- **Ocular surgery:** Nowadays, many types of corneal surgery have been developed to correct for the refractive error by modifying the corneal shape. Such surgeries often lead to a temporary or permanent increase in FLS associated with the healing process. A large number of studies focused on this topic, particularly in relation with Lasik (Laser assisted in-situ keratomileusis). Main studies and findings are reviewed below and refer the reader to the following document for further details (Chisholm, 2003).

Radial Keratotomy (RK) is a technique for the treatment of myopia (Fyodorov and Durnev, 1979) and consists of the creation of radial incisions with a diamond micrometer through 95% of the thickness of the cornea in the mid-peripheral and peripheral cornea, keeping an area of three to four millimetres in the centre (Waring et al., 1985). Intraocular scatter is significant during the next six months after the surgery, decreasing gradually over time (Veraart et al., 1995). After that period, scatter becomes significant only for larger pupil sizes that let surgery incisions to be present in pupil diameter.

In photorefractive keratectomy (PRK), an ablation is produced in the anterior lamina of cornea and just after, a small lamina layer of epithelium is removed. After a few days, keratocytes from the epithelium create a new layer. This migration of keratocytes produces an increase in FLS (Lohmann et al., 1991). The major problem attributed to PRK is the apparition of an opacification known as “haze”, which normally starts after two to
four weeks after surgery and disappears in twelve months in most cases (McDonald et al., 1989, Seiler et al., 1990, Lohmann et al., 1991). At first, the structure of haze is homogeneous but, becomes heterogeneous with time. These heterogeneous areas are subject to present more light scatter than the homogeneous ones (Maldonado et al., 1996). Patients with higher myopia degrees normally show a more intense and persistent haze (Gartry et al., 1992, O'Brart et al., 1994, Maldonado et al., 1996).

Laser assisted in-situ keratomileusis (LASIK) is a technique in which a small flap of the corneal tissue is cut using a microkeratome. A stromal layer is then ablated using an excimer laser and the epithelial flap repositioned. This technique does not allow collagen or extracellular matrix formation due to the preservation on Bowman´s membrane. This is the reason why LASIK adds less light scatter to the cornea compared to other refraction techniques, such as PRK. However, patients with anterior corneal cell density reduced because of LASIK presented greater levels of intraocular straylight than patients without undergoing any type of refractive surgery (Nieto-Bona et al., 2010).

Laser Assisted Subepithelial Keratomileusis (LASEK) involves preserving the extremely thin corneal epithelial layer by lifting it from the eye's surface before laser energy is applied for reshaping. After the LASEK procedure, the epithelium is replaced on the eye's surface. Straylight increases to some degree after hyperopic LASIK and LASEK, but this increase was not found to be statistically significant (Lapid-Gortzak et al., 2010).

- **Stress:** Some protein associated with stress might migrate and generate an aggregation of proteins to the crystalline lens. This continuous aggregation of proteins because of the stress increases the scatter of the lens and eventually might result in the formation of a cataract (Shinohara et al., 2006).
• **Others:** Anterior uveitis or uncorrected myopia greater than -6.00D has been associated with an increase of FLS (van der Heijde et al., 1985, van den Berg, 1986).

### 1.7.3 OPTICAL

In the previous sections, we reviewed the physiological factors related to intraocular scatter, whether natural or pathological. Since 75% of the population in the Western world uses some sort of vision corrections (Vision Council of America, [http://www.thevisioncouncil.org/](http://www.thevisioncouncil.org/), 2014), the scatter properties of such corrections are reviewed hereafter.

- **Ocular lubricants:** There was not found any relationship between intraocular scatter and the use of ocular lubricants (Veraart et al., 1992). However, a recent study found that lubricant eye-drops reduce ocular scatter in patients with mild to moderate dry eye (Diaz-Valle et al., 2012).

- **Spectacles and contact lenses:** The straylight produced by clean spectacle lenses is not significant compared to the total amount of FLS (De Wit and Coppens, 2003). However, there is strong evidence that contact lenses may increase light scatter (Bergevin and Millodot, 1967, Applegate and Wolf, 1987, Elliott et al., 1991, Lohmann et al., 1993). In addition, wearing contact lenses may cause a corneal oedema if the transmissibility of the material of the lens is poor, they are worn for too long, or the lenses have a low permeability (Woodward, 1996). Elliott found in 1991 (Elliott et al., 1991) more intraocular scatter in rigid contact lens wearers than in soft lens wearers, but when the lenses were removed, the trend was reversed. These studies suggest that the increase of intraocular light scatter in a rigid lens is due to the material or the design. The increase in
soft lenses is a result of physiological changes such as corneal oedema and the variation of levels of intraocular scatter is not significant (Elliott et al., 1991).

- **Wavelength dependency**: Intraocular scatter has been proven to be dependent on wavelength (Wooten and Geri, 1987, Whitaker et al., 1993). In one study, the retinal straylight was measured from 625 to 457nm. It was found that well pigmented eyes such as young negroids had nearly clear $\lambda^{-4}$ wavelength dependence. However, in less pigmented eyes (blue eyed Caucasians), the $\lambda^{-4}$ wavelength dependence was not present (Coppens et al., 2006).

### 1.8 METHODS TO MEASURE INTRAOCULAR LIGHT SCATTER

Numerous methods can be found in the literature to measure the scattered light inside the eye. These methods differ widely and, currently, there is no agreement about the parameters (used glare angle, light conditions, diameter of pupil, type of scatter value, and nomenclature, etc.) that should be taken into account when the scatter is evaluated in the human eye (Pinero et al., 2010).

#### 1.8.1 METHODS BASED ON THE MEASUREMENT OF CONTRAST SENSITIVITY

Intraocular scatter has a direct influence on the quality of vision, and a measure of contrast sensitivity can therefore be seen as an indirect method to estimate FLS. Such methods assume that an increase in glare will be due to an increment of light scatter.
However, there is not a simple linear relation between the perception and physical phenomena. Disability glare might be affected by other factors such as optical aberrations, discrepancies with threshold measurements or different light conditions. An advantage of glare tests over a direct assessment of FLS is they can predict better the visual outdoor performances (Holladay et al., 1987). Hereafter is a list of several glare tests that have been compared to straylight measurements in the literature.

- **Nyktotest (Rodenstock GmbH, Ottobrunn, Germany) or Mesotest II (Oculus):** These instruments rely basically on the measurement of the Contrast Sensitivity Function (CSF) in mesopic conditions with a glare source. Although they can be relatively easy to use in a clinical environment, they do not measure glare in real life conditions (both background and testing level of light are predefined), are subjective methods (rely on patient’s input) and do not provide information about the imperfections found in the ocular media that cause glare (van den Berg, 1991). The results given by these instruments have not been found reliable and poorly related with patients complaints (Elliott et al., 1993, Puell et al., 2004, van Rijn et al., 2005).

- **Brightness Acuity Tester (BAT), Marco Ophthalmic Inc, USA:** The BAT consists of a semisphere incorporating a LED and a 12 mm aperture. The instrument is placed against the eye with the patient looking through the aperture at a contrast sensitivity chart. An LED light acts as a glare source. There are three settings for the LED thus allowing measuring the visual performances in three different lighting conditions (BAT Instruction Manual, Marco Ophthalmic Inc.). The best correlation between intraocular scatter and reduction of contrast sensitivity has been obtained using the BAT together with the Pelli-Robson chart (Elliott and Bullimore, 1993).
• **Miller-Nadler Glare Test:** This test is composed of a projector and slides with Landolt rings oriented randomly and that subtend 1.7° at 36 cm. The background of the test is variable (Miller et al., 1972, LeClaire et al., 1982). The drawbacks of this test are the limited number of tests available for each contrast level and the reduction in the intensity of glare if the patient moves off axis (van der Heijde et al., 1985).

• **Vistech MCT8000:** In this test, seven discs containing sine-wave gratings are presented for frequencies ranging from 1.5 to 18 cycles/degree. Its limitations are the reduced number of tests and the application of a criterion-dependent method instead of a forced choice method (Vaegan and Halliday, 1982, Bailey and Bullimore, 1991).

• **Berkeley Glare Test:** This test is based on a low contrast Bailey-Lovie letter chart (18%) collocated on an opaque panel (Bailey and Bullimore, 1991). The visual acuity is measured with and without glare at a distance of 1 metre. The disability glare is calculated by using a method consisting of ten alternative forced-choices.

### 1.8.2 METHODS BASED ON THE EQUIVALENT LUMINANCE TECHNIQUE

• The equivalent luminance technique is a psychophysical method that assumes that the effects of a glare source at an eccentricity theta on the retinal image are similar to superimposing on the observed object a veiling luminance L. The relation between the luminance L, the eccentricity of the source and the illuminance in the plane of the pupil due to the source is given by the Stiles-Holladay equation (Equation 1-1).
Recently, more sophisticated expressions taking into account the age of the subject, glare angle and eye’s pigmentation have been proposed (Vos, 2003). Most instruments based on this method rely thus in modifying the luminance necessary to distinguish foveally an object from the background in the presence of a glare source (Holladay et al., 1987). With a bright glare source, the minimum brightness difference for foveal discrimination increases. In this case, the equivalent luminance ($L_{eq}$) is the required background luminance to create the same adaptive effect on visual sensitivity, as the veil produced by a source of glare at angle $\Theta$ (Walraven, 1973).

The equivalent luminance method assumes that visual effects of glare depend only on FLS reaching the retina. However, other factors have to be considered, such as the different distribution of illuminance produced by a glare source over the retina in two different light conditions (Demott and Boynton, 1958). If this is considered, there will be an error for small glare angles and a bit larger for large glare angles (Holladay, 1926) but, straylight will account for most of the psychophysical effects.

**• The direct compensation technique:** This technique was suggested due to the lack of a widely accepted glare test (Aslam et al., 2007), and allows estimation of the equivalent veiling background ($L_{eq}$) produced by a glare source when using the retina as a detector. This technique is the basis of the van der Berg Straylight meter (van den Berg, 1986, van den Berg et al., 1991). In the instrument there is a uniform grey background with two test zones: a central disk which is initially black and a peripheral annulus. Figure 1.8 represents the van den Berg straylight meter with its main characteristics.
Figure 1.8: The van den Berg straylight meter: The instrument is composed of 3 peripheral annulus acting as glare sources and a central disc. The instrument gives the possibility of measuring straylight at 3.5, 10 and 28 degrees of eccentricity with respect to the eye, depending on the glare source that is used. The glare source has a flickering frequency of 8Hz and uses a wavelength of 570 ± 30nm.

The annulus flickers and acts as glare source. When the annulus is on, it is projected over the pericentral retina and the scattered light also over the fovea. As a result of the scattered light, the central disk would appear as flickering between black and greyish. The luminance of the central spot can then be increased during the annulus “off phase”. This added amount of light is the compensation light, and it will be changed until the flickering perception of the central annulus disappears. The added light that is necessary to achieve the exact compensation is the straylight.

The straylight can be quantified by assessing the straylight parameter “s” from Equation 1-2. The “s” parameter is expressed in log units. The advantage of this method is that the sensitivity of the fovea and the retinal adaptation are not critical due to the lack of
threshold assessment. This technique also avoids adaptation changes and poor fixation effect (van den Berg, 1995).

- The City University (London) scatter program (Figure 1.9) is based on a new flicker-nulling technique implemented by Barbur et al. (Barbur et al., 1995) that allows assessing the full scatter function. In this technique, the scatter index is not constant \( n \neq 2 \) as in the Stiles-Holladay formula. Another difference is that both glare annuli and target are displayed on a high-resolution monitor with a maximum luminance of 100 cd/m\(^2\) viewed from 70 cm. The null point (when both annuli have the same appearance and no flicker is perceived) is evaluated 6 times for every glare angle (five in total). To generate enough illuminance \( E \) in the plane of the pupil (with a CRT monitor) and to keep this illuminance constant for each eccentricity a large annulus of variable size is used (one different for each eccentricity). The program computes the effective eccentricity \( \theta_e \) of each annulus.

![Figure 1.9: The scatter function from the City University Program (Chisholm, 2003).](image-url)

Previous studies that aimed to assess the angular dependence of straylight produced inconsistent results because of the assumption that the angular distribution \( n \) has a constant value of 2; and that any changes between subjects were attributed to the amount
of scatter (k) (Fry and Alpern, 1953, Vos, 1984). Measuring the straylight using the City University Scatter program showed that the scatter index (n) is an important parameter when the straylight parameter (k) is normal (9.3-29.0 for glare angles ranging 0.75°-25°). In addition, the variations of straylight parameter (k) in normal subjects might be due to fluctuations of n, even when the total amount of scattered light within the eye remains constant (Holladay, 1926, Stiles and Crawford, 1933, Fry and Alpern, 1953, Vos and Boogaard, 1963, Vos, 1984).

- **The compensation comparison method** was developed to diagnose patients suffering from large angle light scatter such as early cataract (Franssen et al., 2006a), and to overcome limitations of the direct compensation method (Van Den Berg et al., 2007).

The instrument created for this purpose is the C-Quant Straylight Meter (Oculus, Netherlands). Although very similar to the van den Berg straylight meter (direct compensation method), the main differences are the introduction of a new glare source and the psychometric function. The new glare source is made of a circle divided into two halves. One of them receives the compensatory illumination while the other one remains dark (Franssen et al., 2006a). The peripheral annulus is presented in flickering series. For each series, a determined level of compensatory light is shown during the “off” phase on one of the halves while the other half of the annulus remains black. That black half looks greyish during the “on” phase due to the straylight generated by the peripheral annulus. The stimulus screen of the C-Quant straylight meter is depicted in Figure 1.10.
Figure 1.10: C-Quant stimulus screen. The instrument has a glare source with angle of 5 to 10 degrees with respect to the eye (7 degrees of effective scatter angle). The flickering frequency of the glare source is 8 Hz. The instrument uses an achromatic light. The test field is composed of a circle with two half discs.

Patients have to compare both halves of the central field and decide for each level of compensatory light which half flickers brighter/stronger. If the patient chooses a half with compensation, the score is “1”, and if it is the non-compensated one, the score is “0”. Sometimes, patients will not be able to see any difference and they will have to guess. The two forced-choice psychophysical method (2AF) permits statistical analysis, which confers to the instrument some objective characteristics (Franssen et al., 2006a). A psychometric curve is created with the patient choices using a psychophysical model for the comparison of flickering. The main advantage of this instrument is that a single criterion can be used to check and exclude measurements (Pinero et al., 2010). The measurement can be considered as reliable when the standard deviation of the measurement (ESD) is lower than 0.08 and the quality factor of the psychometric function (Q) is higher than 1. The mean value of “s” in healthy eyes is around 0.9 (Van Den Berg et al., 2007, Gilliland et al., 2008). “s” will increase from 40 years, reaching a mean value of 1.2 for 70 years old, and
1.4 at 80 years old. With cataracts, “s” can reach values of 2 or even higher (Gilliland et al., 2008).

Using the C-Quant, numerous studies have demonstrated the independency between straylight and Snellen visual acuity (Franssen et al., 2007, Van Den Berg et al., 2007, Michael et al., 2009, Van den Berg et al., 2010). In fact, a study carried out in 2003-2004 about the visual function and its impact on driving concluded that a value of “s” of 1.45 (four times more than normal) should be suggested as a limit for driving (van den Berg et al., 2009b, van Rijn et al., 2011). Recent studies have proposed the straylight criterion as a complementary test for cataract surgery (Bal et al., 2011) and for visually demanding jobs, especially to those patients undergoing refractive surgery (van Bree et al., 2011).

- The reaction time is the interval of time between the presentation of a stimulus and the response of the subject. It is believed that this time is significantly affected when high levels of intraocular scatter are present (Aguirre et al., 2007).

- The Halometer is an instrument designed to measure the effect of a halo (Babizhayev et al., 2003, Gutierrez et al., 2003), which is considered to be a consequence of both FLS and wavefront aberrations. For this reason, it is said to measure indirectly the ocular scatter. In this sense, a recent study assessing the relationship between the size of halo induced by a glare source and FLS found that larger halos increase FLS (Puell et al., 2014).
1.8.3 ESTIMATIONS OF FORWARD LIGHT SCATTER FROM BACKWARD LIGHT SCATTER

Estimations of FLS have been carried out based on densitometry of Scheimpflug images from slit lamp or the Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993). Unfortunately, BLS and FLS do no correlate well (Franssen et al., 2007, Vanden Berg et al., 2010). A positive result was however obtained by Bueno et al. (2007) with an instrument based on Purkinje imaging that allowed objective measurements of the scatter from the anterior segment of the eye, avoiding the scatter from the retina (Bueno et al., 2007). The designed instrument recorded and computed the fourth Purkinje image (the one produced by reflection on the back surface of the lens) to separate, by this way, scatter from anterior segment (cornea and lens) and that from retina. The method was first tested in artificial eyes and subsequently, in young eyes with customized contact lenses that added different amounts of scatter to the total of the eye. The sensitivity of the instrument was tested on artificial eyes with a customized contact lens as a cornea (null power contact rigid contact lens with a known concentration of glass microspheres of 1-20µm in the material) in front of biconvex intraocular lens (crystalline). Using this technique, it was possible to measure small increments of scatter (Tabernero et al., 2006, Bueno et al., 2007).

1.8.4 METHODS BASED ON THE DOUBLE-PASS TECHNIQUE

• The double-pass system (DP-SYSTEM) was firstly proposed in 1955 as a method to estimate the quality of the retinal image (Flamant, 1955). A basic DP system is illustrated in Figure 1.11.
Figure 1.11: Double pass system. Basically, a double pass system is an optical instrument where the light passes through the eye twice: light goes inside the eye to the retina and from the retina back to the imaging system. The image created is called double pass image (DP image). To develop a double pass system, a beam splitter is normally used. This beam splitter can reflect and refract the incident light at same time.

