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Optic Nerve Head Image Analysis for Glaucoma Progression Detection

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PhD Thesis

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Declaration

This thesis has been completed solely by the candidate, Neil O'Leary. It has not been submitted for any other degrees, either now or in the past. Where work contained has been previously published, this has been stated in the text. This grants powers of discretion to the University Librarian to allow the thesis to be copied in whole or in part without further reference to the author. This permission covers only single copies made for study purposes, subject to normal conditions of acknowledgement.

Keywords

Abstract

Glaucoma is a leading cause of visual disability across the world and when diagnosed the glaucoma patient will spend the rest of their life receiving treatment in managed clinical care. In the glaucoma clinic, retinal and optic nerve head (ONH) imaging can be used to help the clinician to manage patient treatment appropriately. By providing high resolution images of the optic nerve head structures and identifying changes therein related to disease onset and progression, an objective measure can be obtained as to how well or badly treatment is preventing further disease damage. This thesis contributes to the field of glaucoma progression detection by the analysis of clinical imaging data using confocal scanning laser tomography (CSLT). Primarily it is an investigation of how best to appraise and optimise current algorithms which aim to detect these glaucomatous structural changes in the optic nerve head. This is done by addressing how the performance of these methods can be best assessed in the absence of a gold standard for glaucomatous structural progression.

Glaucoma expert assessment of photographs of the optic disc is the current clinical standard of assessing glaucomatous damage evident in the ONH. This is used in this thesis to act as a reference standard by which these algorithms can be compared. In addition, the statistical principles underpinning trend detection techniques are also investigated along with the performance of these techniques to detect trends in CSLT data in the presence of different types of measurement noise and image quality. A new computer model is developed and validated to simulate stable series of CSLT images, with realistic variability, which can be used to benchmark the false-positive rates of current and future progression algorithms. In conclusion, the main results reported in this thesis show that uncertainties involved in expert assessment of change in ONH photographs limits this as a reference standard for structural change in glaucoma. In addition, since stability in clinical datasets is uncertain, simulation using modelled series is shown to provide a new benchmark for comparing methods of progression detection.

List of Abbreviations and Terms

α-level   statistical significance level (p-value cut-off)
AGIS   Advanced glaucoma intervention study
AUC   Area under the receiver operating characteristic curve
CSLO   Confocal scanning laser ophthalmoscopy
CSLT   Confocal scanning laser tomography
CSM   Cup shape measure
CV   Coefficient of variation
GM   Gradient magnitude
HRT   Heidelberg retina tomograph
IOP   Intraocular pressure
MRI   Magnetic resonance imaging
MPHSD   Mean pixel height standard deviation
NCC   Normalised cross-correlation
NTG   Normal tension glaucoma
OCT   Optical coherence tomography
OHT   Ocular hypertension
OLS   Ordinary least squares
ONH   Optic nerve head
P-DIST   Permutation distribution
PAUC   Partial area under the partial receiver operating characteristic curve
PHSD   Pixel height standard deviation
Power   Probability of detecting change at a given level of statistical significance
RA   Neuroretinal rim area
RALR   Rim area linear regression
RASD   Radial-averaged spectrum density
RGC   Retinal ganglion cell
ROC   Receiver operating characteristic
RHO P-DIST   Permutation distribution of Spearman's rank correlation coefficient
SIM   Statistic image mapping
SLO   Scanning laser ophthalmoscopy
SLP   Scanning laser polarimetry
T-DIST   Student's t-distribution
TCA   Topographic change analysis

1. Background and Aims

This chapter gives an introduction to glaucoma, a brief description of its nature, prevalence and its risk factors. The clinical means to detect and monitor this disease are discussed. Confocal scanning laser tomography, the focus of this thesis, is introduced as a technology which can contribute to clinical decision making by assisting in glaucoma detecting and monitoring.

1.1 Glaucoma

The glaucomas are a group of optic neuropathies, collectively referred to as glaucoma, that have in common a progressive degeneration of retinal ganglion cells (RGC) and their axons. They result in distinct damage to the optic nerve head (ONH) and peripheral vision loss. The mechanism of this RGC degeneration is intrinsically linked with *intraocular pressure* (IOP) and often associated with increased IOP. Glaucoma leads to distinctive changes in the shape or *morphology* of the ONH (Figure 1.1) called 'cupping'. This damage to the ONH causes losses to the *visual field*, which is "that portion of space in which objects are simultaneously visible in the steadily fixating eye" (Spector, 1990). The resulting damage to the visual field is irreversible; though loss can be transitory in the early stages of glaucoma. If untreated, the damage to the affected visual field will most likely intensify and spread until eventually complete loss of vision can occur. It has been estimated that in the year 2000 that at least 67 million people suffered from glaucoma with an a resulting estimated 7 million suffering blindness in both eyes (Quigley, 1996), making it the second leading cause of world blindness (Resnikoff et al., 2004). Given its higher prevalence with increased age and the increasing

longevity of the population, an increased figure of 80 million has been predicted for 2020 with 11 million cases of blindness from glaucoma (Quigley and Broman, 2006). Furthermore, as the World Health Organisation's definition of blindness is based on central vision loss only, the disabling effects of peripheral vision loss are often under-estimated until later stages of the disease have been reached (Quigley, 1996). There is no cure for glaucoma but once detected, appropriate clinical intervention and treatment can help to slow further progression of vision loss – sight cannot be restored but may be maintained making earlier detection all the more important.

Our understanding of the causes, mechanisms and manifestations of glaucomatous damage has been shaped by what is measured and how these measurements are made. Currently three measured features are considered crucial to the recognition of glaucoma, the ONH, the visual field and IOP. Previously it was believed that glaucoma was caused solely by elevated IOP and definitions for glaucoma historically relied on this belief. 'Normal' IOP was defined as that which was within 2 standard deviations of the mean IOP found in the general population of 15.5 mmHg (Colton and Ederer, 1980). Ocular hypertension (OHT) is a condition in which IOP is above this upper limit (greater than 21 mmHg) and historically it became mistakenly synonymous with pre-glaucoma or glaucoma without damage (Phelps, 1977). The association between OHT and glaucoma is now known to be multi-factorial and complex. The prevalence of OHT patients with glaucomatous visual field damage has been reported as approximately 10% (Sommer et al., 1991), though an increased prevalence of glaucoma was shown with increased IOP. In addition, an estimated 10% of untreated OHT patients developed glaucomatous optic nerve or visual field damage within an average follow-up period of 5 years (Kass et al., 2002). It is now understood that glaucoma can occur in eyes with 'normal' IOP (<21 mmHg). Thus it is best to understand that IOP as a risk factor for

glaucomatous damage and that some eyes are more susceptible to the effects of IOP and sustain damage at a lower level. Still, as IOP is the only treatable risk factor for glaucoma, the reduction of IOP remains central to glaucoma treatment.
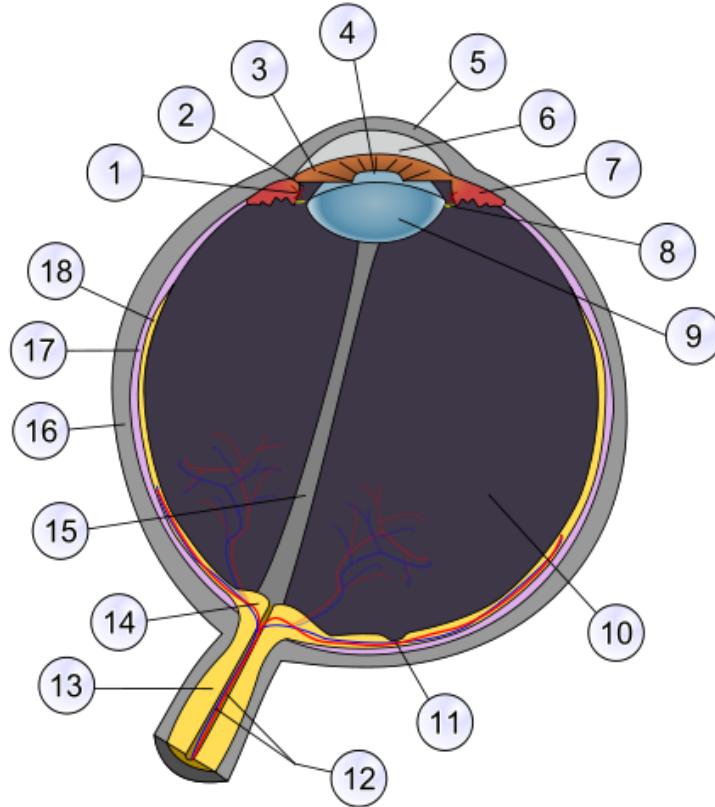
Figure 1.1 Schematic anatomy of the eye. Numbers indicate main or notable features. Important ocular features and those most relevant to glaucoma and ocular imaging include, **2**: posterior chamber, **3**: iris, **4**: pupil, **5**: cornea, **6**: *anterior chamber (aqueous humour)*, **9**: lens, **10**: vitreal chamber (vitreous humour), **11**: fovea, **12**: central retinal blood vessels, **13**: optic nerve, **14**: *optic nerve head* or *optic disc*, **16**: sclera, **18**: retina. (Public domain image from http://commons.wikimedia.org [User: Rhcastillhos])

To understand IOP and its importance in glaucoma, it is crucial to consider the dynamics of the *aqueous humour*, the clear watery fluid secreted into the posterior chamber that circulates through the anterior chamber (Figure 1.1). This fluid is unrelated to tears, or to the denser gel-like substance, the vitreous humour, that is

contained in the rear chamber. The function of aqueous humour is to supply nutrients to the lens and cornea, dispose of the eye's metabolic waste and help maintain eye shape by regulating IOP. To maintain an IOP the inflow of newly produced aqueous humour is balanced by an outflow by drainage between the iris and cornea (Figure 1.1), primarily (80-90%) through a sponge like substance known as the *trabecular meshwork,* the remaining fluid outflow occurs independently through uveoscleral drainage.

Glaucoma is also better understood once the basic principles have been established of how the eye receives and converts light information into neuronal signals to send to the brain. As light enters the eye, it is transmitted and refracted to the retina where it stimulates two different types of *photoreceptor* cells, called cones and rods, which produce electrical signals when activated. Rods become active at low levels of illuminance while cones are active at high levels and so enable human vision to operate over a wide range of stimulus intensities. The RGCs process the signals from these photoreceptors before refining and relaying them to the brain through their axons which exit the eye via the ONH. In humans there are over a million RGCs. The centre of the retina (macula) has a higher concentration of RGCs and cones, where vision resolution is best (Rabbetts, 1998, Purves, 2004). These axons comprise the innermost layer of the retinal nerve fibre layer (RNFL). In mammals the axons of RGCs are guided to the ONH during embryonic development in a process called pathfinding (Oster et al., 2004). These axons converge on the ONH and exit the eye to the brain, passing through the lamina cribrosa - a mesh-like structure of collagen fibres (Figure 1.2). This convergence and exit forms the papillary structure of the ONH consisting of a *rim* of neural tissue and a central depression, known as the *cup* (Figure 1.3) (Weinreb and Khaw, 2004).

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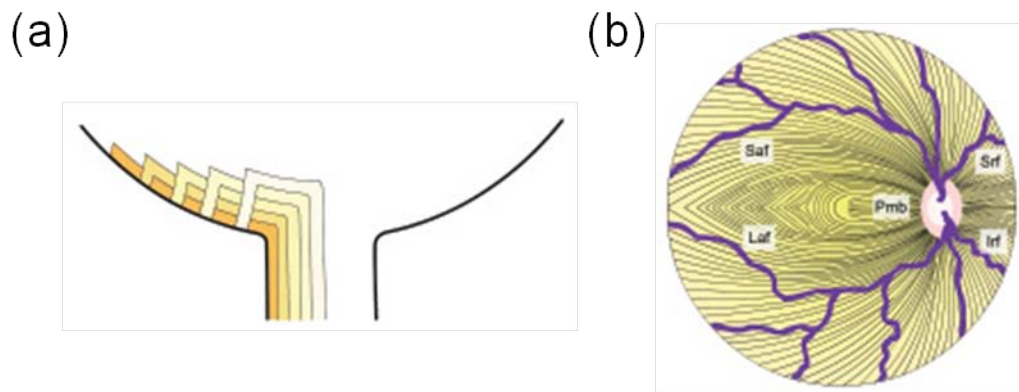
Figure 1.2 Retinal nerve fibre schematics showing (a) exit configurations of retinal nerve fibres leaving the optic nerve head related to the eccentricity of their starting point and (b) the arcuate-path configuration of retinal nerve fibres across the retina. (Images from Khurana, 2007)
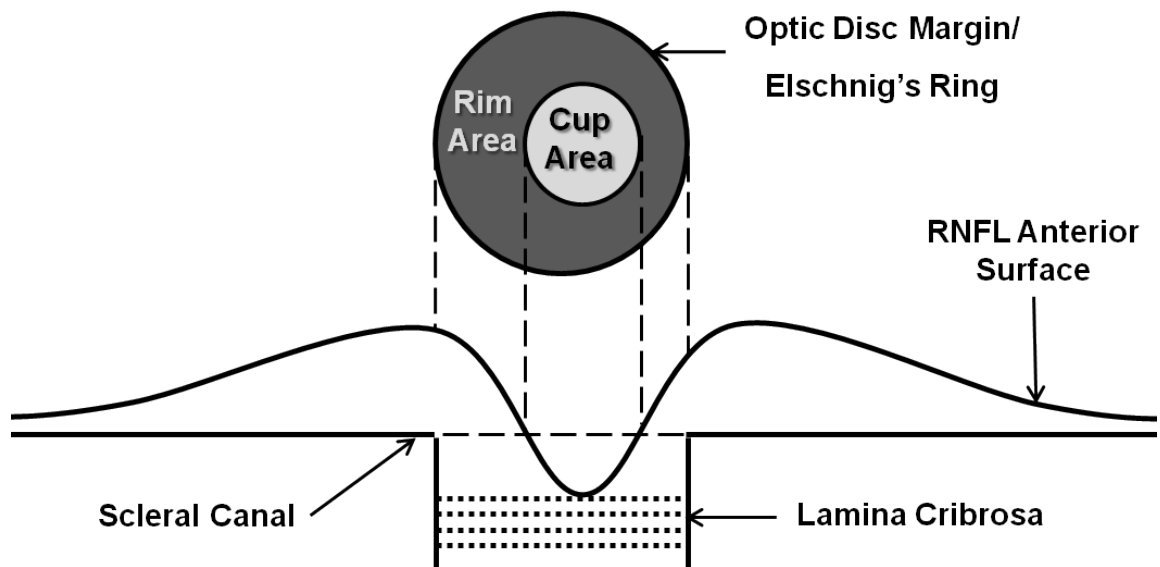
Figure 1.3 Schematic of the ONH and optic disc and relationship to the RNFL.

Glaucoma can be divided into various sub-categories depending on its aetiology and the mechanism of damage (Allingham and Shields, 2005). Glaucoma is defined as secondary or primary depending on whether the glaucoma is associated with some other ocular or systemic disorder or not. Primary or secondary glaucoma can

be broadly categorised further into open-angle glaucoma, closed-angle glaucoma or congenital. Primary open angle glaucoma (POAG) and primary closed angle glaucoma (CAG) are the most prevalent of the glaucomas and their descriptions will follow. Normal tension glaucoma (NTG) is a subdivision of POAG and is characterised by an IOP equal to or below 21 mmHg and thus POAG and NTG appear to represent a continuum of glaucomas with considerable overlap of causative factors. Outside Japan, more than 30% of newly diagnosed cases are NTG (Sommer et al., 1991, Dielemans et al., 1994, Mitchell et al., 1996). The condition may be under-diagnosed in Western countries because of the nature of case-finding for glaucoma. In Japan NTG is the most prevalent form of Glaucoma (Shiose et al., 1991). In CAG the iris is pushed against the trabecular meshwork, sometimes sticking to it and closing off the drainage angle. This angle closure can be an *acute* condition - occurring abruptly and resulting in a large and sudden rise in IOP. CAG may account for up to 50% of glaucoma worldwide as it has a higher prevalence amongst Asians. Congenital glaucoma is a rare glaucoma typically characterised by the improper development and consequent dysfunction of the eye's aqueous drainage channels.

This thesis focuses on POAG; The 2010 American Academy of Ophthalmology Preferred Practice Pattern (American Academy of Ophthalmology Glaucoma Panel, 2010) defines POAG as "a chronic ocular disease process that is progressive, generally bilateral, but often asymmetric." According to their guidelines, it is associated with the following characteristics:

1) Evidence of optic nerve damage from either, or both, of the following:

   a) ONH or RNFL structural abnormalities

b) Reliable and reproducible visual field abnormalities considered a valid representation of the subject's functional status

2) Adult onset

3) Open anterior chamber angles

4) Absence of other known explanations (i.e., secondary glaucoma) for progressive glaucomatous optic nerve change

POAG is the most common form of glaucoma in European, African and North American populations and the second most common form in Asia. To summarise recent studies the prevalence was reported at 1.5-2.4% in Caucasians, 6-8% in Afro-Caribbean's and 1.7-2.0% in Chinese populations (Tielsch et al., 1991, Klein et al., 1992, Coffey et al., 1993, Dielemans et al., 1994, Leske et al., 1994, Mitchell et al., 1996, Wolfs et al., 2000, Foster et al., 2000, Friedman et al., 2004b, Wang et al., 2010). Whereas CAG is often an *acute* disease, POAG is normally a *chronic* disease, resulting in slow progressive damage to the ONH and deterioration of the visual field.

The debilitating effects of glaucoma in everyday visual function are worth considering in light of its prevalence. Though central vision is preserved until the latter stages glaucoma, there is emerging evidence that glaucomatous patients, even with relatively modest visual field defects, may be at increased risk of falls and accidents (Turano et al., 1999, Szlyk et al., 2005, Haymes et al., 2007, Ramulu, 2009). It has also been reported that glaucomatous field defects impact self-assessed disability (Nelson et al., 2003, Noe et al., 2003) and, more recently, objective measures of performance in laboratory-based studies have shown the difficulty patients have with some everyday tasks (Altangerel et al., 2006, Kotecha et al., 2009).

Glaucoma will affect an individual's quality of life when visual field loss makes an individual unable to drive safely, and several studies of varying experimental design have shown that certain glaucomatous visual field defects are not compatible with safe driving (Johnson and Keltner, 1983, McGwin et al., 2004, Haymes et al., 2007, Haymes et al., 2008). These considerations make understanding glaucoma with a view to detecting and treating glaucoma earlier even more compelling.

Glaucomatous neuropathy preferentially damages RGC axons at the vertical poles of the ONH and is influenced to a variable extent by the level of IOP. Though RGC death occurs by apoptosis, the pathogenesis of this is not wholly understood. Underlying theories for axonal loss can be can be grouped by mechanisms of direct *mechanical* effects or those which are *vascular* related - through ischemia. These mechanisms are believed to act in combination rather than one acting at the exclusion of the other. The mechanical theory suggests that IOP acts directly on the lamina cribrosa and, as axons leave the eye through its complex connective tissue, a resulting shearing force is applied. This force causes either direct damage to the axons or disruption to the transportation of neurotrophic factors (Quigley and Addicks, 1980) necessary for survival, can lead to morphological changes in the RGC such as shrinking (Morgan, 2002) and eventually to the death of the cell (Crawford et al., 2000). The lamina cribrosa is less well supported at its inferior and superior margins, offering an explanation for the characteristic damage seen in glaucoma in these locations (Quigley and Addicks, 1981). Furthermore, animal models of short-term IOP increase show corresponding increased pressure gradients across the lamina cribrosa. Histology has shown the laminar structure is not restored to its original state when the IOP is reduced (plastic deformation) and that this structure becomes more easily deformed at the re-application of increased IOP (Bellezza et al., 2003). In the vascular mechanism theory, systemic factors which

can affect blood flow in the ONH may also be a factor in glaucomatous damage.

Changes within the microcirculation of the ONH capillaries are responsible for

axonal loss. Glaucomatous damage can be greater in eyes when the difference

between systemic blood pressure and IOP (*perfusion pressure*) is low (Sommer, 1996).

Perfusion pressure is an important determinant of ocular blood flow (Hayreh, 2001)

and it has been reported to be lower in POAG patients than in OHT patients when

other factors were controlled (Kerr et al., 1998). In glaucoma, RGC apoptosis and

loss of axons, along with the deformation of the lamina cribrosa leads to

characteristic morphological changes of the ONH. Neuroretinal rim decreases in

size (narrowing its surface area) with parallel enlargement of the cup (widening its

surface area) and thus these morphological changes are of particular interest for

evaluating disease state.

The term *optic disc* is often used interchangeably with ONH but in this thesis, to help

with clarity, it is used to refer to the anterior surface and anterior features of the

ONH or that portion of the ONH which is clinically visible by ophthalmoscopy

(Jonas et al., 1999). Understanding the features of the optic disc (Figure 1.3) is

important for glaucoma assessment. Optic disc area and relative rim area have large

between-individual variation. This physiological variability makes glaucoma

identification from these features alone difficult. A healthy neuroretinal rim is

typically widest in the inferior optic disc region, and then in the superior, nasal and

finally temporal regions, termed the 'ISNT' rule (Jonas and Garway-Heath, 2000).

As outlined, glaucomatous damage to the rim is more or less likely in different

regions and this depends on the stage of the disease. Most frequently, the disease

starts with loss in the inferotemporal and superotemporal regions, followed by the

temporal region and lastly in the nasal region (Hitchings and Spaeth, 1977,

Airaksinen and Drance, 1985, Jonas et al., 1999). Optic disc haemorrhages are also

associated with the disease (Drance, 1989) and occur in about 4-7% of glaucomatous eyes. This occurrence is not useful for identifying glaucoma alone due to their occurrence in other optic nerve diseases such as drusen (Hitchings et al., 1976). Diffuse or localised loss of RNFL occurring in glaucoma can also be evident as visible defects in the RNFL which are not present in healthy eyes (Quigley et al., 1992, Jonas and Schiro, 1994). Other features such as vascular changes, peripapillary atrophy and optic disc pallor are also associated with glaucoma. Therefore, examination of the optic disc and surrounding regions is of importance in both diagnosis and detection of progressive damage as will be discussed further.

Risk factors are factors which predispose an individual to disease and are clinically useful to assess the risk of POAG based on the unique characteristics of the patient. POAG risk factors can be separated along demographic and clinical lines though it is likely that a combination of factors increase an individual's risk. It is worth noting that the appearance of the optic disc is not considered a risk factor because its characteristics are part of the definition of glaucoma. Many risk factors have been identified but only a smaller number have strong evidential support (Friedman et al., 2004a). One of the strongest risk factors is elevated IOP, and several studies have demonstrated that the prevalence of POAG increases progressively with higher levels of IOP (Pohjanpelto and Plava, 1974, Sommer et al., 1991). It has been suggested that the overall risk of developing POAG is five times higher with IOP>21 mmHg (Leske, 1983). More recently, a large population study of OHT patients showed higher baseline IOP to remain a leading risk factor for development of POAG (Gordon et al., 2002). Population-based studies of prevalence and incidence of POAG have shown consistently that age is one of the most important risk factors (Tielsch et al., 1991, Klein et al., 1992, Coffey et al., 1993, Dielemans et al., 1994, Leske et al., 1994, Mitchell et al., 1996, Friedman et al., 2004a). As a rule of thumb

these studies reported prevalence rates roughly doubling for each decade after 40. Studies into racial risk factors show that being of African, African-American or Afro-Caribbean origin puts one at a four-fold increased risk of developing POAG over white patients when averaged across age groups (Tielsch et al., 1991, Leske et al., 2004, Girkin, 2004a). Less data are available regarding POAG in other racial groups though results suggest that those from the Indian sub-continent have higher prevalence rates (Ramakrishnan et al., 2003), while those of Hispanic origin likely have intermediate prevalence of POAG between those of African descent and whites (Quigley et al., 2001).

A positive family history of the disease also gives an individual a higher risk of developing POAG, though the disease does not usually exhibit *Mendelian inheritance*. Studies in families with and without cases of glaucoma led to the conclusion that IOP and the aqueous outflow facility are multi-factorial in determination and that POAG is probably multi-factorial also (Armaly, 1968). Evidence of a genetic background comes from studies indicating that the prevalence of POAG in first-degree relatives of POAG patients is 7-10 times higher than in the general population (Becker et al., 1960, Perkins, 1974). There is also a high concordance rate for POAG between monozygotic twins (Goldschmidt, 1973). More recent advances in genetics have led to the mapping of glaucoma genes, however, these genes only account for a small portion of diagnosed glaucoma: a mutation in one of these genes, labelled MYOC, is found in 3-5% of late-onset POAG (Stone et al., 1997). Ethnic risk factors are also significant as has been discussed.

Further risk factors for POAG include myopia and diabetes (Leske, 1983, Wilson et al., 1987), while another study reports a relationship between elevated blood pressure and elevated IOP (Tielsch et al., 1995). A thorough review of risk factors in

glaucoma can be found in (Allingham and Shields, 2005). As IOP is the only treatable risk factor with strong evidence, most treatments of glaucoma focus on reducing IOP and its fluctuation. As glaucomatous neuropathy cannot be reversed, and due to the chronic nature of POAG, treatments can often be considered within the overall context of disease management. Treatments can be broken down into medication, laser surgery and incisional surgery.

The management of POAG usually involves some form of topical and occasionally orally administered treatments that enhance aqueous outflow or reduce aqueous production or both. Prostaglandin analogues are the most commonly prescribed medication for glaucoma and work by increasing uveoscleral outflow (Allingham and Shields, 2005), beta-blockers inhibit aqueous secretion and were commonly used in initial medical management but their use in has declined recently in favour of prostaglandin analogues. Other treatments such as cholinergic agents cause ciliary muscle contraction which stretches the trabecular meshwork (Krieglstein, 2000), carbonic anhydrase inhibitors inhibit aqueous production, adrenergic agonists also inhibit aqueous production and increase trabecular outflow (Allingham and Shields, 2005). As the actions of the various groups of drugs are different, combinations of these agents can be applied to achieve a *target IOP*. Topical medicines containing combinations of treatments are often prescribed to patients who require more than one type of drug for control of their glaucoma. This can help to reduce the burden of self-administered treatment on the patient. These treatments, in isolation or combined with others, have side effects (local to the eye and systemic) of varying severity (Detry-Morel, 2006). The overriding goal of medical treatment is to use the least number of medications necessary to achieve a target IOP with the minimum ocular and systemic side effects.

Laser surgery targeting the trabecular meshwork is known as trabeculoplasty. The two most common methods, Argon laser trabeculoplasty and the newer procedure selective laser trabeculoplasty both reduce IOP by improving aqueous humour outflow and differ in the type of laser used. Both treatments apply laser energy, usually to one half of the angle of the trabecular meshwork at a time. Selective laser trabeculoplasty is a potentially repeatable procedure because of the lack of coagulation damage to the trabecular meshwork, as shown in one study (Kramer and Noecker, 2001). Both treatments are simple, cost-effective and, once performed, do not depend on the compliance of the patient to self-administer medication. Laser trabeculoplasty has been shown to be at least as effective as medical treatment (The Glaucoma Laser Trial Research Group, 1990). Other studies have shown that the effects of laser trabeculoplasty are not always long-lasting however; IOP tends to rise over time in many patients (Schwartz et al., 1985).

The most common incisional surgery performed in adults for glaucoma is *trabeculectomy*. This filtering procedure involves the removal of small part of the trabecular meshwork, specifically of a block of limbal tissue beneath the scleral flap. This creates a passageway for aqueous to escape from inside the anterior chamber of the eye to a pocket created between the conjunctiva and the sclera. Studies have shown trabeculectomy to be more effective than medical and laser treatments at lowering IOP and in preserving visual function in the long-term (Burr et al., 2005). Other surgical techniques, tube-shunt surgery or drainage implant surgery involve the placement of a tube or glaucoma valves to facilitate aqueous outflow from the anterior chamber. Laser and incisional surgeries carry with them low but significant rates of adverse risks such as infection, post-operative transient IOP increases, hypotony and development of cataract which are considered thoroughly elsewhere

(Allingham and Shields, 2005). In the last decade some clinical trials have reported on the effects of treatment over long term patient follow-up.

The Early Manifest Glaucoma Trial (Heijl et al., 2002) compared the effects of lowering IOP using trabeculoplasty combined with medical treatment against no treatment or later treatment. The study showed treatment significantly delays further visual field deterioration with rates of detected further visual field deterioration of 41% in the treated group and 51% in the other group in a median follow-up period of 5 years. The Advanced Glaucoma Intervention Study (AGIS) (Advanced Glaucoma Intervention Study Investigators, 2000) examined the association of visual field deterioration and control of IOP by surgical intervention by both argon laser trabeculoplasty and trabeculectomy. After 5 years of follow-up, the study found a significant relationship between IOP reduction and a lower estimate of visual field loss. The Collaborative Initial Glaucoma Treatment Study has shown that patients randomised to either medical treatment or trabeculectomy at the start of clinical management had similar rates of further visual field damage (Musch et al., 2009). The Ocular Hypertension Treatment Study has demonstrated that, over a follow-up time of 5 years, the rate of conversion to POAG in OHT patients receiving topical glaucoma medication was roughly half of that in those receiving no treatment (Kass et al., 2002). It is worth noting that definitions of 'visual field deterioration' in these studies differed, making comparison between their outcomes difficult. Weinreb and Khaw (Weinreb and Khaw, 2004) provide further consideration of these and other clinical trials. These studies support the view that lowering IOP reduces the rates of further damage in visual fields and damage to the ONH but this view should be tempered by the potential risks and side-effects of treatment. The success of any treatment will be limited by how reliably and early a diagnosis can be established.

1.1 Diagnostic Technology in Glaucoma

Preservation of visual function in glaucomatous patients relies on early detection and appropriate treatment. Detection depends on recognising the early clinically measurable manifestations of glaucoma. A diagnosis of glaucoma no longer relies on the presence of elevated IOP alone and the additional assessment of the visual field and the ONH are now integral to giving a reliable diagnosis. Though these assessments are complementary and a diagnosis is formed in consideration of all factors, these are subsequently discussed individually to give an insight on their operating principles and performance.

Elevated IOP, along with subject age, remain the most important single risk factors. In addition, the periodic fluctuation of IOP or *diurnal variation* throughout the day is another feature which may present a more complex aspect to the risk of glaucomatous damage from IOP (Newell and Krill, 1964). In normal individuals, diurnal variation of IOP typically ranges from 3-6 mmHg with diurnal variations greater than 10 mmHg suggestive of glaucoma - even diurnal IOP fluctuations of greater than 30 mmHg have been reported for some glaucomatous eyes (Newell and Krill, 1964, Sultan et al., 2009). In clinical assessment, *tonometry* is used to measure IOP. This technology measures how much force is required to deform and flatten (*applanate*) an area of the cornea and can be categorised into those methods which are contact or non-contact. Contact tonometers have been shown to have better between-observer agreement (Tonnu et al., 2005b) and of these, the Goldmann applanation tonometer is considered the gold standard for measuring IOP (Sultan et al., 2009). Non-contact tonometers, using an 'air-puff' to deform the cornea, are more portable than contact tonometers and do not require local anaesthesia of the cornea. Corneal thickness is a significant source of error in tonometry resulting in

systematic underestimation or overestimation (Tonnu et al., 2005a, Kotecha et al., 2005). Thick corneas require more applanation force and give artefactually high measured IOP and conversely patients with thin corneas may also have higher IOP than that measured by tonometry (Yagci et al., 2005). This discrepancy can be as much as 10 mmHg between eyes with the same true IOP which are at upper and lower extremes of the distribution of central corneal thickness measurements (Kohlhaas et al., 2006).

Assessing visual function in glaucoma has become central to the management of glaucoma. Loss of sensitivity in the visual field is a correlate with the loss of or damage to signal carrying RGC axons and dendrites and ultimately determines how much effective functional loss a patient has suffered and what the patient can see (Heijl, 2000). *Perimetry* is the technique used to measure the sensitivity (or extent) of the visual field. The technique can therefore help address the real impact of glaucomatous damage on the patient, e.g. changes in the quality-of-life and fitness-to-drive. Automated perimetry, typified by the commercially available Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA), normally measures the central 25-30º of the visual field and this has become a clinical standard. This is performed by presenting light stimuli of varying differential intensity at various retinal locations while the patient fixates on a central target. The location and intensity of stimuli observed by the patient are recorded based on responses from the patient (Heijl and Patella, 2002). Various strategies are used to present stimuli and their intensities depending on the level of accuracy and speed of testing required in clinical assessment. A full threshold algorithm, steps stimulus intensity in fixed increments until a final sensitivity value is recorded for each test location. Alternatively another testing strategy, known as The Swedish Interactive Thresholding Algorithm, has been designed to provide results as reliable as full threshold testing but in a reduced

examination time (Bengtsson et al., 1997, Heijl and Patella, 2002). Perimetry technology can detect large fixation errors, and can estimate false positive and false negative events based on the timing of responses with respect to stimulus presentation. These can be used to give a measure of the reliability of the test. The output from the machine includes a map of the visual field and summary values of the whole field indicating if the field has localised or overall low sensitivity and if this deviates from a set of age matched healthy visual fields - this is especially useful because the variability between visual fields of healthy subjects is less than that of their ONH morphologies. Variability can be caused by the following factors: changes in pupil size, refractive error, ocular media opacities, eyelid artefacts, subject learning, fatigue and fixation errors (Henson, 2000).