The technique is based on the recording of the reflected image from the ocular fundus. The resulting image is called DP (Double-Pass) image. The formation of the DP image from the system starts with a punctual source of light. The plane of this source is conjugated with the retinal plane, configuring by this “first pass” the trajectory of light through the optics of the eye. Part of the light that reaches the retina is reflected and can be recorded in its way out of the eye, conjugating the retina with a plane in which is placed a detector such as a CCD (Charge Coupled Device) camera. The formation of the retinal image in the camera represents the “second pass” and generates the DP image. The DP image is then analysed to extract the information about scatter. This can be done using this image alone as in the OQAS (see below) or by comparing the recorded PSF with the one estimated with a wavefront sensor based on a Hartmann-Shack (HS) (Donnelly et al., 2004).

- The Optical Quality Analysis System (OQAS™, Visiometrics S.L., Tarrasa). OQAS uses a double pass technique. The instrument records the retinal image of a point source. From this image, the instrument is able to calculate the Modulated Transfer
Function (MTF) from the image, thus taking into consideration both high order aberrations and scattered light. The MTF represents the loss of contrast by the optics of the eye as a function of spatial frequency and provides information of the overall performance of the human eye, including diffraction, aberrations and scatter. OQAS gives an Objective Scatter Index (OSI), based on a glare angle between 12 and 20 minutes of arc (Vilaseca et al., 2010). This instrument is perhaps the most objective and repeatable commercial instrument available to assess FLS but presents some limitations. Firstly, it only measures a small glare angle, which is insufficient to give a complete estimation of the retinal straylight (from 1 to 90°). Secondly, the infrared spectrum used by OQAS (780nm) is far from normal viewing conditions of a normal eye. Finally, it only measures the scatter in the most favourable optical conditions such as small glare angle (Liang and Westheimer, 1995, Lopez-Gil and Artal, 1997, Guirao et al., 1999, Rodriguez and Navarro, 2007). In this situation, the interpretation of the PSF gives an estimation of intraocular scatter. In addition, a small part of the effect of high and low order aberrations is present. To develop a DP system, it is necessary to consider middle wavelengths (green-yellow) to asses DP (double-pass) images (Liang and Westheimer 1995; López-Gil and Artal 1997; Guirao, González et al. 1999; Rodríguez and Navarro 2007). All these facts have led to question the validity of some parameters such as OSI (Van den Berg et al., 2010, van den Berg et al., 2013).

- The degree of polarized light emergent from the eye is a method based on the analysis of the degree of polarization of light, from images formed after a double pass through a system with a modified DP imaging polarimeter and a wavefront sensor. This method considers that scatter is related to the depolarization of light. However, it has not been used for clinical purposes yet (Bueno et al., 2004).
• Method to distinguish between higher-order aberrations and intraocular light scatter: In this method, the differences between the modulated transfer function and a double pass system can be considered as an estimation of scatter (Shahidi et al., 2005). A scheme of this model can be seen in the Figure 1.12.

![Figure 1.12: Estimations of intraocular scatter calculated as the difference between MTFs from a DP image and HS (see below) systems (Pinero et al., 2010).](image)

• The Hartmann-Shack wavefront sensor (HS): Is an aberrometer composed of an array of lenslets that samples the wavefront in the pupil plane of the eye and a camera situated at the focal plane of the lenslets. It was proposed in 1960’s by Roland Shack and is
the wavefront sensor most used today, being also the basis of most of the clinical devices measuring aberrations of the eye (Liang et al., 1994, Prieto et al., 2000).

When a distorted wavefront reaches the HS sensor, the pattern of spots created by the lenslet array becomes irregular. The deviation of each spot over the ideal position is proportional to the derivative of the wavefront over the area of each microlens (Figure 1.13).

![Image](image_url)

**Figure 1.13: HS wavefront sensor detector.** A HS detector is composed of an array of microlenses that analyse an incident wavefront by focusing the light passing through each one of the microlenses on a HS image. In case of an optically perfect eye, a plane wavefront projected into the eye would leave the eye (after a double pass) still being a plane wavefront. However, diffraction, aberrations and intraocular scatter in the eye will modify the pattern of an incident plane wavefront to a distorted wavefront (after the double pass). Incident plane wavefronts create HS images with clear dots while distorted wavefronts create HS images with dots and a distribution on light around those dots as a consequence of diffraction, aberrations and light scatter.

The obtained image after each analysis provides information about the wave aberrations and the associated PSF for a certain eye. Because the aim of these instruments (aberrometers) is not to obtain information about light scatter, the difference with the DP-PSF can provide some information on FLS (Cox et al., 2003). Although measurements from the HS wavefront sensor are assessed through the displacement of the spots from
their ideal position, practically, the relative intensity distribution around each spot is also related to the level of scatter induced by ocular structures (Donnelly et al., 2004).

Many studies have tried to extract scatter information from HS images considering the intensity statistics of the HS spot pattern. For this purpose, several metrics have been investigated based on the different values of pixels within a square containing each microlens’ PSF tail and their surrounding light distribution (neighbourhood). See Figure 1.14 for explanation on neighbourhoods.

**Figure 1.14:** A typical spot pattern of a HS image with spot pattern and the neighbourhoods.

In one study, a pixel neighbourhood was defined as a squared perimeter surrounding the pixel with the maximum value (ranging from 0 to 255) of each PSF, of the total pixels M. In a study trying to obtain scatter information from an HS image, it was found that each pixel within the neighbourhood has a value P and a pixel location (i,j). A normal neighbourhood n could be L = 361 (19x19 pixels). 9x9 pixels around the peak were ignored to highlight the tails (where the contribution of light scatter is recorded). The total number of pixels used were M=L-81 (Donnelly et al., 2004).
The influence of exposure time and the size of the pupil on a HS derived metric of forward scatter was studied using a model of nuclear cataract (Donnelly and Applegate, 2005) concluding that the metric of MAX_SD (maximum standard deviation of the PSF lenslets of a HS image) was the one which better explained the results obtained in the study. With respect to the saturation of the HS images, the histograms were used to identify saturated pixels. The image histogram plotted the number of pixels in the HS image (after the removal of the background) against the pixel value. Saturated HS images were observed in the histogram as a third peak near the value of 255 (non-saturated HS images did not present it). It was concluded that to avoid saturation and maximize the dynamic range of the system, the exposure time should be selected before the cataract assessment (Donnelly and Applegate, 2005).

Light scatter from the analysis of the HS images (Figure 1.14) has also been investigated in cataractous, keratoconic and normal eyes. In this study, two different methods were used, the first one measured the contrast of the HS image while the second one measured the width of the PSF. Subsequently, the difference between the measured contrast of the HS image and the widths of PSFs were computed and calculated with the aid of an aberrometer. Twelve neighbourhoods around the centre of the pupil were found to correspond to a pupil diameter of 4mm (Mihashi et al., 2006).

In 2009, Perez Sanchez defined a matrix in which each spot belonged to a small square of 25 pixels of side. In each square, it was calculated a “local parameter” (LP) through the division of the intensity registered in the periphery of the highest value of the square by the intensity of an area of 9 pixels centred on the highest level of the square. This LP provided the scatter of each small square within the HS image. To obtain a global evaluation of the scatter in the plane of pupil, the scatter local parameters were calculated.
for each square. The mean value of all of them was called “global scatter parameter” (GSP) and included the normalization of the highest value of the LP. With this normalization, the value of the GSP ranged from 0 to 10, following the expressions (Perez Sanchez, 2009):

\[
LP = \frac{\text{Sum of all pixel values of 25x25 matrix} - \text{central pixel value}}{\text{Sum of all pixel values of 9x9 matrix} - \text{central pixel value}}
\]

**Equation 1-3:** Local parameter (LP) calculation (also called neighbourhoods) (Perez Sanchez, 2009).

\[
\text{GSP} = \frac{\text{mean LPs}}{\text{Highest value (mean LPs)}} \times 10
\]

**Equation 1-4:** Global scatter parameter (GSP) calculation (Perez Sanchez, 2009).

In addition to the value of GSP, the value of standard deviation associated with the group of LPs provided information relative to the presence of local opacities within the considered pupil diameter. For this reason, the standard deviation of each image was also calculated (Perez Sanchez, 2009).

Finally, Cerviño et al. in 2008 also showed that Max_SD was a repeatable parameter in human eyes. A correlation of 69% was found between the analysis of HS images using Max_SD and the C-Quant straylight meter (Cerviño et al., 2008).

• **The optical reconstruction of the wide–angle PSF in the living human eye** (up to 8 degrees of eccentricity): This instrument (Ginis et al., 2012) based on a double pass system projects a uniform disc of light into the retina. The recorded image from the retina is the convolution of the initially presented disk and also contains information about the PSF of the eye assessed. The dependency straylight – wavelength was found when measuring small scatter angles while it was not possible to obtain same results for wide scatter angle (Ginis et al., 2013). Figure 1.15 shows a scheme of the developed instrument.
**Figure 1.15:** Instrument for the reconstruction of the wide angle PSF in the human eye (Ginis et al., 2013). In the figure, C is a condenser lens, D is diffuser, P is linear polarizers, D1 and D2 diaphragms, LCWS is a liquid crystal selectable bandwidth tunable optical filter, LC-SLM is a liquid crystal modulator and BS a beam splitter.

Basically, the bandwidth tunable optical filter (LCWS) selects different wavelengths between 500 and 650nm and project a series of different discs (50) over the retina. The radius of the ring ranged from 0.18 to 7.3 degrees of eccentricity. Then, the image of the projected disc is compared to the double pass image recorded from the retina and the increase of the intensity in the centre of the disc due to between the two images recorded (Ginis et al., 2013).

### 1.9 RATIONALE

Currently, the most used instrument to obtain FLS measurements is the C-Quant straylight meter. The measuring methodology used by this instrument is the compensation comparison method (Franssen et al., 2006b), which is an evolution from the direct compensation method (van den Berg and Ijspeert, 1992) and that is being widely used prior
to the introduction of C-Quant. However, the direct compensation method is still in use in many laboratories but there is no existing comparison in the literature with its successor.

Some studies have attempted to obtain an objective FLS measurement from commercially available instruments such as the Hartmann-Shack aberrometer, in which FLS information can be extracted from the spot pattern images (Donnelly et al., 2004, Cerviño et al., 2008, Perez Sanchez, 2009). These FLS measurements have been later compared to BLS measurements ((Donnelly et al., 2004). However, the relationship between the objectively measured BLS (through Scheimpflug imaging) and subjective FLS in patients is not yet clear (Bueno et al., 2004, Donnelly et al., 2004).

Researchers have also developed instruments for the objective measurement of FLS (Vilaseca et al., 2010, Ginis et al., 2012). Nonetheless, there is currently no methodology that can relate FLS measurements with psychophysical data. In addition, the C-Quant straylight meter is an instrument based on subjective measurements (based on subject’s input) and designed to measure only wide-angle FLS (van den Berg et al., 2009a). An instrument that could measure FLS at a range of scatter angles would be highly beneficial for clinical diagnosis, considering that size and shape of the analysed particles with respect to the wavelength of the incident light will affect their scattering behaviour: narrow or wide angle scatter.

**AIMS**

The objectives of the present PhD project were to assess whether the FLS measured through objective techniques correlate with the values obtained by the current subjective gold standard instrument of assessing FLS (C-Quant straylight meter), and to develop an objective technique for measuring FLS. More specifically, the project aimed to:
• Compare the two most documented psychophysical techniques to assess FLS (direct compensation and compensation comparison method) (chapter 2).

• Improve the current understanding about the objective FLS information contained on the Hartmanngrams (spot patterns obtained from the Hartmann-Shack aberrometer) and check whether this information can be related to a subjective measurement (C-Quant) (chapter 3).

• Assess the relationship between an objective BLS measurement and a subjective FLS measurement (chapter 4).

• Develop an experimental optical set-up together with an eye model that measures objectively FLS and can relate the values to psychophysical parameters (chapter 5).
2. COMPARISON OF THE COMPENSATION COMPARISON METHOD AND THE DIRECT COMPENSATION METHOD FOR STRAYLIGHT MEASUREMENT

2.1 CONTRIBUTIONS

The study was designed by me and my supervisors, Hema Radhakrishnan and Vincent Nourrit. The data collection was done by my supervisor Vincent Nourrit. The data analysis and the writing of the study were done by me with support from the co-authors.

2.2 PUBLISHING OF THE PAPER

Authors for this study are Pablo Benito Lopez, Hema Radhakrishnan and Vincent Nourrit. This paper will be submitted with the title: “Comparison of the compensation comparison method and the direct compensation method for straylight measurement”. Target journal: Clinical and Experimental Optometry. To be submitted.

2.3 PRESENTATION AT CONFERENCE

2.4 ABSTRACT

PURPOSE: The van den Berg straylight meter was used to assess FLS in a large variety of conditions and pathologies. Although still present in some research laboratories, it was gradually replaced by the more robust C-Quant. The two instruments share similarities (compensation comparison method for the first one, direct compensation method for the second). The purpose of this article is to assess quantitatively the agreement between the two techniques to facilitate comparison of clinical data obtained with each instrument.

DESIGN: Prospective study.

METHODS: FLS values were obtained from 35 subjects (ages ranging from 19 to 77) with the C-Quant and the van den Berg Straylight meter (for each of the three different eccentricities of the scattering source S=3.5°, M=10°, L=28°). Scatter values from both instruments were then compared with two tailed paired sample t-test and Bland-Altman plots. Pearson’s correlation coefficients, coefficients of agreement and limits of agreement were calculated.

RESULTS: A weak linear correlation was found between the two instruments for the three eccentricities (S: $r^2=0.13$, $p=.03$; M: $r^2=0.33$, $p<.001$; L: $r^2=0.36$, $p<.001$). The coefficients of agreement for the C-Quant and Straylight meter at each eccentricity were respectively: S=0.58, M=0.49, L=0.49. The limits of agreement for each eccentricity were found to be a large interval in comparison to the expected scatter value for a normal individual (approximately 0.9).

CONCLUSIONS: Although C-Quant values are approximately of the same order of magnitude as the straylight meter values, no direct equivalence can be made. Comparison
in a clinical study between C-Quant values obtained and previously published straylight values should be done carefully.

2.5 INTRODUCTION

In the last decade, a growing number of papers focused on the assessment and consequences of intraocular Forward Light Scattering (FLS) have been published (see the review papers: (Pinero et al., 2010, van den Berg et al., 2013)). Forward light scattering (FLS) occurs when part of the light propagating through the eye is deviated (scattered) from its original path by less than 90 degrees and does not participate towards the formation of the image. This scattering may induce discomfort or disability glare which can significantly affect the visual performances and quality of life of the patient (de Waard et al., 1992).

FLS is by definition intrinsically difficult to assess objectively and quantitatively and does not correlate well with backward light scatter (Donnelly Iii et al., 2004, Rozema et al., 2011). In this context, the van den Berg Straylight meter (VDB, Observator BV, Ridderkirk, The Netherlands) (van den Berg and Ijspeert, 1992) has been long considered the gold standard to assess FLS and to quantify the discriminative ability, reliability and validity of various glare tests (Elliott and Bullimore, 1993). This instrument was used to quantify FLS in a large variety of clinical conditions, including patients with cataracts (de Waard et al., 1992) and associated posterior capsule opacification (Meacock et al., 2003), but also corneal oedema (Elliott et al., 1993) and corneal dystrophies (Van den Berg et al., 1994, Patel et al., 2008a, Patel et al., 2008b), retinal disorders (Grover et al., 1998), keratoconus (Jinabhai et al., 2012), hypopigmentation (van den Berg and H, 1986), refractive surgery (Veraart et al., 1995), corneal grafts (Patel et al., 2008a, Patel et al., 2008b), or in association with contact lenses (Elliott et al., 1991).
Relatively recently, an improved version of this instrument, the C-quant (Oculus Optikgerate Gmbh, Wetzlar, Germany) (Franssen et al., 2006) has been introduced. This new instrument is easier to use in practice (Cerviño et al., 2008, van den Berg et al., 2013) and based on a more robust approach, the compensation comparison method (Coppens et al., 2006). This method improves repeatability and reliability of the measurement (Coopens et al., 2006, Coppens et al., 2006, Cervino et al., 2008, Cerviño et al., 2008) with respect to the Straylight meter. No reports however can be found in the literature about the correlation between the straylight parameter produced by the C-Quant and the three values obtained with the straylight meter (associated to 3 different glare source eccentricities). Since numerous studies reported straylight measurements in a large variety of conditions and pathologies with the Straylight meter, and since it is still used in some research laboratories, our aim is to assess quantitatively the agreement between the two techniques.

2.6 METHODS

PARTICIPANTS

Thirty five healthy subjects aged from 19 to 77 (41.6±21.7 [mean±SD]) years old were recruited from the student and staff populations of the University of Manchester. Young and older participants were studied as a single group to obtain data reflecting a broad spectrum of straylight value. Contact lens wearers were not included in the study. Any history of ocular disease or ocular pathology was considered as exclusion criteria. This study followed the tenets of the Declaration of Helsinki and was approved by the research ethics committee of the University of Manchester (UK). Informed consent was obtained from all participants after the nature and possible consequences of the study were explained.
FLS values from all subjects were measured monocularly in both eyes. However, only data obtained from the right eye was considered for calculations (Ray and O'Day, 1985).

**VDB STRAYLIGHT METER**

The basics of the Straylight meter are the following. The patient looks into a tube to observe a ring of flickering (8Hz) light-emitting diodes (LEDs) with a wavelength of 570±30nm. The image of the ring is formed on the retina of the patient, and, in the case of a perfect eye, this image would be sharp and there would not be any light in the centre of the ring. In the presence of scatter, some light will be scattered in the middle of the imaged ring. Consequently, the amount of light in the centre of the imaged ring is directly proportional to the intraocular scatter. Since the ring can be equated to a small source of eccentricity θ, the use of various rings allows measurement of the angular dependence of the intraocular scatter (see Figure 2.1). In practice, there is also a spot source in the centre of the ring and the two sources (spot+ring) are flickering in counter-phase. The task of the observer is to adjust the luminance of the spot to cancel the flickering, i.e. to match the veiling illuminance produced by the scattered light. For this reason, this technique is referred to as ‘direct compensation’.