Examination of the ONH is a crucial adjunct to visual field assessment. Ophthalmoscopy is an integral clinical tool for optic disc examination but, apart from summary subjective findings, provides no permanent record of the appearance of the optic disc. Optic disc photography provides a high-resolution permanent record of optic disc appearance. Monoscopic and stereoscopic photographs can be taken with the latter having the added advantage of providing an appreciation of the depth of the optic disc morphology to the clinician. Assessment by trained observers of optic disc photographs alone has been shown to have moderate diagnostic accuracy in differentiating healthy and glaucomatous eyes (Wollstein et al., 2000, Greaney et al., 2002) - of note is the large disagreement between observers (Abrams et al., 1994, Reus et al., 2010, Denniss et al., 2011). The ability to detect changes in the optic disc morphology in follow-up assessments depends on the reproducibility of the method employed; if the method is highly reproducible then small changes in the disc can be detected. However, patients are not always followed by a single clinician and so this large variation between observers can be

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problematic (Garway-Heath, 2000). Flicker-chronoscopy and stereo-chronoscopy (Goldmann and Lotmar, 1977, Barry et al., 1998) improve the identification of small changes between two photographs, but a false-impression of change can be generated by magnification error and parallax (Garway-Heath, 2000). Assessment of simultaneous and sequential stereoscopic optic disc photographs has been demonstrated to be capable of detecting progressive glaucomatous changes (Sommer et al., 1979, Pederson and Anderson, 1980, Odberg and Riise, 1985) but again this determination is often subject to large variation depending on the observer (The European Glaucoma Prevention Study Group, 2003, Jampel et al., 2009). *Planimetry* is the term given to measurements made from photographic images. Some camera and software systems enable viewing of digitised optic disc photographs (Yogesan et al., 1999, Shuttleworth et al., 2000). This facilitates quantitative planimetric assessment of the optic disc but is limited by subjective interpretations of the boundaries of the optic disc and neuroretinal rim (Garway-Heath et al., 1999).

Scanning laser polarimetry (SLP), optical coherence tomography (OCT) and confocal scanning laser tomography (CSLT) form a triad of established semi-automated imaging technologies capable of measuring the posterior segment of the eye and providing quantitative measures of the morphology of structures therein. Unlike optic disc photographs, which require expert training to obtain and examine, these imaging modalities have the advantage of offering relatively easy image acquisition and automated quantification of posterior features, which can help in identifying obvious or suspicious glaucomatous features. Imaging of the RNFL provides surrogate measures by which we can measure the true anatomical changes which accompany the deterioration of the visual field. Both SLP and OCT imaging techniques provide quantitative and objective measures of RNFL thickness.

SLP as typified by the commercially available GDx (Carl Zeiss Meditec, Dublin, CA) is based on the principle of measuring a retardation of backscattered light passing through the presumed form birefringent RNFL (Dreher and Reiter, 1992, Zhou and Knighton, 1997). This retardance is measured around the ONH and can then be translated to the thickness of the scanned RNFL at these locations using a linear conversion factor derived from a histological animal model (Weinreb et al., 1990). The GDx has evolved since its first clinical introduction, and fundamental to each principal stage has been how the scanning system has compensated for the birefringent properties of the cornea which would otherwise distort the retardance readings from the RNFL (Greenfield et al., 2000). The more recent of these approaches, variable corneal compensation and later enhanced corneal compensation rely on estimating the individual's corneal birefringence and compensating for this. Variable corneal compensation uses a variable retarder aligned with the fast axis of corneal polarisation to do this (Zhou and Weinreb, 2002). This technology shows promise in separating normal and glaucomatous eyes (Reus and Lemij, 2004, Tannenbaum et al., 2004). Enhanced corneal compensation adds retardance bias along the slow axis of corneal polarisation, measures the combination of the RNFL and the bias retarder, and extracts from this the RNFL retardance (Zhou, 2006). Theoretically, as the corneal retardance can be better estimated and thus removed, the enhanced corneal compensation mode can improve the signal to noise ratio of the RNFL retardance and thus lead to more accurate and less variable RNFL thickness measurements. Cross sectional studies have shown the diagnostic accuracy of the enhanced corneal compensation mode to be higher (Mai et al., 2007a), produce less frequent atypical retardation patterns (Morishita et al., 2008) and to have a closer relationship to visual field measures

(Morishita et al., 2008, Mai et al., 2007b) when compared to the variable corneal compensation mode.

OCT captures high-resolution, three-dimensional images from within optical scattering media. It uses interferometry to measure the reflectance of light at different depths in the retina and ONH (Hee et al., 1995). RNFL thickness values can be derived by identifying and measuring the depth of the reflectance signal of the anterior and posterior boundaries of the RNFL. Time-domain OCT provides reproducible RNFL thickness measurements at various locations around the ONH (Budenz et al., 2005, Hsu et al., 2006). More recently developed spectral-domain OCT systems with higher resolution and faster image acquisition times provide RNFL thickness measurements with higher reproducibility (Kim et al., 2009). Both have shown reasonable diagnostic performance in differentiating eyes with and without glaucomatous damage (Schuman, 2008, Chang et al., 2009, Rao et al., 2010).

CSLT obtains three-dimensional images of the ONH and other posterior segments of the eye and calculates their topographical surfaces and this technology is the focus of this thesis. The operating principles and features of CSLT are considered in Section 1.2.

1.2 Confocal Scanning Laser Tomography

The scanning laser ophthalmoscope (SLO) (Webb and Hughes, 1981) and later the confocal scanning laser ophthalmoscope (CSLO) (Webb et al., 1987) were developed in the 1980's. The concept of imaging using a scanning laser system originated from the field microscopy in the previous decades (Cremer and Cremer, 1978). SLO and CSLO were designed to image ocular features such as the retina, macula and ONH

and offer advantages over conventional imaging such as direct and indirect ophthalmoscopy and digital fundus photography. Hosts of new applications have followed the advent of SLO and CSLO, such as eye-tracking, SLP, scanning laser Doppler flowmetry, scanning laser fluorescein angiography, scanning laser corneal microscopy and CSLT. A review of these technologies and their clinical applications can be found elsewhere (Sharp and Manivannan, 1997, Ciulla et al., 2003). CSLT, typified by the commercially available Heidelberg Retina Tomograph (HRT, Heidelberg Engineering GmbH, Heidelberg, Germany) is the subject of this thesis. The HRT exists in two versions, the HRT Classic and the more recent HRT II, which differ primarily in their scanning protocols and operating software. What follows is a description of how the HRT acquires images and reviews how the technology is typically used for diagnosis of glaucoma and for detecting progression.

CSLT technology uses CSLO imaging combined with the concept of tomography to give three-dimensional images of the optic disc and peripapillary retina. Scanning laser imaging differs from conventional ophthalmoscopy and digital fundus photography in the method of illuminating the retina to form an image. CSLO does this point-by-point rather than capturing the image as a whole. A low-energy laser is focused on a point on the retina which reflects light back to a detector. A deflector mirror then moves the laser beam horizontally so an adjacent point can be imaged. When one line has been acquired a second deflector mirror moves the beam vertically before acquiring another horizontal line. A two-dimensional image is built up in this raster-like fashion in approximately 32 ms for the HRT Classic and 24 ms for the HRT II. For each image, the HRT Classic acquires the reflected intensity from a total of 256 × 256 equally-spaced points (pixels) in a 10° x 10° scanning angle, and the HRT II acquires a total of 384 × 384 pixels in a 15° x 15° scanning angle.

Another crucial feature of CSLO imaging, and what distinguishes it from non-confocal SLO imaging, is the presence of an aperture after the laser source and another before the detector, both in focal planes conjugate to that of the object being imaged. This is the basis of the confocal optical setup which ensures that only light from the imaged focal plane reaches the image sensor as reflected light from in front of, or behind, the focal plane is rejected at the aperture. Figure 1.4 illustrates a confocal imaging system. The improvement in axial and transverse resolution with CSLO scanning over a non-confocal SLO system is clear in Figure 1.5.
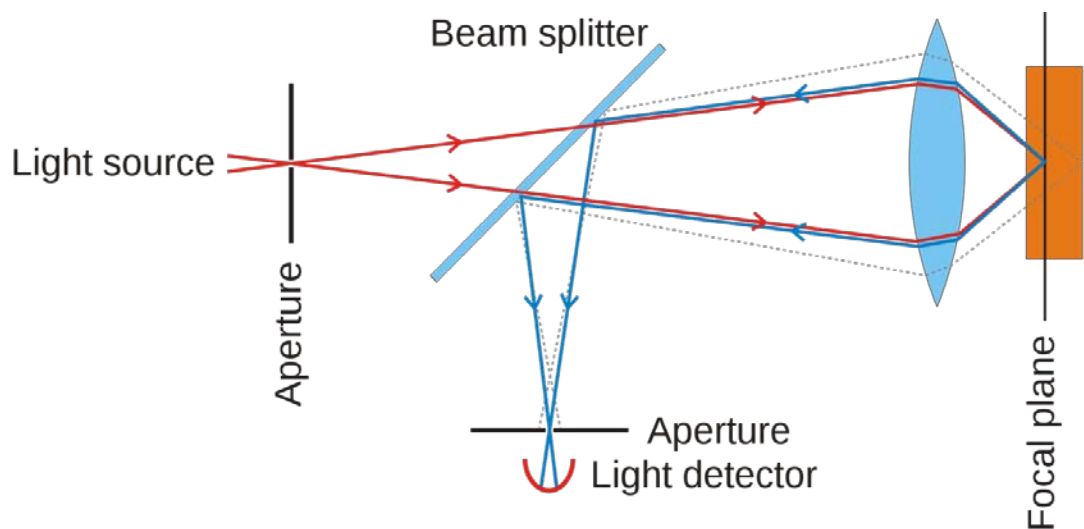
Figure 1.4 Principles of confocal scanning showing the placement of apertures/pinholes in front of the light source/laser and the detector. With an infinitesimally small aperture any light returning from planes posterior or anterior to the focal plane is rejected.
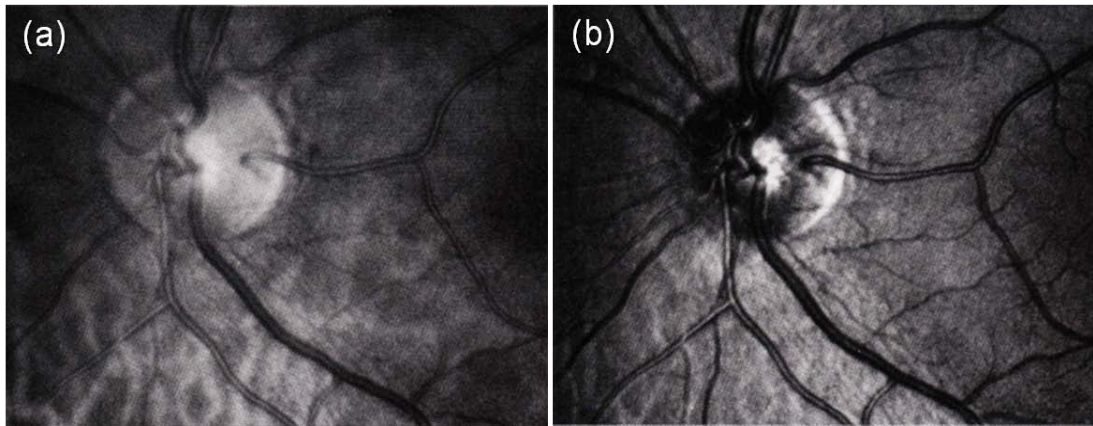
Figure 1.5 Examples of ONH scans of the same eye with a scanning laser ophthalmoscope system in (a) non-confocal and (b) confocal modes. (Images from Plesch et al., 1990)

Tomography implies imaging by sections or slices and involves incrementally changing the plane of focus. This means that CSLT involves incrementally moving the focal plane from anterior to posterior along the optical axis after each two-dimensional CSLO image or *optical section* has been acquired to form a set or confocal stack of optical sections. An example sequence of this is illustrated in Figure 1.6 (a). The descriptive coordinate system for this three-dimensional space fixes measurements along the optical axis (axial) to the z-axis and measurements conjugate to the optical axis (transverse) to the x-y plane. In HRT Classic imaging this confocal stack consists of 32 optical sections spread at equal axial intervals over a scanning depth of 1.5 to 4.0 mm. In HRT II imaging the number of optical sections acquired is automated and depends on the optimum scanning depth for the particular ONH, varying from 16 to 64 with each optical section separated an equal distance of 62.5 μm apart. Each CSLO optical section is successively registered with the previous one to compensate for potential eye movements during scanning. From these aligned, three-dimensional confocal stacks (sizes: HRT Classic 32 x 256 x 256; HRT II [16, 64] x 384 x 384), a two-dimensional topographic height image or

topography is derived. This is done by calculating the approximate axial position of maximum reflectivity signal intensity at each transverse coordinate, along what is referred to as a z-profile (Figure 1.6 (b)). The topography then represents the surface height of the ONH and surrounding papillary retina (Figure 1.6 (c)-(e)) usually but not always at the approximate position of the internal limiting membrane; the intensity at each pixel within the image giving a height in microns. A *reflectance image* is also formed from the maximal reflectance values at each transverse coordinate's z-profile (Figure1.7 (c) & (d)).
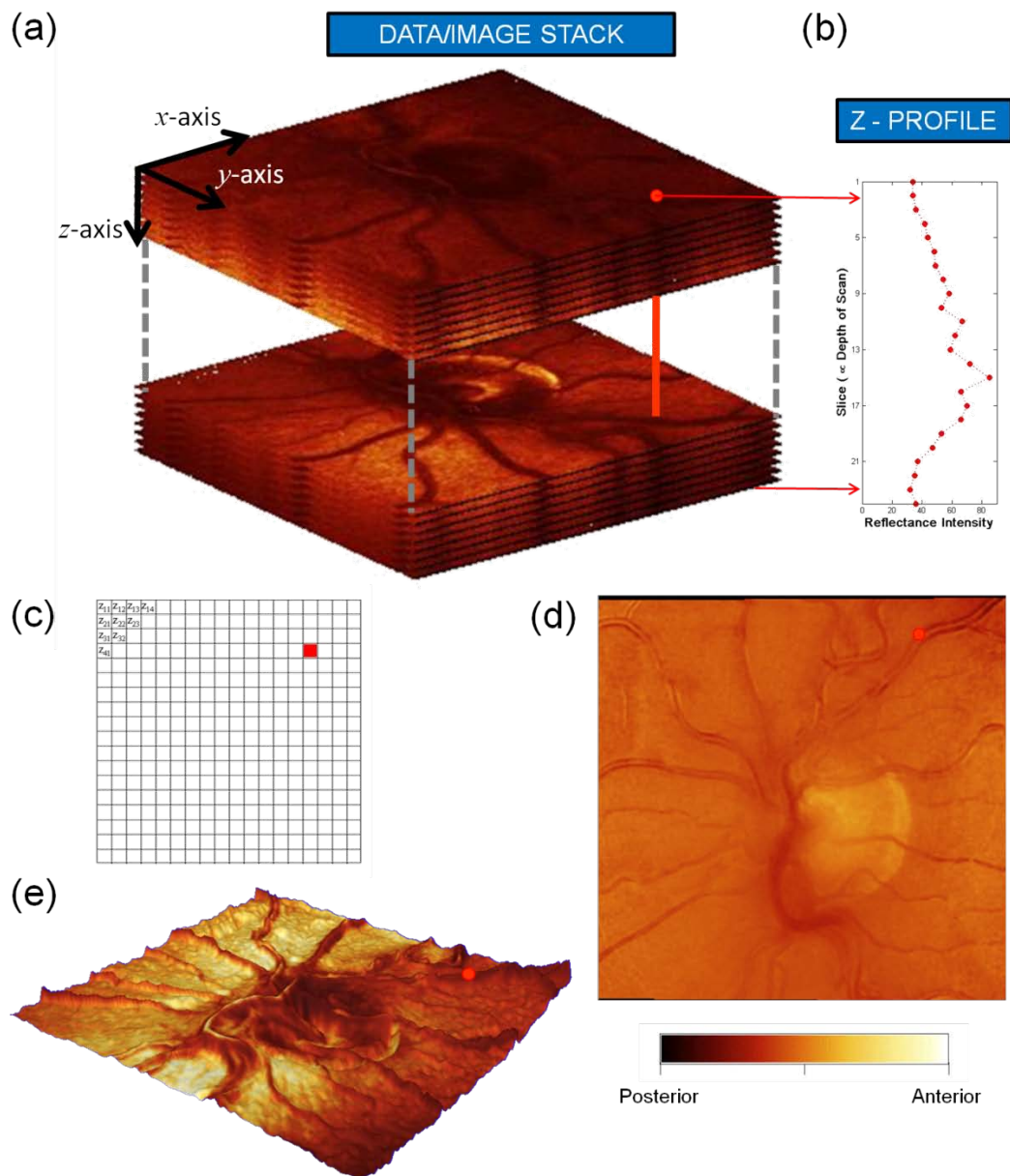
Figure 1.6 Principles of CSLT imaging. (a) "Stack" of confocal scanning laser ophthalmoscope images at incremental focal depths (false colour representation of reflectance intensity). Measurements in the z-axis are referred to as axial and those in the x and y axes are referred to as transverse. (b) The set of axial reflectance values at a given transverse coordinate (x_i, y_j) is known as a z-profile. (c) The axial location of each z-profile maximal reflectance at coordinate (x_i, y_j) is calculated and denoted z_{ij}. (d) Axial locations are mapped to a topographic height image. (e) CSLT three-dimensional representation of (d).
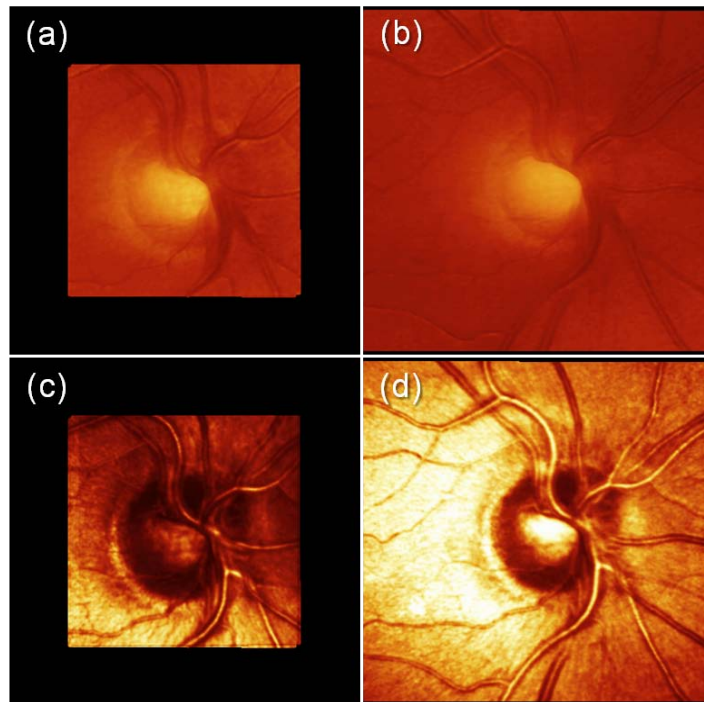
Figure 1.7 Comparison of 10° x 10° HRT Classic topography (a) and reflectance images (c) and 15° x 15° HRT II mean topography (b) and mean reflectance images (d). Images have transverse spatial sampling of 256 x 256 (HRT Classic) and 384 x 384 (HRT II) ensuring that transverse spatial sampling intervals are consistent. (Images from Moorfields Eye Hospital clinic database)

Typically, three *single* topography images are acquired for each examination (this is automated with the HRT II) and averaged to calculate a mean topography. This became the convention after a recommendation from an early study on changes of variability within a mean topography with a varying number of single topographies (Weinreb et al., 1993). Figure 1.7 illustrates the mean topography images and mean reflectance images from a HRT Classic and HRT II examination of the same eye. Image registration algorithms within the HRT software align the single topography images for the within examination and between examination differences in scan positions. The most recent of these uses a technique of matching land-marks common to two images derived from a general methodology developed by Capel

(Capel, 2004). Further details of CSLT are provided elsewhere (Zinser et al., 1989). Though the technology has been shown to be accurate (Dreher and Weinreb, 1991) and to obtain reproducible topography images of the ONH (Chauhan et al., 1994, Rohrschneider et al., 1994), images can be prone to artefacts and noise from a number of sources. These include eye movements, temporary deformations of the ONH, cataract and pupil size. Noise sources and their effects are explored further in Chapters 4 and 5. The repeatability of topography images can be quantified with the mean pixel height standard deviation (MPHSD). This metric is a gauge of the variability of each pixel height measurement across the three topographies used to make up the mean topography (Dreher et al., 1991). Chapter 4 discusses this metric further.
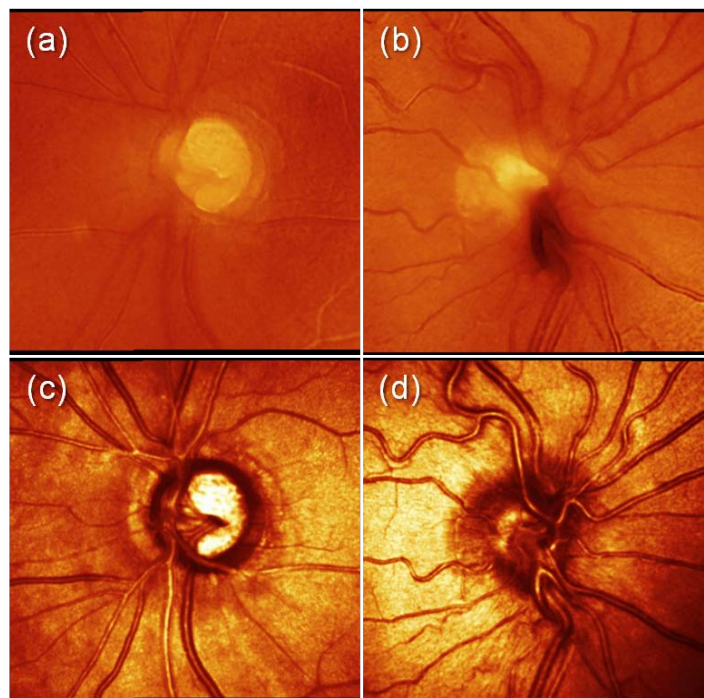
Figure 1.8 HRT II topography and reflectance images of glaucomatous eye (a) & (c) and normal eye (b) & (d). (Images from Moorfields Eye Hospital clinic database)

In Figure 1.8, topography and reflectance images are shown for the right eye of a glaucoma patient and for the right eye of a normal subject. In this example the differences in size and shape of the morphology of the ONH are clear. The HRT software quantifies morphological features of the ONH in glaucoma by calculating a number of three-dimensional *stereometric parameters*. A contour line is first delineated (Figure 1.9 (a)). This is a closed elliptical shape drawn manually using a subjective assessment of the location of the boundary of the optic disc which generally follows the inner margin of the sclera ring. Note that although this input is subjective, its position has been shown to have good between-operator agreement and is better than that of optic disc boundary delineations in other imaging modalities such as planimetry (Garway-Heath et al., 1999).
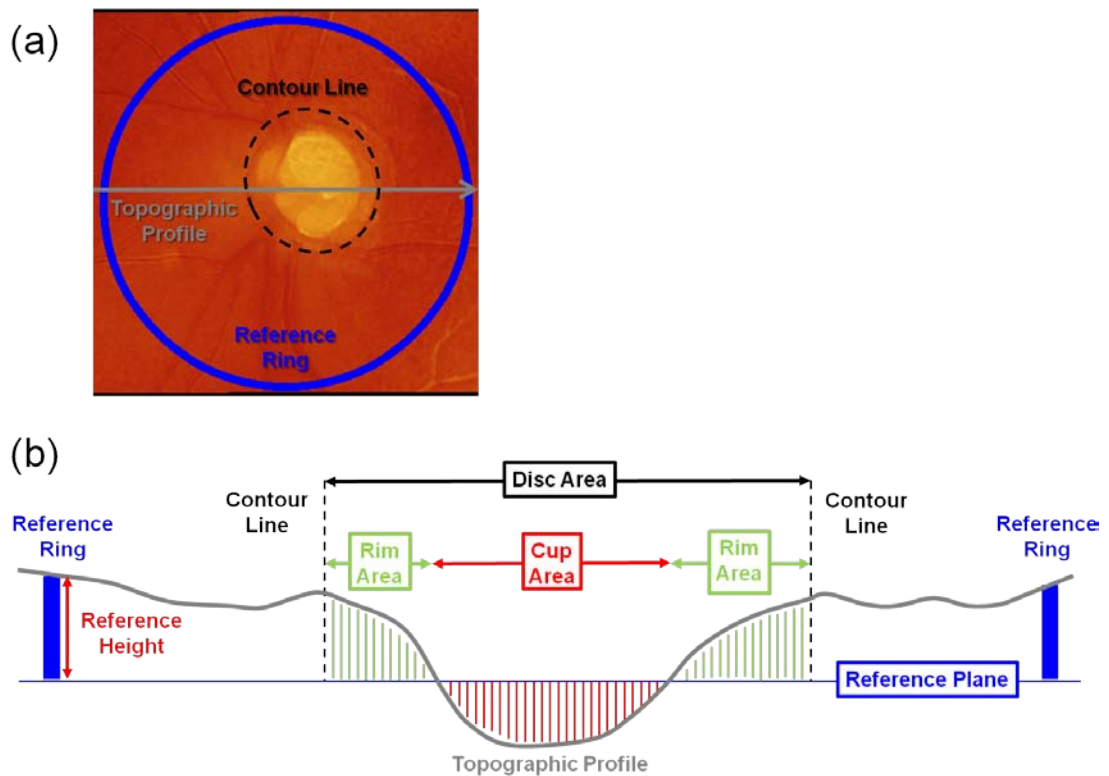
Figure 1.9 (a) HRT topography with manually delineated optic disc boundary (contour line) and (b) illustration of the derivation of topography optic disc, neuro-retinal rim and cup areas from the reference ring and contour line.

The mean peripapillary retinal surface height is found from the topographic height values within a peripheral *reference ring* as shown in Figure 1.9 (a). This reference ring is defined as an annulus, located at the topography image centre, with inner and outer diameters which are 91% and 94% respectively of the topography image width. The topographic image is set to zero at the mean topographic height of the reference ring and adjusted for peripapillary retinal tilt by subtracting a plane fitted to the topographic height values within the reference ring. Once offset and tilt adjustments are made, and after the contour line is drawn, a "reference plane" (see Figure 1.9 (b)) is calculated. This is a plane set parallel to and below the peripapillary retinal surface and it is used to divide the optic disc into neuro-retinal rim and cup (Burk et al., 2000). In Figure 1.10 (a) and (b) neuro-retinal rim area (RA) is simply the sum of the green and blue areas and cup area is measured as the red area. Other stereometric parameters can then be calculated. Rim volume (RV) and cup volume are likewise measures of the total volume between the reference plane and the topographic surfaces within the contour line which are above and below the reference plane respectively. Cup shape measure (CSM) is a measure of the three-dimensional shape of the optic disc cup, also called the third moment, representing the skewness of the distribution of topographic height values within the optic disc cup (Burk et al., 1990). Height variation contour is the retinal surface height variation around the optic disc margin (Hatch et al., 1997). RNFL thickness, as calculated on the HRT, is the mean distance between the reference plane and the topographic height values around the optic disc margin (Iester and Mermoud, 2005). In addition to the output of stereometric parameters for the whole topography image/optic disc (referred to as a *global* measure), when applicable, the HRT provides values in six predefined angular segments: temporal, temporal superior, temporal inferior, nasal, nasal superior and nasal inferior.
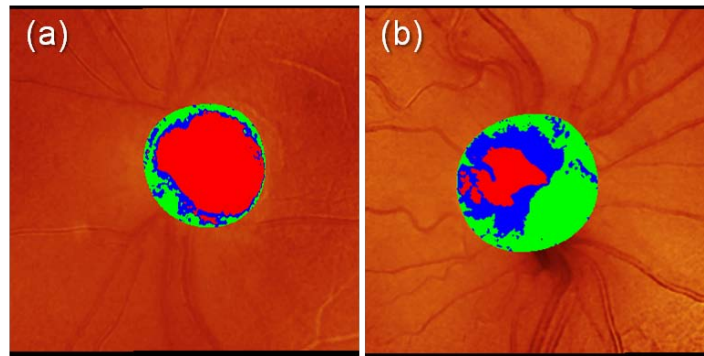
Figure 1.10 HRT II topography images for eyes in Figure 1.8 with overlay of manually delineated optic disc area and colours indicating neuroretinal rim (green and blue) and cup (red). (Images from Moorfields Eye Hospital clinic database)

Stereometric parameters outputted by the HRT are clinically meaningful and are reproducible in varying degrees (Sihota et al., 2002). Because measured cup area and RA are simply the complement of the set of each other and the optic disc area, it is sensible to just consider one of these. RA measurements from stereophotographs have been shown to correlate with visual function (Balazsi et al., 1984). In Figure 1.10 there is a clear difference in RA between the glaucomatous (a) and healthy (b) eye. RA gives a numerical value to a clinically recognisable and meaningful feature; the loss of RA provides a surrogate measure of the loss of retinal ganglion cell axons typical of glaucomatous damage (Yucel et al., 1998). It is the least variable of the stereometric HRT parameters and has been well characterised (Owen et al., 2006, Tan et al., 2003). RA is often examined as a proportion of overall optic disc area to provide a means of adjusting for differences in optic disc size. CSM may help to indicate differences between deeply cupped discs which have many outliers and flat cups which will have fewer outliers; it has been shown to correlate with indices of visual field damage (Brigatti and Caprioli, 1995).

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These stereometric parameters and combinations thereof have thus been used with some success to discriminate between normal optic discs and those with glaucoma. However the observed large between-subject variability of optic disc morphology resulting in large variations of optic disc area, rim area and depth of cupping makes this task non-trivial. A variety of statistical and quantitative techniques applied to the stereometric parameters have been used for this task. Wollstein and colleagues (Wollstein et al., 1998) examined the best parameters that separated patients with early glaucoma from normal subjects. They reported the highest separation between these groups using the 99% prediction interval from linear regression between optic disc area and the logarithm of RA of the normal subjects. This approach has been included into the HRT software and is known as the Moorfields regression analysis (MRA). The MRA suffers from poor performance when optic disc areas are large and the variability of normal and disease free rim areas are most variable and difficult to separate. An ethnicity-specific database has been included in the more recent HRT software versions. This aims to improve the estimation of predictive normative limits by addressing the differences in disc size across ethnicities and has been shown to reduce the specificity but increase sensitivity for blacks while maintaining the similar sensitivity-specificity rates for whites as compared to the non-ethnicity specific database (Zelefsky et al., 2006). More recently when the specificity has been fixed at 90% there was no evident gain in sensitivity from this approach (Roberts et al., 2010). To account for different normative limits of RA at different disc sizes other approaches have used piece-wise linear regression of the logarithm of RA against disc area across four disc area intervals (Hawker et al., 2007) or gone further by deriving the 99% prediction intervals from quantile regression (Artes and Crabb, 2010). The latter of these approaches was successful at reducing the dependence of diagnostic performance on disc area.

In addition an approach derives a probability of an ONH being abnormally-shaped due to glaucomatous damage called the glaucoma probability score (GPS) by automatically approximating the topography with a simple geometrical curved surface and analysing the shape parameters of this surface using a machine learning technique (Swindale et al., 2000). By circumventing the need to draw a contour line this method has the advantage of removing operator variability inherent in the stereometric parameters. However the diagnostic performance of the GPS has been reported to be only similar to that of the MRA and still suffers from more false-positive diagnoses for larger disc areas (Coops et al., 2006). In addition, for approximately 5% of topographies, the analysis algorithm fails to fit an approximating surface. Uchida and colleagues (Uchida et al., 1996) applied neural networks to CSM. Linear discriminant analysis uses combinations of stereometric parameters in optimum proportions to achieve best separation (Mikelberg et al., 1995, Uchida et al., 1996, Bathija et al., 1998, Ferreras et al., 2008). Another method divides RA into thirty-six 10° angular sectors and ranks these to produce a curve which can be used to identify glaucomatous optic discs (Asawaphureekorn et al., 1996, Gundersen and Asman, 2000).

There are further studies which have demonstrated that the HRT discriminates between normal optic discs and those with glaucoma reasonably well (Burgansky-Eliash et al., 2007, Ferreras et al., 2008). However, its diagnostic precision has been constrained by the wide and overlapping ranges of the size and shape of healthy and glaucomatous optic discs. The real promise of HRT technology is in detection of change in ONH structure over time, offering the clinician another tool for glaucoma management given that HRT measurements have been shown to be repeatable. Furthermore, the operational software of the HRT has undergone continuous development and refinement since its introduction in order to specifically improve

its ability to track changes occurring in the ONH (Strouthidis and Garway-Heath, 2008). The main focus of this thesis is on the detection of progressive glaucomatous damage in the ONH in patients by analysis of data from repeated scanning over years of follow-up. Statistical techniques used for this purpose are investigated along with how these can be best compared and optimised. Both HRT Classic and HRT II measurements of the ONH were used for analysis.

1.3 Glaucoma Progression

In the management of glaucoma, preservation of vision is the principal objective. Glaucoma progression - the onset of initial damage in a glaucoma suspect or the occurrence of further damage in an established glaucomatous eye - represents a phase in the disease from which the loss of visual function cannot be restored. Monitoring patients with tonometry, perimetry and ONH inspection is essential to identify this progressive glaucomatous damage. However it is not enough to merely perform these measurements. For example, it has been shown that subjective assessment of follow-up series of visual fields by experts has poor agreement in detecting progressive visual field loss (Werner et al., 1988, Viswanathan et al., 2003). Similar findings have been demonstrated for stereoscopic optic disc photograph assessment (Jampel et al., 2009) and the subjective assessment of graphical outputs from the HRT software (Vizzeri et al., 2009). Furthermore measurement variability is an unavoidable feature of both perimetry and ONH imaging. Statistical techniques have been developed in perimetry to address these issues such as PROGRESSOR (Fitzke et al., 1996, Viswanathan et al., 1997) and Statpac 2 (Heijl et al., 1991). This thesis examines analogous statistical techniques applied to HRT acquired ONH images which involve large amounts of output data.

Estimates of how much damage has occurred to the ONH in a follow-up period and predictions of how much damage will occur are hugely helpful for the clinician when determining whether a surgical intervention or a change in medication is called for. As outlined previously, the HRT can acquire scans of the ONH over time and process the data to provide a series of well aligned topographic images or topographies of the ONH. In order to detect glaucomatous structural change across these topographies with a high diagnostic precision it is necessary to firstly identify *statistically significant* change i.e. change that can be inferred as unlikely to be caused by the inherent measurement noise. Secondly, it is necessary to identify *clinically significant* change, i.e. change that is meaningfully associated with visual field damage and beyond that measured in normal age-related change. This is also true not just for measuring changes in more summary measures such as for the regional and global stereometric parameters provided but in the individual pixels of the topographic images. The clinician may seek to corroborate their subjective assessments of change with statistical inferences as to whether the change estimated from a sequence of observed measurements has a low probability of being caused by measurement noise.

Currently, there is no accepted mathematical model or universally recognised quantitative technique for following the complexity of how a glaucomatous ONH changes over time. Because of ethical considerations, very little data are available on how glaucomatous eyes would change if left untreated and are restricted to visual field measures (Heijl et al., 2009). In the absence of an established model, change is often examined as a series of change *events.* In this framework, when a follow-up measurement exhibits a deviation from baseline measurement beyond some limit and which is confirmed upon repeat testing or scanning, a significant change event is said to have occurred. An alternative approach uses a linear model of change to

derive *trends* providing an easy to interpret average loss (some surrogate for axonal loss such as topographic height or a stereometric parameter) per year. Age-related changes of the optic disc have been quantified both longitudinally and cross-sectionally. Any trend-based model of ONH glaucomatous change should incorporate considerations of these age-related changes, on top of statistical considerations to establish how reliable a measured trend might be. What follows in this section is a review of some of the methodologies for detecting progressive glaucomatous damage to the ONH using the HRT.

1.3.1 Heidelberg Retina Tomograph Progression Detection Algorithms

Methods for detecting change in the HRT can be broadly categorised by whether they analyse summary data of the optic disc morphology (stereometric parameters) or analyse local changes within the image space (pixel or super-pixel methods).

Statistical methods to detect progressive ONH damage have been applied to HRT stereometric parameters. Previous studies (Strouthidis et al., 2005b, Jampel et al., 2006) have shown RA to be the most repeatable parameter, both with between-visit and between-operator variability and it has been demonstrated to have good longitudinal reproducibility (Leung et al., 2008). This suggests it is the best candidate of the stereometric parameters to identify and track glaucomatous changes of the ONH. Studies have quantified the test-retest variability of the HRT stereometric parameters including RA (Mikelberg et al., 1993, Rohrschneider et al., 1994, Miglior et al., 2002, Tan et al., 2003, Strouthidis et al., 2005b, Jampel et al., 2006). These limits have been used to derive limits beyond which change cannot be accounted for by measurement variability and thus considered to represent true morphological change (Kamal et al., 1999a, Kamal et al., 2000). Fayers and colleagues (Fayers et al., 2007) developed a RA event analysis of change based on

this approach but refined the variability limits according to image quality. Tan and

Hitchings (Tan and Hitchings, 2003, Tan and Hitchings, 2004) developed a

technique using 30° angular sectors of RA and an experimental reference plane.

Strouthidis and colleagues performed a simple linear regression of sectoral RA over

time in OHT where progression was defined by an average trend > 1%/year of

baseline RA and a statistical significance level tailored according to series variability

(Strouthidis et al., 2006). In addition age-related change of RA has been well

characterised (Tsai et al., 1992, Garway-Heath et al., 1997, See et al., 2009) as have

the trends in RA changes observed in OHT patients (Strouthidis et al., 2009a) and

these estimates may help to differentiate ageing effects from true disease

progression.

By design, stereometric parameters are summary measures i.e. they are quantified

by summing or averaging data over parts of the topography image for easy clinical

interpretation. This process is highly data reductive and can consequently reduce

the sensitivity of any statistical technique in detecting localised change. However,

when using statistical techniques involving summary measures it is not necessary to

account for any spatial correlation and multiple comparison problems which arise

when assessing change at a pixel-wise level.

The first statistical technique which looked for change on a local scale in series of

HRT images was the topographic change analysis (TCA) (Chauhan et al., 2000). This

analysis is included in the HRT software, and begins by dividing the topographic

image into a 64 × 64 (HRT Classic) or 96 × 96 (HRT II) super-pixel array (each super-

pixel consists of 4×4, or 16 pixels). The topographic height variability in these super-

pixels is compared within a baseline examination to the height change between

baseline and follow-up examinations (Chauhan et al., 2000, Chauhan et al., 2001).

This is done by performing an *analysis of variance* to measure the statistical significance of the average shift in the topographic height across all 16 pixels within each super-pixel from one set of images (3 replicates in the baseline examination) to another (3 replicates in the follow-up examination). For the analysis of variance an F-distribution is used to establish the significance, where the degrees of freedom are adjusted via a correction to account for spatial correlation within each super-pixel. It is worth highlighting that although this correction is used *within* a super-pixel, it does not correct for the spatial correlation *between* neighbouring super-pixels across the whole image. A change map of p-values, indicating the probability of change at each super-pixel, is created. Furthermore contiguous super-pixels showing significant ($p < 0.05$) decreases in retinal height can be clustered together and identified as regions of interest. This allows the generation of various TCA change summary parameters describing the area, volume and location of regions of change. The change map of the most recent follow-up examination is compared with previous examinations and change across time in a super-pixel is confirmed if a significant super-pixel is present in 2 of the last 2, 2 of the last 3 or 3 of the last 4 examinations depending on how many examinations are available and the implementation. The TCA is integrated into the HRT software which produces graphical outputs of the TCA as seen in Figure 1.11 which can be compared to the optic disc display.
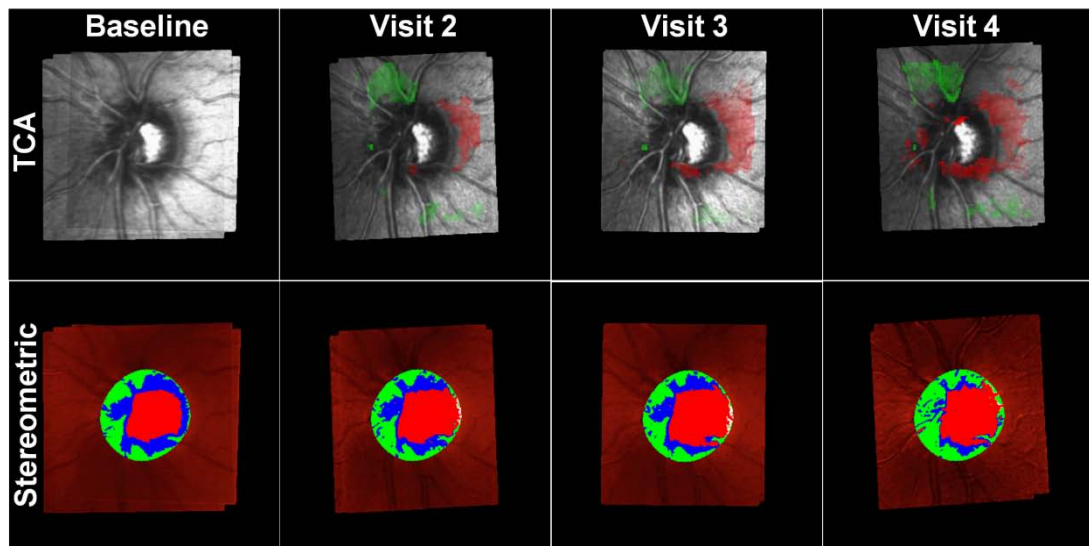
Figure 1.11 Sample HRT Classic topography images series for a sample eye over four visits. TCA outputs are displayed in the top row with statistically significant negative (red) and positive (green) change overlaid. For the same series, overlaid stereometric parameters, rim area and cup area, within the outlined optic disc are shown in the bottom row (colours as per Figure 1.10). A progressive inferior cupping of the optic disc is evident and corresponds with the inferior 'cluster' of red pixels in the TCA map. (Images from Moorfields Eye Hospital clinic database)

Originally an empirical criterion for 'significant' TCA change was established as 20 or more statistically significant, confirmed super-pixels within the optic disc border (Chauhan et al., 2001). This criterion for change was introduced to set the specificity at a particular level, the limits being derived from empirical data (longitudinal data from normal subjects). Subsequently, three criteria for change have been reported to demonstrate the variation in overlap of patients with visual field and ONH progression at different levels of criterion stringency: least conservative (depressed significant super-pixels within the optic disc >6% of the area of the optic disc), intermediate (>10%) and most conservative (>18%) (Artes and Chauhan, 2005). Further criteria for TCA were based on the population limits observed in normal

subjects (Bowd et al., 2009). In this study, the area (in mm^2) of the largest connected cluster of red super-pixels within the optic disc margin was found to be the best measure to separate those progressing patients from non-progressing patients at 90%, 95% and 99% specificities. These guidelines reflect that the TCA has no intrinsic mechanism to account for the multiple comparison problem. The results are also based only on the comparison of, at most, the four most recent follow-up images with the baseline image. Thus detecting change is highly dependent on the quality of the baseline image. In addition because POAG is a slow progressive disease, there are often many years of follow-up and the TCA essentially ignores many interim follow-up examinations. Brett and colleagues (Brett et al., 2003) provide a thorough review of the issues involved in applying statistical techniques to imaging data including how to account for the multiple comparison problem and spatial correlation.

Statistical image mapping (SIM) was adapted and applied to HRT imaging of the ONH by Patterson and colleagues (Patterson et al., 2005) to identify glaucomatous progression and to address some of the theoretical shortcomings of TCA. This technique has an established use in analysing 3D images of the brain acquired using positron emission tomography and magnetic resonance imaging (MRI). In functional MRI, these techniques are used to process and analyze images to determine which parts of the brain are activated by different types of physical sensation, such as sight, sound or movement. A 'so-called' statistic image shows a map of areas of the brain activated by the experiment which can be rendered in three-dimensions. In particular a non-parametric version of these techniques (Nichols and Holmes, 2002, Bullmore et al., 1999, Arndt et al., 1996, Holmes et al., 1996) has been applied to the HRT.

In short, SIM proceeds by calculating the trend of topographic change over time, by performing linear regression on the topographic height for each pixel within the area of the optic disc; this yields a test statistic summarising the amount of change at each pixel. The sequence of the images is then reordered (or 'shuffled') in time and the test statistic is recalculated at each pixel. This step is performed a finite number of times, each time using a unique reordering. At each pixel a distribution of test statistics is generated and the observed test statistic is then compared to this distribution, therefore highlighting whether the observed sequence is something that could have occurred beyond chance. A pixel is flagged as significantly changing if it falls outside the 95th percentile (a probability value of <0.05). The area of the largest cluster of contiguous significantly changing pixels in the observed image series is compared to the distribution of those for the permuted image series. A global probability value for the entire image series is thus derived from this distribution position. This value provides a measure of the spatial extent of the glaucomatous damage which has occurred in the image series and takes account of the similarity that will exist between topography values of neighbouring pixels. The method is wholly based on the patient's own data and the test-retest variability of the image series, while also correctly accounting for the multiple comparison problem which occurs when calculating a test statistic at each pixel across an image space.

1.3.2 Assessing the Performance of Change Detection Algorithms

Apart from theoretical considerations, there is little evidence to suggest that one method for detecting change is better than another and the clinician is left to wonder what method for detecting change is best to use.

Most research assessing how well these techniques can identify and track glaucomatous progression with the HRT has focused on examining agreement between structural and functional measures of progression. In practice, this involves assessing the diagnostic performance of HRT information in predicting visual field changes (Miglior et al., 2001, Artes and Chauhan, 2005, Philippin et al., 2006, Saarela et al., 2010). Studies using visual field changes as a reference standard in this way can be confounded by dissociating factors between structural and functional changes (Hudson et al., 2007). As a consequence, the relative proportions of associated and independent behaviour are not well known and the temporal sequence of structural and functional glaucomatous progression cannot be well-defined.

Exploring alternative, and potentially more useful, reference standards against which the performance of HRT imaging can be assessed may avoid these confounding factors. Further characterising HRT variability will also help to determine an optimum approach to detect ONH progression in HRT images. These investigations will form the main focus of this thesis.

1.4 Objectives

The objectives of this thesis are to assess progression detection algorithms in ONH data from the CLST. In particular, this thesis aims to:

- Compare the performance of three established competing progression detection algorithms using the clinical standard for glaucomatous structural damage to the ONH – expert assessment of optic disc photographs – as the reference standard

- Examine the effects of measurement variability across a range of characteristics on the statistical performance of some basic univariate statistical techniques to detect underlying change

- Investigate image quality metrics of HRT images and their relationships to measurement variability

- Develop and validate a simulation which can reproduce the variability of HRT data and help to provide a test-bed to evaluate the specificity and sensitivity of HRT progression detection algorithms

2. Glaucomatous Progression in Series of Stereoscopic Photographs and Confocal Scanning Laser Tomograph Images

Most research on assessing how well glaucomatous progression can be detected using the HRT has focused on agreement between structural and visual field measures of progression, or on predicting visual field changes based on HRT information (Chauhan et al., 2001, Kamal et al., 1999b, Artes and Chauhan, 2005, Strouthidis et al., 2006, Philippin et al., 2006, Hudson et al., 2007). The fewer investigations comparing longitudinal HRT and stereophotograph series in humans (Chauhan et al., 2001, Kourkoutas et al., 2007, Chauhan et al., 2009) have indicated that agreement between these two structural assessments is moderate with concordances of 65%, 81%, and 44% to 71% (depending on progression criteria and expert observers) respectively. Other research in primate experimental glaucoma showed good agreement between these two imaging methodologies (Ervin et al., 2002).

The aim of this chapter is to examine change in HRT image series identified by three automated statistical analytical methods: TCA, SIM, and ordinary least squares

(OLS) linear regression of rim area (RALR) against time of follow-up. These changes are compared to assessments by glaucoma specialists of change in optic disc stereophotographs from the same eyes in order to determine which method had the highest concordance with expert assessment of stereophotographs. To control the sample specificity and sensitivity of HRT change detection analyses, criteria for change are varied in stringency.

The work in this chapter has formed a paper published in Archives of Ophthalmology (O'Leary et al., 2010b). It was also presented at the Image Morphometry and Glaucoma in Europe Meeting, Rotterdam, The Netherlands on March 12-14, 2008 and the Association for Research in Vision and Ophthalmology Meeting, Fort Lauderdale on April 27 - May 1, 2008.

2.2 Methods

2.2.1 Patients

Data from the Devers Eye Institute Perimetry and Psychophysics In Glaucoma study are analysed and details of the investigation have been previously published (Fortune et al., 2007). All of the patients provided voluntary written consent to participate and to allow their clinical measurements to be securely held for future data analysis. All of the procedures adhered to the tenets of the Declaration of Helsinki and were approved by local ethics committee. Participants were recruited prospectively from the Devers Eye Institute, or other ophthalmic practices in the Portland, Oregon, metropolitan area. At recruitment, all patients were considered to have either high-risk OHT or early POAG. All subjects had a history of untreated IOP of at least 22 mm Hg in both eyes and at least one additional risk factor: a vertical cup-to-disc ratio of at least 0.6 in at least one eye or an interocular cup-to-disc ratio asymmetry of at least 0.2; a positive family history of glaucoma; a personal history of migraine, Raynaud's syndrome, or vasospasm; African-American ancestry; or age older than 70 years. All patients met the following criteria for both eyes: best corrected visual acuity of 20/40 or better and spectacle refraction within ±5.00 dioptre (D) sphere and ±2.00 D cylinder and reliable standard automated perimetry results with MD better than or equal to -6 dB. Patients were excluded if they had any other previous or current ocular or neurological disease, previous ocular surgery (except uncomplicated cataract surgery), or diabetes mellitus requiring medication. Subjects were treated throughout follow-up at the discretion of their managing eye care specialists who were sent a copy of study-related test results yearly.
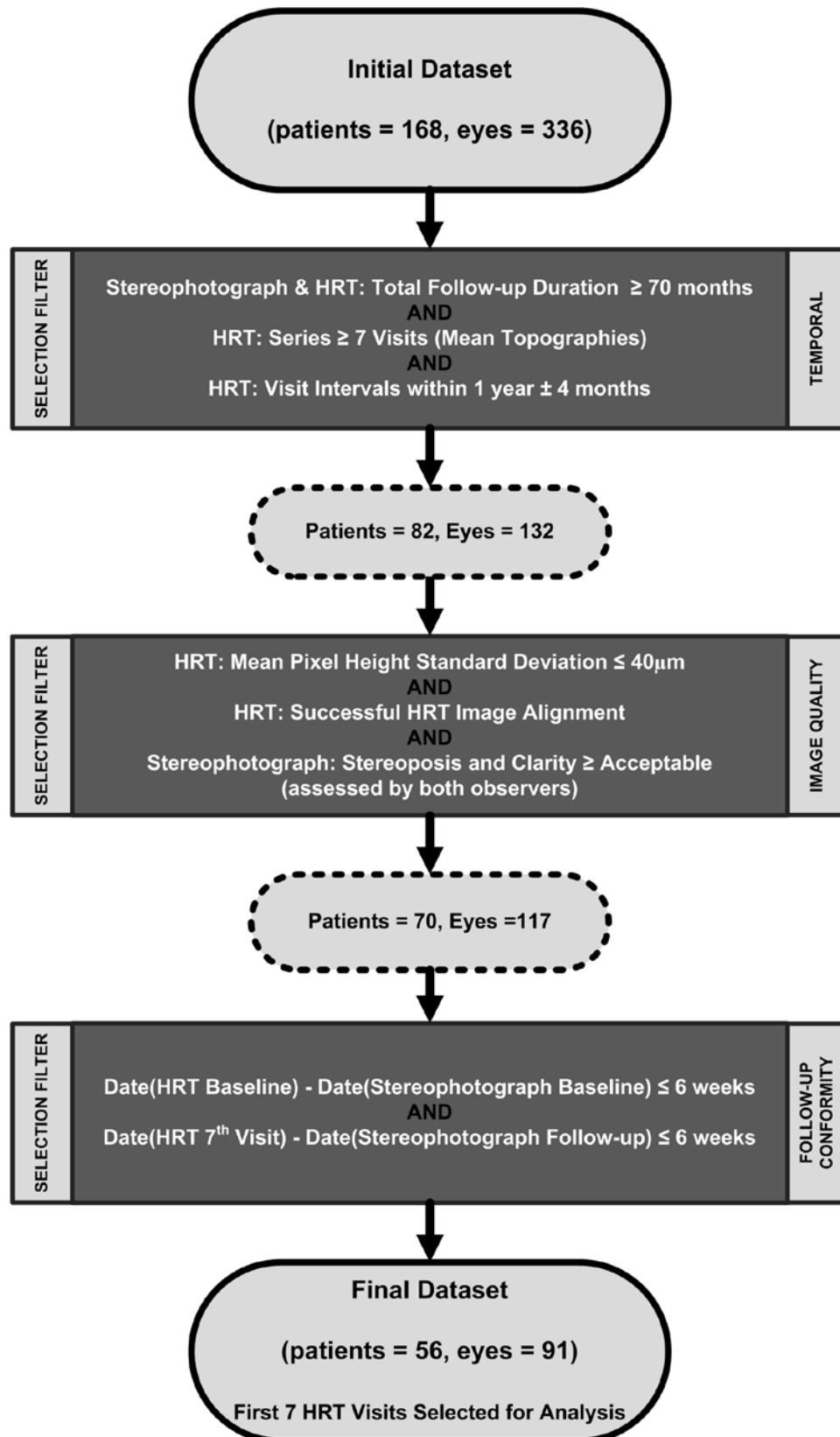
Figure 2.1 Selection criteria applied to stereophotograph and HRT progression study and the resulting sample sizes.

2.2.2 Selection Criteria for Study

Data from an initial dataset of both eyes of 168 patients (336 eyes) with follow-up of at least 4 years (median=6.1 years) were evaluated. Figure 2.1 illustrates the selection criteria from this initial dataset with the numbers of patients and eyes in the study as selection criteria are applied.

2.2.3 Stereo Optic Disc Photography and Grading

Photographs were obtained annually for all of the patients using a simultaneous stereoscopic camera (3-Dx; Nidek Co., Ltd., Gamagori, Japan) after maximum pupil dilation. For each eye, the photographs obtained at baseline and at the most recent follow-up visit were randomly assigned to be labelled as A or B, to mask the temporal order. All other information about the eye and the patient was masked from the graders, including the appearance of the fellow eye. Two fellowship-trained glaucoma specialists independently viewed the baseline and final follow-up photographs sequentially using a Stereo Viewer II (Asahi-Pentax, Tokyo, Japan) and graded them as "changing" or "stable", indicating which photograph showed worse damage (A or B). If there was change, the type of change was recorded, as one or more of the following: increased neuroretinal rim narrowing, increased excavation, new or increased retinal nerve fibre layer defect or new notching. The location of change was recorded in 90 degree sectors (0˚-90˚, 90˚-180˚, 180˚-270˚ and 270˚-360˚). Quality assessments of each image pair were recorded separately for clarity and for stereopsis as 'excellent, 'adequate' or 'unacceptable'.

The reviewers mediated disagreements by re-examining the photographs together to reach consensus; any continuing disagreements between these two graders were adjudicated by a third masked expert. Change identified in the correct temporal

direction (i.e. the follow-up photograph graded as worse) was labelled 'true' (glaucomatous change). Change identified in the 'wrong' temporal direction (i.e. the baseline photograph graded as worse) was labelled as 'false progression'.

1) *Sample specificity*, 2) *sample sensitivity*, and 3) the *reproducibility* of the assessment method are estimated by presenting the graders with three additional sets of photographs:

1) A second set of stereophotographs obtained on the same day of a subset of 10 cases from the larger study cohort were presented again. These 10 cases were randomly assigned a unique identification number and were inserted into the study set. Sample specificity (i.e. the rate of correctly identifying no change) is defined as the proportion of these 10 eyes that the graders determined to have remained stable.

2) Two glaucoma experts selected 10 examples of 'definite' glaucomatous change from their private practices that are separate from the study cohort. Temporal order was masked using the same A and B labelling scheme, and the photographs were randomly inserted into the study set. Sample sensitivity (i.e. the detection rate of true glaucomatous change) is defined as the proportion of these cases that the graders identified as progressing in the correct temporal order.

3) Reproducibility is determined by duplicating the photograph pair for 10 eyes and reassigning each pair with a second unique identification number.

The graders were unaware that these 30 cases were not part of the study cohort.

2.2.4 Confocal Scanning Laser Tomography

The CSLT images were obtained using the HRT Classic. Several single topographies per examination were acquired and the three best-quality images (by subjective assessment) were combined to create a mean topography for each eye. An experienced operator outlined the optic disc margin. Images were analysed using the latest available software (version 1.6.1.0) but are not imported to HRT III software. The manual land-marking facility was used to correct obvious failures of the automatic alignment algorithm to adequately register images across time.

2.2.5 Statistic Image Mapping

SIM (as discussed in Chapter 1) is applied to topographic image series in this study. Its full methodological details can be found elsewhere (Patterson et al., 2005). Unlike the TCA, SIM is not integrated into the HRT software as a tool for progression analysis. The SIM algorithm was reproduced entirely in MATLAB (R2009a, The MathWorks Inc, Natick, Massachusetts) utilising an image-wise linear regression for each re-ordered image series which was programmed to take advantage of the platform's more efficient matrix operations. A map of significant change is produced at each final follow-up examination along with a significance value for the largest connected cluster of significantly negatively changing pixels. For an image series of length 10 examinations processing time is approximately 6 minutes on a 2.66 GHz dual core processor comparable to the reported 3 minutes of the original algorithm coded in the C computing language (Patterson et al., 2005).

2.2.6 Topographic Change Analysis

TCA was performed on the data as per the description in Chapter 1. This is implemented as follow: using the super-pixel change probability maps and mean difference from baseline maps, exported from the HRT software, a significant change map is constructed for each follow-up examination. This is done by identifying super-pixel locations with significant decrease in the retinal height from the baseline examination (i.e., the locations with negative height change in the mean difference image and change probability < 0.05). Change across time is confirmed by comparing the most recent follow-up examination findings with those of the previous 2 examinations producing a final TCA map.

2.2.7 Linear Regression of Rim Area

As detailed in Chapter 1 of this thesis RA has been shown to give information which is clinically meaningful and also more reproducible than the other stereometric parameters from the HRT. Consequently is likely to be a better candidate among these for detecting and tracking glaucomatous structural changes. RA analysis for this study is trend-based. Global RA and RA for the six pre-defined sectors are analysed across time using OLS linear regression and p-values obtained for the null-hypothesis that the trend of the linear fit is $<0\text{mm}^2$ per annum. The fixed $320\mu\text{m}$ reference plane is used for all RA calculations (Burk et al., 2000) because it has been shown to improve the repeatability of RA measurements (Strouthidis et al., 2005a, Breusegem et al.).

2.2.8 Analysis: Measures of Change and Sliding Scale

For SIM, the measure of change is the probability value of the largest cluster of 'active' (red) pixels. In the TCA, the measure of change is the area of largest cluster of red super-pixels as a percentage of disc area. In the case of RA, the measure of change is the lowest p-value (most significant) obtained by LR of the 7 (global and 6 sector) RA linear trends. Using expert-assessed stereophotographs as the reference standard, the aim is to vary the criteria for change for each method of HRT change analysis and compare proportions identified as changing. Thus, receiver operating characteristic (ROC) curves are generated to measure the diagnostic precision of each HRT change-analysis method in predicting glaucomatous optic disc changes assessed on stereophotographs. ROC curves are discussed further in Chapter 3. Agreement between HRT methods and stereophotograph change, at equal rates of glaucomatous progression classification, is examined and illustrated using area proportional Venn diagrams. This entailed fixing discriminant criteria to classify the same number of eyes as changing in HRT analyses as in the stereophotograph assessment. Analysis was carried out in MATLAB (R2009a, The MathWorks Inc, Natick, Massachusetts)

2.4 Results

Ninety-one eyes of 56 patients from the original 336 eyes of 168 patients met the chronological and quality criteria (Figure 2.1). The proportion of glaucomatous and OHT patients was 87.5% and 12.5% respectively. Measurements from 7 annual, mean HRT scans (composed of 3 single HRT scans) for each eye in this study are used for analysis. Mean patient age at baseline was 56 years (range 35-82 years) and the male-female ratio was 52:48. The racial mix was as follows: 54 (96%) white; 1 Hispanic and 1 American Indian.

In the patient dataset, 33 eyes (36%) were assessed as exhibiting glaucomatous change using the stereophotograph reference standard. In 47 out of 91 instances (52%) the assessment required adjudication by the third grader (Table 2.1).

The mean interval between baseline stereophotograph and baseline HRT scan acquisition was 8 days and the mean interval between follow-up stereophotograph and final follow-up HRT scan acquisition was 11 days.

Of the same-day stereophotograph set, 2 of 10 eyes were judged to be changing by graders, giving sample specificity of 80% (95% CI, 44%-98%). Of the definite glaucomatous change set, 8 of 10 eyes were judged to be changing. Of the repeated set, 2 of 10 stereophotograph pairs resulted in different assessments on repeated presentation. Thus sample sensitivity and reproducibility are both estimated to be 80% (95% CI, 44-98%).

Figure 2.2 shows the ROC curves for TCA, SIM and RALR. Areas under the ROC curves (95% CIs) are as follows: 0.61 (0.56 - 0.66) for TCA; 0.62 (0.57 – 0.67) for SIM; and 0.66 (0.61 – 0.71) for RALR. Using the method of Hanley and McNeil (Hanley

and McNeil, 1983b) to compare areas under two ROC curves resulted in p=0.79, 0.26 and 0.24 for pair-wise comparisons of TCA/SIM, TCA/RALR and SIM/RALR respectively. At a fixed specificity of 90% for all three methods, sensitivities are 25% for TCA, 27% for SIM and 40% for RALR, respectively.

Table 2.1: The summary of stereophotograph assessment of progression in the study with the proportion of assessments reached by consensus and the proportion requiring adjudication.

		Progression		
		Yes	No	Total
Mode of Decision	**Consensus**	11 (12%)	33 (36%)	44 (48%)
	Adjudication	22 (24%)	25 (27%)	47 (52%)
	Total	33 (36%)	58 (64%)	91 (100%)
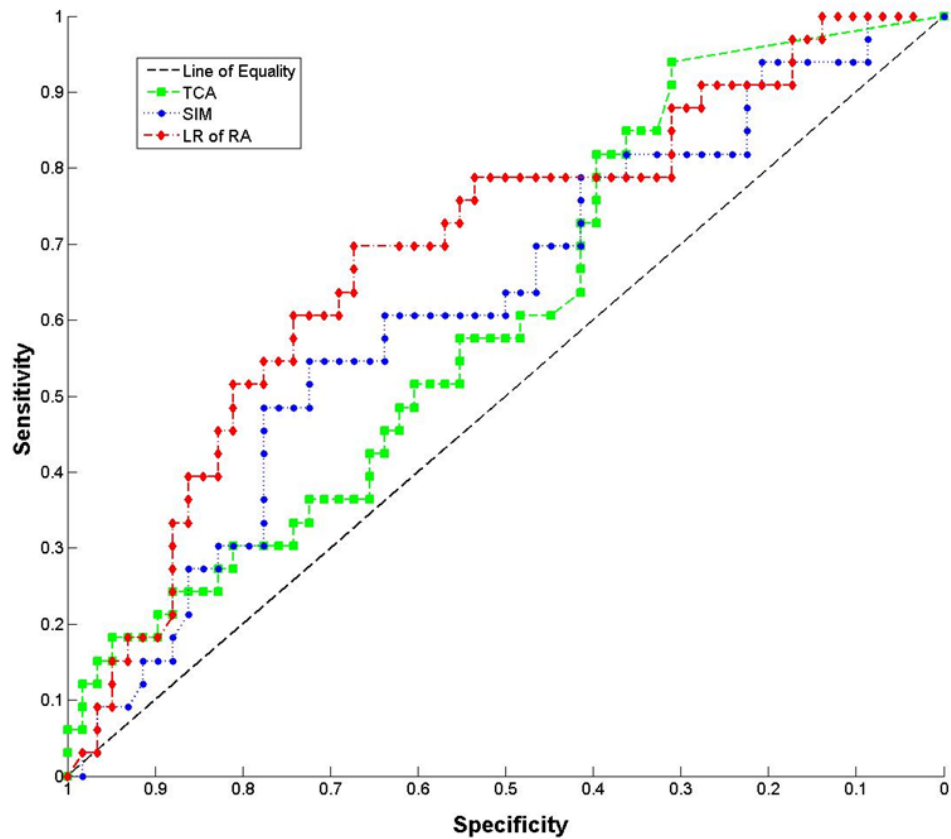
Figure 2.2 ROC curves for HRT progression algorithms using stereophotograph-assessed glaucomatous change as the reference standard for TCA, SIM and RALR. Areas under the ROC curves are 0.62 for SIM, 0.61 for TCA and 0.66 for RALR.