Six measurements were taken in total for each participant, two measurements for every eccentricity of the scatter source (3.5°, 10° and 28°) (van den Berg and Ijspeert, 1992). Measurements for each one of the eccentricities were averaged. Subjects with refractive correction were measured with their habitual spectacles. The spectacles transparency was assessed before measurement (clean, no scratches that might affect the measurement (De Wit and Coppens, 2003)).
C-QUANT

The compensation comparison method used by the C-Quant straylight meter (Franssen et al., 2006) is similar to the direct compensation technique. However, in this case, the inner test field is divided into two flickering half-discs inside of another flickering ring. The ring represents the glare source (achromatic light) and has an effective angle of 7 degrees of eccentricity (van den Berg, 1995). The psychophysical test consists of series of 2-forced choice test where the participant has to decide which one of the two half discs is flickering the most (see Figure 2.1). The psychometric function used by this method (Franssen et al., 2007) decides which comparisons are shown to the participant. The test provides a measurement of the FLS, the standard deviation of the measure (ESD) and a quality factor of the psychometric function (Q) (Coopens et al., 2006). Reliability of the measurements is considered acceptable when ESD is lower than 0.08 and Q higher than 1.

Figure 2.1: Van den Berg straylight meter and C-Quant straylight meter. The van den Berg straylight meter has three different glare sources to measure at 3.5, 10 and 28 degrees of eccentricity while the C-Quant straylight meter has only one at 7º of effective eccentricity. Glare sources in both instruments have a flickering frequency of 8Hz. In the van den Berg straylight meter the testing field is a central disc while C-Quant has a circle with two testing halves. C-Quant straylight meter uses a achromatic light while van den Berg straylight meter uses LEDs of 570±30nm.
The measurements were obtained in scotopic light conditions after the correct positioning of the participant with respect to the instrument was checked (van den Berg, 2014). The test was repeated until two reliable measurements were obtained (ESD<0.08 and Q>1). The instrument allows inserting lenses to correct for the refractive error (spherical equivalent) of the subject so subjects did not have to wear their spectacles (van den Berg, 2014).

DATA ANALYSIS

The distribution of the data was analysed with the Kolmogorov-Smirnov test for normality. Relations between C-Quant measurements and Straylight meter values for each one of the eccentricities (S, M and L) are assessed with two tailed paired sample t-test and Pearson’s correlation coefficient ($r$), considering a $p$-value less than 0.05 as significant.

The relations between instruments values were determined with Bland-Altman plots (Bland and Altman, 1986, Bland and Altman, 1999) and the coefficient of agreement (COA) obtained for each two sets of data compared. In the present study, limits of agreement were calculated as the mean value of the differences between the two methods ± 1.96 times the standard deviation of the differences between two sets of measurements (C-Quant and one of the Straylight meter eccentricities (S, M or L)). It represents the interval where the differences in measurements from 2 instruments can be expected to fall with 95% probability (Bland and Altman, 1986).

A multiple regression analysis was performed in order to assess the relationship between C-Quant values (dependent variable) and the three eccentricities measured with the Straylight meter S, M and L (independent variables). The absence of multicollinearity is one of the assumptions to perform a multiple regression analysis. In this sense,
correlation coefficients ($r$) between variables (C-Quant, Straylight meter S, M and L) and Variance Inflation Factor (VIF) were calculated. The multiple regression analysis is considered viable when the correlation coefficients relating dependent with independent variables are preferably be above 0.3 while relationship between independent variables are not too high (normally less than 0.9). VIF, as an indicator of multicollinearity, it explains how much the independent variables of a multiple regression are linearly related. A VIF value higher than 10 was considered as an indication of multicollinearity (Pallant, 2010).

The results of this analysis were obtained using SPSS Statistics 21 (IBM, Chicago, IL) and MedCalc 12 (MedCalc Software, Mariakerke, Belgium).

### 2.7 RESULTS

The data summarised in Table 2.1 represents the values obtained with the C-Quant and the three different eccentricities of the Straylight meter ($S = 3.5^\circ$, $M = 10^\circ$ and $L = 28^\circ$). It is appreciable that mean straylight values obtained from the Straylight meter decrease as the measured scatter angle increases ($S = 1.12$, $M = 1.04$ and $L = 0.99$). This tendency has been previously reported (Jinabhai et al., 2012).

<table>
<thead>
<tr>
<th>C-Quant (Log(s))</th>
<th>Straylight meter (log(s))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Mean</td>
<td>1.08</td>
</tr>
<tr>
<td>SD</td>
<td>0.29</td>
</tr>
<tr>
<td>Min</td>
<td>0.72</td>
</tr>
<tr>
<td>Max</td>
<td>1.93</td>
</tr>
</tbody>
</table>

*Table 2.1: Summary of the mean values, standard deviation (SD), minimum and maximum values obtained for C-Quant and the 3 different eccentricities of Straylight meter (S, M, L).*
The data distributions obtained from the study and assessed with the Kolmogorov-Smirnov test were found to be normal for C-Quant ($p > 0.10$), Straylight meter (S) ($p > 0.10$), Straylight meter (M) ($p > 0.10$) and Straylight meter (L) ($p > 0.10$). Boxplot of the distribution of data is presented in Figure 2.2.

![Boxplot diagram representing the distribution of data for C-Quant and the three Straylight meter eccentricities measured (S, M and L). Boxes contain 50% of the data (from lower to upper quartile) while the line inside represents the median value. Vertical lines cover from the minimum to the maximum value, excluding the outside values (circles) and the far out values (black squares).](image)

In Figure 2.3, the Straylight meter values for the three different eccentricities (S, M and L) are plotted against the C-Quant (CQ) values. Pearson’s correlation coefficient for each pair of data set showed a weak linear relationship between FLS values obtained with the two instruments ($r^2 = 0.13$ and $p = 0.03$ for Straylight meter(S) and C-Quant, $r^2 = 0.33$ and $p < 0.001$ for Straylight meter(M) and C-Quant, $r^2 = 0.34$ and $p < 0.001$ for Straylight meter(L) and C-Quant). T-tests results for C-Quant and each one of the eccentricities S, M
and L were respectively: \( t(34) = 0.77, p = 0.45 \); \( t(34) = -1.14, p = 0.26 \) and \( t(34) = -2.2, p = 0.04 \).

Figure 2.3

Straylight meter(S) and C-Quant

\( r^2 = 0.13, p = 0.03 \)
Figure 2.3: Scatter value from C-Quant is plotted against the three different tested eccentricities ($S = 3.5^\circ$, $M = 10^\circ$, $L = 28^\circ$) of the Straylight meter ($A = \text{Straylight meter}(S)$ and C-Quant, $B = \text{Straylight meter}(M)$ and C-Quant, $C = \text{Straylight meter for } L$ is plotted against the mean CQ). Pearson’s correlation coefficient and p value are shown on the graphs. A weak relationship can be observed between values obtained from both instruments.
In order to gain a better understanding of the relation between the C-Quant and Straylight meter data sets, the associated Bland-Altman plots (difference C-Quant-straylight results plotted against the associated mean) are represented in Figure 2.4. The spread of the data representing differences of the values between the two instruments with respect to the mean does not show any direct relationship between the two instruments for each one of the eccentricities.
Figure 2.4: The graphs show the differences of the measurements between the two methods plotted against their mean values for each one of the three different tested eccentricities (A: S=3.5°, B: M=10°, C: L=28°). Mean value of the differences and limits of agreement (defined as mean of the differences ± 1.96xSD) are also presented.
Table 2.2 shows the statistics obtained for the differences of C-Quant values and the three eccentricities measured with the Straylight meter.

<table>
<thead>
<tr>
<th></th>
<th>S-CQ</th>
<th>M-CQ</th>
<th>L-CQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.04</td>
<td>-0.05</td>
<td>-0.09</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.29</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Min</td>
<td>-0.54</td>
<td>-0.54</td>
<td>-0.58</td>
</tr>
<tr>
<td>Max</td>
<td>0.62</td>
<td>0.44</td>
<td>0.4</td>
</tr>
<tr>
<td>COA</td>
<td>0.58</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>Limit of agreement Max</td>
<td>0.62</td>
<td>0.44</td>
<td>0.40</td>
</tr>
<tr>
<td>Limit of agreement min</td>
<td>-0.54</td>
<td>-0.54</td>
<td>-0.58</td>
</tr>
</tbody>
</table>

*Table 2.2: Statistics for the differences of C-Quant (CQ) values minus straylight data for each one of the three eccentricities of the Straylight meter (S-CQ, M-CQ, L-CQ) including the Coefficients of agreement (COA).*

To perform the multiple regression analysis, multicollinearity is assessed. Table 2.3 shows the correlation coefficients (r) relating the four variables (C-Quant and eccentricities S, M and L of the Straylight meter).

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>C-Quant</th>
<th>Straylight meter S</th>
<th>Straylight meter M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straylight meter S</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Straylight meter M</td>
<td>0.57</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Straylight meter L</td>
<td>0.58</td>
<td>0.66</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Table 2.3: Correlation coefficients (r) relating C-Quant values and the three eccentricities measured with the Straylight meter (S, M and L).*

To calculate VIF, linear relationship between independent variables is evaluated while another variable acts as dependent one. To do this, each one of the variables (C-Quant value, Straylight meter S, M and L) will act as dependent variable while VIF is calculated for the remaining three variables. This process is repeated as many times as variables are evaluated (four in this case). Table 2.4 shows the results of this analysis. VIF
values lower than 10 indicate the absence of multicollinearity and the viability of the multiple regression analysis.

<table>
<thead>
<tr>
<th>DEPENDENT VARIABLE</th>
<th>CQ</th>
<th>S</th>
<th>M</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDEPENDENT VARIABLES</td>
<td>S</td>
<td>M</td>
<td>L</td>
<td>CQ</td>
</tr>
<tr>
<td>VIF</td>
<td>2.56</td>
<td>4.52</td>
<td>3.15</td>
<td>1.56</td>
</tr>
</tbody>
</table>

*Table 2.4: Calculation of VIF for the variables C-Quant (CQ) and the three eccentricities of the Straylight meter S, M, and L.*

The multiple regression analysis is depicted in Table 2.5. In this analysis, the dependent variable is C-Quant while the three eccentricities of the Straylight meter act as independent variables. The coefficient of determination $R^2$ obtained for this analysis reveals that independent variables (three eccentricities measured with the Straylight meter) explain the 38.03% of the dependent variable (C-Quant straylight value). However, this result cannot be considered statistically significant ($p=.0018$) as p value for the calculation of $R^2$ should be less than 0.001. Coefficients obtained for each one of the independent variables analysed showed an influence over the dependent variable (C-Quant) with -0.28 for Straylight S, 0.54 for M and 0.41 in case L. However, as with the coefficient of determination, none of the coefficients obtained for the independent variables are statistically significant ($p > 0.05$ for a 95% confidence interval).

<table>
<thead>
<tr>
<th>INDEPENDENT VARIABLES</th>
<th>COEFFICIENTS</th>
<th>STD. ERROR</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Straylight meter S</td>
<td>-0.28</td>
<td>0.3</td>
<td>-0.95</td>
<td>0.35</td>
</tr>
<tr>
<td>Straylight meter M</td>
<td>0.54</td>
<td>0.36</td>
<td>1.49</td>
<td>0.15</td>
</tr>
<tr>
<td>Straylight meter L</td>
<td>0.41</td>
<td>0.3</td>
<td>1.39</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*Table 2.5: Multiple regression analysis using C-Quant as dependent variable and the three eccentricities of the Straylight meter as independent variables. Coefficients, standard error (Std. Error), t-value and correspondent p-value are also shown.*
2.8 DISCUSSION

The aim of the present study was to assess the agreement between the direct compensation (van den Berg Straylight meter) and the compensation comparison technique (C-Quant). Both techniques have been proven useful. While the Straylight meter was the gold standard for several years in techniques to assess FLS and led to many publications (Elliott and Bullimore, 1993, Veraart et al., 1995, Jinabhai et al., 2012) the C-Quant has probably replaced it as the most used instrument to measure FLS (Van Den Berg et al., 2007, Nischler et al., 2010, Montenegro et al., 2012).

The results from this study are in agreement with straylight values published in the literature for both van den Berg straylight meter (Jinabhai et al., 2012) and C-Quant (van den Berg et al., 2013). However, the correlation between the straylight meter and the C-Quant was shown to be weak for each eccentricity, particularly for the lowest eccentricity (3.5deg $r^2 = 0.13$, $p < 0.03$; 10deg; $r^2 = 0.33$, $p < 0.01$; 28deg; $r^2 = 0.34$, $p < 0.01$). Theoretically a higher correlation with ring M should have been obtained but this can be explained by the limited number of subjects and the variability of the measure.

However, further data analysis suggests that the values obtained with the two instruments are not directly interchangeable. The limits of agreement for each eccentricity were respectively equal to or larger than 0.49 (Table 2.2). This means that for a given measurement with the C-Quant, the interval where we can expect the straylight meter value to fall is too large to be clinically useful (e.g. for the M eccentricity, straylight meter ’s results should be -0.54 smaller or +0.44 greater than the C-Quant value (Table 2.2)).

Absence of multicollinearity between the four variables (C-quant, Straylight meter S, M and L) indicates the viability of the multiple regression analysis. To perform a multiple regression analysis, correlation coefficients ($r$) relating the four variables need to
be above 0.3 and below 0.9 and VIF (Table 2.4) values lower than 10 (Pallant, 2010). However, the coefficient of determination ($R^2$) and coefficients obtained for each one of the predicting variables (Table 2.5) were not statistically significant ($p > 0.001$ and $p > 0.05$ respectively). Hence, C-Quant straylight values cannot be predicted as a contribution of the straylight values obtained with the Straylight meter for the three different eccentricities measured (S, M and L). These results were not expected as the C-Quant straylight value is proportional to the square of the angle measured (van den Berg et al., 2013) and the distribution of scattered light over the retina. This lack of linear relationship between C-Quant values and Straylight meter (S, M or L eccentricity values) might be due to the different measured eccentricities in the van den Berg Straylight meter ($3.5^\circ$, $10^\circ$ and $28^\circ$) and C-Quant ($7^\circ$), and leads us to think that straylight values obtained from C-Quant should possibly be compared to a combination of the straylight values obtained for different Straylight meter eccentricities. In this sense, the straylight produced by a glare source of $7^\circ$ of eccentricity (C-Quant) was supposed to be comparable to a combination of the straylight produced by the three eccentricities ($3.5^\circ$, $10^\circ$ and $28^\circ$) of the Straylight meter, or at least two of them (e.g. $S = 3.5^\circ$ and $M = 10^\circ$).

The differences between the measurements obtained using the two techniques may be due to a variety of factors. Firstly, the use of the psychometric function in C-Quant provides the measurement with a reliability (Coopens et al., 2006) that is not available in the van den Berg straylight meter. Secondly, the eccentricity of the glare source is not strictly identical ($3.5$, $10$ and $28$ degrees for the Straylight meter (van den Berg and Ijspeert, 1992) and $7$ degrees for the C-Quant (van den Berg, 1995)) but also the type of glare source used also varies. The Straylight meter is based on a ring of LEDs when the C-Quant uses two half discs and a ring. Perhaps more importantly, both instruments rely on a subjective estimation of scattering and the measured values cover slightly different ranges.
As a result, the standard deviation of the difference between the two measurements is increased which can negatively affect the agreement between the two methods. This is particularly true for the straylight meter which does not benefit from the improvements present on the C-Quant (computerized 2-choices forced tests, measure of reliability).

As a conclusion, although C-Quant values are approximately of the same order of magnitude as the straylight meter ones, no direct equivalence can be made. Comparison in a clinical study between C-Quant values obtained and previously published straylight values should be done carefully.
3. COMPARISON OF FORWARD LIGHT SCATTER ESTIMATIONS USING HARTMANN - SHACK SPOT PATTERNS AND C-QUANT

3.1 CONTRIBUTIONS

The study was designed with the help of my co-authors. Both co-authors Hema Radhakrishnan and Vincent Nourrit provided important contributions to the study. The data collection and the writing of the study were done by me with useful advice from the co-authors.

3.2 PUBLISHING OF THE PAPER


3.3 PRESENTATION AT CONFERENCE

This research was presented in part as a paper presentation at the 6th EOS Topical Meeting on Visual and Physiological Optics (EMVPO 2012). Held at University College Dublin (UCD), Dublin, Ireland (20 August 2012 - 22 August 2012).
3.4 ABSTRACT

PURPOSE: To assess if a non-modified commercial wave front sensor can be used to estimate forward light scatter and how this assessment matches the one obtained from C-Quant.

DESIGN: Prospective comparative study between measurements obtained with C-Quant and Hartmann-Shack (HS).

METHODS: Objective scatter data were derived using an HS metric based on previously reported methodology. The method was first validated using a model eye, by spraying an aerosol over 4 contact lenses to generate various levels of scatter. Measurements with both methods (HS-based and C-Quant) were subsequently obtained in 33 healthy participants (mean age of 38.9 ± 13.1 years).

RESULTS: A good correlation was observed (r = 0.97, p < 0.05) between the density of droplets over the contact lenses and the objective scatter value extracted from the hartmannograms. The mean C-Quant values for participants were in agreement with those reported in the literature for healthy subjects (0.87±0.18 [mean±SD]). However, no significant relationship was found between the measurements obtained from C-Quant and the metric derived from HS (r = 0.13, p = 0.46).

CONCLUSIONS: Although the hartmannograms were found to provide a valid objective measurement of the light scatter from model eyes, the measurements on human eyes were not significantly correlated with the subjective and clinically accepted C-Quant light scatter meter. The difference could be caused by the fact that C-Quant assesses large
angle scatter while HS method collates information from a narrow angle around the centre of the point spread function.

3.5 INTRODUCTION

Intraocular light scatter occurs when the light passing through the ocular media suffers a deviation from its original trajectory due to the presence of inhomogeneities in the optical media (e.g. corneal scarring or cataract). In contrast to backward light scatter, forward light scatter (FLS) reaches the retina. Its influence on the retinal image can be equated to a superimposed veiling luminance (Fry and Alpern, 1953) and its main effect is the impairing of the functional visual performances (i.e. reduced contrast sensitivity and glare (Paulsson and Sjostrand, 1980, De Waard et al., 1992, Artal et al., 2001)). Recent studies have suggested the use of intraocular light scatter as an additional test for patients undergoing cataract surgery (Bal et al., 2011) or refractive surgery, particularly if they have visually demanding professions (Van Bree et al., 2011).

The measurement of intraocular FLS is a difficult but important issue. Since the beginning of the 20th century (Holladay, 1926, Stiles and Crawford, 1937), numerous methods have been suggested for assessing FLS. Instruments to measure intraocular scatter can be divided into two broad categories (subjective and objective) depending on whether they rely on the patients’ input.

Several types of subjective methods have been developed (cf. Van Den Berg et al. (van den Berg et al., 2012) for a recent survey of ocular straylight measurement). While some techniques compare contrast sensitivity measurements in the presence and
absence of a glare source (Miller et al., 1972, Vaegan and Halliday, 1982, Bailey and Bullimore, 1991, Van Den Berg, 1991, Elliott and Bullimore, 1993), perhaps the most studied and documented instrument (C-Quant straylight meter, Oculus GmbH, Wetzlar, Germany) uses the compensation comparison method (Franssen et al., 2006). It was developed to diagnose patients with large angle light scatter in the eye (e.g. in patients with early cataract) (Franssen et al., 2006). The C-Quant has good reliability and repeatability for assessing FLS (Cervino et al., 2008). In addition, C-Quant has also been successful in providing good correlation between measurements of FLS and reduction of visual function (Van Den Berg et al., 2007, Michael et al., 2009).

The optical quality of the eyes is usually only described in terms of aberrations, although the uniformity of the ocular media also affects the retinal image. Retinal image and quality of vision (in part) can be explained and assessed qualitatively by the retinal point spread function (PSF) (Montés-Micó et al., 2008, Van Den Berg et al., 2009). For this reason, instruments providing objective measurements of FLS are usually based on the double pass (DP) technique (analysing the light reflected by the retina) (Santamaría et al., 1987, Artal et al., 1995, Kuroda et al., 2002, Donnelly lli et al., 2004). Since Hartmann-Shack wavefront sensors record an array of PSFs, several authors have suggested different approaches for the extraction of light scatter values from the hartmanngrams (Applegate RA, 2000, Kuroda et al., 2002, Applegate RA, 2003). The displacement of PSF spots on the hartmanngrams is due to aberrations and the scatter value is retrieved by calculating the intensity of PSF’s tails (Applegate RA, 2000, Donnelly lli et al., 2004). Using this method, Donnelly et al. (Donnelly lli et al., 2004) evaluated five different metrics referred to the intensity around the centre of each PSF of the hartmanngrams (PSFlet).
The maximum standard deviation of the intensities of each PSFlet of the hartmanngram was found as the best metric explaining FLS (Donnelly Iii et al., 2004).

As several wavefront sensors have been developed for applications in ophthalmology and optometry, the development of a numerical tool that can extract a measure of FLS from the analysis of the hartmanngrams could extend the range of applications of commercial wavefront sensors. However, this method has been reported mainly with research instruments and without any comparison to any results obtained by a subjective technique. Only one study addressed this issue but with a limited number of measurements (Cerviño et al., 2008). For this reason, the aim of this study is to assess if a non-modified commercial wave front sensor could be used to estimate FLS (via the development of a numerical tool) and how this assessment would match the one obtained with a method sometimes considered as the gold standard (C-Quant straylight meter).

3.6 METHODS

This study is comprised of two experiments:

1. **Measurement of FLS with customized contact lenses:** Six customized contact lenses with different amounts of scatter were placed in front of a model eye. After obtaining the hartmanngram from both contact lens + model eye, a mathematical algorithm extracts the scatter value from it.
2. **Measurement in subjects with both HS and C-Quant:** After validating the algorithm to extract the scatter value from the hartmanograms, measurements from thirty three subjects are obtained from both Hartmann-Shack and C-Quant, thus comparing, objective with subjective measurements of FLS respectively.

**MEASUREMENT OF FLS FROM THE HS IMAGES**

The irox3 wavefront sensor (see Figure 3.1 for information about HS sensors) uses the HS technique with an infrared light of 780nm to measure aberrations and a lenslet array of 32x32.

**Figure 3.1:** HS wavefront sensor detector. A HS detector is composed of an array of microlenses that analyse an incident wavefront by focusing the light passing through each one of the microlenses on a HS image. In case of an optically perfect eye, a plane wavefront projected into the eye would leave the eye (after a double pass) still being a plane wavefront. However, diffraction, aberrations and intraocular scatter in the eye will modify the pattern of an incident plane wavefront to a distorted wavefront (after the double pass). Incident plane wavefronts create HS images with clear dots while distorted wavefronts create HS images with dots and a distribution on light around those dots as a consequence of diffraction, aberrations and light scatter.
The array of lenslets produces the hartmanngram spot pattern (Figure 3.2A). In such an image, the displacement of the spots with respect to their reference position is due to the aberrations. The width of each spot (or PSFlet) is related to FLS (Figure 3.2B). The lenslet and detector properties of the irx3 wavefront sensor are not publicly available and it is thus difficult to provide an exact value of the pixel size in minutes of arc. However, according to previous publications (e.g. Leroux et al. (Leroux and Dainty, 2010) and Nam et al. (Nam et al., 2011)) a value around 3 arcminutes could be expected.
Figure 3.2: A) Hartmanngram spot pattern from one of the participants. In this picture, each square represents an area of 13x13 pixels around the centroid; squares containing more than two saturated points or whose intensity pixels were under the threshold (obtained to analyse the images with Matlab) were not considered for calculations and look missing. For this particular hartmanngram, the mean, standard deviation, minimum and maximum of the standard deviations of all PSFlets were respectively $8.9 \times 10^{-3}$, $6.87 \times 10^{-2}$, $7.7 \times 10^{-3}$, 0.17. The associated HS value is equal to the maximum standard deviation. B) An enlarged PSFlet showing the centre of the PSF and the surrounding intensity. The sampling distance in the pupil plane is 0.23mm.

A custom written Matlab (MathWorks®, Natick, MA) program (program code is available in the Annexes of this thesis) was developed to extract the scatter information from the HS images following the approach described by Donnelly et al. (Donnelly III et al., 2004) The Matlab program included the following steps:

- Rejection of images of poor quality (defocused, highly irregular spots pattern due to blinking or poor centration).

- Rejection of overexposed images. Due to the short dynamic range of the HS CCD camera (8 bits), HS images are easily overexposed. Overexposed images are
difficult to analyse, as information around the centre of the PSFlet is lost by clipping. To avoid this problem, images where image histogram showed a third peak were considered overexposed and removed from calculations (Donnelly Iii and Applegate, 2005). In addition, some of the PSFlet may present local saturation. It was considered that PSFlets with more than 2 saturated pixels i.e. pixel value of 255 (the maximum value possible to be recorded on the HS sensor), do not contribute to the scatter calculations for the whole image.

- Extracting a metric for FLS from the Hartmanngrams: Hartmanngrams from the HS are converted into an intensity image. To reduce noise (fluctuation in the signal produced by all electronic instruments) the lowest intensity value of the image is subtracted to all pixel values of the hartmanngram. Subsequently, the intensity image is thresholded to find the location of the centroids of each PSFlet and to index them. Thresholding of the images was done empirically in order to assess the lowest intensity pixels of the neighbourhoods (most information on FLS within the neighbourhood is stored near the borders of the neighbourhood, where intensity pixels are lower) after removing the noise of the image. A 13x13 pixel neighbourhood is defined around each centroid. PSFlets with very low intensity values with respect to the threshold or those with more than two saturated points are not considered for calculations. Finally, the standard deviation of the pixel intensities from each PSFlet of the Hartmanngram is calculated and the maximum one is used as a measure of scatter. This is the metric suggested by Donnelly et al. (Donnelly Iii et al., 2004) and used in Cerviño et al. (Cerviño et al., 2008).
EXPERIMENT 1: MEASUREMENT OF FLS WITH CUSTOMIZED CONTACT LENSES

In order to validate this program, measurements were obtained with a model eye (RME - http://www.optomshop.co.uk/Retinoscopy_Model_Eye.htm, January 2013). To simulate different levels of scatter, different concentrations of aerosol containing aluminium chlorohydrate (refractive index = 1.51) were sprayed over six RPG contact lenses (Figure 3.3). The lenses were sprayed for approximately 0 (none), 0.5, 1 and 1.5 seconds at a distance of 40 centimetres. The concentration of the aerosol particles was then measured by obtaining an image of the central zone of each lens (representing 60% of the whole lens area) and processing it with Matlab to estimate the droplets’ density. Images of the lenses 1 hour after spraying were also taken to assess that the density and opacity of the droplets over the lenses remained stable.

To measure the magnitude of light scatter, the model eye was collocated in front of the aberrometer with clamps. The end of the model eye was covered with a black rough surface to reduce the amount of light reflected and to avoid overexposing the HS sensor. The recording of the hartmanngrams was executed in scotopic light (less than 0.5 lux), changing only the lens holder with the contact lens in front of the model eye and leaving the rest of the set-up steady. The HS images were obtained for the non-sprayed lenses first and then the lenses were changed in incremental order with the most sprayed lenses being tested in the end. Three hartmanngrams were recorded, processed and averaged for each contact lens per level of scatter. Therefore, there were 12 images in total, obtained with the HS sensor for each lens. The whole process was repeated for all the six lenses.
Figure 3.3: Photos of one 9mm RPG contact lens (left) for each density of aerosol droplets (simulating different scatter conditions). Right: photo of the central zone of the lens (60% of the lens = 38.06mm²) after processing with Matlab to assess objectively the droplets density. First line of photos shows the non-sprayed lens. Consecutive lines correspond to same lens with added quantities of spray (without removing the previous spraying). Pixel size of the photos (obtained with a normal camera) is 1.4 microns and the mean droplet’s size was 31.2 ± 21.52 pixels.
EXPERIMENT 2: MEASUREMENTS IN SUBJECTS WITH BOTH HS AND C-QUANT

Thirty three subjects took part in the study after providing informed consent. The study was approved by the University of Manchester Research Ethics Committee. The mean and standard deviation of our patients’ age was 38.9 ± 13.1 years with the minimum and maximum age being respectively 24 and 62 years. Mean spherical equivalent was -1.22 diopters ± 3.07 SD (max = 6.75, min = -9.00). Pupil size of each participant was measured in the dark (6.29±0.61mm (mean±SD); min= 5mm; max=6.8mm) and set mechanically to 5 mm with the Hartmann-Shack aberrometer. The criteria of exclusion were the following: ocular pathology, history of ocular diseases, and contact lens wear in the last 24 hours. Subjects wearing glasses (17) were measured without them on both instruments, using lenses for the correction of refraction (spherical equivalent) in case of C-Quant (Van Den Berg, 2014). Participants were selected from a relatively wide range of age groups so that the data would span a broad range of straylight values (Hennelly et al., 1998).

The measurement technique with the C-Quant (Oculus) has been described by Franssen et al. (Donnelly lll and Applegate, 2005). In summary, the subject looks into a tube at two flickering half-discs, surrounded by a flickering annulus simulating a glare source of achromatic light at 7 degrees of eccentricity. The test consists of approximately 20 forced choices where the subject has to decide which half-disc is flickering the most. At the end of the test, one measure of straylight is produced, together with the standard deviation of this measure (ESD) and a quality factor (Q). Measurements can be considered reliable when the ESD is lower than 0.08 and the quality factor higher than 1. Monocular measurements were taken in scotopic light conditions. The correct position of the patient
was also carefully monitored as it may influence the measurement (Van Den Berg, 2014). Two measures were taken for each eye. If both measures were reliable, the mean value of these 2 measures was taken as the scatter log value; if not, the measurement was repeated until 2 acceptable measurements were obtained (ESD<0.08, Q>1) where ESD is the estimated standard deviation and Q is the fit quality parameter of the psychometric function (Coopens et al., 2006).

The irx3™ wavefront sensor was used to obtain the HS images. Three measurements were obtained for each eye of the patient in scotopic light conditions. If any of the images was thought not to be valid (e.g. blinking), a new one was taken. The test was done on each eye monocularly, without refractive corrections and using an occluder to cover the non-tested eye.

STATISTICAL ANALYSIS

The Pearson’s correlation coefficient (r) and two tailed paired sample t-test are obtained to find an association in both experiments. Data sets were checked for normality using the Kolmogorov-Smirnov test and a p-value lower than 0.05 was considered significant.

The results of this analysis were obtained using SPSS Statistics 21 (IBM, Chicago, IL) and MedCalc 12 (MedCalc Software, Mariakerke, Belgium).
3.7 RESULTS

EXPERIMENT 1: MEASUREMENT OF FLS WITH CUSTOMIZED CONTACT LENSES

Figure 3.4 shows the FLS values extracted from the hartmanngrams of the customized contact lenses in comparison with the calculated density of droplets over the same lenses (cf. Methods). Figure 3.4 illustrates a significant correlation between the two sets of data ($r = 0.97$, $P < 0.05$).

![Scatter on contact lenses](image)

*Figure 3.4: Relationship between the amount of FLS for each lens (extracted from the hartmanngrams) and the associated concentration of scatter droplets.*
EXPERIMENT 2: MEASUREMENTS IN SUBJECTS WITH BOTH HS AND C-QUANT

C-Quant measurements are plotted against the corresponding scatter value extracted from the hartmanngrams in individual participants (each one of the 33) in Error! Reference source not found.. No significant correlation was found between the 2 sets of measurements ($r = 0.13, p = 0.46$).

Figure 3.5: Relationship between the FLS values obtained from the HS (abscissae) and those from the C-Quant (ordinate).

The measurements of FLS from C-Quant were compared directly with the HS FLS measurements for each participant to facilitate comparison with a previous study (Cerviño et al., 2008). The data provided by the C-Quant corresponds however to the logarithm of the scatter value. Hence the scatter data from the C-Quant (scaled
linearly) was compared to the HS data. No significant correlation was found ($r = 0.11$, $p = 0.53$).

### 3.8 DISCUSSION

**EXPERIMENT 1: MEASUREMENT OF FLS WITH CUSTOMIZED CONTACT LENSES**

The results from this experiment agree with those obtained in a previous study (De Brouwere, 2007) in which different concentrations of microspheres with variable diameter and known refractive index were used to simulate increased corneal scatter. The study (De Brouwere, 2007) used a commercial wavefront analyser (WaveLight Wavefront Analyser, WaveLight Technologie Inc., Erlangen AG) and the results showed an increased scatter as the concentration of microspheres became higher. The use of microspheres in contact lenses to simulate corneal scatter was validated and found reliable for the comparison and calibration of optical devices measuring ocular scatter. In this study, using an artificial eye and six contact lenses with pre-determined amount of droplets containing aluminium chlorohydrate, the irx3™ Wavefront Aberrometer returned proportionally higher scatter values as concentration raised.

**EXPERIMENT 2: MEASUREMENTS IN SUBJECTS WITH BOTH HS AND C-QUANT**

The lack of correlation observed between the CQ data and the one estimated from the HS spot patterns can be at first surprising. However, it can be explained by different factors. Firstly, both instruments are intrinsically different in design and objectives. The C-
Quant is mainly sensitive to large angle scatter while HS measures a very narrow angle scatter around the centre of the PSF (Van Den Berg et al., 2009). This large dimensional difference in angular domain could be related to different physical processes. The PSFlet spread may be related to irregularities causing "micro-aberrations" (Nam et al., 2011) (i.e. aberrations on a spatial scale less than the diameter of an individual lenslet) rather than an increase in smaller scatter particles.

Only one study (Cerviño et al., 2008) investigated the relation between C-Quant scatter values and the one extracted from a commercial HS. The metrics used in this study were similar to ours but the instrument was different (Hartmann-Shack analyser, WASCA, Zeiss/Meditec), and therefore, different size and number of lenslets and different size of PSFlet. The authors reported that no correlation could be found if using all of their data (10 eyes with a mean age of 27.5 years ± 2.9 SD and a spherical equivalent of -1.61 ± 2.59 SD diopters) but could find a good relation after rejecting 4 images due to defects and omissions on the hartmanngram image (Cerviño et al., 2008). The difference with our data (33 eyes with a mean age of 38.9 years ± 13.1 SD and a spherical equivalent of -1.22 ± 3.07 SD diopters) can also be due to the larger sample size, the larger range of FLS values considered or the differences in instrumentation.

Another possible explanation is that commercial wavefront sensors such as the one used in this study (or in Cerviño et al. (Cerviño et al., 2008)) usually incorporate a confocal aperture between the two relay lenses to reduce high angle scatter that would otherwise reduce the sensor’s performances (this aperture is not present in the system used by Donnelly III et al. (Donnelly Iii et al., 2004)). This confocal aperture avoids unwanted light reaching the CCD sensor of the HS, minimizing the detection of high angle
scatter angle, and maximizing the detection of aberrations, which are focused on the CCD through a narrow beam of light. This aperture may however not prevent to assess scatter in a case similar to our model eye (significant scatter originating near the pupil plane) but should limit possibilities to measure it in a human eye.

Other factors that could explain the absence of correlation are the relatively low level of scatter present in our subjects compared to our artificial eye and psychophysical factors such as light conditions. HS is always used in low light conditions, trying to achieve a pupil size as large as possible. However, psychometric measurements take place with a glare source in front of the eye, producing miosis of the pupil. In this sense, Luuk Franssen et al. (Franssen et al., 2007) found that for normal pupils (2-7 mm of diameter), the dependence intraocular scatter – pupil diameter is very weak; while in large angle scatter and small pupil diameters, the contribution of the translucency of the eye wall to scatter is significant, depending on pigmentation and wavelength factors. In this study, none of the subjects had a pupil size outside of the 2-7mm of diameter range. In addition, overexposed images widen the size of the PSF, making more difficult the evaluation of the images to extract scatter values (Donnelly Iii and Applegate, 2005). Cerviño et al. (Cerviño et al., 2008) showed that processing overexposed images would allow optimizing the calculation of intraocular scatter. As HS images are typically overexposed, (Donnelly Iii and Applegate, 2005) another criteria was added: if the PSF contains more than two saturated points (pixel value over 254), the PSFlet does not count for the total calculation of scatter.