Figure 2.3 shows the agreement of TCA, SIM and RALR identified change with stereophotograph change, after rates of identified progression are matched to those of the stereophotograph assessment (36%). Concordances are 0.54 for TCA, 0.65 for SIM and 0.67 for RALR and associated Cohen's κ values are 0.05, 0.23 and 0.30 respectively. Figure 2.4 shows the agreement among the HRT change-detection methods at equal rates of identified change (36%). This reveals concordances among the HRT change-detection methods to be 60% and pair-wise concordances of between 71% - 76%.
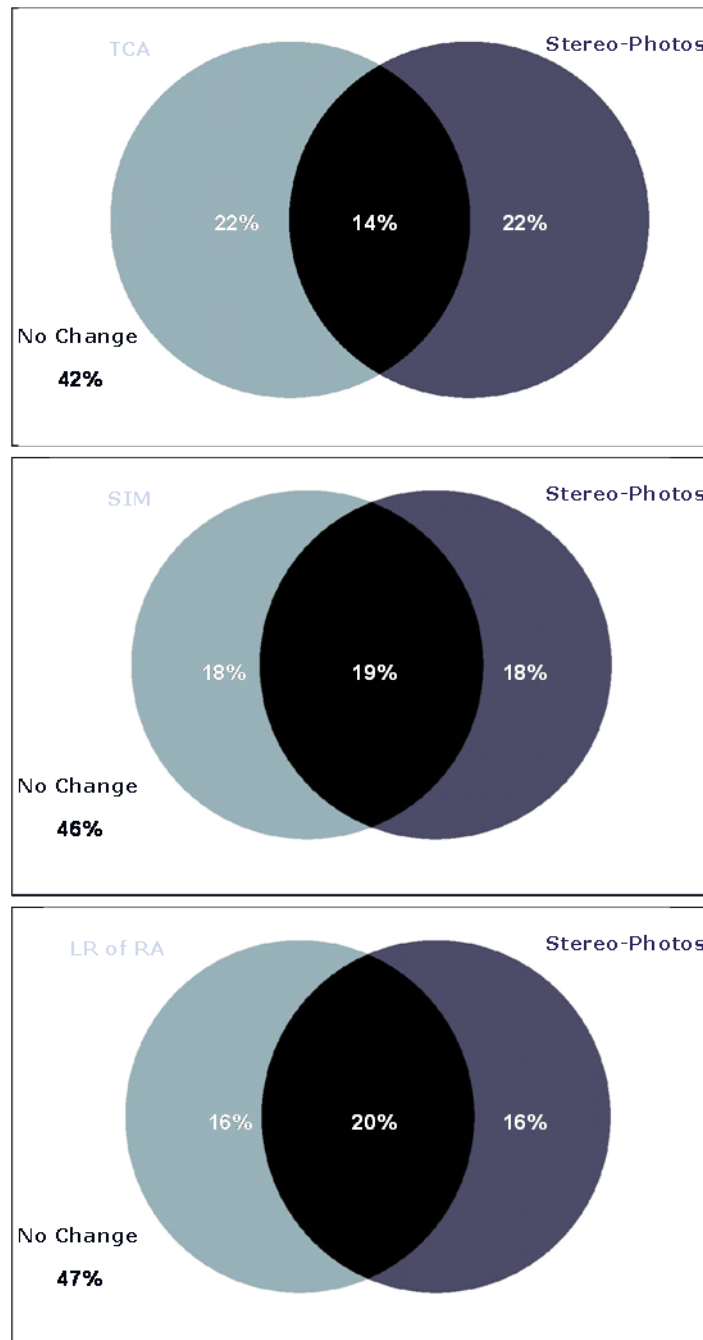
Figure 2.3 Area proportional Venn diagrams representing the agreement of TCA, SIM and RALR with stereophotograph assessment. Equal rates of identified progression mean that the circles in each diagram are equal in area.
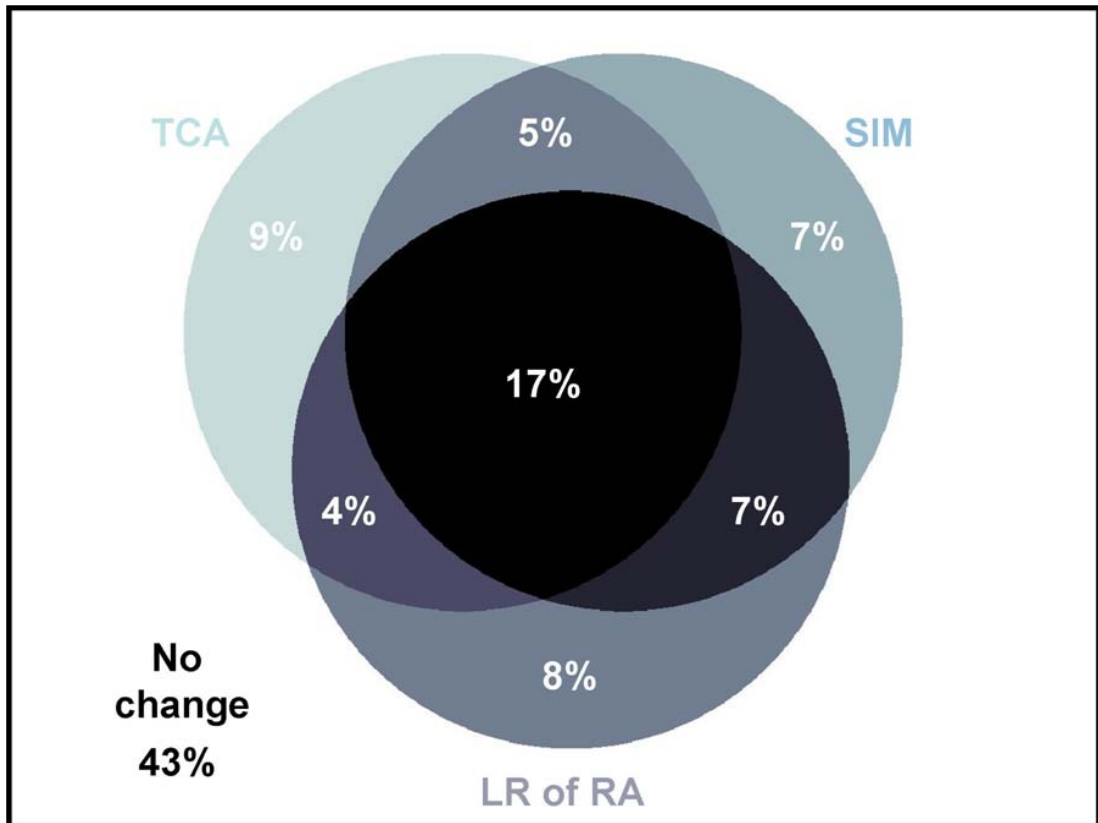
Figure 2.4 Area proportional Venn diagrams representing the agreement of TCA, SIM and ordinary RALR with each other in determining glaucomatous progression. Equal rates of identified progression mean that the circles are equal in area.

Figures 2.5-2.8 show 4 cases to illustrate different levels of agreement between HRT analyses and stereophotograph assessment when criteria for progression are fixed for equal classification rates. The stereophotograph decisions were reached by consensus in case 1 and case 4 but required adjudication in cases 2 and 3.
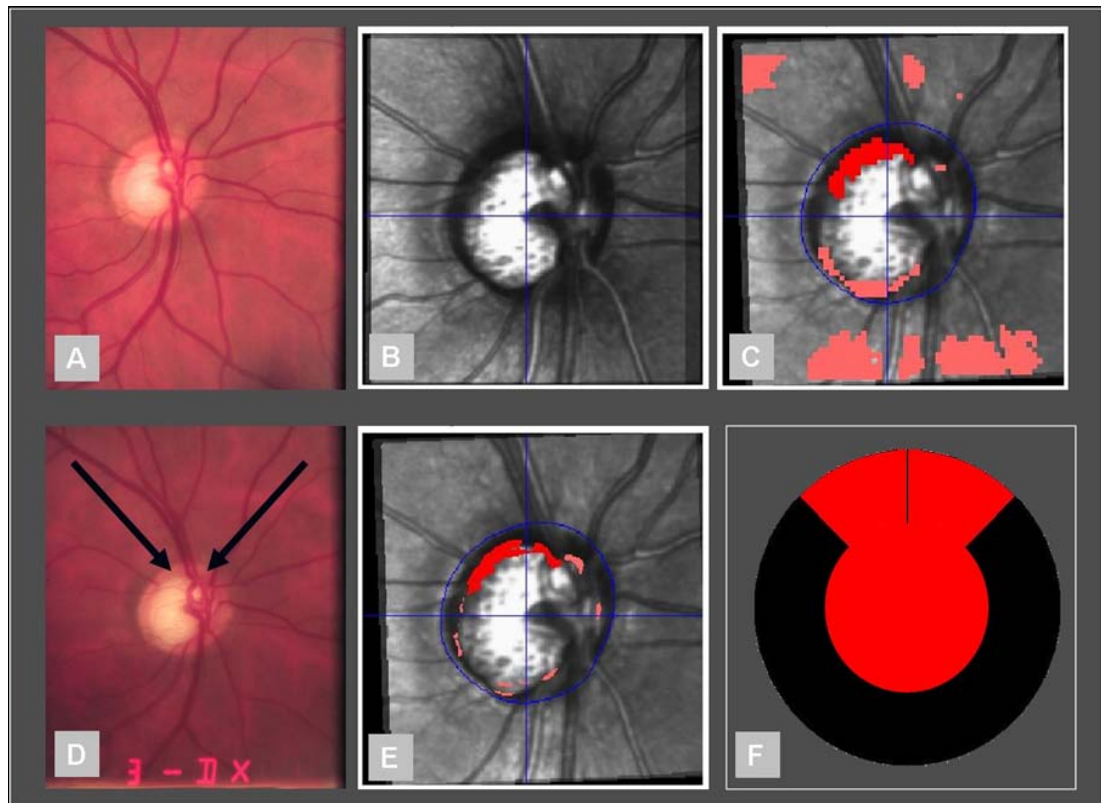
Figure 2.5 Case 1. A Single baseline (April 1998) and D single final follow-up (April 2005) photographs from stereophotograph pairs, with excavation and rim-narrowing indicated supero-temporally and supero-nasally (arrows). B Baseline HRT mean image (April 1998). Final follow-up HRT mean image (April 2005) with C TCA (progression flagged) and E SIM (progression flagged) outputs (the dark red pixels represent the largest cluster of pixels within disc). F Output for RALR (red sectors represent significant p-values for negative trend of RA).
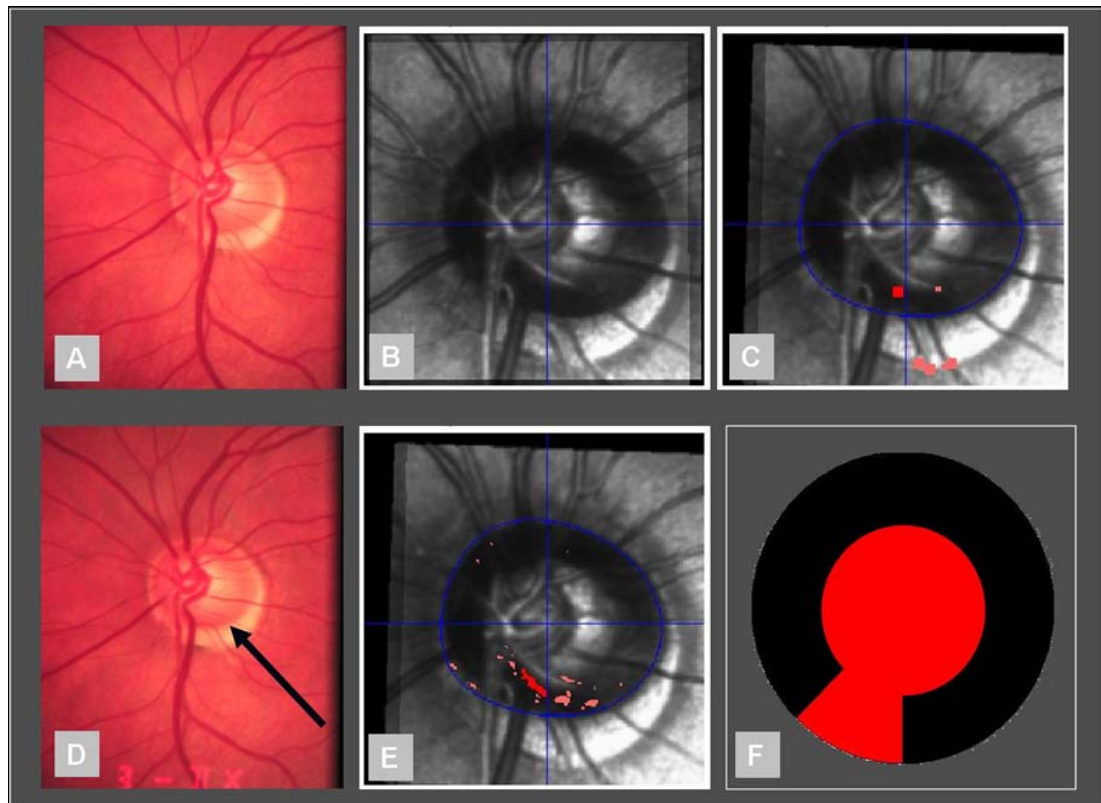
Figure 2.6 Case 2. A Single baseline (August 1998) and D single final follow-up (August 2005) photographs from stereophotograph pairs with excavation indicated infero-temporally (arrow). B Baseline HRT mean image (August 1998). Final follow-up HRT mean image (August 2005) with TCA C (no progression flagged) and SIM E (no progression flagged) outputs (dark red pixels represent largest cluster of pixels within disc). F Output for RALR at (red sector and centre represent significant p-value for negative trend of RA).
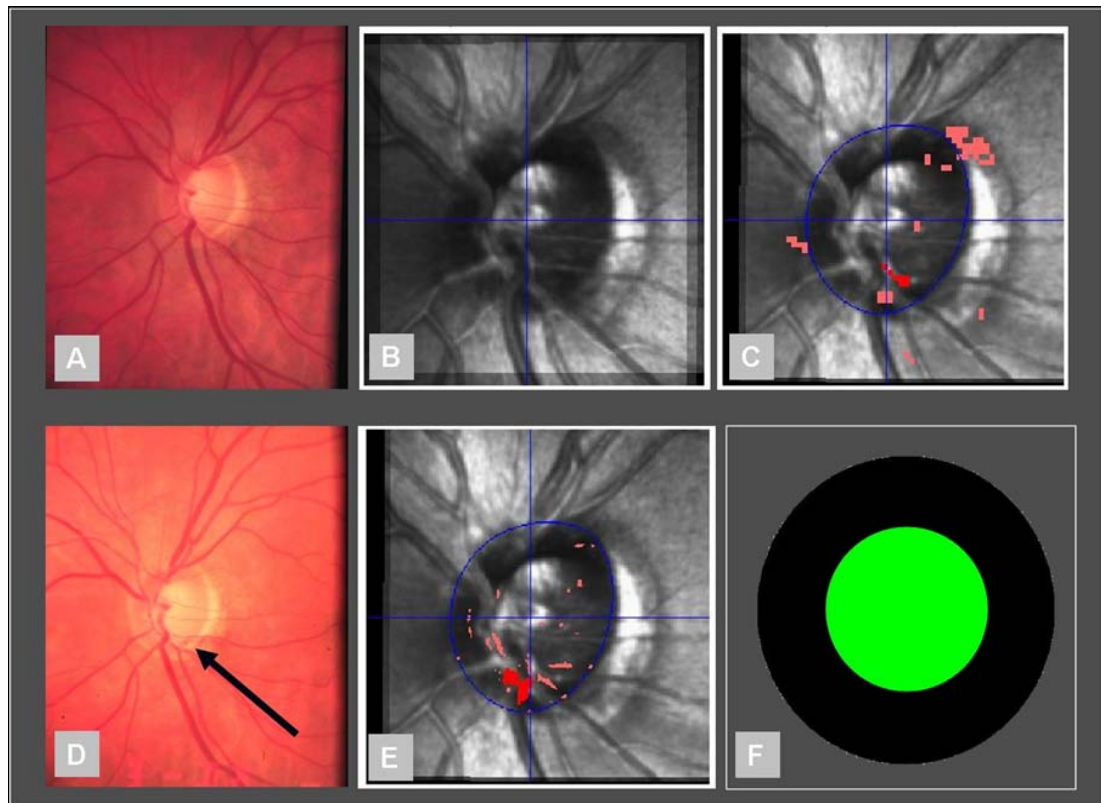
Figure 2.7 Case 3. A Single baseline (October 1998) and D single final follow-up (August 2005) photographs from stereophotograph pairs with excavation indicated infero-temporally (arrow). B Baseline HRT mean image (October 1998). Final follow-up HRT mean image (August 2005) with TCA C (no progression flagged) and SIM E (no progression flagged) outputs (dark red pixels represent largest cluster of pixels within disc). F Output for RALR (green centre represents no significant p-values for negative trend of RA).
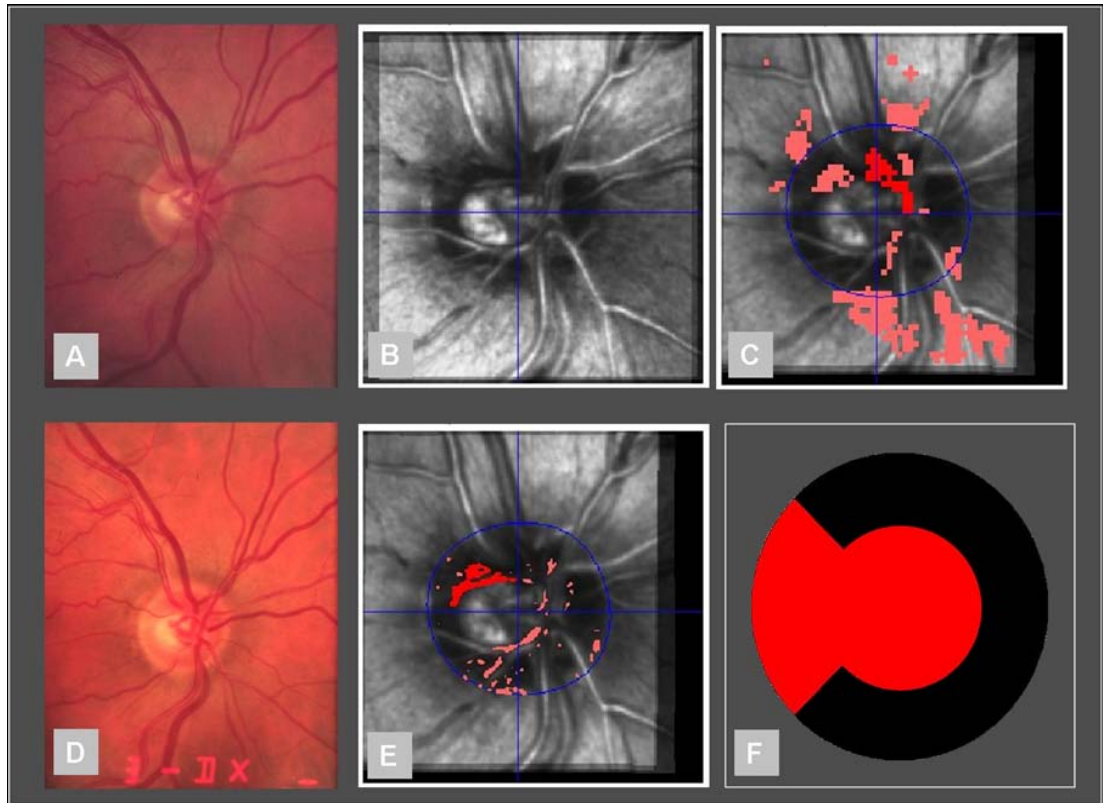
Figure 2.8 Case 4. A Single baseline (July 1998) and D single final follow-up (July 2005) photographs from stereophotograph pairs with no observed change. B Baseline HRT mean image (July 1998). Final follow-up HRT mean image (July 2005) with TCA C (progression flagged) and SIM E (progression flagged) outputs (dark red pixels represent largest cluster of pixels within disc). F Output for RALR (red sector and centre represent significant p-value for negative trend of RA).

2.5 Discussion

The CLST, as typified by the HRT, has been shown to give a repeatable measure of optic disc structure (Dreher et al., 1991, Cioffi et al., 1993, Chauhan et al., 1994). The HRT does reasonably well in distinguishing glaucomatous eyes from healthy eyes (Mikelberg et al., 1995, Asawaphureekorn et al., 1996, Bathija et al., 1998, Wollstein et al., 1998, Swindale et al., 2000, Zangwill et al., 2001, Coops et al., 2006). The real promise of the technology may be in offering a reliable method for tracking structural change, potentially providing useful clinical management information about disease stability. A method for quantifying change is required to realise this potential and there has been much research activity in developing an appropriate technique (Chauhan et al., 2001, Tan and Hitchings, 2003, Patterson et al., 2005, Strouthidis et al., 2006, Fayers et al., 2007) but there is little evidence to suggest that one method is better than another.

Studies using functional progression (visual field deterioration) (Girkin, 2004b, Sample et al., 2002, Artes and Chauhan, 2005, Strouthidis et al., 2006) are confounded by aspects of the relationship between structural and functional changes. The relative proportions of associated and independent behaviour are not well-known and the temporal sequence of structural and functional glaucomatous change is not well-defined. Because change identified by structure and function do not seem closely related, it may be more likely that progression identified by glaucoma experts from optic disc stereophotographs would provide a better reference standard against which to assess the performance of another structural measurement for progression (CSLT images).

This study is one of few examining agreement of HRT change analyses with expert assessed stereophotographs (Chauhan et al., 2001, Ervin et al., 2002, Chauhan et al., 2009, Kourkoutas et al., 2007). A variety of statistical methods are considered for detecting change in HRT images and the stringency of the criteria for HRT change was varied to give a measure of sample sensitivity across the full range of sample specificity. This study took advantage of data from a carefully collected prospective longitudinal study across a relatively long period, and strict image quality criteria were applied. Previous research (Morgan et al., 2005) has shown better between-grader agreement and better reproducibility from stereophotographs than from monoscopic photographs when discriminating between glaucomatous and healthy discs. The estimate of reproducibility for expert assessed progression in stereophotographs in this study, at 80%, is comparable with that of previous studies that have obtained κ values of 0.62-0.89 (Parrish et al., 2005) and 0.80-1.00 (The European Glaucoma Prevention Study Group, 2003) for within-observer reproducibility.

The ROC analysis suggests that, when using stereophotograph assessment as the reference standard, automated HRT methods have only moderate precision to predict change. The ROC curves revealed poor sample sensitivities for clinically relevant regions of high sample specificity. At equal rates of classification, poor agreement is found between the expert-assessment of stereophotographs and the HRT analyses. Both stereophotographs and HRT images are assumed to give an accurate and repeatable measure of the structure of the optic disc but the false-positive and false-negative rates of both the reference standard and the HRT methods may largely explain this poor agreement. This is illustrated in cases 1-4 (Figures 2.5-2.8). The false positive rate of photograph grading from this study (estimated to be 20%) is a major factor. This rate falls within the range of previous

studies (Ervin et al., 2002, Chauhan et al., 2009) (ranges of 0% - 50% and 5% - 77% respectively depending on stringency of progression criteria and observers). Furthermore, grader agreement, even between 'experts', in other tasks, such as separating healthy and unhealthy optic discs, (Reus et al., 2007) or assessing progression events in series of optic disc stereophotographs (Jampel et al., 2009, Chauhan et al., 2009) and visual fields (Viswanathan et al., 2003) is not good. Owing to the nature of this dataset (generally early glaucoma) there is likely to be a wide range in the magnitude of changes and agreement may be much worse when changes are of small magnitude, partly evidenced by the 52% of stereophotograph assessments that required adjudication by a third expert.

Further reasons for the difference may be that features sometimes implicitly attributed to glaucomatous change in stereophotographs (e.g. colour changes) may not be apparent in HRT images which are simply estimates of the topographic height of the optic disc surface and surrounding areas. Other contributing factors are that certain optic disc configurations (such as hypoplastic and tilted discs) may present greater difficulty for either HRT analysis (image registration, contour line fitting and RA calculation) or stereophotograph assessment.

Concordance among all three statistical methods for detecting HRT change is 60% and pair-wise concordance are 71% to 76%. It is not surprising that agreement among HRT analyses is better than is agreement between stereophotograph assessment and HRT analyses. Differing statistical methods such as those used in this study will never have perfect concordance even on the same data. However the level of disagreement between HRT analysis methods is probably amplified by the low stringency of the change criteria (fixed to identify equivalent 36% - proportions), and the related (likely) high false-positive rate. These criteria are less

stringent than those used in previous studies (Chauhan et al., 2001, Patterson et al., 2005).

The results of this study expose the limitation of using grader-assessed stereophotographs alone as a reference standard for structural glaucomatous progression. Future studies to assess the effectiveness of HRT change analysis methods may require a more innovative approach to establishing a reference standard. Using an accumulation of information from a variety of measurements (visual field, IOP and optic disc) and presenting this as a continuous scale for evidence of change might be useful (Artes and Chauhan, 2005). Another approach might be to simulate series of images with known properties, using a virtual platform in which the CSLT image formation process is simulated computationally from a baseline scan. Automated analysis of the reconstructed optic disc from stereophotographs may also be useful (Xu et al., 2008). Imaging devices, such as spectral domain OCT, which may give surrogate measurements closer to what is really required to detect real glaucomatous change, may also help refine reference standards for progression.

Though this is outside the scope of this study, it is worth remembering that progression detection is not necessarily a binary classification between changing and not changing. There are a small proportion of patients who are likely to suffer large glaucomatous damage in a short space of time while the typical patient experiences slow changes over a long period of time with a continuum in between. This continuum has been observed in visual field data of untreated patients (Heijl et al., 2009). So it is important to distinguish this complementary approach and address the issue of reporting the best estimates of optic disc changes along with the statistical confidence in these.

There are other limitations to this study. Varying criteria for progression in stereophotograph assessment would have been useful in determining various diagnostic strengths of the HRT analyses (Chauhan et al., 2009) but may also have led to even less agreement between graders. The setting of a cut-off criterion to determine progression is not trivial: because the true specificity of a criterion cannot be known, setting the cut-off value to result in equal rates of progression provides an opportunity to compare the agreement between the HRT change detection methods when the 'hit-rate' is the same. Times to progression were not examined, in part due to stereophotographs being assessed only at baseline and final follow-up. Therefore there are no estimates to report as to which HRT analyses detected change earliest, although one could question the value of such analyses when agreement between methods is poor. The number of eyes in the definite glaucomatous change stereophotograph set and the same-day (no glaucomatous change) set are low, resulting in large CIs for the estimates of sample specificity and sensitivity for the expert-assessed change. However, as already discussed, these estimates are similar to those of previous studies. Because only 'depressed' change is examined in HRT topographies, glaucomatous change resulting in elevation of the optic disc surface (if it occurs), would have been overlooked. However, given the nature of glaucoma, this approach seems reasonable given that an elevation may be more indicative of measurement noise or morphological change independent of glaucoma than a depression and may have resulted in a higher rate of falsely detected glaucomatous change. Moreover, the ability to accurately detect changes, or lack of changes, in HRT longitudinal image series will depend enormously on the ability of the software to register the images appropriately and thus these results are limited by the constraint of the HRT alignment algorithm. Only HRT Classic (10° x 10°) images were available in the study which although wide enough to contain the optic disc in

all cases, have less available features for registration than HRT II (15° x 15°) resulting in poorer alignment in the latest HRT software (Bergin et al., 2008). Also HRT Classic topography series unlike HRT II series, are not corrected for parabolic error between mean topographies caused by axial movement of the eye between exams (Balasubramanian et al., 2011), potentially leading to further topographic variability.

A separate analysis of glaucomatous and OHT patients may have revealed differences in the rates of change in stereophotograph assessments and HRT algorithms but the number of OHT patients was so low as to limit the reliability and utility of any inferences from this comparison. Finally the resulting proportion eyes progressing by stereophotograph assessment should be used with caution when extrapolating to groups of non-white patients and groups with more established or later stage glaucoma.

In conclusion, this study revealed poor agreement between progression detection using a variety of HRT statistical methods and expert-assessed stereophotographs of the optic disc. Using stereophotograph-assessed change as the reference standard does not help determine which HRT change algorithm best identifies glaucomatous change in this group of patients with high risk OHT and those with early glaucoma. This does not imply that stereophotographs are not integral to the assessment of glaucomatous change. Indeed they are a clinically well-accepted standard that have been used in major clinical trials. However, the diagnostic precision associated with observer stereophotograph-assessed change precludes it from being a stand-alone benchmark by which to evaluate alternative change detection tools. The practical benefits of being able to observe change using automated or semi-automated digital image analysis, and other recent evidence, (Chauhan et al., 2009) suggest that it is an

important tool for assessing disease progression, especially if a statistical method for

best detecting the change can be established. The extent to which these algorithms

measure real ONH changes across time in HRT image series and their related

stereometric parameters will need to be established in other ways and this will be

addressed in the following chapters.

3. Trend Detection in Series of Univariate Measurements

It is well established that POAG is a slow progressive disease which requires continuous management by the clinician. This means that, once diagnosed, POAG patients will need clinical follow-up examinations for the rest of their lives. The advantage of an approach for measuring change as a *trend* will be that it can incorporate information from the often long series of follow-up measurements more readily than an event analysis and give a clinician a better insight on the success (or failure) of certain treatments with a particular patient. The estimation of trends in metrics such as global RA and mean RNFL thickness has an important role as a summary measure by which to quantify optic disc changes measured by imaging. RA and RNFL are surrogate measures of axonal health and trends in these are relevant in that a general decrease over time can serve as a measure of glaucomatous axonal loss. They also lend themselves readily to clinical interpretation: change in an area can be easier to appreciate visually and interpret than a change in a topographic height. When attempting to detect true glaucomatous structural change it is necessary to firstly identify statistically significant changes before determining if the change represents an amount of loss to warrant a change in treatment or further or more thorough examinations. A statistical model which describes just one variable is known as a *univariate model* and it is the purpose of this chapter to examine univariate models for detecting trends in an individual stereometric parameter (in this case RA) with equal application to detecting change in other stereometric parameters and much more localised measures such as in the individual pixels or super-pixels of topographic images.

Statistical models of univariate trends in glaucoma have mostly taken the simplest of forms. Linear models of change with simple (but strong) assumptions about the measurement noise are frequently used in modelling longitudinal series of both localised and global visual field (Hitchings et al., 1994, Fitzke et al., 1996, Viswanathan et al., 1997, Gardiner and Crabb, 2002a, Heijl et al., 2009) and structural measures (Strouthidis et al., 2006, See et al., 2009). The linear model requires fewer parameters than more complicated models and crucially provides an easy to understand average linear *trend* across time at any point in time which is equivalent to the average change per unit time over the whole follow-up. Because of the strong assumptions it makes, it is known as a *parametric* model. Another model of change, by ranking measurements, has been used before to establish the evidence of change in sectoral RA measurements (Artes and Chauhan, 2005). This type of easily understood model makes very few assumptions of the data – and is known as a *non-parametric* model. As a result, this type of method can be more appropriately applied to a wider range of data series where change may not follow a linear trend and it is potentially more robust to outliers. This rank-based approach does not however provide a measure of change but merely indicate if there is evidence for a trend occurring over time. Other approaches such as those assuming a linear model but drawing statistical inferences by non-parametric means can be classified as *semi-parametric* methods such as that used in SIM pixel-wise analysis for detecting significantly changing topographic locations over time (Patterson et al., 2005). Along with the strength of the underlying trend, the measurement variability of any data, will govern how well this trend can be detected by any statistical technique.