Although HS scatter value has been found reliable for patients with high levels of scatter, i.e. cataracts (Donnelly Iii et al., 2004), the signal intensity recorded on the
hartmanngrams from patients with normal levels of scatter is very similar to the instrument noise ratio (Nam et al., 2011), adding an extra difficulty to the scatter extraction process from the hartmanngrams.

Straylight is a functional measure, it is not directly a measurement of retinal light distribution and with this respect some optical approaches (such as the Hartmanngram based approach) may be limited. In this context, the new optical method reported by Ginis et al. (Ginis et al., 2012) may be a step toward a better optical evaluation of FLS.
4. FORWARD AND BACKWARD LIGHT SCATTER MEASUREMENTS IN THE HUMAN EYE

4.1 CONTRIBUTIONS

The study was designed by me. Both co-authors Hema Radhakrishnan and Vincent Nourrit provided important contributions to the study. The data collection was done by me and my co-author Hema Radhakrishnan. The data analysis and writing of the study was done by me with useful advice from the co-authors. The writing of the paper was done by me and both co-authors Hema Radhakrishnan and Vincent Nourrit.

4.2 PUBLISHING OF THE PAPER

Pablo Benito Lopez, Hema Radhakrishnan and Vincent Nourrit. Predicted straylight values based on Scheimpflug densitometry. Target journal: Clinical and Experimental Optometry. To be submitted.

4.3 PRESENTATION AT CONFERENCE

This paper has not yet been presented at any conference.
4.4 ABSTRACT

PURPOSE: To assess how backward light scatter (BLS) measurements using the Oculus Pentacam are compared with forward light scatter (FLS) values as measured with the C-Quant straylight meter.

DESIGN: Prospective comparative study between measurements obtained with C-Quant straylight meter and Pentacam density values.

METHODS: Twenty-six subjects were included in the study with ages ranging from 19 to 35 (29.92 ± 4.06 [mean±SD]) years old and spherical equivalent ranging from 0 to -8 (-1.9 ± 2.27 [mean±SD]) dioptres. Backward light scatter measurements (Oculus Pentacam) were obtained and compared with forward light scatter measurements (C-Quant straylight meter values).

RESULTS: No significant relationship was found between corneal BLS and FLS ($r^2 = 0.10, p = 0.11$); or crystalline lens BLS and FLS ($r^2 = 0.010, p = 0.56$).

CONCLUSIONS: BLS measurements based on the Oculus Pentacam images do not provide information that can be compared to FLS values as measured with the C-Quant straylight meter.

4.5 INTRODUCTION

The transparency of the ocular media can be affected by various pathological conditions that increase the level of light scatter towards the retina (forward light scatter, FLS) or backwards (backward light scatter, BLS). Clinically FLS is more important than BLS as it reduces the contrast of the retinal image (Fry and Alpern, 1953) and visual
performance (Paulsson and Sjostrand, 1980, de Waard et al., 1992, Artal et al., 2001). However, the assessment of FLS has been proven to be a difficult task (Bettelheim and Ali, 1985) while BLS measurements can be obtained by Scheimpflug imaging (Drews, 1964). Hence assessing the relationship between FLS from BLS measurements would be of significant interest. This relationship was found in excised human lenses (Bettelheim and Ali, 1985) but was not possible to be demonstrated with in vivo subjects (de Waard et al., 1992). This lack of agreement has been attributed to the difference of the processes causing FLS and BLS (van den Berg, 1997, van den Berg and Spekreijse, 1999), where the size of the particles (e.g. keratocytes and multilamellar bodies) with respect to the incident light wavelength characterizes the forward or backward light scatter behaviour of the intraocular particles (Born and Wolf, 1980). In this sense, major ocular structures contributing to intraocular scatter, the cornea and crystalline lens, are sources of BLS (Olsen, 1982, Bettelheim and Ali, 1985, Smith et al., 1990, van den Berg, 1997, van den Berg and Spekreijse, 1999, Wegener et al., 1999, Donnelly et al., 2004, Wang et al., 2004, Patel et al., 2007, Hillenaar et al., 2011) and FLS (Bettelheim and Ali, 1985, van den Berg, 1996, van den Berg, 1997, van den Berg and Spekreijse, 1999, Koh et al., 2014).

FLS can be measured with the aid of C-Quant (Franssen et al., 2006) (Oculus Optikgerate GmbH, Wetzlar, Germany). The instrument has been used to assess in vivo FLS in a great variety of situations, especially when the transparency of the crystalline lens (e.g. cataracts (de Waard et al., 1992)) and cornea are altered (e.g. Fuchs dystrophy (Trousdale et al., 2014)). BLS can be measured by observing the light reflected back from the ocular media when a slit light is projected against the eye. An example of BLS measuring device is the Pentacam® (Oculus Optikgerate GmbH, Wetzlar, Germany).
A relatively recent instrument based on Purkinje imaging allowed the objective measurement of the scatter from the anterior segment of the eye, avoiding the portion of scatter attributed to the retina (Bueno et al., 2007). The instrument measuring the fourth Purkinje image (the one produced by the reflection of the back surface of the crystalline lens) was first tested on artificial eyes and subsequently on human eyes with customized contact lenses (with certain amount of microspheres simulating FLS) (Bueno et al., 2007). The instrument successfully extracted the BLS contribution of both cornea and crystalline lens but was also able to detect the small BLS increments due to the FLS simulations (customized contact lenses) (Bueno et al., 2007).

In another study, a certain level of correlation was found between nuclear opalescence (NO) and the standard deviation of the tails around the PSF lenslets of a Hartmann-Shack spot pattern (Applegate et al., 2004). NO is one of the four grading scales of the LOCS III cataract gradient system, and grades the opacity of the crystalline from a transparent lens (NO value of 0.1) to a dense cataract (NO value of 6.9) in steps of 0.1. NO can therefore be considered as an indirect measure of BLS in the eye. On the other hand, the tails around the point spread functions (PSF) obtained from the lenslets imaging of a Hartmann-Shack aberrometer contain information of FLS in the eye (Donnelly et al., 2004). Donnelly et al. (2004) proposed a metric in which FLS (obtained from the Hartmann-Shack spot patterns) and BLS (NO) could predict up to 51.2% of the visual acuity (logMAR) of the subject, thus establishing a dependent relationship between FLS and BLS.

Despite the partly negative results from these studies, it is relatively difficult to imagine that the physiological changes leading to increased BLS will not also increase FLS. For instance existence of keratocytes in the cornea are one of the causes of FLS
(Jester et al., 2007) and a study found that increased presence of keratocytes would also increase corneal density measurement (Patel et al., 2001). For this reason, the present study investigates whether BLS obtained as density measurements from the Oculus Pentacam can be linked to the assessment of FLS from the C-Quant straylight meter.

4.6 METHODS

In order to evaluate the relationship between BLS and FLS, density analysis of the cornea and the crystalline lens using Pentacam were assessed for a possible relationship with C-Quant values. Therefore, the study evaluated the correlation between corneal density (both peak and 3D), crystalline density (3D) and C-Quant straylight values. Corneal thickness, although found previously unrelated to increased scatter (Olsen, 1982), was included in the study and was also compared to C-Quant scatter values as different corneal thicknesses were thought to affect the average density measurement obtained with Pentacam (Olsen, 1982).

PARTICIPANTS

Twenty nine subjects were recruited from The University of Manchester student and staff population. Ocular abnormalities (i.e. visible defects in eye structures), previous ocular surgery and being a contact lens wearer (Nio et al., 2003) were considered as exclusion criteria. The Pentacam PNS (Pentacam Nucleus Staging) tool was also used to define the inclusion criteria. PNS was found useful to evaluate the density of nuclear cataracts and its correlation to LOCS III up to a nuclear opalescence value equal to 5 (Magalhaes et al., 2011). In the present study, a PNS value equal to 0 was mandatory for all the participants. PNS values equals to or greater than 1 considered the participant excluded.
from the study as this value means a level of crystalline density unusually high for healthy eyes, which might affect the analysis of the data. After applying that criterion, 26 subjects were included in the analysis, with ages ranging from 19 to 35 years ($29.92 \pm 4.06$ [mean±SD]) and spherical equivalent ranging from 0 to -8 (-1.9 ± 2.27 [mean±SD]) dioptres. Cylindrical power of the subjects ranged from 0 to -2 (-0.61 ± 0.57 [mean±SD]) dioptres. Although both eyes were tested, only right eyes were taken into consideration for statistical analysis in order to minimize errors caused by the presence of strong correlation between the two eyes (Ray and O'Day, 1985). The study followed the Declaration of Helsinki Ethical Principles. Ethics approval was obtained from the University of Manchester's ethics committee and written consent was obtained before participation in the study. All measurements were made with natural pupil diameters under dim lighting conditions.

MEASUREMENT WITH C-QUANT

Measurements with the C-Quant straylight meter are based on the compensation comparison method (Franssen et al., 2006). This method consists of two half discs surrounded by a flickering ring. The flickering ring is the glare source of achromatic light and has an effective eccentricity angle of 7 degrees (van den Berg, 1995). The two halves also flicker and the participant has to decide which half is flickering stronger. In this way, when one of the halves flickers, flickering will also be perceived on the other half by participants with certain levels of scatter in the eye. During the test, a two forced-choice assessment and a psychometric function decides on the comparisons that will be presented to the participant (Franssen et al., 2007). The results from this assessment are: the ocular straylight value ($\log(s)$) (van den Berg et al., 2013), the standard deviation (ESD) of successively measured values and the quality factor of the psychometric function (Q)
(Coopens et al., 2006). An ESD value lower than 0.08 and Q value higher than 1 indicates good reliability of measurements.

Measurements were obtained under scotopic light conditions. Participants’ position with respect to the instrument was checked (Van den Berg, 2014). Two valid measurements (ESD<0.08 and Q>1) were obtained and averaged for each participant. Participants’ refraction was corrected with their spherical equivalent refraction (Van den Berg, 2014) when needed.

**MEASUREMENT WITH PENTACAM**

Pentacam (Figure 4.1) is an instrument based on Scheimpflug imaging that uses a 360° camera with a blue LED light source (475nm) to obtain 25 Scheimpflug monochromatic photographs (1003x520 pixels) of the eye. Each one of these photos corresponds to a different meridian (ranging from 0 to 360° with steps of 14.4°) of the eye. Pixel intensities (densities) of these monochromatic photos are presented in grey scale units and range from 0, for a maximum transparency and minimum light scatter detected (black pixel), to 100 for maximum light scatter and minimum transparency (white pixel). Measurements were considered as valid when the quality coefficient Q was equal to “OK” and none of the 25 photographs presented any defects (part of the image missing or not well aligned). Measurements took between 1 to 2 seconds and were obtained in scotopic light conditions. Two valid measurements were obtained for each one of the participants. The basic version of Pentacam was used in this study.
Figure 4.1: Scheimpflug image obtained with Pentacam. Peak corneal densitometry value is the highest intensity pixel obtained from a histogram of the cornea for a certain measured meridian (top right corner, in green). 3D corneal densitometry assessment divides the cornea in concentrical zones around the pupil centre and provides with an average density value for each zone. The optical axis is represented with the dotted red line.

The Pentacam instrument comes with a basic densitometry software that can be improved upon through premium add-ons (Table 4.1).
<table>
<thead>
<tr>
<th>MEASURED ZONE OF THE EYE</th>
<th>BASIC PENTACAM SOFTWARE</th>
<th>PREMIUM PENTACAM SOFTWARE ADD-ONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>Peak density (peak corneal density value)</td>
<td>3D corneal density (average density value measured at concentric zones from corneal apex (0-2, 2-6, 6-10 and 10-12mm))</td>
</tr>
<tr>
<td></td>
<td>Corneal thickness (measured at 0, 2, 4, 6, 8 and 10mm of eccentricity from corneal apex)</td>
<td>X</td>
</tr>
<tr>
<td>Crystalline</td>
<td>Peak density (peak crystalline density value)</td>
<td>PNS (Pentacam Nucleus Staging) density tool: Provides with a cataract grading scale and a 3D crystalline density which gives an average density level measured at concentric zones from corneal apex (PDZ1=0-2, PDZ2=0-4 and PDZ3=0-6mm)</td>
</tr>
<tr>
<td>Any part</td>
<td>Average linear density (average density level measured between 2 user selected points on the Pentacam density image)</td>
<td>X</td>
</tr>
</tbody>
</table>

|Table 4.1: Types of density measurements and corneal thickness measurements available in the basic version of Pentacam (software version 1.20r29).|

Corneal density can be measured either using the Peak density analysis which is integrated into the basic software package and gives a single peak density value for the cornea at a measured meridian around the corneal apex (for a total of 25 meridians); or the 3D corneal density measurement (provided as a premium add-on) which gives an average density value for a concentric volume around the corneal apex (0-2, 2-6, 6-10 and 10-12mm of eccentricity) (Greenstein et al., 2010, Takacs et al., 2011, Otri et al., 2012) and provides an average grey scale unit value for each one of them. The 3D corneal density tool also subdivides the cornea into anterior layer (first 120µm of corneal thickness), centre layer and posterior layer (last 60 µm of corneal thickness). Centre layer does not have a fixed thickness as it depends on individual corneal characteristics and is the result of deducting the sum of the anterior and posterior layers from the total corneal thickness of the measured participant. Many studies have obtained measurements from subjects using
the peak corneal density analysis (Greenstein et al., 2010, Takacs et al., 2011, Otri et al., 2012, Elflein et al., 2013). However, this method has been found to be less repeatable compared to the 3D corneal density measurement (Kirkwood et al., 2009).

Crystalline lens density can be assessed by using the Peak density analysis which is integrated into the basic Pentacam software and provides the highest density value for the lens, for a meridian and around the corneal apex. Crystalline lens density can also be measured using the PNS (Pentacam Nucleus Staging) premium add-on density tool. The PNS tool provides a cataract grading scale similar to the one provided by the Lens Opacification Classification System (Kirkwood et al., 2009, Magalhaes et al., 2011) (LOCS III) (Chylack et al., 1993). The PNS tool also comes with a 3D crystalline density measurement which provides an average density value measured at concentric volumes around the corneal apex. These volumes are defined between: 0 and 2mm (PZ1); 0 and 4mm (PZ2); and 0 and 6mm (PZ3). In the Pentacam software, values for corneal thickness are shown for 0, 2, 4, 6, 8 and 10mm of eccentricity.

Two valid measurements were obtained and averaged for each participant with both C-Quant and Pentacam. In case of Pentacam, each valid measurement gives a total of 25 Scheimpflug images providing:

- Peak densitometry: 1 value per image.
- 3D corneal densitometry: 1 value per each eccentricity (0-2mm, 2-6mm, 6-10mm, 10-12mm) and region (anterior, centre and posterior).
- Corneal thickness: 1 value per eccentricity (0, 2, 4, 6, 8 and 10mm).
- 3D crystalline density: 1 value per each of the eccentricities (PDZ1 = 0-2mm, PDZ2 = 0-4mm and PDZ3 = 0-6mm)
STATISTICAL ANALYSIS

The distributions of the data from Pentacam and C-Quant were analysed for normality with the Kolmogorov-Smirnov test (normality accepted for p value higher than 0.1 using the Lilliefors significance correction). The relationships between corneal thickness, corneal density, crystalline density and C-Quant were assessed with two tailed paired sample t-test and Pearson’s correlation coefficient (r). A linear regression (least squares) was performed to assess the contribution of BLS data from Pentacam density measurements to FLS (C-Quant FLS values). A p-value of less than 0.05 was considered as significant.

The results of this analysis were obtained using SPSS Statistics 22 (IBM, Chicago, IL) and MedCalc 12 (MedCalc Software, Mariakerke, Belgium).

4.7 RESULTS

Normality was found for all the analysed data sets (p > 0.1). The participants’ pupil diameter measured with Pentacam (averaged from two valid measurements) ranged from 2.4 to 4.35mm (2.95 ± 0.52mm [mean±SD]). Values for the C-Quant straylight meter (averaged from two valid measurements) ranged from log(s) = 0.73 to 1.27 (1.02 ± 0.06 [mean±SD]).

Peak corneal density: Average values ranged from 24.50 to 30.71 (28.15 ± 1.65 [mean±SD]). The correlation between these values and C-Quant values was not statistically significant ( r = 0.31; p = 0.11).
**3D corneal density**: Average values are presented in *Table 4.2*. As it can be observed, higher density values correspond to the 10 to 12 mm radial eccentricity follow by the radial zone measured around the corneal apex (0 to 2 mm). Focussing on the corneal area, the anterior part of the cornea shows the highest density value for all the eccentricities, followed by the centre one. Higher standard deviation values for the measured eccentricity between 10 and 12 mm might be due to the proximity of this area to the eyelid.

<table>
<thead>
<tr>
<th>3D CORNEAL DENSITY VALUES</th>
<th>Radial eccentricity measured from corneal apex</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal area</td>
<td>0-2 mm</td>
<td>2-6 mm</td>
</tr>
<tr>
<td>Anterior</td>
<td>24.7 ± 1.28</td>
<td>21.8 ± 1.06</td>
</tr>
<tr>
<td>Centre</td>
<td>21.1 ± 0.98</td>
<td>17.97 ± 0.82</td>
</tr>
<tr>
<td>Posterior</td>
<td>17.27 ± 0.79</td>
<td>15.05 ± 0.75</td>
</tr>
<tr>
<td>Total</td>
<td>21.03 ± 0.91</td>
<td>18.33 ± 0.84</td>
</tr>
</tbody>
</table>

*Table 4.2*: Values (grey scale units) for the 3D corneal density for each one of the radial eccentricities measured from the corneal apex and for each corneal area (mean±SD).

Anterior corneal zone comprises the first 120 µm of corneal thickness and posterior corneal covers the last 60 µm of corneal thickness. The centre of the cornea does not have a fixed thickness and is calculated as the result of resting both anterior and posterior corneal thickness from the total corneal thickness of the measured participant. Total values are also shown for each of the eccentricities and corneal areas.

**Corneal thickness**: Averaged values obtained for the different eccentricities measured (0, 2, 4, 6, 8 and 10 mm) from the corneal apex are presented in *Table 4.3*. In *Table 4.3*, corneal thickness value increases from the corneal apex (0 mm) to 10 mm of radial eccentricity.

<table>
<thead>
<tr>
<th>CORNEAL THICKNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eccentricity measured from corneal apex</td>
</tr>
<tr>
<td>Mean (µm)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>SD (µm)</td>
</tr>
</tbody>
</table>

*Table 4.3*: Average corneal thickness values and standard deviation (SD) obtained for the participants at different radial eccentricities measured from the corneal apex. Values are expressed in µm.
The correlation found between corneal thickness and C-Quant FLS values for each of the eccentricities measured was: 0mm ($r = 0.06, p = 0.76$), 2mm ($r = 0.06, p = 0.77$), 4mm ($r = 0.06, p = 0.77$), 6mm ($r = 0.07, p = 0.73$), 8mm ($r = 0.07, p = 0.75$) and 10mm ($r = 0.03, p = 0.89$).

**3D crystalline density:** Pentacam was not able to capture the density value of the crystalline lens for the larger measured eccentricity (PDZ3=0-6mm) in most participants and hence it was not taken into consideration. PDZ1 values ranged from 8 to 11.35 ($9.61 \pm 0.77$ [mean±SD]) while PDZ2 values ranged from 8.5 to 11.45 ($9.75 \pm 0.72$ [mean±SD]).

Graph 1 shows the BLS (average 3D corneal density values (Total 0-2 mm) and the average 3D crystalline density (PDZ1)) as a function of C-Quant straylight meter values (FLS). In both cases, a weak and statistically insignificant relationship was found: average 3D corneal density values (Total 0-2 mm) and C-Quant ($y = 2.16x + 18.84$, $r^2 = 0.1$, $p = 0.11$); average 3D crystalline density (PDZ1) and C-Quant ($y = 0.68x + 8.92$, $r^2 = 0.01$, $p = 0.56$).
Figure 4.2: Average 3D corneal density values (Total 0-2mm) and the average 3D crystalline density (PDZ1) in grey scale units as a function of C-Quant (log(s)).

4.8 DISCUSSION

The study assessed whether a relationship that could be found between density maps due to BLS (obtained with Pentacam) and FLS as measured with the C-Quant. The study is based on a young healthy population with very low levels of crystalline lens.
density (PNS levels equal to 0 were considered as inclusion criteria) and non-dilated pupils.

In this study, BLS measurements with Pentacam were performed using the peak density and 3D density analysis in case of the cornea. Crystalline density was assessed using the 3D density analysis. Another study suggested that repeatability improves with the averaging of 3D density values and decreases when using single peak values (Kirkwood et al., 2009). Furthermore, high repeatability and validity of the 3D density analysis of ocular lenses was found in subjects with and without cataracts (Kirkwood et al., 2009).

**Corneal density** results obtained for this study differ depending on the type of measurements used: peak or 3D analysis. For the peak density analysis, 28.15 ± 1.65 (mean ± SD) grey-scale units while 20.20 ± 1.79 (mean ± SD) for the 3D one. These results are in agreement with a previously published study (Ni Dhubhghaill et al., 2014) and make sense considering the way of measurement: the value obtained by the peak density analysis is the result of a single peak value measured for the corneal lens along one meridian, while the 3D measurement provides an average value of a cylindrical volume of the cornea around the corneal apex, which covers 0-2, 2-6, 6-10 and 10-12 mm of eccentricity.

**Peak corneal density** measurements have been found to be related to the LOCS III cataract grading system (Pei et al., 2008). However, the limitations of this measurement (peak value for a single line along the cornea and for certain meridian) compared to the 3D corneal density measurement showed also less repeatability (Kirkwood et al., 2009).

Results obtained from the **3D corneal density** analysis indicate that higher density levels for the anterior corneal layer in every measured eccentricity coincide also with a higher density of keratocytes for that layer (Patel et al., 2001) and therefore with an
increased level of intraocular scatter (Jester et al., 1999). However, if the relationship between BLS and FLS existed, measurements from the 3D corneal density would be expected to correlate better with FLS values (Figure 4.2) as crystalline lens density values were intended to be as low as possible for the study (PNS = 0). This fact should also have conditioned the C-Quant values, making the scatter contribution from the cornea a larger part of it.

In one study (Wu et al., 2014) results obtained from the 3D corneal density analysis and compared to C-Quant values showed a slightly better yet negative agreement for the eccentricities of 0-2 ($r = 0.13, p = 0.04$), 2-6 ($r = 0.15, p = 0.03$) and 6-10mm ($r = 0.16, p = 0.02$) (Wu et al., 2014). These results (Wu et al., 2014), which have also different 3D corneal density values from the ones obtained in this study (and a larger previous study with similar results to the present study (Ni Dhubhghaill et al., 2014)), might be due to differences in the software used for the density measurement of the Pentacam images, units conversion or saturation of the processed density images.

**Corneal thickness** measurements obtained in this study are in agreement with a previous study in which the repeatability of the corneal thickness measurements was positively assessed using Pentacam (Miranda et al., 2009). Corneal thickness was previously found not to affect FLS measurements (Wu et al., 2014).

In this study, the **peak crystalline lens density** was measured, but not taken into account for calculations as it was found to be dependent on the amount of light reaching the crystalline lens for each one of the meridians. The selection of participants with low levels of crystalline density (PNS = 0) for this study might be the reason for the change of shape of the crystalline density map obtained for each one of the meridians. In addition, due to this study assessing a small radial zone of cornea and crystalline around the corneal
apex, pupils were not dilated. This fact might have limited the validity of the 3D crystalline density measurements (even when the quality factor Q equals to “OK” and none of the 25 photographs presenting any defects) of the crystalline lens with non-dilated pupils to values PDZ1 = 0-2mm and PDZ2 = 0-4mm (excluding PDZ3 = 0-6mm) of eccentricity. Furthermore, one study showed that measurements of the posterior capsule of the crystalline lens can be difficult even if the pupil is totally dilated (Fujisawa and Sasaki, 1995). To our knowledge, no studies are available in the literature evaluating whether Pentacam density values in dilated and non-dilated pupils differ. In this sense, a study has suggested this comparison to ensure that Pentacam can be used for mass screening (Lim et al., 2014). This lack of illumination is not only appreciable at the back of the crystalline lens, but also when measuring near the eyelids or eyelashes. This effect produces a great variability on the 3D corneal density measurements as seen with the increase of the standard deviation, especially for the 10-12mm eccentricity (Table 4.2).

Density values (grey scale units) obtained for the 3D crystalline density measurements are almost half the value of the 3D corneal density values. This comparison contradicts the purpose of this study which is to compare BLS and FLS, since scatter contribution of the crystalline lens to the total FLS is supposed to be higher than that of the cornea (Vos and Boogaard, 1963). The weak agreement observed between 3D crystalline density values and C-Quant values (Figure 4.2) might be due to the lack of pupil dilation.

The study has considered the two most important ocular FLS sources which are the cornea (30%) (Vos and Boogaard, 1963) and the crystalline lens (40%) (Bettelheim and Ali, 1985) (cornea and crystalline). BLS from cornea and crystalline lens should have accounted with similar amounts in case of agreement. This lack of agreement may, however, be explained by various factors. Firstly, the BLS value obtained by Pentacam
might be a measure of light reflection (Rosales and Marcos, 2009). Our hypothesis that the structure causing this reflection/BLS would also produce significant FLS may be incorrect. FLS is mainly a combination of Mie/geometric scatter rather than Rayleigh scatter while BLS is the opposite (Born and Wolf, 1980, van den Berg, 1997, van den Berg and Spekreijse, 1999). In this sense, multilamellar bodies, which are the main FLS source in the crystalline lens, can be explained by the Mie theory (van den Berg, 1997, van den Berg and Spekreijse, 1999, Vos, 2003, Costello et al., 2007). Secondly, density values of both cornea and crystalline lens have not been considered with respect to their thicknesses. The thickness of the ocular scatter particles defines their scatter behaviour (FLS or BLS) (van den Berg, 1997, van den Berg and Spekreijse, 1999). This analysis of the scatter particles using Pentacam BLS images would allow the corneal and lens correction algorithms of Dubbelman et al. (Dubbelman and Van der Heijde, 2001, Dubbelman et al., 2001, Dubbelman et al., 2006) and might give a better approximation to FLS using BLS images. Unfortunately, Pentacam raw images can only be analysed by using the software provided by the manufacturer (Rosales and Marcos, 2009) and the tools available with such software. Extraction of raw images for extra analysis are also limited due to proprietary limitations and only custom made programs can give access to such images (Rosales and Marcos, 2009).
5. INSTRUMENT AND COMPUTERIZED BASED MODEL FOR THE OBJECTIVE MEASUREMENT OF FORWARD LIGHT SCATTER

5.1 CONTRIBUTIONS

The study was designed by my co-author (Vincent Nourrit) and me. The optical set-up was initially suggested by J. Barbur (City University, London). The data collection and the analysis of the study were done by me with useful advice from my co-author. The writing of the study was done by me and my co-author.

5.2 PUBLISHING OF THE PAPER

Target journal: Journal of Biomedical Optics. Authors: Pablo Benito Lopez and Vincent Nourrit. To be submitted.

5.3 PRESENTATION AT CONFERENCE

This paper has been submitted to the Visual and Physiological Optics meeting, held in Wroclaw (Poland) from the 25th to the 27th of August 2014 (EMVPO) before being
withdrawn. However, the EMVPO organizing committee still published it in the proceedings (Benito Lopez et al., 2014).

5.4 ABSTRACT

BACKGROUND: In the last decade, several instruments have been introduced to measure objectively and accurately intraocular forward light scatter (FLS). Unfortunately, these measurements cannot be related precisely to any physiological factors.

PURPOSE: To develop an instrument together with a model with a view to obtain accurate FLS measurements that can be related to the sources of scatter.

METHODS: An optical set-up was developed where a subject or an artificial eye looks at an LED ring, placed at a fixed distance, and the retinal image of the ring is recorded with a CCD camera (double pass configuration). Firstly, the validation of the instrument was conducted by an artificial eye, where the scatter was produced by a microscopy plate containing a layer of silica microspheres. A non-sequential Zemax model of this set-up was also developed in order to relate the experimental measurements to the parameters of the optical system. In both – the optical set-up and Zemax model – the amount of forward light scatter was assessed from the distribution of light inside the ring of imaged LEDs on the CCD. Secondly, ten participants were tested with the optical set-up. The scatter measurements in human eyes were compared with the FLS values obtained from the C-Quant straylight meter. A Zemax model of the human eye, including scatter structures, was also developed and compared to the measurements obtained on participants with the optical set-up.
RESULTS: The amount of light scattered in the artificial eye was successfully recorded by the instrument and was in good agreement with the Zemax model of the optical set-up. Human eye measurements also related well with C-Quant straylight meter values. Scatter from the Zemax eye model could not be positively related to the human eye measurements.

CONCLUSIONS: Results with the artificial eye suggested that the proposed optical set-up can provide the means to assess FLS objectively and that the FLS measurement can be related to a physical parameter through the Zemax model (e.g. scatter particle density). Results in human eyes correlated well with measurements obtained with the C-Quant ($r^2 = 0.75; p < 0.05$) but cannot yet be explained by the developed Zemax model.

5.5 INTRODUCTION

Our vision is often described as limited by three optical factors: aberrations, diffraction and light scatter. While diffraction is inherent to any optical system and aberrations can be successfully corrected through various means (e.g. spectacles, surgery), scatter increases with age and irremediably reduces our visual performances. The importance of the FLS lies in its effect on the quality of the retinal image because of the reduction of contrast sensitivity and the increase in disability glare (De Waard et al., 1992). As a result, the amount of FLS could be for instance the deciding factor in cataract surgery (Bal et al., 2011) or limiting in visually demanding jobs (Van Bree et al., 2011).

Despite the importance of FLS there is currently no instrument to measure it objectively and to relate it to physiological parameters. The purpose of this study is to address this lack. In section 5.6 the literature related to the measurement and modelling
of FLS is reviewed before the methods used for this study are presented (5.7). Results and discussion are shown in sections 5.8 and 5.9 respectively.

5.6 REVIEW OF FLS MEASURING AND MODELING

5.6.1 MODELLING FLS

5.6.1.1 Modelling the physiological sources of scatter

In contrast to low order aberrations that can be related to few simple physiological parameters, the origin of FLS is complex. FLS in the healthy human eye corresponds to 1-2% of the incoming light (Vos, 1984). For a healthy eye, up to 30% of the FLS comes from the contribution of the cornea (Vos and Boogaard, 1963), 40% from the crystalline (Bettelheim and Ali, 1985), 10% from the vitreous (Koenz et al., 1995) and another 20% is due to the retina (Vos and Bouman, 1964). A small fraction of the FLS (≤1%) can also be attributed to transillumination through the iris (van den Berg et al., 1991).

Regarding the cornea, the anterior surface of the epithelial cell layer and the posterior surface of the endothelium were found to be the major sources of scatter when using in-vivo confocal microscopy (Jester, 2008). Scatter from the stroma is limited to Keratocytes nuclei (Jester, 2008) as demonstrated by the good relationship between decrease in the density of keratocytes and the reduction of FLS (Jester et al., 2007). These keratocytes occupy a volume of 9-17% within the stroma and have a density of 23000 cells/mm³ in human eyes (Hahnel et al., 2000, McLaren et al., 2005). Keratocytes nuclei
have a thickness of 1µm (Hahnel et al., 2000) and an average refractive index of 1.401 (Patel et al., 2001).

Regarding the crystalline lens, scatter is due to the aggregation of protein molecules in the crystalline’s nucleus (Bettelheim and Ali, 1985) and mainly because of the posterior migration of cells from the epithelia creating large organelles called multilamellar bodies (Gilliland et al., 2001, Gilliland et al., 2004). Multilamellar bodies have a density of 556 cells/mm³ for healthy crystalline lenses, a diameter of 2.7±0.7µm and an average refractive index of 1.49 (Gilliland et al., 2004, Costello et al., 2007).

5.6.1.2 Modelling light propagation within the eye

The absence of an instrument to relate the FLS measurements to a physiological parameter is probably due to the structural complexity of the human eye, but also the associated difficulty to describe light propagation within the eye.

Over the last 150 years, numerous eye models have been proposed with increasing complexity (aspheric surfaces, GRIN, etc. (see (Navarro, 2009) for a review). Some of the models allow taking into account scatter elements (e.g. (Kelly-Pérez et al., 2011, Chen et al., 2012, Kelly-Perez et al., 2013) when the scatter sources are arbitrarily defined (e.g. number and distribution of scatter particles in the crystalline lens (Kelly-Perez et al., 2013).

Due to the geometric dimensions of the eye and the limited influence of diffraction, propagation through such models has usually been carried out using
geometrical optics. When field propagation is considered, calculations are usually limited to the measurement of the Fourier transform of the wavefront in the pupil’s plane. Mas et al. proposed an interesting model to simulate wave propagation inside the eye and along any distance using Fractional Fourier transform (Mas et al., 2009). Although theoretically superior to the geometrical models, the main limitations of these approaches are: the discrepancy between the complexity of the calculations and the lack of accuracy to characterize the different ocular elements. For instance, the characteristics of the crystalline lens: tilt, decentring, GRIN, etc. are known with limited accuracy and thus discourage researchers from pursuing a sophisticated model.

5.6.2 MEASURING FLS

Methods to assess FLS are usually divided into two groups depending on whether they require or not active participation from the tested participants.

Methods that automatically (without the subject’s input) assess FLS rely on the analysis of a retinal image while a glare source is being presented to the eye (usually the assessment of the point spread function (PSF) through double pass techniques (Santamaría et al., 1987, Artal et al., 1995, Kuroda et al., 2002, Donnelly Iii et al., 2004)). The main issue with this approach is the difficulty to measure small angle scatter due to the large dynamic range of the signal and the fact that the centre of the PSF is mainly controlled by aberrations. In this context, a recent study from Ginis et al. presents a promising instrument that can reconstruct the wide-angle PSF up to 8 degrees (Ginis et al., 2012).
The methods based on subjects’ response assess the contrast sensitivity in the presence of a glare source. The two most successful methods are probably the direct compensation technique (van den Berg, 1986) and the compensation comparison method (Franssen et al., 2006). The latter is based on the direct compensation technique but with a psychophysical analysis that improves the repeatability substantially. On the contrary to the methods not relying on the subject’s input, these methods assess the domain of the PSF corresponding to large angle scatter (Van Den Berg et al., 2009) as depicted in Figure 5.1.

![Figure 5.1: PSF plot the human eye and domains covered by the different FLS measuring techniques (Van Den Berg et al., 2009).](image)

The information obtained by the current instruments to measure FLS does not always correlate well with physiological factors (cf. previous chapters of this PhD thesis).
The aim of this study was therefore to develop an instrument together with a model to obtain accurate and reliable FLS measurements and to account for the source of scatter.

5.7 METHODS

The study was divided into two parts. Firstly, an optical bench was set up where a subject or an artificial eye, looks at a LED ring placed at a fixed distance, and the retinal image of the ring is recorded by a CCD camera (in a double pass configuration). The artificial eye was made of a mirror and a lens. Determined levels of light scatter were produced with a microscopy plate and a layer of silica microspheres whose properties are known (index of refraction, diameter, and density). Four microscope plates containing increasing density levels of silica microspheres were used to simulate increased levels of scatter. A non-sequential Zemax model of this set-up was also developed. Mie scatter was introduced in the Zemax model to simulate the scatter produced by the silica microspheres. In both – the optical set-up and the Zemax model – the light distribution within the retinal image of the ring was analysed. Details of the optical set-up are introduced in sub-section 5.7.1.

Once the agreement between experimental set-up and model was validated, scatter measurement from human eyes was obtained with the prototype and compared with scatter values from the C-Quant straylight meter. The eye model and recruitment criteria for patients are described in section 5.7.2.
5.7.1 EXPERIMENTAL SET-UP USED FOR EXPERIMENT 1 AND 2

The optical double pass instrument is depicted in Figure 5.2.

**Figure 5.2:** Optical set-up for the objective measurement of FLS. Light from the light source (LEDs ring) is reflected by the beam splitter into the eye. This light is then reflected by the retina and transmitted by the beam splitter into a series of lenses (L1, L2 and L3) and apertures (D1 and D2) and finally recorded into a CCD camera. CCD, retina and light source are conjugated. D1 eliminates diffuse reflection from the cornea while letting FLS pass through. D2 blocks specular reflexions from the cornea.