The variability of ONH imaging data is frequently reported but rarely characterised beyond the most summary of descriptors such as the standard deviation or coefficient of variation. These measures are used to helpfully distinguish if one area

of the ONH has greater measurement variability than another (Tan et al., 2003, Strouthidis et al., 2005b) or if one device has greater measurement variability than another (Kim et al., 2009). Though it is useful for these comparisons the standard deviation is sufficient only in characterising variability from a normal distribution. Other characteristics of measurement variability such as the skewedness (the tendency of a distribution to be smeared in one particular direction) or the kurtosis (the tendency of a distribution to have heavier tails) of the measurement variability, and ideally an empirical fitting of different distributions to measurement variability distributions are desirable. There is a notable exception to this in analysis of HRT RA data (Owen et al., 2006). This analysis by Owen and colleagues has demonstrated that both the test-retest and longitudinal measurement variability of RA are non-normally distributed.

Furthermore, in many fields, the dependence of the variability of a measurement on the underlying value of the measurement is not always uniform. This phenomenon is termed *heteroscedasticity* and the case where variability remains uniform is called *homoscedasticity*. This is especially relevant for modelling change as by definition the measurement will change over time. It is well known that the variability of visual field sensitivity is associated with loss of sensitivity (Henson et al., 1999, Spry et al., 2001). No theory that test-retest or longitudinal RA variability change with RA itself has been established. There is evidence both for (Tan et al., 2003, Leung et al., 2008, Lin et al., 2009) and against (Strouthidis et al., 2005b, Jampel et al., 2006) stage of disease being related to same-eye variability of HRT topographic and morphological measures though it is likely that these latter studies did not have a sufficient range of RA to show any relationship. For those studies reporting changes in RA variability with disease stage, an increase has been consistently shown for more

advanced disease stage. Thus it is worthwhile investigating the potential effects of changes in RA variability over time.

Another potentially difficult factor for any statistical change detection technique to address is that measurements in a series (or the measurement errors) may have some relationship to each other. This relationship of serial measurements with each other is known as *autocorrelation*. This can be periodic as in the case of the circadian changes in IOP (Hughes et al., 2003) and is often seen on a longer timescale in the form of seasonal effects (Giuffre et al., 1995, Wu and Leske, 1997). No studies have systematically investigated any periodic changes in ONH data from the HRT (or any other devices) beyond the short time-scale of the cardiac cycle (Chauhan and McCormick, 1995). A single case study has shown an large variation in RA of a patient with a large circadian IOP amplitude (Lee et al., 1999) and another study has shown that induced acute IOP increases result in increased optic disc diameters (Poostchi et al., 2010) which may affect RA .

One way to investigate the false positive detection rate from statistical methods is to use real data in which any detection of statistically significant changes or trends will be due to measurement noise alone. A 'stable' dataset in which it is highly unlikely for any measured change to be associated with glaucomatous pathology; in particular, a series of many repeated measurements acquired over a short period of time can be useful for this. This circumvents the need for an external gold standard of stability in serial ONH measurements. The use of a Monte Carlo simulation (Metropolis and Ulam, 1949) has, with the advent of increased computing power, offered applied statisticians an accurate tool to compare and contrast statistical methods where analytical solutions are not easily available. This approach is taken to assess the ability of statistical algorithms to detect underlying trends buried in

noisy data without confounding effects from an uncertain reference standard of change. In a simulation setting, measurement variability can be designed to be representative of that of real data with the further advantage that it is also possible to simulate series where an underlying trend is present and known in advance. Given that we know so little about the trends governing RA change over time and the measurement noise characteristics, it seems wise to experiment with a range of 'scenarios' in which different combinations of measurement variability and trends are used to assess the performance of change detection techniques of interest.

The aim of this chapter is to assess the performances of different parametric, semi-parametric and non-parametric univariate statistical techniques for detecting trends using both short-term 'stable' series and simulated series. Simulation allowed the design of multiple 'scenarios' of series with different measurement variability and magnitudes of underlying change (known by design) which approximate to real data.

3.1 Trend Detection Techniques

In assessing a series of univariate measures over time, the clinician, clinical scientist or computer algorithm is presented with a series of n measurements (in the case of this analysis, RA measurements) $y_i = (y_1, y_2 \ldots, y_n)$ at corresponding time points $t_i = (t_1, t_2 \ldots, t_n)$. To detect an underlying trend in this data involves devising a model of change over time, fitting this model to the data and testing that the fitted model has a significant trend with a hypothesis test.

One of the simplest approaches to this might be to assume a linear trend model along with other assumptions (below) and to perform an OLS linear regression. In general terms, OLS regression is an approach to quantifying the relationship between a scalar variable y to one or more predictor variables denoted X by optimally fitting the observed variables to an assumed model giving "best-fit" parameters for this model. In OLS linear regression, a linear model and its estimated parameters are used to estimate this relationship. In the case of trend analysis the OLS linear regression approach would be to model time as a predictor variable and its linear relationship with whatever measurements are being observed over time.

The principal assumptions of OLS are that there are no errors in the predictor variable (time) with the following additional assumptions (Salkind and Rasmussen, 2007):

1. Measurement errors about each point are sampled from a normal distribution with a mean of zero (*normally distributed*)
2. Distributions from which measurement errors are sampled do not change across the predictor variable (*identically distributed/homoscedastic*)

3. Successive measurement errors are independently sampled (*independent/non-autocorrelated*)

These assumptions are strong and it is likely that scenarios exist in serial measurement of clinical data such as RA measurements where these are violated - these have been discussed in the introduction to this chapter. Under assumption of the normality of the error terms, the estimator of the slope coefficient will itself be normally distributed (Salkind and Rasmussen, 2007). As a result, the hypothesis-test regarding the significance of the trend measured by OLS linear regression (after an appropriate adjustment for the measurement noise) can be inferred from a theoretical *student's t-distribution*. For this reason OLS linear regression trend detection is referred to as a parametric method. Other means of modelling trends (see (c) below) and other means of performing hypothesis tests in a non-parametric fashion are readily implemented and easily understood for trend detection and will be used in the analysis of this chapter along with OLS linear regression.

A *permutation test* is a hypothesis test which infers the significance of a test-statistic from distribution of test-statistics under reordering of measurements. Evolving from the work of Pitman in the 1930s (Pitman, 1937), these tests have come to form a subset of non-parametric statistical methods best used when it is unknown how this a test-statistic is distributed (Good, 2005). For a trend analysis, it involves permuting or reordering the time labels in a series of observed measurements, calculating a test-statistic such as a linear regression slope or Spearman's ρ and replicating this to cover all possible unique reorderings. The null hypothesis is simple: "there is no underlying trend in the observed series" and if so then the trend test-statistic in the observed series will not be different to the permutation distribution. The position of the observed test-statistic in the permutation distribution will govern its inferred statistical significance. The *exchangeability* of a sequence ensures the correct

inferences are made from permutation testing (Good, 2005). The advantage of this approach is in removing the assumption about the underlying distribution of the test-statistic by replacing it with the permutation distribution thus making it more robust in datasets which do not adhere to parametric assumptions. For practical purposes, the number of reorderings is limited in this analysis of this chapter at a maximum of 1000.

In this analysis the following particular three trend detection techniques are chosen and classified as (a) fully parametric, (b) semi-parametric and (c) non-parametric techniques:

(a) OLS T-DIST: OLS regression with significance of slope inferred from the position of the parametric test statistic in the student's t-distribution. Linear regression attempts to describe the association between two independent variables and can serve as a form of trend analysis when one of the variables is time. (This is the same technique used in Chapter 2 for RA analysis - labelled RALR.).

(b) OLS P-DIST: An OLS linear regression method (parametric) with the significance of the observed slope inferred non-parametrically from permutation distribution of slopes (OLS SL P-DIST). This method has been used before to detect the significance of observed change in series of images at a pixel level to identify glaucomatous progression (Patterson et al., 2005) and also applied to the area of functional neuro-imaging (Holmes et al., 1996). Appendix A provides a derivation for the monotonic relationship of the slope and the slope/standard error test-statistic distributions within a permutation test used by Patterson and colleagues. This simply means that the standard error of each permuted slope estimate need not be calculated: the slope alone can be permuted to obtain the same rank (position) in a permuted distribution as the slope-standard error ratio. By parametrically fitting the

underlying linear trend by OLS, and as it does not rely in a significance estimate from the Student's t-distribution, this method can be termed semi-parametric.

(c) RHO P-DIST: Spearman's rank correlation coefficient ρ is a non-parametric measure of the association between two variables – essentially quantifying how well the relationship between the two variables can be described by a monotonic function. This is performed by comparing the rankings of each variable in pairs. By letting one of these variables be time, this becomes a measure of trend of the other variable. Spearman's ρ is the test statistic for underlying trend. For a given sample of n paired data $(t_1, y_1), ..., (t_n, y_n)$, let $R_i = \text{rank}(t_i)$ and $S_i = \text{rank}(y_i)$ this is defined by:

$$\rho = \frac{\sum_{i=1}^{n}(R_i - \bar{R})(S_i - \bar{S})}{\sqrt{\sum_{i=1}^{n}(R_i - \bar{R}) \sum_{i=1}^{n}(S_i - \bar{S})}}$$

where $\bar{R} = \bar{S} = \frac{(n+1)}{2}$ is the average rank. If some values of R or S are tied, these values are simply assigned the average of the ranks to which they would have been assigned. A permutation test is used to derive the significance of the test statistic from the observed series. Rank based methods such as this have been applied to detecting underlying trends in areas such as environmental and hydrological sciences and an review of these and other trend detection techniques can be found in Hipel and MacLeod (Hipel and McLeod, 1994).

For all methods, hypothesis tests are one-sided i.e. testing if trends are significantly less than zero. As discussed in Chapter 2, given that an underlying negative trend of RA damage is expected to be indicative of an optic disc with progressive glaucomatous, the investigation of statistically significant positive trends in RA series seems unnecessary.

3.2 Data

Both real and simulated datasets of 'stable' RA series are used to assess the performance of change detection techniques. Without knowledge of the underlying signal in many real datasets, simulation will serve to generate data with known trends of measured RA change over time. Underlying trends are simulated according to estimates in a previous study examining OHT patients developing significant glaucomatous damage (Owen et al., 2006). These trends are assumed to be characteristic of those found in progressing glaucoma patients though differences in the type and stage of glaucoma may well differ.

3.2.1 Real Data

Seventy-four eyes of 74 subjects recruited from the OHT clinic at Moorfields Eye Hospital were included in an HRT test-retest study detailed elsewhere (Strouthidis et al., 2005b). In short, 43 eyes with OHT and 31 with POAG were selected. The subjects had no previous history of intraocular surgery and had all experienced ONH imaging using the HRT. This study adhered to the tenets of the Declaration of Helsinki and had local ethics committee approval and the subjects' informed consent. OHT is defined as IOP greater than 21 mmHg on two or more occasions and a baseline Humphrey 24–2 full threshold AGIS score of 0. POAG is defined as a consistent AGIS visual field score greater than 0, and a pre-treatment IOP greater than 21 mmHg on two or more occasions. Each eye was selected on the basis that it had a refractive error less than 12 dioptres of spherical power and no history of previous intraocular surgery. In subjects with lens opacity, the eye with the greater degree of opacity was preferentially selected although the presence of lens opacity itself was not a criterion for subject selection. HRT Classic images (10° scan width)

and HRT II images (15° scan width) were acquired across two visits within 6 weeks. A total of five HRT Classic mean topographies and five HRT II mean topographies were generated for each eye. Three HRT single topographies were used to generate a mean topography for both HRT Classic and HRT II data. After satisfying the device checks of minimal eye movement, acceptable focus and illumination during acquisition there were no criteria placed on image quality. Thus the ranges of media opacity and image quality are large. The mean age of patients at baseline was 68.2 years (standard deviation 10.2 years) and the male:female ratio was 41:33.

These data are separated into those with HRT Classic data only and those with HRT II data only. This minimises any potential artefact which may be induced by combining these two data types (Balasubramanian et al., 2011, Wang et al., 2011). A contour line was drawn onto each baseline mean topography and automatically exported to the follow-up mean topographies ensuring a constant disc area for all mean topographies in the same series. A manual alignment facility optimised contour line position on an image-by-image basis if automatic alignment was judged to have performed poorly. The contour line was placed at the inner margin of the highly reflective halo at the boundary of the disc in the reflectance image (Strouthidis et al., 2009b). RA measurements were derived using the Moorfields fixed, standard reference plane (Poli et al., 2008, Asaoka et al., 2009).

For this analysis, patient data are treated as pseudo time-series data without any pathology related changes and age-related changes, serving to assess the false-positive rate in change analyses due to measurement noise alone. If the interval is uniform between measurements in a series, the significance of the trend is unaffected by the interval itself given a fixed number of measurements. Thus an

arbitrary 6 month interval is imposed between successive mean topographies in a series - representative of common clinical follow-up.

3.2.2 Simulated Data

One of the most straightforward methods of simulating longitudinal glaucomatous changes in a series of n univariate measurements $y_i = (y_1, y_2 \dots, y_n)$ at corresponding time points $t_i = (t_1, t_2 \dots, t_n)$ involves assuming a linear change of visual field or structural measures over time. This is chosen because it is the most easily specified trend; a univariate model with an underlying linear trend can be described with the following equation

$$y = a + bt + \varepsilon .$$

Simulation of longitudinal global RA series is implemented in practice by propagating underlying series of (changing or stable) RA 'measurements' through time, sampled at equal intervals, and adding noise to each of these measurements. As with the real data, an arbitrary 6 month interval is placed between each follow-up measurement. For progressive RA change in glaucoma, a linear model is used as the simplest (having the least governing parameters). Thus a series of measurements over time can be described by the following equation:

$$x_i = a + b\, t_i + \ \varepsilon_i ,$$

where x_i is the measured RA value at the ith time point, a is the RA value at time t=0 and b is the trend of RA with time. The measurement errors are described by the term $\varepsilon_i \sim D$ sampled from a specified probability distribution. These error distributions and how they change with time are allowed to deviate from the

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assumptions of OLS linear regression and are specified according to sections 3.2.2.2 and 3.2.2.3.

3.2.2.1 Linear Trend and Stable Data

Underlying linear RA trends, derived by OLS, have been reported by Owen and colleagues (Owen et al., 2006) in a group of 44 OHT patients who developed confirmed visual field loss in a follow-up period of between 2 to 7 years. The median, lower and upper quartiles were reported for these patients to represent weak, moderate and strong trends. It is these reported trends which are used for our simulation model before the addition of measurement variability. Thus there were three categories for the underlying trends:

(i) No change data: These stable series are series with no underlying change over time. They have been given a uniform global RA measurement in time given by an average RA of 1.6 mm². They will be used to establish the numbers of false positives flagged by each statistical method. This is represented in the model by setting $b = 0$.

(ii) Moderate trend data: Underlying RA change was added to the stable series. The linear trend is taken to be -0.012 mm²/year. This is the median trend reported in OHT converters by Owen and colleagues (Owen et al., 2006). This is implemented in the model by setting b = -0.012 mm²/year.

(iii) Strong trend data: Underlying RA change is added to the stable series. The linear trend for these series is -0.021 mm² /year: the upper quartile trend reported in OHT converters by Owen and colleagues (Owen et al., 2006). This is implemented in the model by setting b = -0.021 mm²/year.

3.2.2.2 Measurement Noise Distribution

Measurement noise is then added to stable and changing RA signals. In RA measurement, values can be highly reproducible but noise sometimes increases dramatically because of image acquisition artefacts or processing difficulties such as alignment failure between single topographies forming a mean topography. It has been shown that the hyperbolic distribution can better model the fatter tails observed in the distributions of RA measurement errors for test-retest and longitudinal data (Owen et al., 2006). It is hypothesised for this analysis that, in addition to the standard normal distribution, two other distributions: the Laplace and hyperbolic distributions could serve to model observations of HRT measurements. Measurement variability took the form of these three different distributions (representing three different scenarios) with controllable parameters which could be manipulated to give low, medium and high levels of variability to fit data according to the characteristics of a simulated RA scenario.

(i) Normal distribution: In approximating medical data, the normal distribution is typically used because of its broad applicability and mathematical tractability. Observational error in an experiment is usually assumed to follow a normal distribution. It is a symmetrical distribution (also referred to as the Gaussian distribution) whose shape is determined by two parameters, location (mean) and spread - standard deviation.

(ii) Laplacian Distribution: Like the normal distribution, the Laplacian distribution is symmetrical and can be characterised by a location parameter (mean) and a spread parameter. It can be thought of as two decaying exponential curves spliced together back-to-back with the spread parameter controlling the magnitude of decay of these curves. It is more "peaked" meaning more observations fall directly on the average

than are seen in a normal distribution and has a heavier tail than an equivalent normal distribution. The difference between two independent identically distributed exponential random variables is governed by a Laplace distribution (Dodge, 2008).

(iii) Hyperbolic Distribution: This distribution belongs to the family of 'stable' distributions; stable refers to the property of distributions that retain shape when added together. These distributions are generalisations of the normal distribution; they can be manipulated to be more peaked and have heavier tails than the normal distribution. In contrast to the normal and Laplace distributions, hyperbolic distributions have four parameters each defining location, scale, peak, and symmetry. They are used widely in financial mathematics for modelling stable random variables with extreme values that occur more frequently than in the normal distribution (Eberlein and Keller, 1995). They were first used to describe the hyperbolic shapes of the log of empirical distributions of the size of wind-blown sand deposits (Barndorff-Nielsen, 1977, Tsoar, 1994).

Figure 3.1 shows these three distributions in three settings of equal variance and mean but with different shapes.
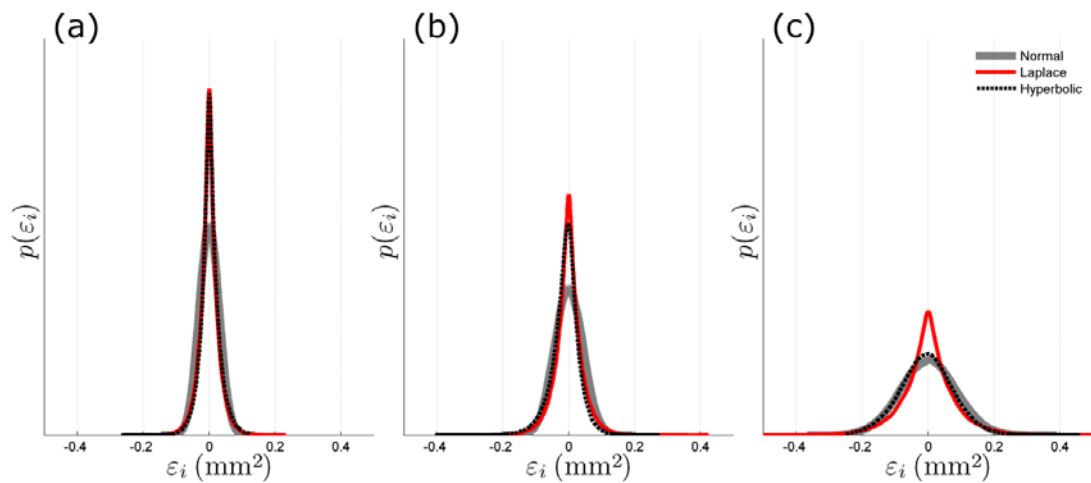
Figure 3.1 (a) Low, (b) medium and (c) high levels of RA measurement noise ε_i with normal, Laplace and hyperbolic probability distributions. Within each level, these distributions have all been adjusted to have equal variances. Shape parameters of the hyperbolic distributions are consistent with those fitted to observed RA measurement error distributions from Owen et al. (Owen et al., 2006).

3.2.2.3 Measurement Variability Levels

The parameters controlling these three types of error distribution are chosen by fitting the three distributions to the data of longitudinal RA measurement error for topography series of different image quality obtained from Owen and colleagues (Owen et al., 2006). These quality measures were categorised as good, acceptable, and poor image quality with variability increasing with decreasing image quality. (Chapter 4 provides a further investigation into the relationship of image quality with RA variability.) Controlling parameters of these distributions are set to give the following noise levels:

(i) *Low level noise*: The noise parameters derived from fitting each distribution to the noise of longitudinal series of good image quality - see Figure 3.1 (a).

(ii) *Medium level noise*: The noise parameters derived from fitting each distribution to the noise of longitudinal series of medium image quality - see Figure 3.1 (b).

(iii) *High level noise*: The noise parameters derived from fitting each distribution to the noise of longitudinal series of poor image quality - see Figure 3.1 (c).

3.2.2.4 Changes in Within-Series Variability

As has been outlined, there is some evidence to suggest that RA variability may change with increasing ONH damage and the consequences should be investigated for a 'pattern' of noise over time. In consideration of this, distributions of measurement noise are modified to change over time (or remain uniform) in seven different sub-scenarios to achieve the following patterns of measurement variability (illustrated in Figure 3.2).

(a) Low uniform noise: Measurement noise at each point is independent of time and has a magnitude at the low noise level (see Figure 3.2 (a)).

(b) Medium uniform noise: Measurement noise at each point is independent of time and has a magnitude at the medium noise level (see Figure 3.2 (b)).

(c) High uniform noise: Measurement noise at each point is independent of time and has a magnitude at the high noise level (see Figure 3.2 (c)).

(d) Medium increasing noise: Measurement noise increases monotonically with time and has a magnitude (when averaged over the whole series) equivalent to medium level noise (see Figure 3.2 (d)).

(e) Medium decreasing noise: Measurement noise decreases monotonically with time and has a magnitude (when averaged over the whole series) equivalent to medium level noise (see Figure 3.2 (e)).

(f) Medium uniform noise with random outliers: This noise model consists of medium level uniform noise with an additional randomly chosen outlier measurement with high level measurement noise. The series averaged magnitude noise is then normalised to be equivalent to medium level noise (see Figure 3.2 (f)).

(g) Medium autocorrelated noise: Temporal autocorrelation is the correlation of a signal with itself as a function of time; measurements or indeed measurement noise closer together in time or periodically separated can be more likely to be similar depending on the autocorrelation. In regression analysis using time series data, autocorrelation of the residuals ("error terms") is a problem. This violates the OLS assumption that the serial error terms are independent. While it does not bias the OLS coefficient estimates, the standard errors tend to be underestimated (and the t-scores overestimated) especially when the autocorrelations between the errors separated by a low time-lag (separation in time between measurements) is positive (Salkind and Rasmussen, 2007). What time-scale this autocorrelation occurs in for these data however is unknown, as is its strength. For the purposes of examining the worst effects of autocorrelation on trend detection techniques, a large positive autocorrelation effect over a period of several visits has been chosen. Measurement error is set to a mean magnitude equivalent to the uniform medium uniform noise scenario but includes a lag filter function such that measurement noise closer in time is more likely to be similar. This is implemented by a backwards filter. This autocorrelation filter adds information to a given measurement error ε_i at time t_i based on previous ε_j such that:

$$\varepsilon_i = \frac{d_1\varepsilon_{i-k} + d_2\varepsilon_{i-k+1} \dots + d_{k-1}\varepsilon_{i-1} + d_k\varepsilon_i}{\sum_1^k d_m}$$

where the coefficients:

$$d_{i-k} = \frac{1}{2^{k+1}}$$

are fitted to achieve a small magnitude autocorrelation effect measureable across four visits (see Figure 3.2 (g)).
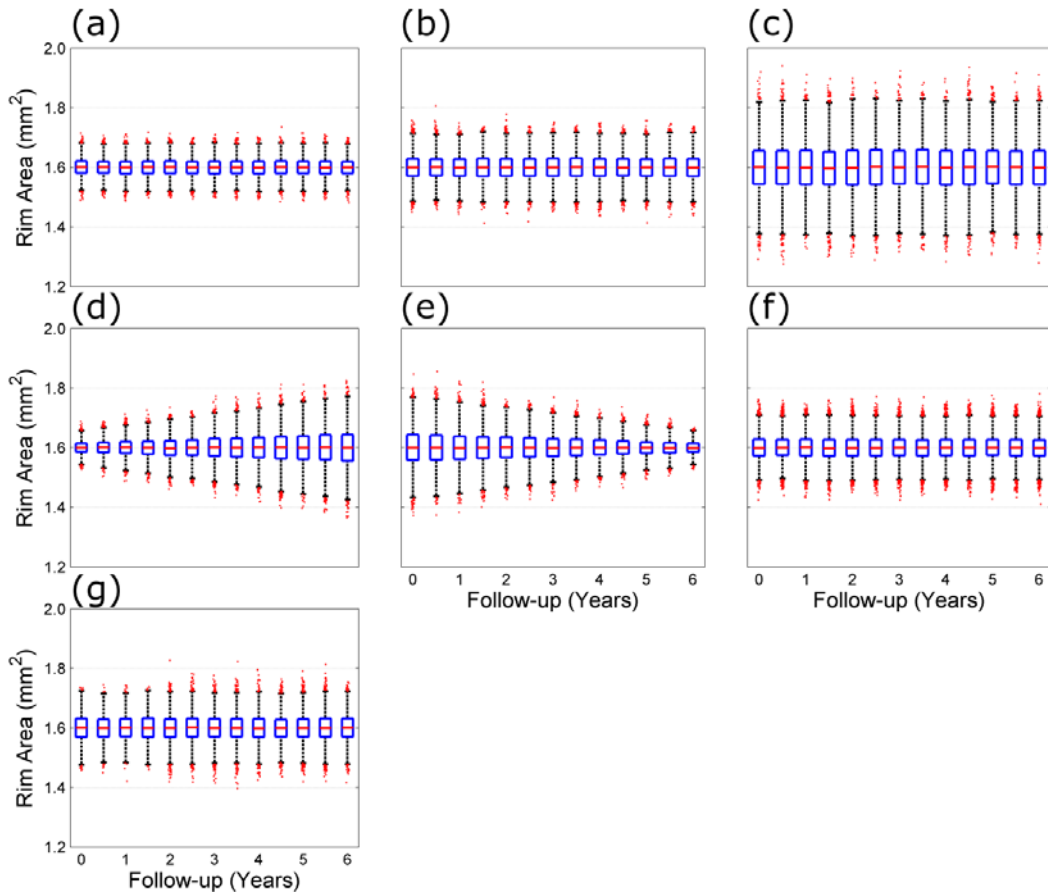
Figure 3.2 Box-whisker plots showing the spread of normally distributed measurement variability at each follow-up examination for stable series. Each panel represents a scenario of measurement variability with the following average magnitudes and its change across the follow-up period: (a) uniform low noise, (b) uniform medium noise, (c) uniform high noise, (d) medium increasing noise, (e) medium decreasing noise, (f) medium noise with an outlier present, and (g) medium noise with autocorrelation.

In summary, scenarios involving Laplacian and hyperbolic noise involve data series which violate the OLS fitting procedure. In addition, scenarios were generated with noise structures (d), (e), (f) and (g) where violations of the assumptions of the OLS fitting procedure and the exchangeability assumption in permutation testing were permitted.

A total of 315,000 time series are simulated: 5000 Monte Carlo simulations for each of the 63 'scenarios' of every possible combination of trend (3 possible), measurement error distribution (3 possible) and time-wise error pattern (7 possible). These large sets of data enable statistically powerful inferences about and comparisons between the diagnostic performances of change detection techniques at given trends and number of measurements and for given levels and patterns of measurement noise. The summary performances of the different change detection analyses were compared using metrics averaged across all of these scenarios. Individual cases of interest were also examined to identify circumstances of noise distribution, noise pattern and signal when one of the change detection algorithms described in the following section may be preferable over others.

3.3 Performance Measures of Change Detection Techniques

The low prevalence of a disease such as open-angle glaucoma requires that screening or case finding methods in an at risk population have high specificity (>95%) to avoid unacceptably high numbers of false positive test outcomes. A formulation for appropriate specificity limits is less clear for tests of glaucomatous progression; it depends primarily on the proportion of glaucoma suspects being monitored or glaucoma patients already in treatment who will experience the onset of new or further glaucomatous damage respectively during a given observation period. Nevertheless it is hypothesised that only a minority of glaucoma suspects or patients will have sufficient damage occurring in an average follow-up interval (e.g. 6 months) to warrant a treatment intervention. As a result, emphasis on the diagnostic strength of change detection methods is placed on decision making with low false positives.

In the real data, it is extremely unlikely for true glaucomatous damage to have occurred during the short follow-up period. Thus any changes detected by each method can be considered false positives – the proportion of series flagged as progressing by each method referred to as the *false-positive rate*. The relationship between the false positive rate and the p-value 'cut-off' which binarises series into negatively changing or not negatively changing is investigated at the final follow-up examination (5th time point in the series). P-value cut-offs were recorded at 10% and 5% false positive rates. These values are also intended to be presented as cut-offs for future studies or meta-analyses which may use RA as a measure of progression and for which no reliable measurement of specificity can be readily ascertained.

In the simulated setting, the trend detection performances of methods were examined at only the 5th examination (≥ 2 *pseudo*-years follow-up) and afterwards.

3.3.1 Statistical Power

Because simulated series were set to be both stable and changing, the probability of detecting true positive changes, referred to as statistical *power* of each method, could be examined (Cohen, 1988). Again, emphasis is placed on those areas of low false positive detection, thus power is measured in each noise scenario set (of 5000 simulated series) for each method at fixed 5% false positive detection levels in the corresponding stable scenario set (of 5000 simulated series). Power to detect change at this low level of false positives is examined at each follow-up visit when stable data and data with underlying trends (either medium or high magnitude) were matched according to noise scenarios.

3.3.2 ROC and Partial ROC Analysis

Many diagnostic tests give quantitative results - such as a p-value or a trend over time - rather than binary outcomes and so different thresholds of these results can be used to binarise a diagnosis into normal or abnormal. An ROC curve is simply the plotted values of a diagnostic test's true positive rate against its false positive rate for a range of thresholds, continuous or discrete. Analysis using ROC curves was originally developed for evaluating radar signal detection and has been used in general signal detection theory since then (Egan, 1975). In medicine it has also been adopted in the evaluation of diagnostic tests (Altman and Bland, 1994, McNeil et al., 1983). ROC curves are used accordingly in this analysis to assess the performance of trend detection methods across a range of false positive rates. The area under the ROC curve (AUC) is used to quantify the performance of the test. The maximum

possible value for the AUC is 1 with a practical minimum value of 0.5

corresponding to the average value obtained by a guessing strategy. For this

analysis, the area under the partial ROC curve (PAUC) metric is more important.

The PAUC is calculated as the area under some restricted portion of the ROC curve

corresponding to false positive values of clinical interest. It is particularly useful, as

in the case of this analysis, when comparing the two ROC curves, with close

attention given to minimising false positive decisions.
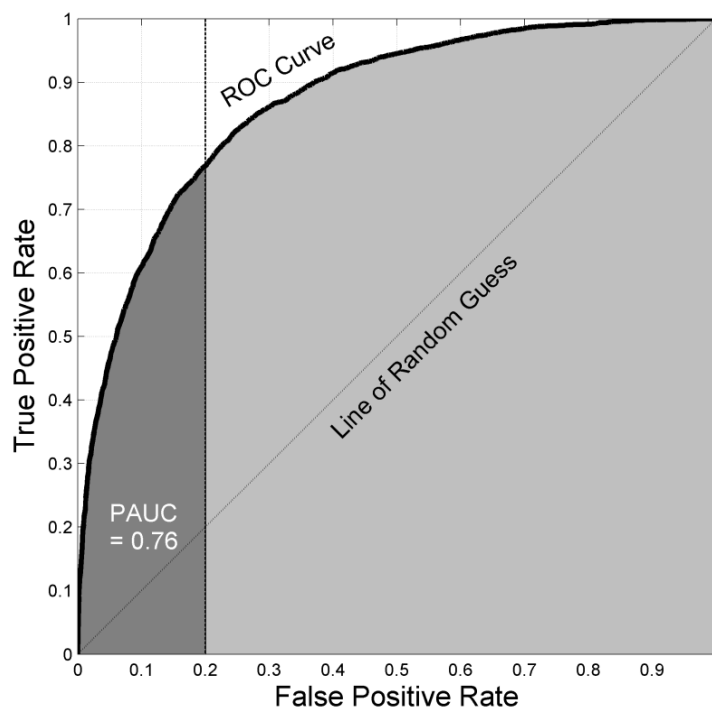
Figure 3.3 Example of ROC curve and the dark grey PAUC within a region of the

ROC curve with false positives ≤ 0.2. True positive rate is also known as *statistical*

power.

An example of an ROC curve with its corresponding PAUC for a false positive rate

≤ 20% is shown in Figure 3.3. For this analysis, PAUC are examined for those partial

ROC curves with a false positive rate ≤ 20%. PAUC can usefully be used as an

accurate summary measure of these power estimates in a range of different false

positive rates. PAUC values have been transformed, for interpretation purposes,

giving a minimum of 0.5 and maximum value of 1. Thus, the transformation lets one view the PAUC on the same scale as the AUC.

The statistical significance of differences between AUCs of testing strategies at each visit, noise pattern and trend over time are established using the method of Hanley-McNeil for paired data (Hanley and McNeil, 1983a). Similarly the method of McClish (McClish, 1989) is used to examine differences between PAUCs. The Bonferroni correction is used to compensate for the multiple comparisons involved in this testing.