In summary, the eye looked at the light source (LEDs ring) through the beam splitter (BS) (Thorlabs BP108 - Ø1” pellicle beam splitter, uncoated for a reflexion:transmission ratio of 8:92). The light from the LED ring was then reflected by the retina and captured by the CCD camera after passing again through the pellicle beam splitter (BS) and a series of lenses (L1-L2-L3) and apertures (D1-D2). The CCD, the retina and the light source (LEDs ring) were conjugated. The source of light (the chromaticity coordinates are X:0.287-0.330, Y:0.295-0.318) was composed of 16 white LEDs (Nischia LED 5mm white 44000mcd NSPW500GS-K1) of 5mm width each, forming a circle of 1.78 centimetres of radius. The LEDs ring could be moved mechanically to vary the distance.
light source - eye. The smallest angle of observation had to be greater than 7 minutes of arc in order to be outside the PSF (Westheimer and Liang, 1995). With this LED circle, different eccentricities with respect to the optical axis (eye - LED ring) could be measured for each distance of the LED ring with respect to the eye. D1 was conjugated with the retina and thus stopped unwanted signals such as diffuse reflection on the cornea and retina. On the contrary, D2 allowed passing only light coming with an angular eccentricity larger than the centred black circle. D2 was made of an uncoated transparent plastic film with a black dot at the centre and its aim was to block the incoming light caused by specular reflection from the cornea.

The amount of light captured with the CCD in the centre of the annulus was proportional to the level of scatter inside the eye. In the absence of scatter, all the light originated in the LEDs should be concentrated in their respective image. The image of the LEDs ring on the retina was recorded using a CCD camera (Rolera XR, Qimaging, BC, Canada) and processed with the program Q-Capture version 2.901 (Qimaging, BC, Canada), obtaining an image size of 696x520 pixels and a pixel size of 12.9 x 12.9µm. The optical path of the set-up was protected with a black flocking material in order to avoid unwanted reflections or the apparition of artefacts.

MEASUREMENT OF FLS

Noise from the system, mainly due to back reflections from the pellicle beam splitter, was measured at the beginning of each measuring session in scotopic conditions. The pixel intensity produced by the noise was measured and later deducted from the recorded CCD image. The intensity profile across a diameter of the LED ring is presented in Figure 5.3 where the blue line represents the signal recorded by the instrument and
the red line corresponds to the noise signal. The two peaks correspond to the LEDs profile image. The fact that the instrument signal between the two peaks is not reduced by the noise level illustrated the presence of scatter.

![Image](image.jpg)

**Figure 5.3:** Pixel intensity profile recorded by the CCD. Red line corresponds to the instrument noise. Blue line shows the recorded LEDs pixel intensity (peaks) and the amount of scatter (intensity recorded within the two peaks). Intensity per pixel measured in this simulation at the centre of the two peaks is about 5 times higher than the level of measured noise. The pixel intensity produced in case of aberrations due to subjects’ eye should have given a homogenous scatter pattern for all the CCD image (and not only in the centre of the ring, also outside) and with a similar intensity signal to the one produced by the noise in that graph.

The CCD exposure time was chosen (empirically) to maximize the intensity detection at the centre of the LEDs ring at the camera sensor.
A customized software was developed in Matlab to analyse the images obtained from the CCD camera. The software took the images recorded from the CCD (centre of the LED ring reflected from the retina) and corrected them from the noise signal (deducted an image obtained prior to measurements with artificial/human eye). Subsequently, the average intensity per pixel in the central area of the LED ring was calculated.

5.7.2 FIRST EXPERIMENT: VALIDATION OF THE PROTOTYPE WITH CUSTOMISED LENSES AND ZEMAX MODEL

An artificial eye was used for this part of the experiment. The eye consisted of a lens of 62.5D (F=16mm, diameter=1”) and a mirror. This specific lens was selected for the experiment as the size and the dioptric power were similar to the ones presented in a human eye. The mirror was used instead of retina in order to reduce the modelling complexities.

The source of scatter was created with an uncoated microscope plate which was covered with a layer of a polymer solution and a microscope plate cover on top. The polymer solution contained a determined percentage of silica microspheres whose properties are known (Monodisperse Silica Microspheres, mean diameter 4.08um, index of refraction = 1.45, Cospheric, U.S.A.). The diameter of microspheres was chosen in order to simulate the diameter of the scatter sources that can be normally found within the human eye (i.e. keratocytes). Four microscope plates with increasing levels of microspheres density were used to simulate increased levels of scatter. These microscope plates where placed between the lens and the mirror. A distance of 50 cm between the light source and
the artificial eye was considered. All images were taken in scotopic light conditions. The ‘noise’ image was recorded before each measurement.

In summary, three series of pictures were recorded for each of the following scatter sources (i.e. a total of 12 images):

- Microscopy slide alone (index of refraction n=1.51) + polymer (n=1.30) + cover (n=1.51).
- Microscopy slide (n=1.51) + 1% concentration of microspheres (n=1.45) + polymer + cover.
- Microscopy slide + 2% concentration of microspheres + polymer + cover.
- Microscopy slide + 3% concentration of microspheres + polymer + cover.

The modelization of the prototype was made by using the optical design software Zemax_EE 2009 (Radiant Zemax, Redmond, U.S.A.) in non-sequential mode, together with a scatter library, Mie.dll (provided by Zemax software) to simulate MIE scatter. Values of density (percentage concentration of microspheres per volume), index of refraction and average microsphere diameter (4.08 µm) were added to the design for each one of the microscope plates. For comparison between simulations (Zemax) and experimental results (microscopy plates in the artificial eye), the following ratio was calculated for each one of the microscopy slides:

\[
\frac{\text{Intensity of scatter light within the centre of the LED ring's image in the presence of scatter microspheres}}{\text{Intensity of scatter light within the centre of the LED ring's image in the absence of scatter microspheres}}
\]

The intensity of scatter light recorded in the absence of scatter microspheres (microscopy slide alone) by the CCD corresponded to a number of analysis rays defined by
the user in Zemax. In this study, $5 \times 10^6$ analysis rays were sent into the Zemax model of the artificial eye for the simulation.

5.7.3 SECOND EXPERIMENT: RESULTS FROM PARTICIPANTS, COMPARISON RESULTS PROTOTYPE / C-QUANT AND PROTOTYPE / ZEMAX SCATTER EYE MODEL

In order to maximize the alignment eye - optical system during measurements with participants, a chin rest was added to the set-up. Moreover, an additional aperture was placed in front of the chin rest with the adequate diameter to facilitate the task of alignment by the subject to the optical system.

The pupillary diameter (assessed with a pupillary ruler), distances from the light source to participants’ pupil and light intensity level of the light source were measured for each one of the participants (Bergamin and Kardon, 2003).

The measuring technique was the following:

- A first image in the absence of human eye is taken to calculate the noise of the instrument.

- After placing the chin of the subject on the chin rest and adjusting the distance eye – light source and the intensity level of the light source, the subject was asked to look at the centre of the ring. Two valid measurements (images without artefacts and well-aligned images) were obtained and averaged for a determined distance and intensity level of the light source. Subsequently, average intensity per pixel
recorded by the CCD camera was calculated for the ten participants and compared to FLS values from C-Quant.

All measurements were done under scotopic light conditions. The distance eye – light source was set to 50 cm. Participants were asked to focus on the centre of the LEDs ring.

**MEASUREMENT WITH C-QUANT**

The C-Quant straylight meter (Figure 5.4) uses the compensation comparison method (Franssen et al., 2006) for the assessment of FLS. Essentially, a test field is presented to the subject consisting of two half discs surrounded by a ring. This ring is the glare source of achromatic light and has an effective eccentricity of 7 degrees (van den Berg, 1995). During the assessment, a series of 2-forced choice tests are presented to the subject. The subject has to decide, while the glare source flickers, which one of the two inner half discs is flickering the most. FLS will be perceived by the subject as a flickering on the non-flickering half disc. A psychometric function (Franssen et al., 2007a) adapts the type of test presented to the subject and the results for this assessment are three values: the straylight parameter (log(s)), the standard deviation of the measurement (ESD) and a quality factor (Q) (Coopens et al., 2006).
The assessment with C-Quant is performed under scotopic light conditions after checking that the subject’s position with respect to the instrument is correct. Participants with any refracted error were corrected with their spherical equivalent correction (Van den Berg). A valid measurement of FLS (log(s)) is considered when the standard deviation of the measurement is lower than 0.08 and quality factor higher than 1. Two valid measurements were obtained for each one of the participants and averaged.

For this study, ten participants underwent the experiment. Visible defects in the eye structures (with slit-lamp examination), history of ocular diseases or previous ocular surgery were considered as exclusion criteria. Due to the lack of a mechanism to control the pupillary miosis of the eye in bright conditions (LEDs), only participants with pupillary diameters (in miosis) larger than the measured eccentricity (2.04") were considered. Age of the participants ranged from 29 to 35 (32 ± 2.16 [mean±SD]) years. Spherical equivalent ranged from -3.25 to 1.25 (-0.8±1.57 [mean±SD]) dioptres. Only right eyes were tested and taken into consideration for statistical analysis.
ZEMAX EYE MODEL OF SCATTER AND MEASUREMENT

The model of scattering in the human eye was done in Zemax non-sequential mode and using the MIE scatter tool to simulate bulk scatter within the lenses.

The model was based on the physiological parameters of the eye obtained by Liou & Brennan (Liou and Brennan, 1997). This model was chosen because it offers a good compromise between simplicity and anatomic realism. For instance, it allows taking into account factors such as aspheric surfaces for the cornea and lens, a curved surface for the retina, or a gradient refractive index for the crystalline lens. Major characteristics of this model are shown in Table 5.1.

<table>
<thead>
<tr>
<th>Ocular Surface</th>
<th>Radius (mm)</th>
<th>Thickness (mm)</th>
<th>Asphericity</th>
<th>Refractive index (555nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>7.77</td>
<td>0.50</td>
<td>-0.18</td>
<td>1.376</td>
</tr>
<tr>
<td>Aqueous</td>
<td>6.40</td>
<td>3.16</td>
<td>-0.60</td>
<td>1.336</td>
</tr>
<tr>
<td>Front crystalline</td>
<td>12.40</td>
<td>1.59</td>
<td>-0.94</td>
<td>Gradient A</td>
</tr>
<tr>
<td>Back crystalline</td>
<td>Infinity</td>
<td>2.43</td>
<td>------</td>
<td>Gradient B</td>
</tr>
<tr>
<td>Vitreous</td>
<td>-8.10</td>
<td>16.27</td>
<td>0.96</td>
<td>1.336</td>
</tr>
</tbody>
</table>

Gradient A = 1.368 + 0.049057 * z - 0.015427 * z^2 - 0.001978 * r^2
Gradient B = 1.407 - 0.006605 * z^2 - 0.001978 * r^2
n(λ) = n(0.555μm) + 0.0512 - 0.1455 * λ + 0.0961 * λ^2

Table 5.1: Main characteristics of Liou-Brennan model eye for each of the ocular surfaces.

The same Zemax model of the optical set-up from the previous section was used for this part of the experiment. However, the artificial eye was changed by the human eye model. Intraocular scatter within the eye model was due to the bulk scatter added at the level of the cornea and crystalline lenses. Keratocytes and multilamellar bodies were added as scatter sources for the cornea and crystalline lens respectively. The data for the simulation of these scatter sources (density, index of refraction and size) is presented in section 5.6.1.1.
Two different measurements of the scatter were obtained from this eye: the total scattered light captured by the CCD and the average intensity per pixel recorded in the central area of the CCD ring image. In order to measure the total light scatter captured by the CCD, a transparent filter was introduced to the current Zemax model optical set-up. The filter was placed between D2 and L3 (Figure 5.2) and its function was to block any rays coming from the eye that are not perpendicular to its surface. According to the design of the optical set-up, rays from the light source passing through an eye without scatter will be perpendicular to D2 and L3 in their trajectory to the CCD. In the presence of intraocular scatter within the model eye, rays not perpendicular to D2 and L3 will be due to scatter and will not pass this filter. A percentage of FLS within the model eye was obtained by recording the amount of rays captured by the CCD camera and the ones blocked by the filter. Both measurements were the result of averaging 5 simulations.

STATISTICAL ANALYSIS

The Kolmogorov-Smirnov test was used to assess the normality of the data. Relationships between variables were assessed with two tailed paired sample t-test and Pearson’s correlation coefficient. A $p$-value inferior to 0.05 was considered as significant.

The results of this analysis were obtained using SPSS Statistics 22 (IBM, Chicago, IL) and MedCalc 12 (MedCalc Software, Mariakerke, Belgium).
5.8 RESULTS

5.8.1 FIRST EXPERIMENT: VALIDATION OF THE PROTOTYPE WITH CUSTOMISED LENSES AND ZEMAX MODEL

In order to compare the results obtained from the CCD sensor of the prototype and the ones obtained from the Zemax model, the dimensions of the images of the light source (LEDs ring) were checked. The image of both rings (CCD sensor and Zemax) had a diameter of 1.3 mm (measured from the centre of the LEDs). Noise obtained from the optical system compared to scatter signal had a ratio of about 1:5.

Figure 5.5 shows an image recorded by the CCD obtained as a result of the measurement of a microscopy plate with certain level of scatter. The diameter of the central area used for calculation (inside blue circle) was fixed to 1mm. This size allowed measuring just the contribution of light scatter obtained in the centre of the LED ring and not intensity from the LEDs. This area (one millimetre of diameter) corresponded also to the area inside the LEDs circle (on both CCD image of the optical set-up and Zemax model) where no pixel intensity is detected in absence of a scatter source (artificial eye with microspheres or a human eye) and when the noise is removed.

![Figure 5.5: Recorded image from the optical prototype. The central area used for calculations (inside blue circle) has a diameter of 1mm. Brightness changes of LEDs correspond to the particular radiation patterns (narrow viewing angle) of the LEDs and the difficulty of alignment.](image)

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Figure 5.6 represents the results obtained in Zemax for each one of the microscopy slides tested. As scatter increases, the intensity per pixel distribution of light spread on the central measuring area rises, making the peaks corresponding to the cross section profile decrease in height.

![Figure 5.6](image)

**Figure 5.6:** Light distribution obtained in Zemax for each one of the microscopy slides tested. A) Microscopy slide alone. B) Microscopy slide + solution of polymer with 1% of concentration of microspheres. C) Microscopy slide + 2% of microspheres concentration. D) Microscopy slide + 3% of microspheres concentration. Cross sectional profiles are also shown above.

Figure 5.7 depicts a comparison of the different intensity profiles obtained for each of the microscopy slides with different concentration of microspheres (microscopy slide alone, 1, 2 and 3% density of microspheres) using Zemax. Only the central area between the two peaks is measured for the calculation of light scatter.
Figure 5.7: Comparison of the cross sectional profiles obtained for the 4 microscopy slides tested using Zemax. Red line correspond to the microscopy slide alone, orange = 1% density of microspheres, blue = 2% and green = 3%. The intensity of the central part between the two peaks (measuring area) increases in accordance to light scatter. Profiles have been smoothed to make the visual comparison easier. In the absence of scatter sources, the recorded intensity is null in the central zone (1mm of diameter).

Table 5.2 represents the average intensity per pixel obtained from the central part of the CCD image after being processed with Matlab for each one of the microscopy slides.

<table>
<thead>
<tr>
<th>Microscopy plate</th>
<th>Average intensity pixel value (optical set-up)</th>
<th>Zemax measurements (intensity ratios)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.9 ± 2.36</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>59.27 ± 2.66</td>
<td>1.88 ± 0.032</td>
</tr>
<tr>
<td>3</td>
<td>100.74 ± 3.25</td>
<td>3.72 ± 0.026</td>
</tr>
<tr>
<td>4</td>
<td>140.32 ± 4.56</td>
<td>5.44 ± 0.03</td>
</tr>
</tbody>
</table>

*Table 5.2: Scatter values obtained from the measuring areas for the optical set-up and ratios from Zemax for the different microscopy plates.*

Figure 5.8 depicts the results of the comparison between scatter obtained through the optical prototype and its Zemax model. A good relationship was found between measurements ($r^2 = 0.98; p < 0.05$). In Figure 5.8, the amount of “scatter” due to the
microscopic plate alone is due to multiple reflections within the plate or small particles on the plate.

Figure 5.8: Plot comparing scatter values obtained with Zemax (intensity ratios) and average intensity pixel from the prototype. Results from the three series for the four different amounts of scatter on microscopy slides are shown.

5.8.2 SECOND EXPERIMENT: RESULTS FROM PARTICIPANTS, COMPARISON RESULTS PROTOTYPE / C-QUANT AND PROTOTYPE / ZMAX SCATTER EYE MODEL

The ratio scatter measurement/noise observed in the measurement of scatter from subjects and using the optical prototype was higher than the one observed when measuring scatter from microscopy slides. Figure 5.9 shows a retinal image recorded from the CCD of the prototype.
Figure 5.9: Image of the LEDs ring. Light distribution over the image is a consequence of FLS. With FLS, the amount of light participating to the LEDs image decreases and the amount of light at the centre of the ring increases. Green circle marks the area used for calculations.

The pupillary diameter of the participants of the study was $3.1 \pm 0.39$ (mean ± SD) in miosis and $5.49 \pm 0.66$ (mean ± SD) in mydriasis.

Figure 5.10 shows the comparison between the average intensity per pixel obtained inside the LED ring and C-Quant values. Both values showed a good relationship ($r^2 = 0.7515$, $p < 0.05$).
Figure 5.10: Comparison of the scatter values measured with the optical prototype (at 50cm distance at an angular eccentricity of 2.04 degrees. A good relationship was obtained.