3.3.3 Imaging Follow-up Period

The effect of the length of follow-up on the performance of each statistical trend detection technique in different noise and trend scenarios is assessed with PAUC values.

3.3.4 Students t-Distribution for RA Series Test Statistic

In a given series under OLS T-DIST analysis, the measured test-statistic is assumed to be sampled from the student's t-distribution and its statistical significance is inferred from this distribution. In order to determine if this assumption is appropriate in series which do not adhere to the assumptions of OLS, the test-statistic of OLS T-DIST is permuted. This shape of this permutation distribution is compared to the relevant student's t-distribution for each scenario and follow-up examination.

3.4 Results

The relationship between p-values and the number of false positives for RA values from mean topographies acquired with the HRT Classic and the HRT II are shown in Figure 3.4 (a) and (b) respectively. Table 3.1 displays the α-levels which resulted in $\lesssim$ 10% (7/74 patient series) and $\lesssim$ 5% (3/74 patient series) false positive progressions in HRT Classic and HRT II data.
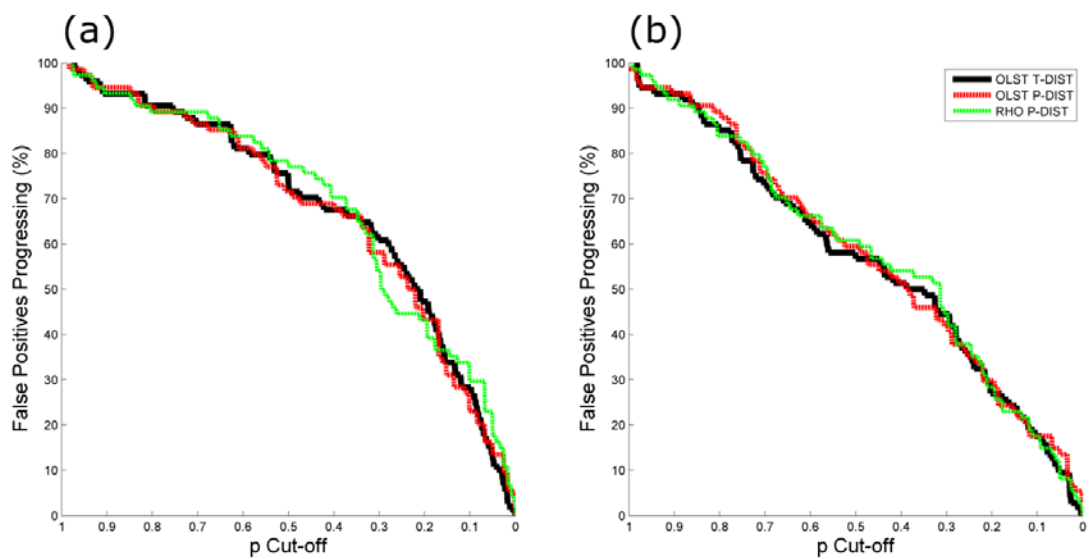
Figure 3.4 (a) HRT Classic data: Percentage of falsely flagged progressing series at fifth and final examination for the p-value cut-off of each change detection test (b) HRT II data: Percentage of falsely flagged progressing series at fifth and final examination for the p-value cut-off of each change detection test.

The mean AUCs associated with statistical change detection techniques across all visits, signals, noise distributions and patterns were 0.825 for OLS T-DIST, 0.814 for OLS SL P-DIST and 0.815 for RHO P-DIST. The mean PAUCs associated with statistical change detection techniques across all visits, signals, noise distributions and patterns were 0.738 for OLS T-DIST, 0.744 for OLS SL P-DIST and 0.736 for RHO P-DIST.

Table 3.1 Real no-change RA data: the α-level (p-value cut-off) of the hypothesis test of negative change over time for each change detection test given a fixed/anchored percentage of falsely flagged progressing rim area series at fifth and final examination.

		Data (False Positive Rate)			
		HRT Classic ($\leqslant$10%)	HRT Classic ($\leqslant$5%)	HRT II ($\leqslant$10%)	HRT II ($\leqslant$5%)
α-level of Method	OLS T-DIST	0.039	0.023	0.056	0.022
	OLS P-DIST	0.034	0.017	0.034	0.017
	RHO P-DIST	0.034	0.026	0.034	0.026

Statistical power estimates are shown in Tables 3.2 and 3.4 for a moderate trend of RA loss after 3 and 6 years of follow-up respectively. Statistical power estimates are also shown in Tables 3.3 and 3.5 for a high trend of RA decrease after 3 and 6 years of follow-up respectively. Colour-coded arrays indicate the overriding patterns. Comparing across rows, all techniques across all distribution types (normal, Laplace, hyperbolic) show consistently lowest power for high noise series followed by medium noise with autocorrelation. Comparing across columns enables comparison between trend detection techniques within each scenario.

Table 3.2 Power levels for three change detection methods of detecting change at **medium trend of RA deterioration** (-0.012mm²/year) at 7th visit after 3 years of follow-up in different noise scenarios. Total RA decrease during this follow-up period: 0.036mm². Entries are colour coded: red corresponds to lower power values, yellow to moderate power values and green to higher power values.

	Power: 1-β (at α = 5%)								
	Noise Distribution								
Change Detection	Normal			Laplace			Hyperbolic		
	OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST
Low Uniform	0.24	0.23	0.22	0.29	0.29	0.27	0.28	0.28	0.28
Medium Uniform	0.15	0.15	0.15	0.20	0.20	0.20	0.19	0.18	0.18
High Uniform	0.10	0.10	0.10	0.11	0.11	0.10	0.10	0.10	0.09
Medium Increasing	0.20	0.19	0.19	0.25	0.24	0.26	0.25	0.24	0.23
Medium Decreasing	0.12	0.12	0.12	0.15	0.15	0.15	0.15	0.14	0.15
Medium with Outlier	0.18	0.17	0.17	0.21	0.21	0.20	0.19	0.19	0.20
Medium Autocorrelated	0.13	0.12	0.12	0.15	0.15	0.15	0.14	0.14	0.13

Noise Pattern

Table 3.3 Power levels for three change detection methods of detecting change at **<u>high trend of RA deterioration</u>** (-0.021mm²/year) at 7th visit after 3 years of follow-up in different noise scenarios. Total RA decrease during this follow-up period: 0.063mm². Entries are colour coded: red corresponds to lower power values, yellow to moderate power values and green to higher power values.

		Power: 1-β (at α = 5%)								
		Noise Distribution								
		Normal			Laplace			Hyperbolic		
Change Detection		OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST
Noise Pattern	Low Uniform	0.49	0.48	0.45	0.56	0.56	0.52	0.55	0.54	0.52
	Medium Uniform	0.30	0.29	0.28	0.36	0.36	0.35	0.37	0.35	0.34
	High Uniform	0.15	0.15	0.14	0.17	0.17	0.17	0.13	0.13	0.12
	Medium Increasing	0.42	0.41	0.39	0.51	0.49	0.50	0.50	0.49	0.45
	Medium Decreasing	0.22	0.22	0.20	0.27	0.26	0.27	0.27	0.26	0.27
	Medium with Outlier	0.32	0.32	0.31	0.39	0.39	0.37	0.36	0.35	0.35
	Medium Autocorrelated	0.23	0.21	0.21	0.26	0.25	0.25	0.28	0.27	0.25

Table 3.4 Power levels for three change detection methods of detecting change at **<u>medium trend of RA deterioration</u>** (-0.012mm^2/year) at 13th (and final) visit after 6 years of follow-up in different noise scenarios. Total RA decrease during this follow-up period: 0.072mm^2. Entries are colour coded: red corresponds to lower power values, yellow to moderate power values and green to higher power values.

		Power: 1-β (at α = 5%)								
		Noise Distribution								
		Normal			Laplace			Hyperbolic		
	Change Detection	OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST
Noise Pattern	Low Uniform	0.80	0.79	0.77	0.82	0.82	0.83	0.82	0.82	0.83
	Medium Uniform	0.57	0.56	0.53	0.59	0.60	0.63	0.58	0.57	0.59
	High Uniform	0.23	0.22	0.22	0.25	0.26	0.27	0.24	0.25	0.23
	Medium Increasing	0.49	0.48	0.46	0.57	0.55	0.59	0.57	0.56	0.60
	Medium Decreasing	0.52	0.52	0.50	0.57	0.56	0.60	0.57	0.57	0.61
	Medium with Outlier	0.56	0.55	0.55	0.59	0.58	0.63	0.60	0.60	0.63
	Medium Autocorrelated	0.37	0.37	0.35	0.40	0.39	0.38	0.39	0.38	0.39

Table 3.5 Power levels for three change detection methods of detecting change at **<u>high trend of RA deterioration</u>** (-0.021mm²/year) at 13th (and final) visit after 6 years of follow-up in different noise scenarios. Total RA decrease during this follow-up period: 0.126mm². Entries are colour coded: red corresponds to lower power values, yellow to moderate power values and green to higher power values.

	Change Detection	**Power: 1-β (at α = 5%)**								
		Noise Distribution								
		Normal			**Laplace**			**Hyperbolic**		
		OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST	OLS T-DIST	OLS P-DIST	RHO P-DIST
Noise Pattern	Low Uniform	1.00	1.00	0.99	0.99	0.99	0.99	0.99	0.99	0.99
	Medium Uniform	0.94	0.93	0.91	0.91	0.91	0.92	0.92	0.91	0.92
	High Uniform	0.47	0.46	0.46	0.51	0.51	0.54	0.47	0.47	0.45
	Medium Increasing	0.88	0.88	0.87	0.89	0.88	0.89	0.89	0.88	0.90
	Medium Decreasing	0.89	0.89	0.87	0.89	0.88	0.90	0.89	0.89	0.90
	Medium with Outlier	0.93	0.92	0.91	0.92	0.91	0.92	0.92	0.92	0.92
	Medium Autocorrelated	0.76	0.76	0.73	0.78	0.76	0.74	0.78	0.77	0.77

Differences were found for the between trend detection techniques within scenarios at each visit for AUCs, PAUCs, and power at 5% and 10% to be highly statistically significant (P<0.0001). Significant statistical differences were found between all PAUCs of testing strategies at each visit, change magnitude and noise (P<0.0001). The high sample sizes (N=5000) in each scenario render these statistical inferences trivial however as the obtained PAUCs are close to their true underlying (asymptotic) values. Rather in this case the pair-wise differences in between technique AUCs and PAUCs should be emphasised. The median differences between PAUC values between methods were as follows: PAUC(OLS T-DIST) – PAUC(OLS P-DIST) < 0.001; PAUC(OLS T-DIST) – PAUC(RHO P-DIST) = 0.002; PAUC(OLS P-DIST) – PAUC(RHO P-DIST) = 0.002 with respective interquartile ranges of 0.003, 0.010 and 0.011. In comparison, values of PAUC (across all scenarios, visits and techniques) decreased by a median of 10% when uniformly distributed measurement variability was increased from low to medium, and decreased by a median of 8% when increased from medium to high. Similarly PAUC values decreased by a median of 8% when autocorrelation was added to uniformly distributed measurement variability.

The changes in PAUC values with increasing follow-up examinations in different scenarios with OLS T-DIST, OLS P-DIST and RHO P-DIST trend detection techniques are shown for moderate and high trends in Figure 3.5 and 3.6 respectively. The median of the increase in power values at each 6 monthly-interval for each scenario and technique was approximately 4%.
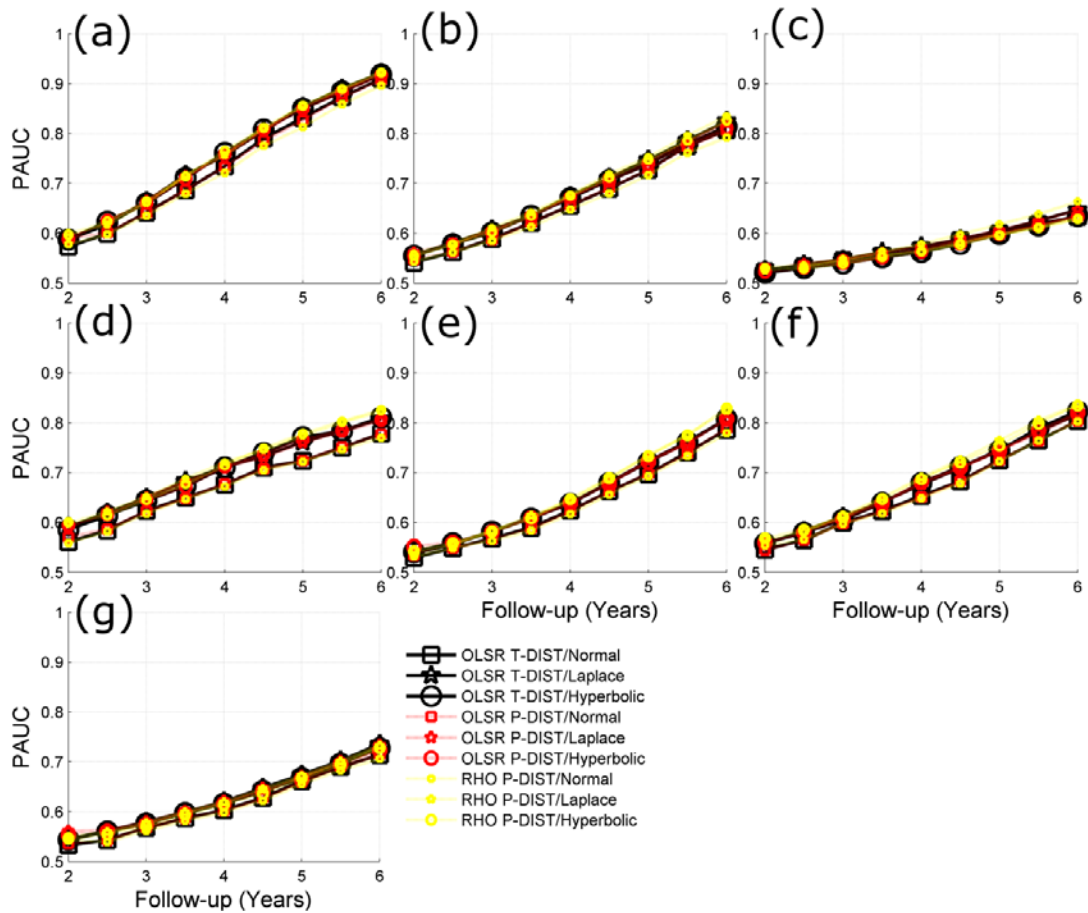
Figure 3.5 Changes with follow-up period of PAUC values (false positive rate <
20%) for the three change detection methods for the three indicated noise
distributions in series of a **medium trend of RA deterioration** (-0.012mm²/year). The
seven scenarios of noise pattern over time are (a) low uniform noise, (b) medium
uniform noise, (c) high uniform noise, (d) medium increasing noise, (e) medium
decreasing noise, (f) medium noise with an outlier measurement and (g) medium
noise with autocorrelation. See Figure 3.2 for illustration of noise patterns.
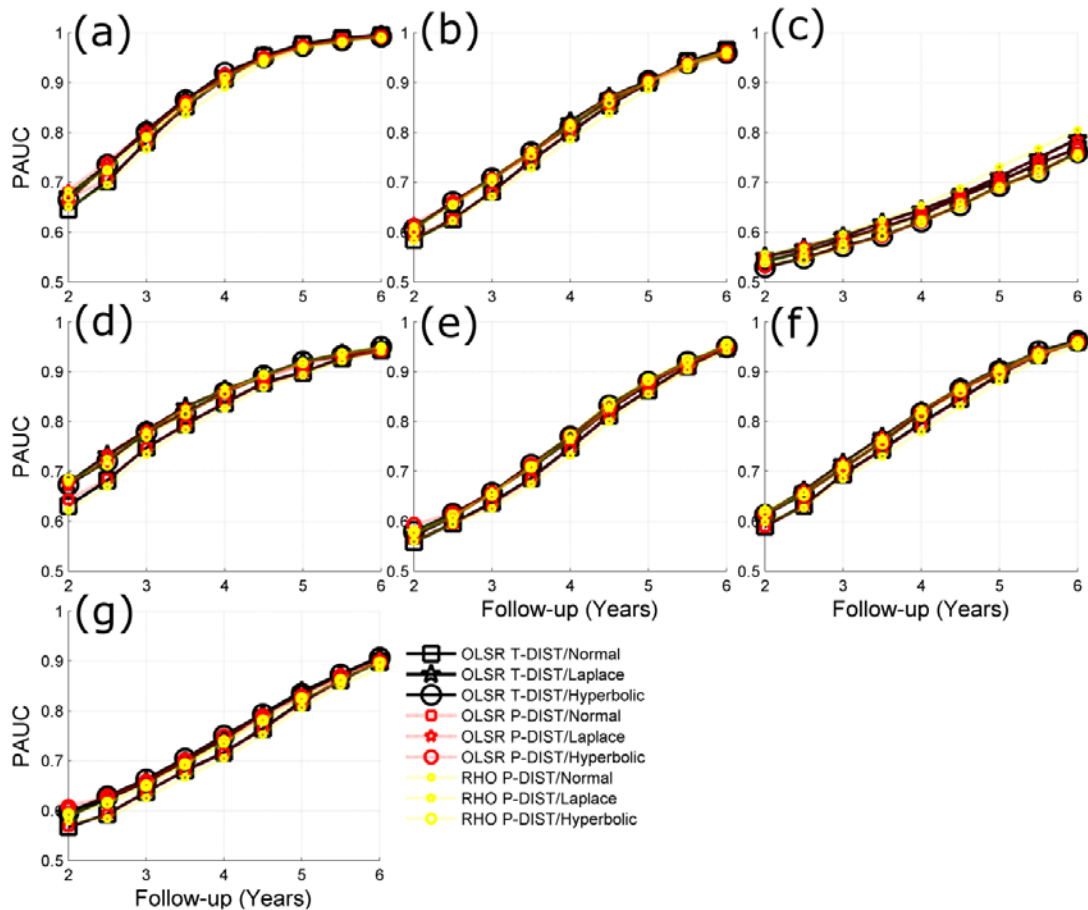
Figure 3.6 Changes with follow-up period of PAUC values (false positive rate < 20%) for the three change detection methods for the three indicated noise distributions in series of a **high trend of RA deterioration** (-0.021mm²/year). The seven scenarios of noise pattern over time are (a) low uniform noise, (b) medium uniform noise, (c) high uniform noise, (d) medium increasing noise, (e) medium decreasing noise, (f) medium noise with an outlier measurement and (g) medium noise with autocorrelation. See Figure 3.2 for illustration of noise patterns.
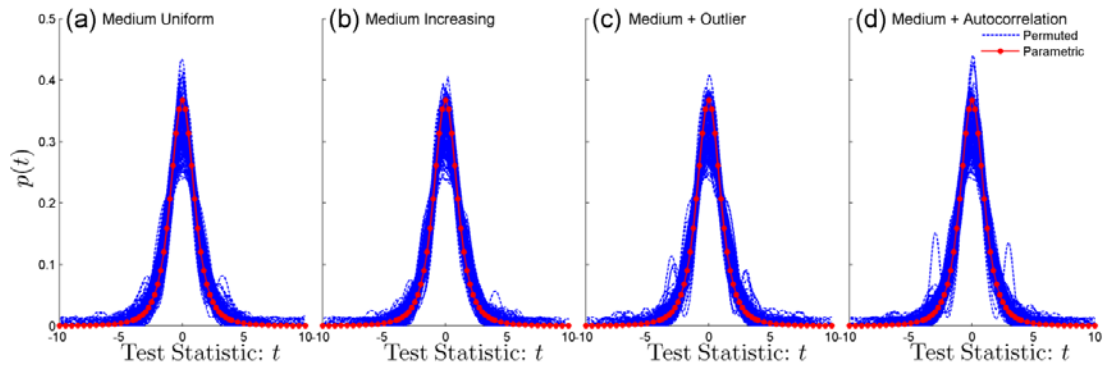
Figure 3.7 Two-hundred randomly sampled permutation distributions of OLS linear regression test-statistics with the parametric Student's t-distribution (3 degrees of freedom) overlaid for 4 scenarios of noise pattern: (a) medium uniform noise, (b) medium increasing noise, (c) medium noise with an outlier and (d) medium noise with autocorrelation. These distributions are for stable simulated series Laplace measurement noise at 5th RA measurement (2 years). The average p-value for the two-sample Kolmogorov-Smirnov test is for (a) 0.78, (b) 0.81, (c) 0.81 and (d) 0.82.
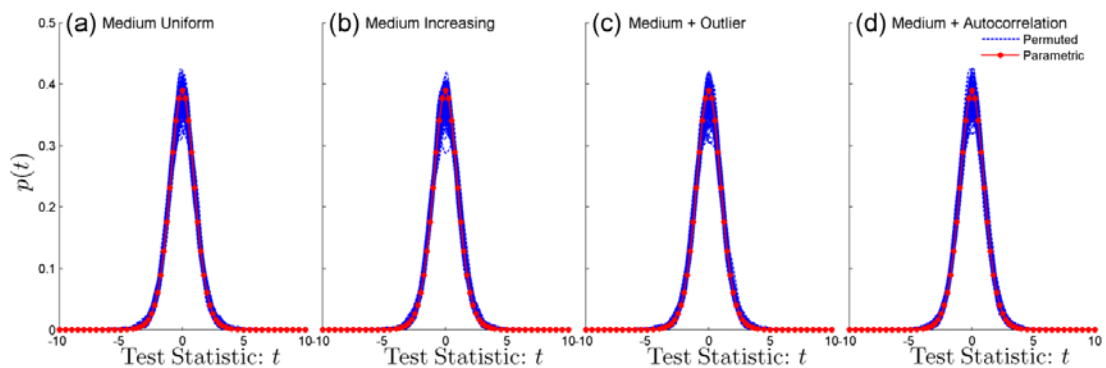
Figure 3.8 Two-hundred randomly sampled permutation distributions of OLS linear regression test-statistics with the parametric Student's t-distribution (11 degrees of freedom) overlaid for 4 scenarios of noise pattern: (a) medium uniform noise, (b) medium increasing noise, (c) medium noise with an outlier and (d) medium noise with autocorrelation. These distributions are for stable simulated series hyperbolic measurement noise at 13th RA measurement (6 years). The average p-value for the two-sample Kolmogorov-Smirnov test is for (a) 0.52, (b) 0.50, (c) 0.53 and (d) 0.54.

3.5 Discussion

In glaucomatous progression, the ability to detect and track trends in univariate measures such as RA, RNFL thickness, visual field MD and IOP is essential. It is also important to know how best to detect and measure trends in point-wise analysis both as pixels in imaging or as testing points in visual fields. Appropriate techniques should be able to optimally distinguish series of measurements which exhibit statistically significant trends over time (those series with more reliably measured deterioration respectively) from those which do not before the clinician can decide if change is clinically significant or not. The performance of these techniques will depend on factors such as the number of measurements, length of follow-up time, the magnitude and structure of measurement variability and the underlying change. To this end, simulated series were derived with a range of change and variability parameters in order to establish how well various trend detection techniques can measure the underlying trends or stability of the series. Short-term time series of HRT Classic and HRT II derived RA measurement data were also used as an approximation to stable data to measure the p-value thresholds of each methods hypothesis test which produce clinically relevant false-positive rates.

In assessing the alpha-level of these change detection methods, OLS T-DIST yields a consistently higher α at the ~5% and ~10% false positive levels compared to OLS P-DIST. This means that a 'less strict' alpha level needs in OLS T-DIST to be set to achieve the same level of false positives in this sample of 74 and could infer that OLS P-DIST is less powerful. The results of the analysis comparing the AUCs and PAUCs of competing methods show statistically significant differences throughout between all methods in all noise and signal scenarios at each visit. This is almost

wholly due to the high number of virtual patients which reduces the standard error of the AUC and PAUC estimates. These differences, though statistically significant, are relatively small for all scenarios. It is not surprising that average AUCs and PAUCs for all methods increase with an increasing number of measurements because the power of any test will increase as the magnitude of sampling increases.

There is little literature on the precise nature of RA measurement noise and almost none on the precise change over time in series of RA. As a result this simulation model and its interpretation may well only capture some of this information. However the methodology here has examined a wide spectrum of noise and signal scenarios using empirical noise parameter estimates where available. Linear and rank based models of RA change only were examined in this analysis. It may be argued that a linear model is inadequate to describe the complex process of RA changes occurring in glaucomatous damage. However, without any evidence of a more suitable model, it is difficult to justify exploration of an unlimited range of alternate, more complex models. These models, by design, require more parameters to define the dependence of the output variable - in this case, RA - on the predictor variable(s) - in this case, time. This can result in poor statistical power at low sample sizes and will be particularly at risk of over-fitting models to the data. This is a modelling error where the model is freer to be closer to each observed point and ends up fitting the underlying signal in the series but also the measurement noise itself. In addition these extra parameters may not lend themselves to an instant and immediate interpretation in a clinic setting.

Though this methodology is very transferrable to measurements derived from other imaging devices or visual fields, the exact results presented here are those relating directly to real and simulated longitudinal RA series and should only be interpreted

as such. It may be useful in time to model RNFL thickness as derived from OCT or SLP rather than RA from HRT. The RNFL thickness may well have differing noise and signal characteristics over time and this should be the subject of further investigation. It is worth looking at these findings however in comparison to another example in visual field glaucoma progression research. Monte-Carlo simulation has been used previously to answer clinically relevant questions in glaucoma progression detection. Chauhan and colleagues (Chauhan et al., 2008) reported on the minimum number of equally-spaced visual field examinations required to detect (with a power of 0.80) a linear trend of -4 dB/year in visual field mean deviation (summary measure of sensitivity) using estimates of different degrees of visual field variability. This relied on assumptions that the measurement errors were identically and normally-distributed and independent. Although intended to examine RA changes, the analysis of this chapter shows that violations in these assumptions can considerably affect these reported measurements and that this is consistent across the change detection techniques investigated. In particular the effects of autocorrelation, with no-measurable increase in the degree of variability, on the power to detect an underlying trend are comparable to the effect of increasing the variability considerably and would change the estimates provided by Chauhan and colleagues in their report.

The calculated relationship and its significance between the dependent variable and independent variable (in this case time) in an OLS linear regression (OLS T-DIST) are based on the assumptions of uniformly, normally distributed measurement errors and independence of serial measurements. This analysis has tested the performance of trend detection techniques under violations of these assumptions: changing magnitude of variability with time, correlations between the measurements over time and non-normal distributions of noise. The autocorrelation

of serial measurements as modelled in this analysis had a large effect on the performance of all methods. This is to be expected for the OLS T-DIST method as the standard error of the slope/trend will be systematically underestimated. This also occurs for the permutation framework for both linear and rank based methods. Previous research has shown that resampling methods which do not take into account the serial correlation of the dependent variable can underestimate the standard error of Spearman's ρ (Park and Lee, 2001) which would again decrease the performance.

If the number of data points is small, it may be difficult to detect assumption violations. With small samples, violation assumptions such as non-normality or heteroscedasticity of variances are difficult to detect even when they are present. With a small number of data points linear regression offers less protection against violation of assumptions. With few data points, it may be hard to determine how well the fitted line matches the data, or whether a nonlinear function would be more appropriate. Since parameter estimation is based on the minimization of squared error, a few extreme observations can exert a disproportionate influence on parameter estimates. Calculation of confidence intervals and various significance tests for coefficients are all based on the assumptions of normally distributed errors. If the error distribution is significantly non-normal, confidence intervals may be too wide or too narrow.

The results of this analysis suggest that for data which can be modelled as presented there is little difference in the three methods of analysis investigated. One crucial assumption, that progressive RA damage followed a linear trend, was not altered in this simulation and this may offer more promise for a rank-based method which shows relative efficiency almost equal to linear trend detection methods. In cases of

non-linear trends, a rank-based method is likely to prove more powerful in detecting this underlying trend. In consideration of these factors, a rank based approach may provide the best option of these three approaches. If these time series are representative of longitudinal RA measurements of a large set of virtual patients then these inferences can also be applied to real data scenarios. Further characterisation of RA and topographic variability are required as are predictors of these.

4. Assessment of Quality in HRT Images

Image quality assessment gives important clinical information on the fidelity of rendered images. The quality of an image is important and sometimes crucial in clinical decision making as the example case of Figure 4.1 shows. From a neuro-radiological study (Phal et al., 2008), this case involved a patient with a brain lesion symptomatic of epilepsy and normally indicated by a higher intensity (higher tissue density) scan value in MRI. This lesion was not identified in an initial lower quality scan but was evident in a secondary improved quality scanning modality. The first scan was acquired using an MRI scanner with a magnet of lower flux (units in Tesla or T) than the scanner used for the second scan. Image quality has been shown in other studies to vary considerably between MRI scanners with magnets of differing operating flux (Beyersdorff et al., 2005, Kataoka et al., 2007).

In the context of examining retinal images with application in glaucoma detection and tracking over time image quality metrics can inform the clinician or automated algorithm how much or how little confidence to place in a measurement derived from a particular image. In assessing the overall quality of a medical image factors such as the optical resolution of the scanning or imaging system, the noise inherent in the measuring system, the contrast of the image and the presence of artefacts in the image must be accounted for.
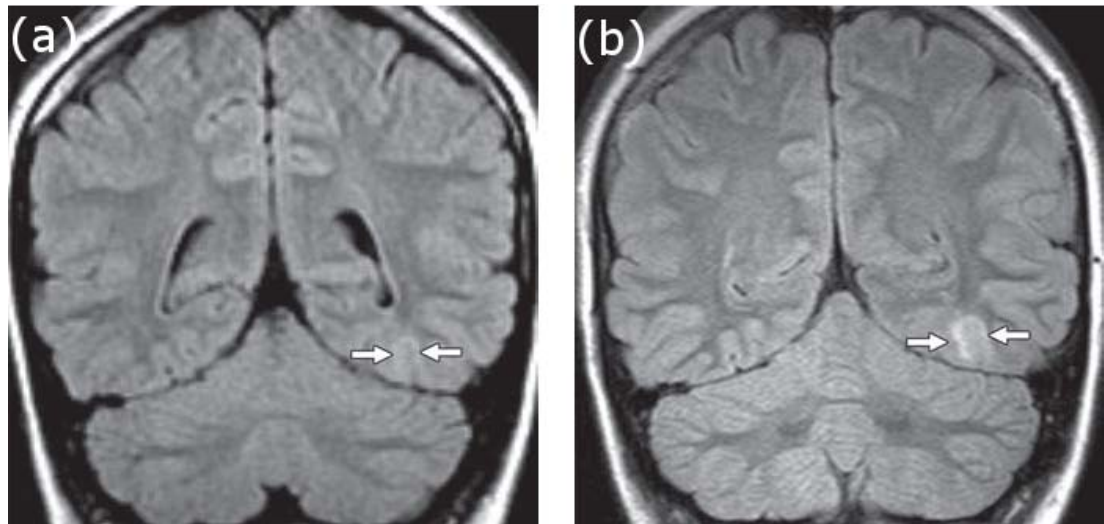
Figure 4.1 MRI scans of patient with intractable nocturnal seizures. (a) Coronal image using 1.5-T magnet MRI showing questionable curvilinear focus of high signal intensity (arrows). Abnormal signal intensity was missed at first review of images. (b) Coronal image using 3-T MRI showing curvilinear band of high signal intensity (arrows) white matter without apparent mass effect. (Reproduced from (Phal et al., 2008))

The image quality of a given HRT mean topography is measured with the MPHSD; a metric provided by the HRT software as an assessment of the within examination variability across three single topographies. The HRT software also gives other assessments of image quality at the time of acquisition. It provides a list of 'pass' or 'fail' checks for appropriate accommodation and camera distance, for sufficient image brightness and image illumination and for levels of eye movement, blinks, fixation loss and eye drift below tolerable levels (Figure 4.2). Once a scan has passed these criteria, it is the MPHSD which dictates the post-acquisition assessment of image quality. The manufacture provided guidelines for interpreting the quality of a resultant MPHSD value of a mean topography are summarised in table 4.1.
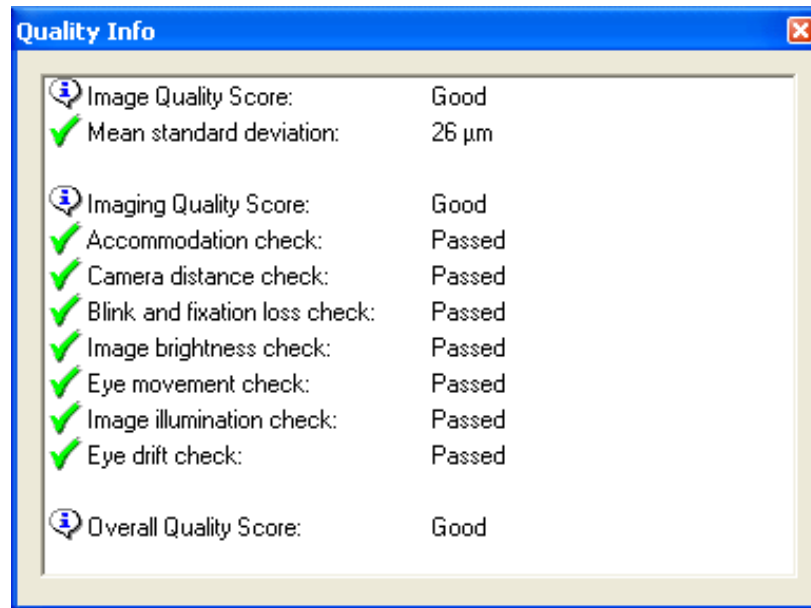
Figure 4.2 Example output of image quality assessment by HRT software of the constituent images in a HRT mean topography.