With regards to the simulation of the human eye model with added scatter structures, the total amount of rays scattered forward represented 0.49±0.003 (mean ± SD) per cent of all the rays recorded by the CCD of the optical set-up Zemax model. Average intensity per pixel within LED ring (inside the circle) was 68.02±0.05 (mean ± SD).
5.9 DISCUSSION

5.9.1 FIRST EXPERIMENT: VALIDATION OF THE PROTOTYPE WITH CUSTOMISED LENSES AND ZEMAX MODEL

The good results obtained from the comparison between the experimental set-up and the Zemax model validated the optical instrument and allowed for the measurement of increased levels of light scatter. The results obtained from the measurement of light scatter from the microscopy plates are in agreement with another previously published study in which light scatter produced by customized contact lenses was assessed through a double pass system (De Brouwere et al., 2007). Furthermore, the good results observed in Figure 5.10 allow for the association of measurements to a particular source of scatter (i.e. plate number and associated microsphere concentration).

5.9.2 SECOND EXPERIMENT: RESULTS FROM PARTICIPANTS, COMPARISON RESULTS PROTOTYPE – C-QUANT AND PROTOTYPE / ZEMAX SCATTER EYE MODEL

Results from participants, comparison results prototype – C-Quant

The first results obtained in patients with the experimental set-up are in good agreement with C-Quant results ($r^2 = 0.75; p < 0.05$). The optical set-up is designed to measure scatter angle as large as psychophysical methods do. These results contradict a previous study in which the measurement of large angle scatter was limited to psychophysical methods (Van Den Berg et al., 2009, Benito Lopez et al., 2015). However,
they are in agreement with another recent study which utilises an optical method to assess large angle FLS (Ginis et al., 2012).

In this study, human eyes were tested using the optical set-up and a scatter angle of 2.04° and compared with C-Quant, that measures with an effective eccentricity of 7° (van den Berg, 1995). Although the differences between the designs of the scatter sources used by the two instruments must be considered, the agreement obtained with two different instruments measuring FLS at different scatter angles might be explained in part due to the definition of the straylight parameter used by C-Quant:

\[ s = \Theta^2 \times PSF \] (van den Berg et al., 2012)

In this formula, the straylight parameter \( s \) is equal to the scatter angle to the power of 2 and multiplied by the PSF of the human eye. In this sense, the agreement between the amounts of FLS measured by the two instruments tested in this study might be explained by FLS being proportional to the square of the measured scatter angle.

These results suggest that the presented optical set-up could allow measuring objectively FLS. However, the optical set-up could benefit from several possible improvements to make it more robust and lead to its application as a clinical instrument. For instance, a central LED in addition to the LEDs ring light source could be introduced to match the veiling illuminance created by the LEDs ring and act as an adaptation of the nulling flickering technique (van den Berg and Ijspeert, 1992). In the classic instrument, the subject adjusts the luminance of the central light source to match the retinal illuminance caused by the LEDs ring (van den Berg and Ijspeert, 1992). In this set-up, the patients input would be replaced by an analysis of the retinal image recorded by the CCD for different level of luminance of the central LEDs ring. Moreover, in combination with
the flicker cancellation technique it would allow the estimation of the full scatter function of the eye if different scatter angles are measured (Barbur et al., 1993).

Another improvement would be the addition of a system to control the pupillary diameter while measuring. Short exposures times of the eye to bright lights produce the activation of the pupil response. Introducing a system that allows for the quick presentation of the light source to the tested eye and the measurement would avoid the pupil response (Bergamin and Kardon, 2003). Franssen et al. found that for natural pupils (between 2 and 7 mm of diameter), FLS is weakly affected by the pupillary diameter. However, smaller pupillary diameters produce a reduction of the measured amount of FLS (Franssen et al., 2007b).

A critical task while doing the measurements is the alignment of the eye with the optical system. A small movement of subject’s head can mean a loss of important information when the LEDs ring image is recorded. The need of an instrument that automatizes and tests the alignment of the prototype with the tested eye would be of great benefit. Finally, the lack of a fixation point is another reason for multiple repetitions of measurements. When the eye is not fixating correctly, the images on the CCD appear distorted and need to be repeated. In this study, participants were asked to fixate one of the LEDs from the LED ring, and when the person doing the measurements said, the subject rapidly fixated on the centre of the ring and the measurement was taken. However, this method frequently finished with the acquisition of a non-valid image (with artefacts). This alignment complement would increase the accuracy of the measurement.

**Prototype - Zemax scatter eye model**

The percentage of FLS obtained from the simulation with the Zemax model of the human scatter eye is 0.49% and thus lower than the physiologically estimated FLS
corresponding to a human eye, which is usually between 1 and 2% (Vos, 1984) of the incoming light. These results are however in agreement with previous studies in which the lack of biometric data with respect to corneal scatter parameters was solved by varying the quantity of scatter structures until FLS in the model agreed with the values calculated by the CIE (Chen et al., 2012) disability glare formula (Vos and van den Berg, 1999). In this study (Chen et al., 2012), scatter particles were aimed to mimic the scatter characteristics of the corneal lens and produce the percentage of light scatter usually attributed to this lens. In another study, random distributions of particles where added to a model eye trying to simulate the scatter produced by cataracts in the crystalline lens (Kelly-Pérez et al., 2011). The lack of biometric data of scatter particles in the eye might explain the results of the current study (0.49% of FLS rather than 1-2%) where the intensity per pixel obtained in the model eye was only 68.02±0.05 (mean ± SD) while the average intensity per pixel measured for the ten participants was 165.4±17.94 (mean ± SD).

In view of our results and previous studies (Kelly-Pérez et al., 2011, Chen et al., 2012) a first step for achieving a more realistic simulation of intraocular light scatter could be to increase the complexity of the model (e.g. shape, multiple sizes, locations of scatter structures) and to be able to insert these elements into an optical design program (basically creating custom object libraries with Zemax or CAD models). Unfortunately, such steps would further increase the difficulty of associating an experimental measurement to a scatter parameter of the model.

In summary, the presented optical set-up has been validated as an objective method to assess large angle FLS. The FLS measurements from the optical set-up can also be related to physical parameters through the Zemax model (e.g. scatter particle density).
Human eyes large angle FLS measurements from the optical set-up were highly correlated with data obtained with C-Quant. However, they cannot yet be explained by the developed Zemax model of the human eye.
6. FINAL SUMMARY AND FUTURE WORK

6.1 FINAL SUMMARY

The measurement of the effect of intraocular forward light scatter in the human eye has been the main motivation of this project. The main effect of forward light scatter on vision is the decrease of contrast on the retinal image. This reduced contrast may become a significant issue, particularly when a good visual performance is not only required but obligatory (e.g. plane pilot).

This PhD thesis started with the comparison between the instrument considered the current gold standard to measure FLS (C-Quant) and its predecessor (the Van der Berg Straylight meter) (chapter 2). Despite the importance of the two instruments in the literature, the relationship between measurements obtained with the two techniques had never been quantified. Results from our study are slightly surprising since no significant relationship could be found between the two measurements (even with a multivariable function; chapter 2) but illustrate the difficulty in quantifying precisely FLS and the importance of the angular domain.

Following this study, we took interest in commercial instruments (Hartmann-Shack in chapter 3 and Pentacam in chapter 4) by measuring FLS and BLS. The work presented in chapter 3 allowed us to clarify the relation between FLS assessed with the C-Quant and FLS assessed from Hartmanngrams to demonstrate that a commercial Hartmann-Shack wavefront sensor cannot be used to assess FLS without significant modifications. The work presented in chapter 4, allowed us to illustrate the lack of direct correlation between forward and backward scattering.
Finally, an innovative prototype to measure objectively the FLS in the eye together with its Zemax model is presented in chapter 5. This optical prototype is tested on a model eye as well as a human eye. Scatter measurements obtained with this prototype are comparable to the ones obtained with the C-Quant straylight meter. A Zemax scatter model of the eye is also presented in this chapter but the simplicity of the model lead to a lack of agreement between the model and real FLS data obtained with the prototype.

6.2 FUTURE WORK

- **Objective forward light scattering assessment through Hartmann-Shack aberrometer spot patterns**: As described in chapter 3, commercial Hartmann-Shack aberrometers usually incorporate a confocal aperture to reduce high angle scatter that increases the sensor’s performance (Donnelly Iii et al., 2004), but limits the measurement of light scatter in a human eye. The study presented in chapter 3 might give different results if scatter measurements are obtained from an aberrometer that has been modified in this way to receive the maximum amount of scatter light from the human eye. In addition, a patent presenting a method to assess the scatter from the space situated between the PSF lets of a Hartmann-Shack spot pattern has been presented (Levecq and Harms, 2011) and that could possibly be of interest in order to give an additional application to aberrometers by assessing intraocular forward scatter.

- **Prototype for the objective measurement of forward light scattering**: Improvements and future research for this prototype have already been outlined in chapter 5. After such improvements, many different studies can be performed in order to validate
the instrument for human eyes in a great variety of situations (e.g. different cataract grades or ocular diseases), for different age groups and wavelengths, but also to assess the effect of some topical and intraocular drugs.

- **Human eye model of intraocular scatter:** Scatter sources within the ocular media that are described in the current literature cannot explain the amount of forward light scatter attributed to a healthy eye (1-2%) as shown in chapter 5. Deeper understanding of the scatter characteristics of the human eye could be provided with a model of the human eye for intraocular scatter that simulates the different eye clinical cases and their evolution. This model could include different types of intraocular structures, with different shapes and sizes that mimic in a more realistic way the scattering complexity of the human eye. The eye model presented in chapter 5 was developed in Zemax but could also have been developed in a specially developed modelling software for the eye that might introduce some scatter modelling advantages (Donnelly, 2008).
7. REFERENCES


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ANNEXUS

Program to analyse the HS spot patterns

% PREPARING MATLAB TO RUN OUR PROGRAM

clear all; close all; more off; clc; % Cleans all variables, close all opened programs and
clean the main window
wd=cd; %Sets the folder of the Matlab file as a default folder

% OPENING OUR HS IMAGE FROM THE FOLDER WHERE IT IS STORED,
% PREPARING IT TO WORK WITH IT AND DISPLAYING IT

[FILE,PATH] = uigetfile('*.*','Select all the image files','MultiSelect', 'off'); % Opens the
% file and saves it in "FILE" following the route "PATH". Deactivates multi-selection of files
% (only one can be selected at a time)

% Sets the folder of the Matlab file as a default folder

wd=cd(PATH) % Tells Matlab where it located our HS image
name=char(FILE); % Sets the variable "name" with the name of the file
im = double(imread(name)); %Reads the image and converts into "double" (type of
% numerical variable). From here, the image is a numerical matrix with a value associated to
% each pixel. The matrix is now called "im"
im=im(:,:,1); % The reading of the image with the command "imread" gives back a 3
% dimensional matrix. The three of them are the same. With this line, we choose only the first
% one

im=im/255; % Divides the matrix between the maximun value than a pixel can have. 255
% corresponds to a saturated pixel. 0 correspond a pixel without intensity, a pixel with black
% color. This line makes the pixel values smaller and easier to work with.

% DISPLAYS THE ORIGINAL IMAGE WITH JET BACKGROUND.

imagesc(im) % Shows the image (im)
colormap jet % Sets the background of the showed image as "jet" (pre-designed colour of
% matlab)
figure % Asks Matlab to open a new window in case that other image needs to be shown
% (keeps the one opened two lines before)

% PREPARATION FOR THE ANALYSIS, SET OF NEIGHBOURHOOD SIZE, SET
% THE THRESHOLD AND FIND OF NEIGHBOURHOODS AND CREATION OF AN
% ADDITIONAL MATRIX TO CHECK OVERLAPPED NEIGHBOURHOODS

rimo2=im; % Sets the new variable "rimo2" with the value of the variable "im" (our
% numerical matrix)

r=6; % Sets the radius of the neighbourhood. That means that around the central peak
% (pixel with highest value) of each neighbourhood, there will be, inside the neighbourhood,
% 6 pixels to the top, 6 pixels to the bottom, 6 pixels to the left and 6 to the right. Each
neighbourhood will be a matrix of 13x13 pixels (169 pixels in total) with the centre located in the pixel with the highest value (pixel number 84 within the neighbourhood)

\[ b = \text{im2bw}(\text{im}, 0.3); \] % The function that finds the centres of the neighbourhoods is pre-defined in Matlab. To work with it, the image needs to be converted into binary (0 or 1), black or white. This is what this function does and saves it to the variable "b". Here the threshold is set to 0.3 (this value was obtained empirically).

\[ \text{labeledImage} = \text{bwlabel}(b, 8); \] % This function associates the variable "labeledImage" to a matrix in black and white and values 0 or 1 with labels on each pixel of the image with information about pixels with same value surrounding others. The parameter is sets to 8 to specify 8 connected objects

\[ \text{blobMeasurements} = \text{regionprops}(\text{labeledImage}, \text{'all'}); \] % The variable blobMeasurements takes the information about the neighbourhoods found on the image (this information is given with the function "regionprops" that only works in images in white and black.

\[ \text{numberOfBlobs} = \text{size}(\text{blobMeasurements, 1}); \] %the variable "numberOfBlobs" takes the number of neighbourhoods found on the image.

\[ \text{matrixzeros} = \text{zeros}(1000,1000); \] % Creates a additional matrix called "matrixzeros" of 1000x1000 with all the elements with value 0. This matrix will be used to check that the neighbourhoods are not overlapped. The size of the matrix (1000x1000) is a bit bigger than the HS images from our aberrometer (644x492), but when analyzing images from other aberrometers (sometimes with larger size), this size is needed.

%THE ANALIZATION OF THE IMAGE STARTS

\[ \text{for } k = 1 : \text{numberOfBlobs} \] % Starting the loop, we are going to check all the neighbourhoods from the first to the last one (numberOfBlobs) in +1 steps.

\[ k \]

% GETTING THE POSITION OF THE CENTRE OF THE NEIGHBOURHOOD.

\[ \text{currentblob} = \text{blobMeasurements}(k).	ext{PixelIdxList}; \] % Get list of pixels in current blob

\[ \text{xn} = \text{blobMeasurements}(k).	ext{Centroid}(1); \] % Gets the coordinate (xn) of the highest value of pixel within the neighbourhood K. Each pixel has a coordinate (xn,yn)

\[ \text{yn} = \text{blobMeasurements}(k).	ext{Centroid}(2); \] % Gets the coordinate (yn) of the highest value of pixel within the neighbourhood K

% CHECK IF THE NEIGHBOURHOOD OF A PART OF IT HAS ALREADY BEEN USED (OVERLAPPED)

\[ \text{if max((matrixzeros(yn-r:yn+r, xn-r:xn+r))) < 1} \] % Check if the neighbourhood (K) has already been used. If K has been used, or a part of another neighbourhood has used a piece of K, there will be a value 1 in that part of "matrixzeros", and the rest of the loop will not apply to that neighbourhood and it will be rejected. Only a neighbourhood K, with an area (yn-r:yn+r, xn-r:xn+r) on "matrixzeros" and a value "0" will pass to the next step of the loop.

\[ \text{matrixzeros(yn-r:yn+r, xn-r:xn+r)= 1}; \] % If the neighbourhood K was not used, the first thing to do, is to give a value 1 to the area (yn-r:yn+r, xn-r:xn+r) on "matrixzeros" to make sure this area is not used again for the calculations.
zone2=im(yn-r:yn+r, xn-r:xn+r);  % Defines zone2 as a small square around the centre of the neighbourhood.

% CALCULATION OF SATURATED PIXELS WITHIN THE NEIGHBOURHOOD

j=zone2>254;  % Creates a binary vector with the elements of zone2 which values are higher than 254. Since 255 is the maximum value that a pixel can take, a pixel with this value will be saturated. Saturated pixels within the neighbourhood will have a value of 1 and non-saturated ones, a value of 0.
isa(j, 'logical');  % Converts the vector "j" into logical.
saturatedpixel = length(zone2(j));  % Gives to the variable "saturatedpixel" the number of saturated pixels within the neighbourhood.

% CALCULATES IF THE NEIGHBOURHOOD IS SATURATED.

if saturatedpixel<3  % Check if there are more than 2 saturated pixels within the neighbourhood. If so, the loop finish and the neighbourhood is rejected.

% SUPERIMPOSE OF A SQUARE ON THE RAW AREA (NEIGHBOURHOODS DRAWING ON THE IMAGE.

aa=1.5;  % Defines a thickness of 1.5 pixels.
azone=zone2;  % Gives to azone the value of the variable zone2.
azone(1,:)=aa;  % Paints the line around the neighbourhood, only neighbourhoods used will have a superimposed square.
azone(:,1)=aa;  % Paints the line around the neighbourhood, only neighbourhoods used will have a superimposed square.
azone(:,end)=aa;  % Paints the line around the neighbourhood, only neighbourhoods used will have a superimposed square.
azone(end,:)=aa;  % Paints the line around the neighbourhood, only neighbourhoods used will have a superimposed square.
rimo2(yn-r:yn+r, xn-r:xn+r)=azone;  %Copies the superimposed square over the original image rimo2 (= im).

% CONVERSION OF MATRIX TO ALLOW REMOVING THE CENTRAL PIXEL

reformazone2=reshape(zone2,169,1);  % Converts the matriz zone2 (of 169 elements) into a vector called reformazone2 (of 169 elements).
ind=[84];  % Selection of the central element of the vector (84), that corresponds with the central element of the previous matrix.
reformazone2(ind)=[];  % Removal of the element 84 from the vector "reformazone2".

% Storage of the neighbourhood

matriztotal(:,:,k)=reformazone2;  % Stores the vector "reformazone2" into a vector of k dimensions called "matriztotal".
end
end
% CALCULATION OF NOISE AND REMOVAL

ruido = min(min(matriztotal)); % Calculates the minimum value of the minimum value of the vectors within "matriztotal" and saves it in the variable "noise".
matriztotalsinruido = matriztotal - ruido; % Rest the noise to matriztotal.

% CALCULATION OF STRAYLIGHT

straylight = std2(max(matriztotalsinruido)); % Gets the straylight of the image by calculating the maximum standard deviation of the "matriztotalsinruido"

% DISPLAY THE FINAL IMAGE.

imagesc(rimo2); % Displays the original image with the superimposed squares.
colormap gray; % Sets the background of the picture to gray.

% SHOW FINAL RESULTS (NAME OF THE FILE + STRAYLIGHT VALUE).

display(name); display (straylight); % Display the straylight of the image and the file name of the image.