MPHSD as provided by the HRT software is actually the calculated *geometric mean* (not the *arithmetic mean*) of the sample standard deviation of each pixel in the mean topography image across the three constituent single topographies. As an illustration, if a sample contains n measurements a_i the more familiar arithmetic mean μ_a is calculated as follows:

$$\mu_a = \frac{a_1 + a_2 + a_3 + \cdots a_n}{n},$$

and the geometric mean μ_a is calculated as follows:

$$\mu_g = \sqrt[n]{a_1 a_2 a_3 \dots a_n}.$$

The geometric mean is less affected by long tails in negatively and positively skewed distributions (Altman, 1999).

Table 4.1 Instrument guidelines categorising MPHSD (courtesy of Heidelberg Engineering, Heidelberg, Germany)

MPHSD (µm)	Category	Description
< 20	1	Very good
20 - 30	2	Good
30 - 40	3	Acceptable
40 - 50	4	Look for way to improve
> 50	5	Low quality (do not use as baseline image)

MPHSD has been shown to be affected by media opacities due in part to aging and cataracts (Zangwill et al., 1997, Strouthidis et al., 2005b). This has been quantified with significant correlations of the MPHSD to scores of the severity of opacities of various types and at various locations in the eye using the Lens Opacities Classification System III (Chylack et al., 1993). In the first of these studies (Zangwill et al., 1997), an observer panel assessed, on a ten point scale, the quality of the 3 x 32 constituent CSLO optical sections composing a mean topography on 39 subjects. Here, significant correlation of the subjective image quality of the scanned pre-processed CSLO images to the MPHSD was strong (r^2=0.76). This is because in cases with high noise (light scatter) during the acquisition of CSLO optical sections, there will be inherit difficulties in accurately determining the position of maximum reflectance, and hence calculating the topographic height. Another study has shown MPHSD to be strongly correlated to the camera sensitivity of the device and spherically corrected astigmatism but not with age however (Sihota et al., 2002) though subjects with media opacities were excluded from this study.

Previous studies have used the MPHSD as a metric to evaluate the repeatability of the technology in normal subjects and glaucoma patients (Chauhan et al., 1994,

Rohrschneider et al., 1994). Most previous studies of detection of glaucoma in HRT images have used this metric as a criterion in selection of data for analysis. Below the 50μm threshold for acceptable images (table 4.1) evidence suggests that the diagnostic capability of the HRT is unrelated to MPHSD (Sung et al., 2009). Many studies have used 50μm as a cut-off for including or excluding images (Medeiros et al., 2006, Coops et al., 2006, Saito et al., 2009, Burgansky-Eliash et al., 2007) and others have used 40μm (Harizman et al., 2006, Zelefsky et al., 2006). The problems of screening a normal elderly population (aged 65 to 89 years) with HRT have been highlighted in a study containing 880 individuals which reported that 29% of images from subjects in a normal elderly population had MPHSD >50μm and were thus excluded from the study (Hawker et al., 2006). In this same study 53% of 95 glaucoma patients were also excluded due to MPHSD values >50μm.

Studies of glaucomatous progression detection in HRT images have employed MPHSD as an image quality selection filter (Kourkoutas et al., 2007, Bowd et al., 2009) using 50μm as a cut-off for selection. Another study has used a MPHSD of <35μm and iterative change in MPHSD between visits of <10μm as selection criteria for analysis (Saarela and Airaksinen, 2008) accounting for an exclusion of 18% and 16% respectively of eyes from the study.

MPHSD has been shown to be a good predictor of variability in stereometric parameters (Sihota et al., 2002) and in particular of variability in longitudinal and test-retest RA measurements (Owen et al., 2006) and as a means of stratifying and optimising progression analyses of these parameters (Fayers et al., 2007). The interpretation of MPHSD as with any image quality metric should be clear and consistent. Achieving a better understanding of factors influencing it and its

implications for the reliability of mean topography derived data is worthwhile both in clinic and for research studies.

The aim of this chapter is to investigate if the characteristics of topography and reflectance images before alignment and averaging affect the resulting variability of mean topography images. To do this an examination of simulated data in addition to an examination of real data is performed. Frequency domain (using Fourier analysis) and image gradient metrics are compared in both these simulated and real data to MPHSD values. Independent subjective grading of topography and reflectance image pairs by a group of experienced users of the HRT are also compared to MPHSD values. The agreement of the subjective assessments between graders was also quantified to measure the need for an objective quality metric of HRT images.

The work in this chapter was presented at the Image Morphometry and Glaucoma in Europe Meeting, Mannheim, Germany on January 13-14, 2006 and the Association for Research in Vision and Ophthalmology Meeting, Fort Lauderdale on April 30 - May 4, 2006.

4.1 Measurements of Image Variability

For this study, global variability is quantified using the MPHSD and local variability is quantified using PHSD maps and distributions. Real data acquired across a large range of imaging conditions are used for analysis. Simulated data are also used to test the specific hypothesis that the same misalignment noise applied to different images produces variability dependent upon characteristics of the initial image. This hypothesis has important clinical implications because two different eyes may have images that exhibit exactly the same amount of measurement noise but may yield very different MPHSD values because the features in the images are different. In HRT topography images these characteristics can be related to the underlying morphology of the ONH and the imaging quality of the optics of the eye.

4.1.1 Real Data

In examining quality of HRT image data, the topography and reflectance images of 74 eyes of 74 subjects from the same study detailed in Chapter 3 are analysed. In particular HRT Classic, baseline mean and (three) single topography and reflectance images of each selected eye are examined. As discussed previously, this dataset of images was deliberately enriched with eyes having a range of media opacities and thus the image quality range would also be expected to be large. The MPHSD values of all 74 topographies are calculated and outputted using the HRT Classic software. The PHSD maps of all 74 topographies are also calculated from the raw single topographies. Inter-quartile ranges are calculated for all PHSD distributions to quantify the 'spread' of these distributions. Examples of PHSD maps are shown for given mean topographies in Figure 4.3 along with the distribution of PHSD values.
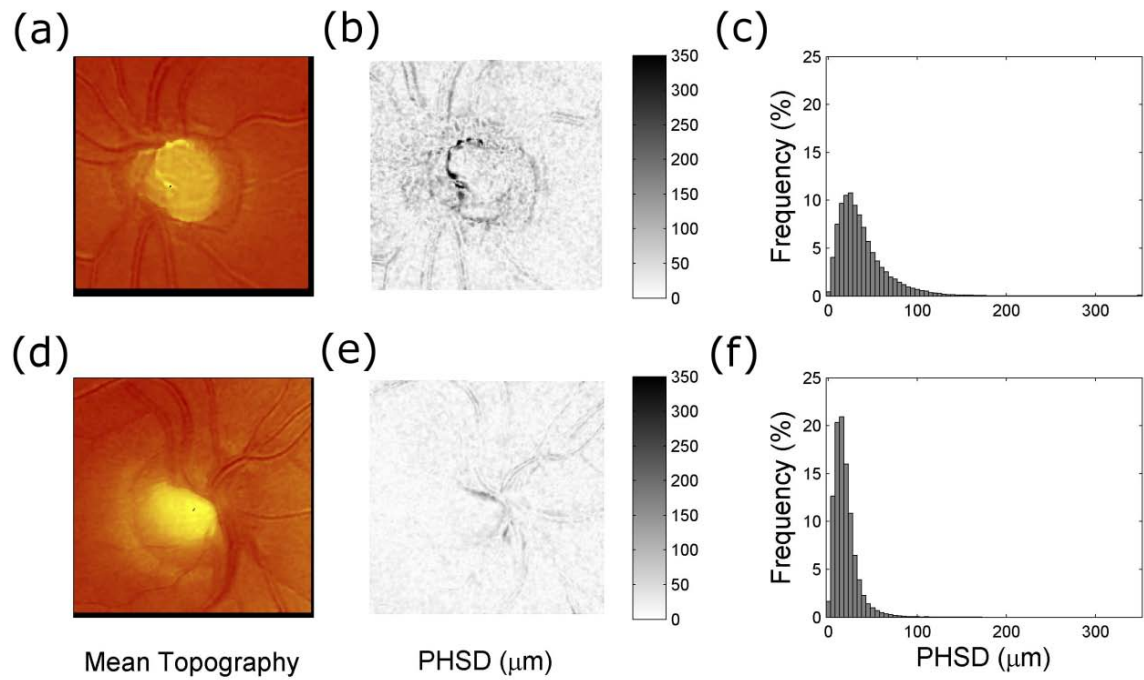
Figure 4.3 Sample mean topographies (a) and (d) with respective PHSD maps (b) and (e). PHSD distributions (c), (f) are also displayed. MPHSD values are 15μm and 30μm for (a) and (b) respectively.

MPHSD is also further examined as a predictor for two types of RA variability: overall series variability and the contribution of an individual topography's RA deviation to the variability of RA measurements in a series. The relationship of MPHSD to overall series variability is assessed by simply comparing the standard deviation of the 5 RA measurements to the mean MPHSD in the 5 mean topographies for each patient. Secondly, the predictive value of MPHSD values in identifying outlier measurements in a given series is examined. Firstly, the mean of the 5 values of RA is calculated and used as a best available estimate of the true RA for each patient. This mean is subtracted from each of the 5 individual RA measurements to give 5 deviations from the mean for each patient. These deviations are then normalised (dividing by the standard deviation of the 5 RA measurements) as a way to quantify the proportion or contribution of each RA measurement to the

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overall series RA variability. The five normalised deviations are then compared to the 5 matching MPHSD values normalised by the average MPHSD.

4.1.2 Simulated Data

The simulated images are derived in a similar fashion to a previously published approach (Patterson et al., 2005) Here, post-processed single topographies are used as seeds to propagate simulated single topographies and from these serial mean topographic images are derived. Noise, approximately mimicking the noise inherent in mean topography formation, is applied to the single seed topography producing simulated single topographies in a given series. The method for simulating images with known properties involved synthesising new single topographies from one 'seed' single topography by applying various types of noise. Firstly, random, small transformations composed from translational, rotational, shear and scaling parameters are applied to a seed single topography. Some of these transformations are visualised in Figure 4.4.

White noise (spatially independent pixel Gaussian noise) is added to each misaligned single topography with each pixel chosen from a zero-centred, normal distribution: $N(0, \sigma)$. The small random transformations along with white noise can be used to approximately model the main noise (sources of variability) at the image. The spread of each distribution from which each randomly sampled transformation parameter is sampled is estimated by trial and error. This is because it is impossible to measure the residual movement error that remains after the HRT alignment algorithm has been applied to all single topographies when forming a mean topography. Further details of how each transformation is implemented and

the probability distributions for parameters involved in each transformation are given in Appendix B.
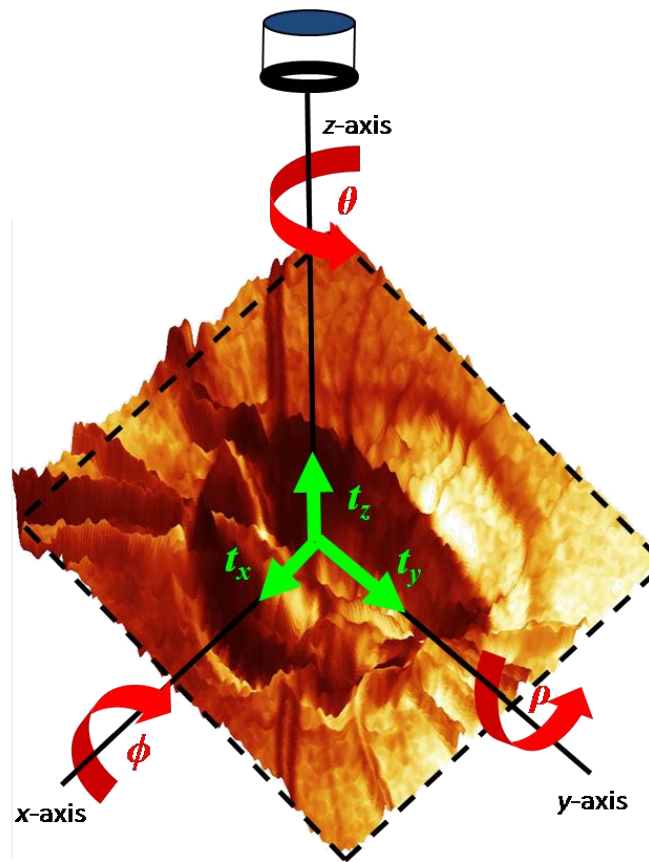
Figure 4.4 Sample HRT Classic topography displayed in three dimensions with reflectance intensity colour mapping. Misalignments due to translations (t_x, t_y, t_z) along and rotations $(\emptyset, \rho, \theta)$ about the (x, y, z) axes are shown. Measurements and translations in the z-axis are referred to as *axial* and those in the xy plane as *transverse*.

This simulated noise is applied to 74 single topography images, each from the baseline scan of an eye included in the real dataset. A simulated set of 3 single topographies is generated for each ONH by the application of an identical set of 3 noise components to each of the single topographies. It is from these 74 sets of 3 single topographies that 74 mean topography images are generated. The associated

individual pixel height standard deviation (PHSD) values are calculated at each pixel in all mean topographies. From these, a MPHSD value is calculated for the corresponding mean topography. For the purposes of examining the effect of misalignment and white noise on mean topography formation, noise applied across all seed single topographies is, therefore, controlled to be the same for each generation of a simulated single topography. This is demonstrated in Figure 4.5 for two sample series/seed single topographies.

The simulation was performed 50 times, creating 50 mean topographies for each eye. Thus, when averaged over many simulations, it becomes possible to examine the dependence of the MPHSD metric on the characteristics of the seed topographies alone.

Two types of simulated noise will produce variability independent of the image itself. These are axial translations and white noise. In an attempt to identify a minimal level of noise which is independent of the optic disc morphology, different combinations of white noise and axial translational noise (by varying parameters σ and t_z) are applied to 'blank' images (pixel values=0) the same size as HRT Classic single topographies (256 x 256) to produce MPHSD values. The assumption is made that the minimum MPHSD values observed in the real mean topography data will be mostly occurring in well-aligned, 'low feature' images. Under this assumption, values for σ and t_z are selected to produce this minimum MPHSD. Different MPHSD values for varying parameters σ and t_z are plotted in Figure 4.6. The minimum observed MPHSD value (see section 4.3 and Figure 4.10) observed in this study's real mean topographies would thus be used to determine what values to choose for parameters σ and t_z along with the results in Figure 4.6.
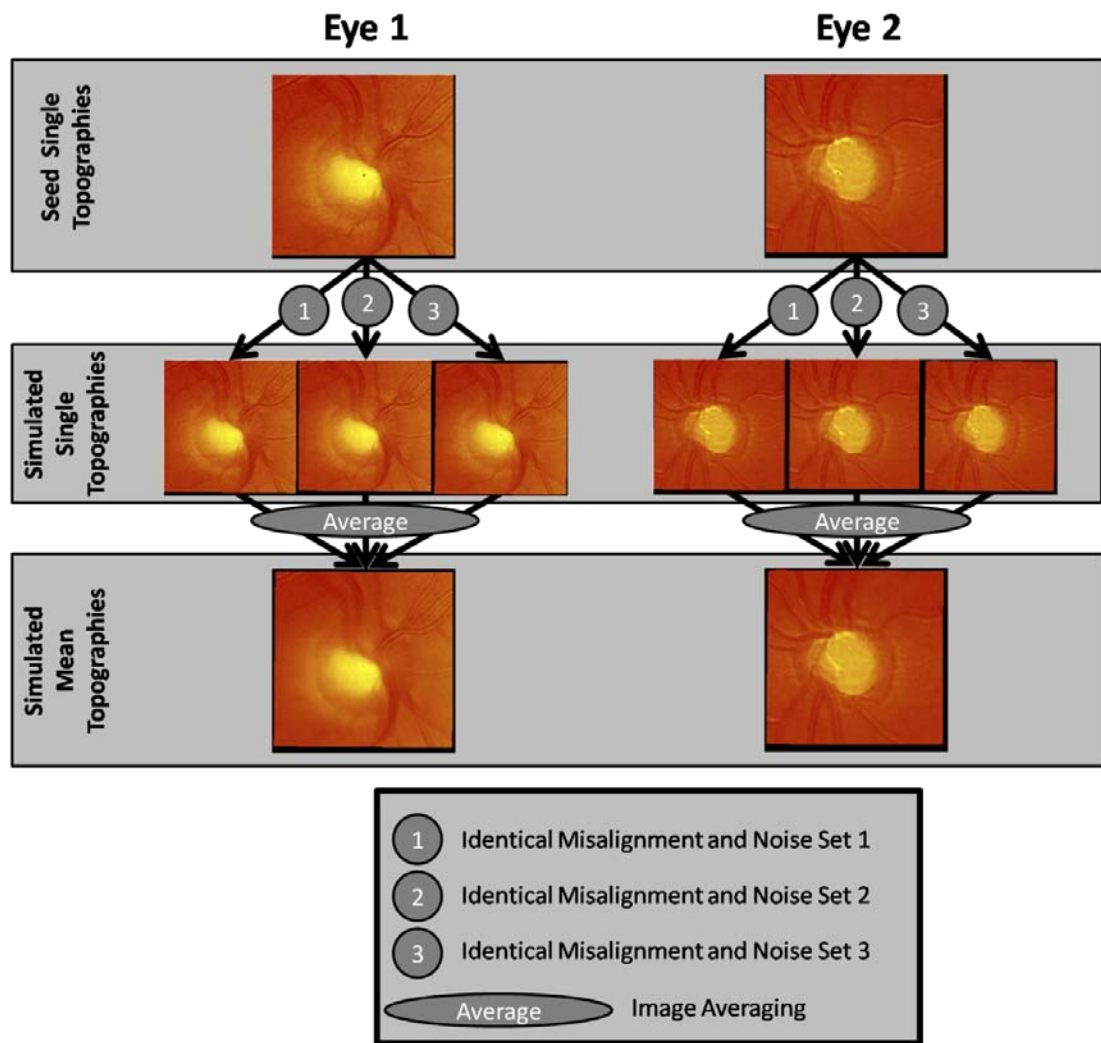
Figure 4.5 The simulation schematic with three different, random misalignment and noise sets applied to a seed single topography to produce three simulated single topographies for two sample eyes. In this example each set applied is **_identical_** for both eyes and this is the case across all seed topographies in the analysis.

As previously stated, it would be impossible to measure any post-alignment remaining misalignment. The simplifying assumption is made that the simulated misalignments and noise are close to the residual misalignment after registration between real single topography and reflectance images. Thus these transformations are small enough to not require re-alignment as would be the case for real data. As single reflectance images are only used in the alignment stage (see Section 1.2) of

image formation of the mean topography and thus the MPHSD, they are not

analysed for simulated data.
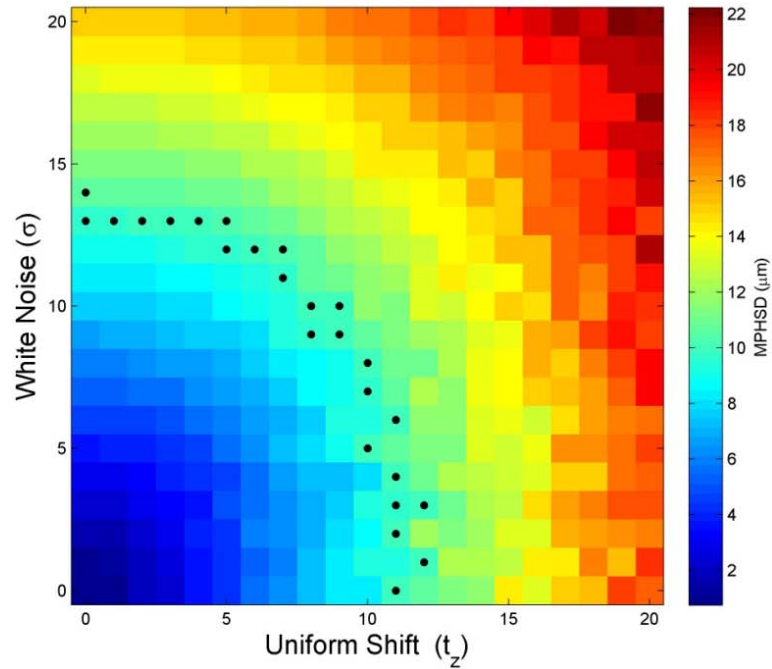
Figure 4.6 The relationship of MPHSD of a 256 x 256 'flat' image with different

levels of morphologically independent noise added. Boundary points where

MPHSD values are produced corresponding with the minimum MPHSD observed

in real data (10μm) are indicated.

4.2 Measurements of Image Characteristics

Three approaches are used in measuring the relationship of the MPHSD to image characteristics of the reflectance and topography images. These approaches use information about (i) the underlying spectrum in the images, (ii) the gradients in the images and (iii) the quality assessment of a panel of experienced HRT users.

4.2.1 Image Frequency Analysis

In image processing, Fourier analysis is a standard tool for examining, modifying and characterising images. Its theoretical basis is that any signal can be decomposed, using a *Fourier transform*, into a series of component sinusoidal signals of different frequencies and amplitudes and this can be represented in the frequency domain. In the frequency domain the amplitudes of the signal's various component frequencies can be represented in a *power spectrum* and this allows an examination of the relative amounts of each frequency component in a signal. In a one-dimensional signal, such as a sound or an electrical signal, the power spectrum is one-dimensional and the frequencies related to time (the signal changes with time). For a two-dimensional signal, as in the case of an image, the power spectrum is two-dimensional with the direction of the frequency component indicated along with its amplitude (see Figure 4.7 and Figure 4.8 (a)-(d)). These frequencies are related to spatial units (the image changes with position). An illustrative simple case is shown in Figure 4.7 where (a), (b) and (c) are images of vertical bars, a face and a galaxy respectively. In (a), there are no features in the vertical axis so the power spectrum (d) contains frequency amplitudes on the horizontal axis only. It is often easier to objectively characterise the type and number of features and the noise present in images and to quantify underlying differences between images in the frequency

domain and this is illustrated in Figure 4.7. More technical details on Fourier

analysis in image processing can be found in (Bovik, 2009) and (Smith, 2003).

Fourier analysis is performed on each of the three constituent single topography

images of a mean topography image for real data and on the seed single topography

images for simulated data. As the reflectance image is used for alignment in the

HRT software, Fourier analysis is also performed on the three constituent single

reflectance images of a mean reflectance image for real data. It has been shown that

different types of images can be categorised by their Fourier power spectra

(Torralba and Oliva, 2003). In particular, images of natural scenes have been shown

to have characteristic power spectra (Ruderman and Bialek, 1994). The summed

spectral magnitude density of each image is calculated (average of the power

spectrum across all pixels except the 0th frequency representative of the average

intensity of the image). The radial-averaged spectrum density (RASD) of each

appropriate image is calculated. This involves averaging all possible directional

power spectra in discrete radial intervals and provides a convenient means to

compare two dimensional spectra in one dimension. The RASD centroid is

calculated to indicate the frequency at which 50% of the spectral energy is

distributed on either side; it is analogous to a median of a distribution or the 'centre

of mass' of the RASD and has previously been used for dimensional signals used as

a means of characterisation in music perception (Schubert et al., 2004), where it has

been shown to correlate well with the perceived timbre in experiments using

computer-generated multiple instrument sounds. It is also used, among other

measures, as an indicator of the depth of anaesthesia when monitoring patient

electroencephalogram (EEG) readings during surgery (Schwender et al., 1996,

Nieuwenhuijs et al., 2002, Drummond et al., 1991); in this field it is referred to as the

spectral edge frequency. Examples of topographic and reflectance images, their

respective power spectra and RASD along with the centre of mass analysis are given in Figure 4.8.
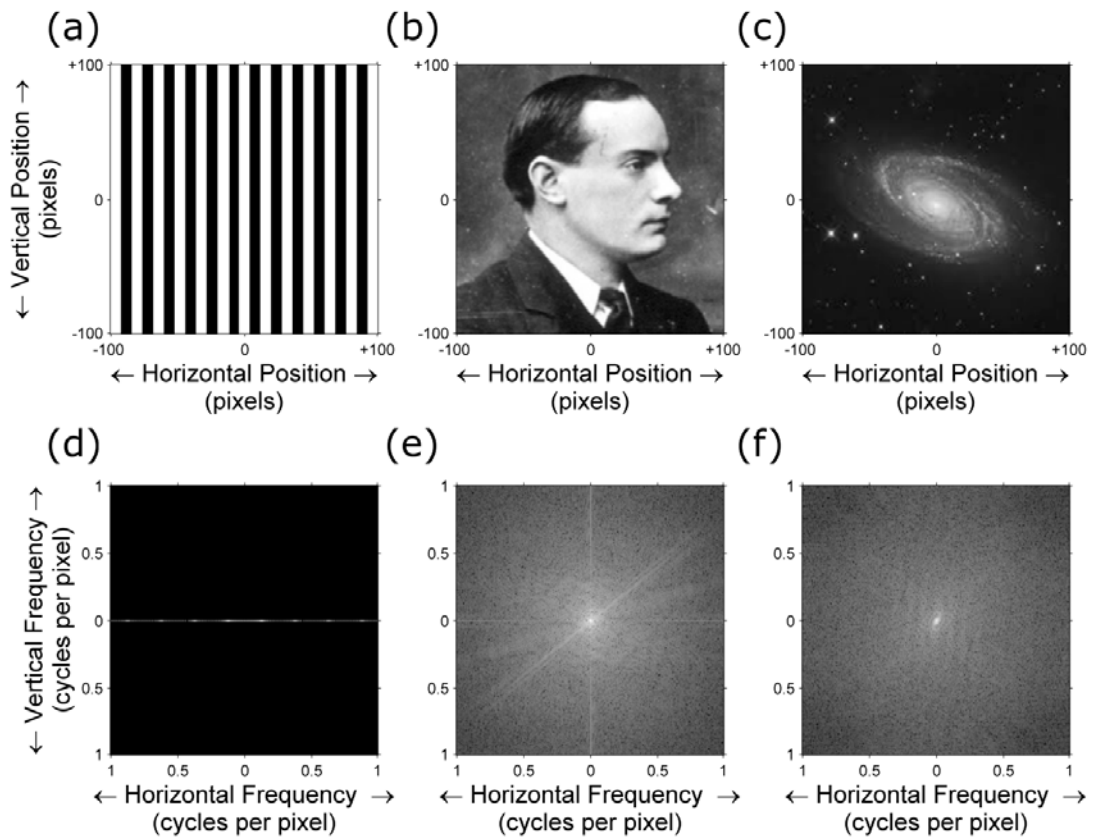
Figure 4.7 Fourier analysis example of (a) an image with periodic vertical stripes, (b) photograph of Pádraig Mac Piarais in side profile, (c) telescope image of the M91 galaxy, (d) power spectrum of (a), (e) power spectrum of (b), (f) power spectrum of (c). The brighter the points in (d), (e) and (f) indicate the higher amplitude of a given frequency - lower frequencies are located towards the centre of these images and higher frequencies towards the edges. Note that log scales for the intensity are used for (d), (e) and (f) as the proportions of frequency components at the centre (representing the average of the signal) and at key characteristic frequencies are much higher than elsewhere.
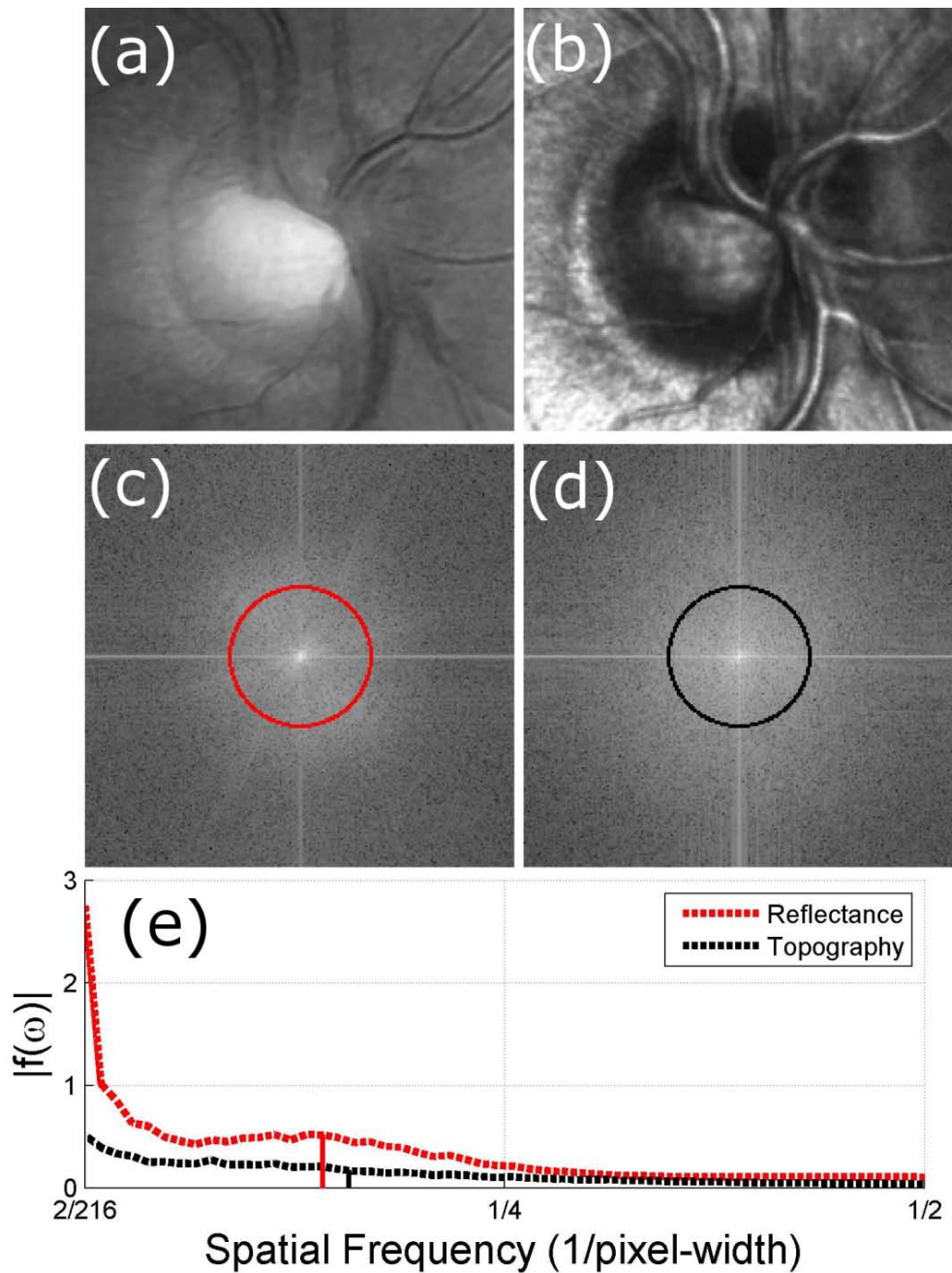
Figure 4.8 Fourier analysis, example of (a) single topography image, (b) single reflectance image, (c) magnitude of frequency spectrum of single topography image, (d) magnitude of frequency spectrum of single reflectance image, (e) RASD (dashed lines) with solid vertical lines representing the centroid for the reflectance and topography images. The position of each RASD centroid is also marked on images (c) and (d).

4.2.2 Image Gradient Analysis

Fourier analysis will provide an estimate of how global characteristics of the image influence MPHSD measures. The occurrence of higher or lower variability at certain spatial locations in a HRT image should also be described and accounted for. Previous studies have qualitatively noted the matching locations of anatomical features such as vessels and the ONH cup margin in HRT topographies with relatively higher locations of variability across single topographies composing a mean topography (Chauhan et al., 1994, Chauhan and McCormick, 1995).

One study (Brigatti et al., 1995) calculated "steepness" maps measuring local spatial variability and variability across single topographies (in a 3 x 3 x 3 pixel neighbourhood) at each pixel. Some correlation between the matching pixel locations of the steepness maps and the PHSD maps was reported. The calculation of these steepness maps however was limited as it ignored the potential spatial nature of the variability in the pixel neighbourhood. In addition by including the variability across single topographies in the steepness calculation it did not make a valid calculation of the local spatial variability.

A preferable approach would be to calculate the gradients along transverse x and y axes at each pixel for each single topography and reflectance image. The combined square root of the mean squared sum of these two gradient components is calculated to provide a scalar value representing the local gradient magnitude (GM) about each pixel. The GM can be visualised as the maximum slope magnitude of a plane fitted to the 3 x 3 pixel neighbourhood about each pixel.
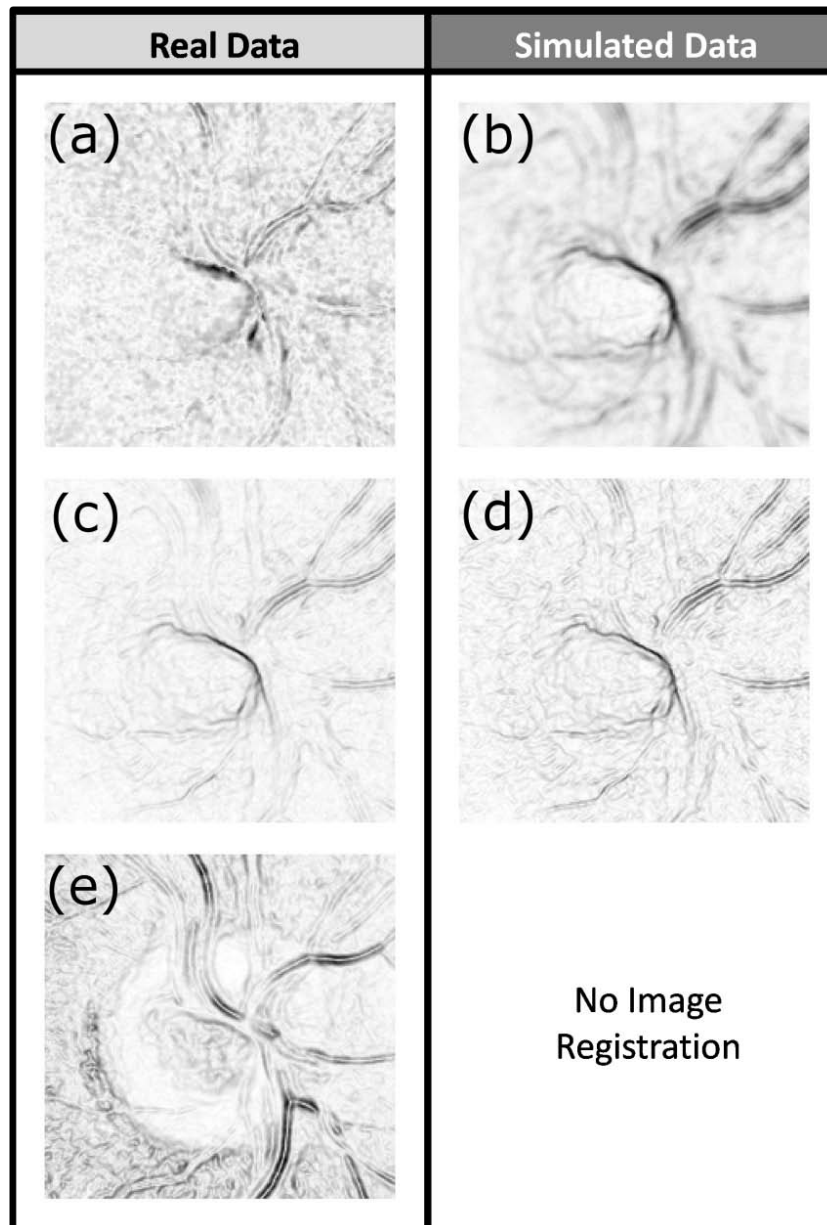
Figure 4.9 Gradient analysis, example of (a) mean real topography PHSD map, (b) mean simulated topography PHSD map, (c) average GM map of constituent real single topography images, (d) GM map of seed single topography image, (e) average GM map of constituent real single reflectance images. For real data, cross-correlation coefficients are 0.41 between maps (a) and (c) and 0.25 between maps (a) and (e). For simulated data, cross-correlation coefficients are 0.64 between maps (b) and (d). Note: Grey-scale maps have equal ranges across rows of this Figure but not along columns.

For a given mean topography, the GM maps for all constituent single topographies and related single reflectance images are compared to the related PHSD map using image cross-correlation. The similarity of two images by cross-correlation is quantified by the maximal, normalised cross-correlation coefficient. For simulated data, as no reflectance images are involved in the generation of a simulated mean topography, only GM maps of the seed single topographies are compared to PHSD maps. An example of this analysis for real and simulated data is given in Figure 4.9.

4.2.3 Subjective Assessment

Six experienced users of the HRT, including glaucoma fellows and glaucoma specialists (referred to as *graders*) were presented with 28 PowerPoint slides, each containing a sample HRT reflectance and topography image displayed as a pair. These 28 image pairs were chosen on a semi-random basis - it was required that they be representative of the image quality distribution in the overall sample - and the MPHSD distribution in this sub-sample was compared to the MPHSD distribution in the overall sample. Graders were asked to classify the quality of each image pair on a discrete scale of 1 to 5. Score 1 is described as 'Best' quality and score 5 as 'Worst' quality. No other criteria were given for image quality assessment. The graders were asked to repeat this scoring with the same series in a randomised order and blind to their previous scores. A linearly weighted Cohen's κ score is used to measure the pair-wise agreement between all observers and within observers.

A panel score is also calculated for each image pair from the mean of each observer's mean score. These panel scores are compared to MPHSD values to assess the correspondence between the clinician's confidence in image quality and the objective measurement of MPHSD. Frequency and gradient metrics as described

previously are also calculated for these 28 mean reflectance and topography image pairs. These metrics are combined equally (normalised so that reflectance and topography images would have equal weighting when their respective metrics are averaged). These averaged metrics for each pair are compared to the panel subjective scores of quality.

4.3 Results

Figure 4.10 shows the distribution of MPHSD values for the HRT Classic real dataset. Values of MPHSD are observed over a large range 10 - 119μm. Also shown is the distribution of MPHSD values for the sub-sample of HRT Classic real data used for subjective observer analysis.
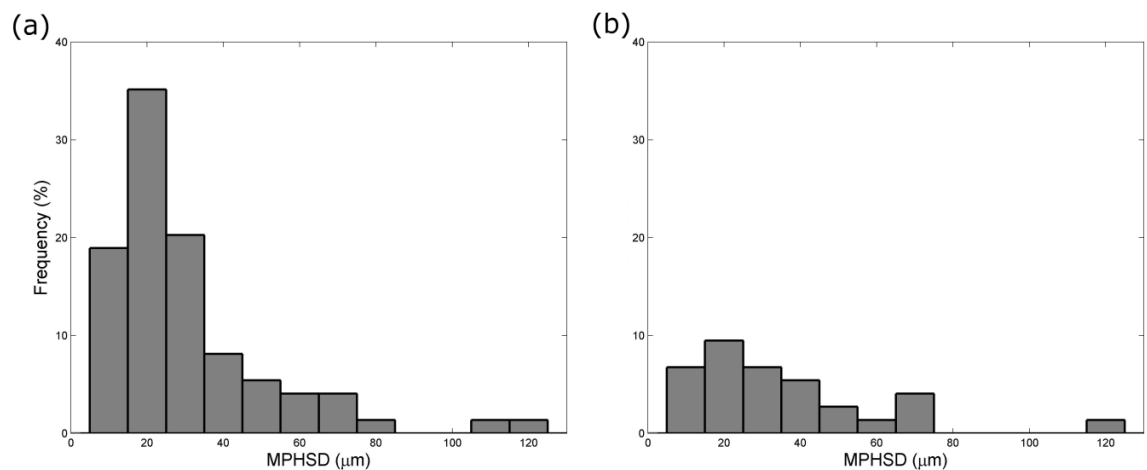
Figure 4.10 (a) Distribution of the MPHSD values for all 74 HRT Classic baseline mean topographies. (b) Distribution of the MPHSD values for a subset of 28 randomly selected topographies from all 74 HRT Classic mean topographies.

All distributions of PHSD values for all mean topographies are bounded below by zero. As such, all distributions are found to be positively skewed. Mean topographies with MPHSD values in the upper ranges exhibited PHSD distributions

with a larger spread (as assessed by the IQR) than those of lower MPHSD values
(Figure 4.11).
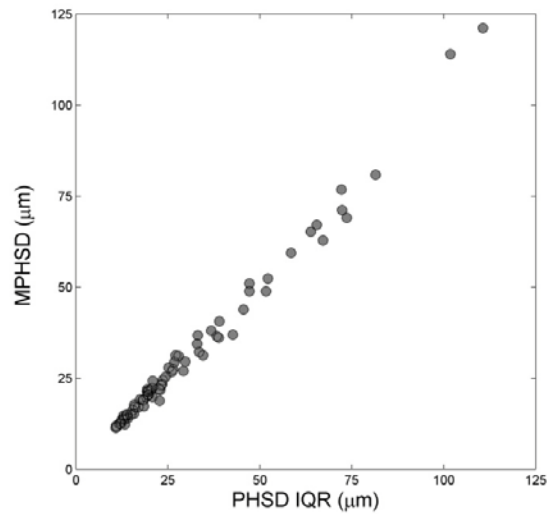
Figure 4.11 MPHSD values compared to inter-quartile range (IQR) values for PHSD
distributions.

Figure 4.12 shows the effects of MPHSD on RA variability. For the series average
setting (a), a strong linear relationship is observed between series RA standard
deviation and the series averaged MPHSD: Pearson's sample correlation coefficient,
r=0.59 (p<0.001). For the individual image setting (b), only a very weak linear
relationship is observed between the individual image MPHSD as a fraction of the
mean series and the image deviation from the RA best available estimate (series
mean RA) as a fraction of series RA standard deviation (Pearson's rank correlation
coefficient, r= 0.15 p=0.003).
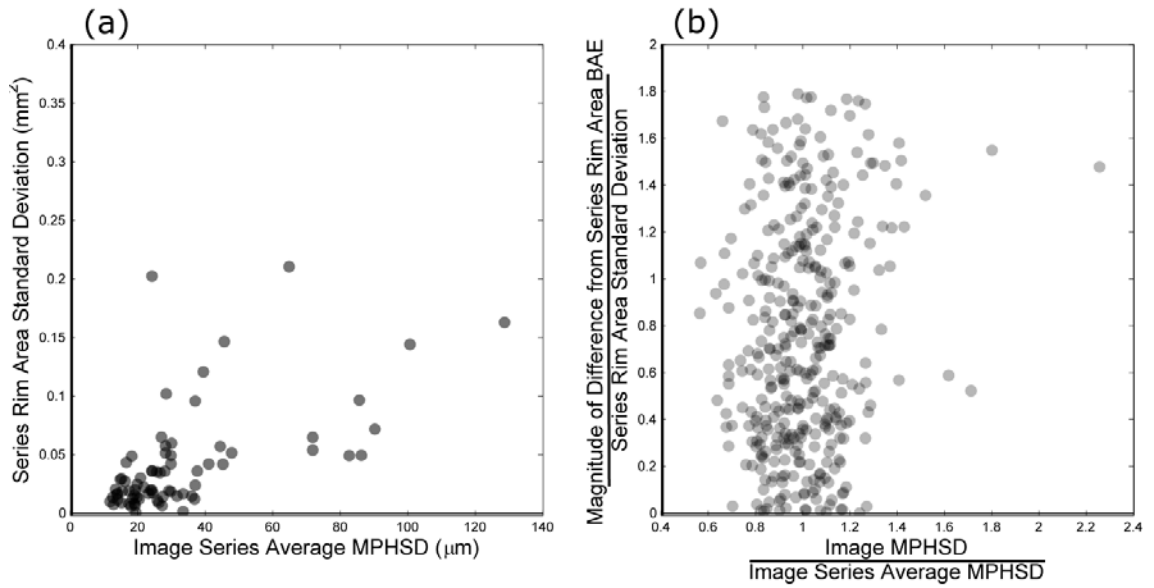
Figure 4.12 (a) Series RA standard deviation values plotted against series-averaged MPHSD values. (b) The magnitude of difference of individual RA measurements from the series RA best available estimator (BAE) - as calculated by the series average RA measurements – as a fraction of series RA standard deviation plotted against MPHSD values as a fraction of series-averaged MPHSD values. Areas of a higher density of points are represented by darker shading.

For the simulation, the range of MPHSD values varied considerably ($18 - 116\mu$m) despite identical noise being applied to generate all mean topographies. The range of MPHSD values for simulated data is similar to that of real data but poor agreement is observed with increased real mean topography MPHSD values as seen in Figure 4.13.
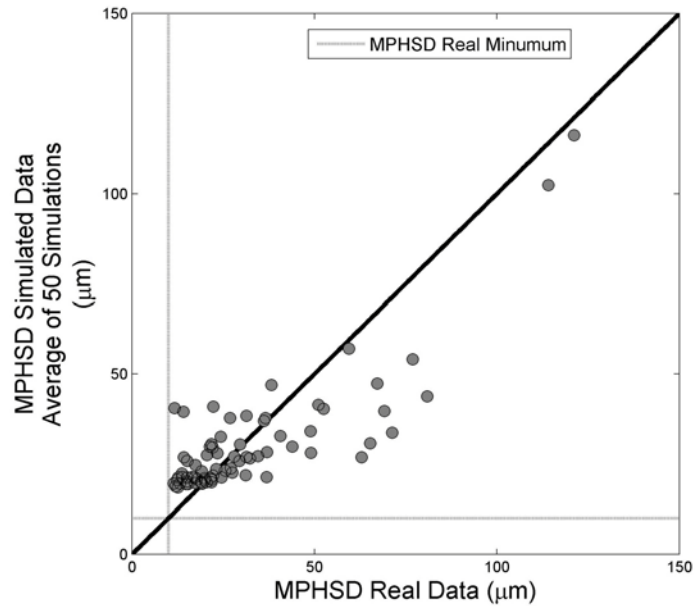
Figure 4.13 MPHSD values for real and simulated mean topographies. The minimum observed MPHSD (10μm) in real mean topographies is plotted as a lower bound for simulated mean topography MPHSD. Pearson's sample correlation coefficient r: 0.79 (p<0.001), MPHSD real – MPHSD simulated mean: 7.6 μm, standard deviation: 13.7 μm.

Fourier analysis revealed a strong significantly positive dependence of the MPHSD value on the RASD centroid and the summed spectral magnitude density of "pre-averaged" single topography for both real and simulated data. Simply put the MPHSD of mean topographies is affected by the characteristics of the component images. In addition, for real data, there is a strong significantly positive dependence of the MPHSD value on the RASD centroid and a negative dependence of the MPHSD on the summed spectral magnitude density of "pre-averaged" single topography for both real and simulated data. Figure 4.14 illustrates these relationships.

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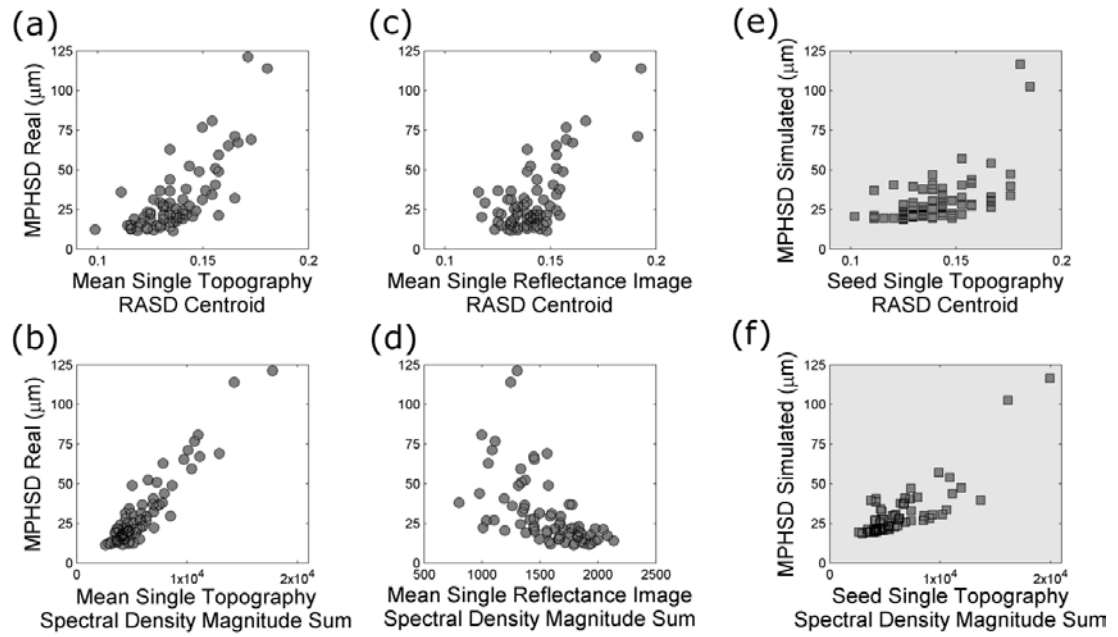
Figure 4.14 Fourier metrics of constituent single images compared to MPHSD values. Pearson's r correlation coefficients are for (a) 0.74, (b) 0.93, (c) 0.69, (d) -0.55, (e) 0.58 and (f) 0.58.
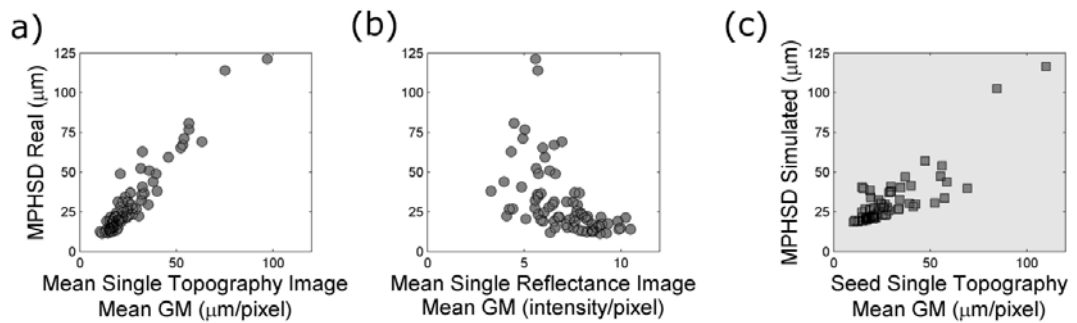
Figure 4.15 Measure of the average GM of constituent single images compared to MPHSD values. Pearson's r correlation coefficients are for (a) 0.95, (b) -0.51 and (c) 0.85.
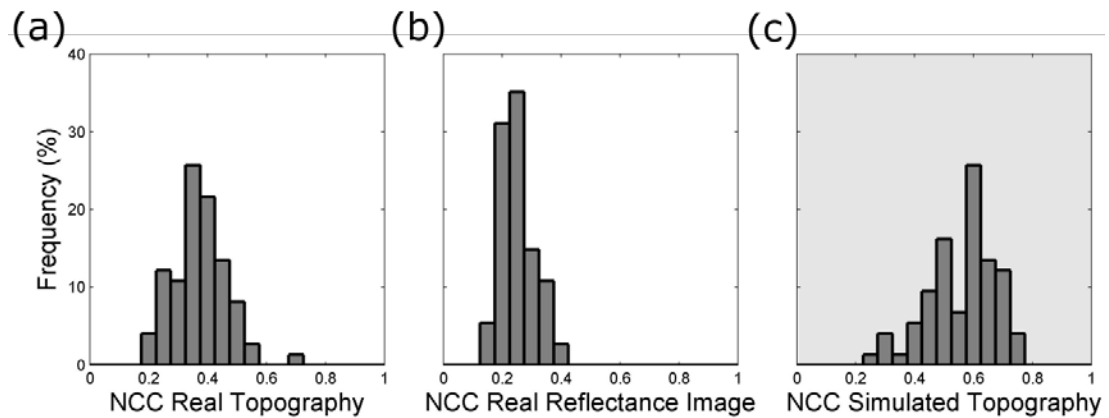
Figure 4.16 Distributions of (a) averaged measure of the NCC of real mean topography PHSD maps and GM maps for each constituent single topography (b) averaged measure of the NCC of real mean topography PHSD maps and GM maps for each constituent single reflectance image and (c) averaged measure of the NCC of multiple simulated mean topography PHSD maps and GM maps for single seed topographies. Means of distributions are 0.375, 0.25 and 0.56 for (a), (b) and (c) respectively.

Both locally and globally, there is a measurable relationship of MPHSD to image gradient measures of "pre-averaged" single topographies. On a global scale, for simulated data and real data, there is a strong positive relationship between GM mean values averaged across single topographies and MPHSD values. For real data, there is also a negative relationship between the GM mean values averaged across single reflectance images and the MPHSD. Figure 4.15 displays these relationships. On a local scale means of NCC values in comparing maps of PHSD maps and GM maps of single topography and reflectance images are 0.37 and 0.25 respectively for real data. For simulated data, the mean of NCC values for comparisons of PHSD maps and GM maps of single topographies is 0.45. NCC distributions for all 74 scans are presented in Figure 4.16.

Within and between-observer agreement among clinicians quantified by linearly weighted κ values are shown in table 4.2 including mean between observer agreement and mean within observer agreement (repeatability).

Table 4.2 Agreement between the subjective assessments of the image quality of HRT mean topography and reflectance image pairs by 6 observers (A – F). Quality assessments were made over a subset of 28 image pairs. Agreement is quantified by use of linearly weighted κ values. Observers were asked to repeat their quality assessments on the same (but reordered) subset (1 – 2). Within observer agreements are in indicated by the italic font. The strength of agreement is indicated by colour - red represents the lowest agreement and green the highest.

Observer												
	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
A1		*0.78*	0.58	0.75	0.37	0.51	0.50	0.61	0.61	0.53	0.61	0.52
A2			0.61	0.78	0.49	0.59	0.62	0.64	0.55	0.57	0.65	0.65
B1				*0.67*	0.45	0.49	0.63	0.66	0.37	0.36	0.54	0.48
B2					0.54	0.60	0.58	0.70	0.52	0.59	0.81	0.66
C1						*0.76*	0.51	0.53	0.26	0.46	0.64	0.61
C2							0.59	0.60	0.41	0.62	0.79	0.69
D1								*0.82*	0.38	0.41	0.55	0.48
D2									0.48	0.51	0.66	0.50
E1										*0.62*	0.47	0.42
E2											0.64	0.58
F1												*0.75*
F2												

Median Within Observer
0.76

Median Between Observer
0.58

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The relationship between observer image quality panel scores and MPHSD values is displayed in Figure 4.17 (a). Equally weighted combined Fourier metrics (RASD centroid) and gradient metrics (mean GM) for reflectance and topography image pairs are plotted in Figures 4.17 (b) and (c) respectively. Examples of four mean topography and reflectance image pairs are given in Figure 4.18. The associated panel scores, the standard deviation of observer scores (averaged over repeat assessments) and MPHSD values are also included.
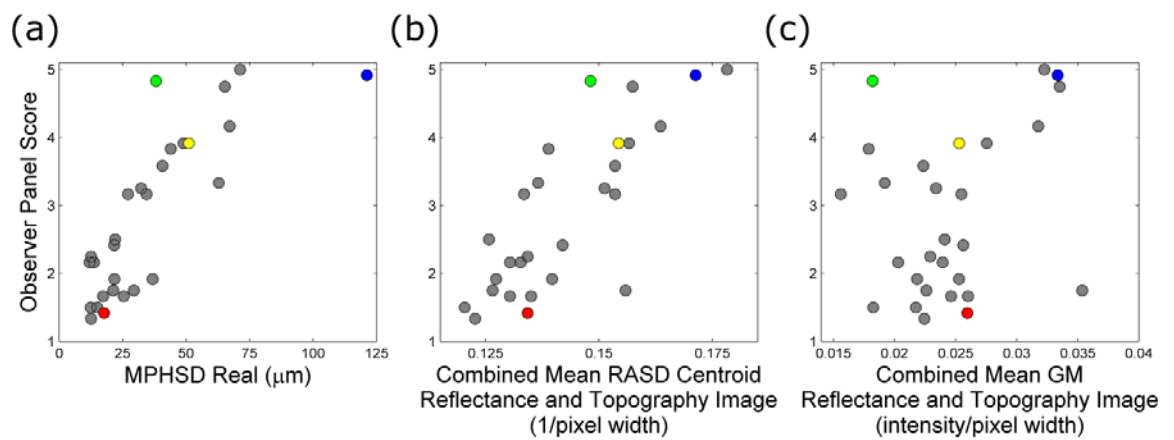
Figure 4.17 Subjective observer assessed panel scores of image quality of mean topography and reflectance image pairs compared to (a) MPHSD, (b) reflectance/topography image combined RASD centroid measurement and (c) reflectance/topography image combined mean GM. Coloured points on figures correspond to those examples in Figure 4.18. Pearson's r correlation coefficients are for (a) 0.81 (b) 0.81 and (c) 0.28.
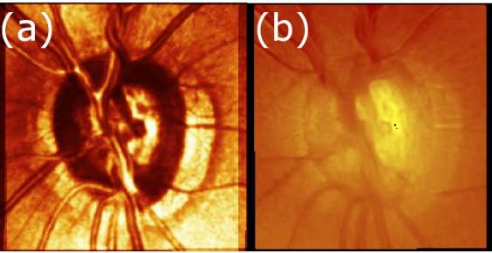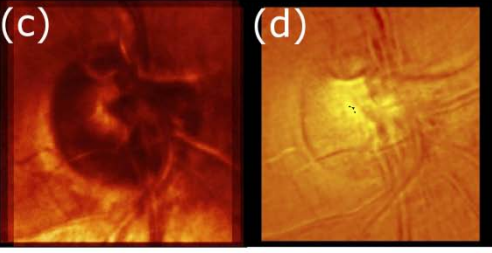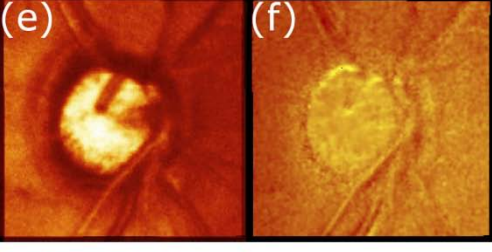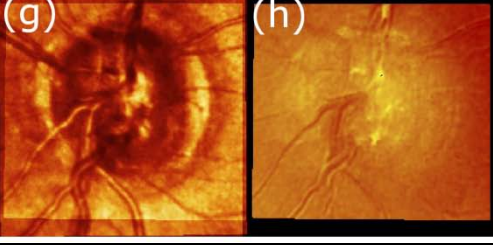
Reflectance-Topography Image Pair	Mean Panel Score (Rounded)	MPHSD (Category)	Panel Score SD	Plot Symbol
(a) (b)	1.42 (1)	18μm (1)	0.51	🔴
(c) (d)	4.83 (5)	38μm (3)	0.39	🟢
(e) (f)	4.92 (5)	121μm (5)	0.29	🔵
(g) (h)	3.92 (4)	51μm (5)	0.90	🟡

Figure 4.18 Examples of reflectance-topography image pairs (a)-(b), (c)-(d), (e)-(f) and (g)-(h) presented to experienced Heidelberg Retina Tomograph operators with mean panel scores (rounded to nearest category) and standard deviation of scores across all observers. MPHSD values and manufacturer supplied categories for these values are displayed. Coloured symbols are used to represent these examples in Figure 4.15. (SD: standard deviation).

4.4 Discussion

In case finding using medical images, a measurement of image quality is necessary to assess how adequate any evidence (or lack thereof) in the image is for diagnostic purposes. The effect of a degraded image quality on clinical decision making is illustrated in the opening example of Figure 4.1, a case no doubt where a metric showing poor image resolution would have perhaps led to an immediate second scan in the first review of the patient. In the assessment of glaucomatous progression, the confidence in any change detection algorithm will need to incorporate an objective, easily understandable measure of image quality which is robust and accurate on a local and global scale.

Below the 50μm threshold for acceptable images evidence suggests that the diagnostic capability of the HRT is unrelated to MPHSD (Sung et al., 2009). Image quality assessments in glaucoma detection and monitoring of structural changes in the retina and ONH are not just relevant to HRT imaging. RNFL assessments using OCT have been shown to be significantly affected by image quality; measurements of RNFL thickness have been shown to be correlated to image quality metrics (Sung et al., 2009, Balasubramanian et al., 2009).

The results of this and previous studies (Owen et al., 2006, Sihota et al., 2002) show that high average MPHSD values for a series of HRT derived RA measurements are predictive of higher RA variability. However this study also found that, in a series itself, a mean topography with a relatively higher MPHSD value than the other mean topographies had little relatively higher contribution to RA variability in that series. In the context of glaucoma progression studies, these findings mean that a series of RA measurements can be more helpfully assessed with the MPHSD as an

indicator of the overall series variability; this concept has been used in a previous study to optimise a HRT RA progression analysis (Fayers et al., 2007). Conflictingly, a MPHSD measurement of a mean topography may serve little use as an individual weighting parameter for its associated RA measurement in analyses of change such as a weighted least squares regression or a Chi-squared regression of RA over time or in the identification of outlier RA measurements in a series.

The MPHSD values of mean topographies of different ONH and surrounding area morphologies in different scanning conditions (due to different media opacities and aberrations) fall within a large range. Significantly, a large component of this variability is due to the underlying characteristics of the pre-averaged single reflectance and topography image sets. In short, the MPHSD is affected by the features in the pre-averaged images and is therefore not a wholly independent measure of image quality. MPHSD values are higher for mean topographies whose constituent single topographies produce higher image metrics indicating the amount of signal 'energy' (spectral density magnitude sum), and the image-averaged localised gradient about each pixel (mean GM). This has implications for the clinical use of this metric, especially when it is used, for example, in population based studies as a threshold upon which patients are included or excluded.

A simulation was developed to illustrate that, when equal misalignment noise is used to generate new single topographies, a systematically large range of MPHSD values of the resulting simulated mean topographies values is demonstrated. This confirms that misalignment noise interacts with the single topography structure in producing MPHSD values: MPHSD is not a true measure of misalignment noise between single topographies. Quantitative evidence is also provided of the relationship of pixel neighbourhood gradient maps of single topographies and the

PHSD maps i.e. evidence of the correspondence between local areas with high gradients (or edges) and high PHSD areas. This leads to the somewhat obvious conclusion that 'movement'   applied to less 'flat' images, with less homogeneity, will result in higher global variability due to increased local variability in the locations of higher gradients.

Where the directionally-independent median frequency of the spectral density magnitude distribution (RASD centroid) is higher in single topography and reflectance images the MPHSD of mean topographies is also higher. From this one can infer that the higher the proportion of high frequencies in a single topography set, the higher the MPHSD for the resulting mean topography. These high frequencies can come from real structures such as the sharp edges of vessels and the cup/rim margin but also from image acquisition background noise from light scatter in eyes with high levels of media opacity.

The HRT alignment software uses the features in single reflectance images to assess how best to align single topographies before averaging to form a mean topography. Some reflectance image metrics (spectral density magnitude sum and mean GM) are negatively correlated to MPHSD values. This suggests that, the higher the values of these metrics, the higher likelihood that features in reflectance images are correctly detected and single topographies are better aligned reducing the MPHSD.

Observers, even those trained to use the HRT and interpret its output data, can have poor agreement with each other (weighted Cohen's κ lower limit of 0.26 and median of 0.58) in assessing the quality of output HRT topography and reflectance image pairs. Moreover, the repeatability of image quality judgements by expert observers is only moderate (median κ=0.76) as measured by agreement with a

second grading of the same set of image pairs by each observer. The between-observer median κ value in this study is comparable to the between-observer median *unweighted* Cohen's κ of 0.55 in a study of clinicians assessment of the quality of 100 fundus photographs (Bartling et al., 2009). Both between-observer and within-observer agreement measures for HRT image quality grading are no better than the decisions of clinicians when assessing monoscopic and stereoscopic photographs for the presence of glaucomatous damage in another study (Varma et al., 1992). Here, the median weighted κ for within-observer agreement was 0.79 for both monoscopic and stereoscopic conditions. For between-observer agreement, the median weighted κ values were 0.57 and 0.67 in monoscopic and stereoscopic conditions respectively. This reinforces the need for an objective and robust quality metric to help the HRT user. The panel-averaged scores had a good correlation with the MPHSD values and metrics of the spectral domain (r^2=0.66 for both) showing that these metrics can make similar assessments of image quality as the pooled expertise of observers.

The simulation used in this analysis is developed using a heuristic approach aimed at generating mean topographies with MPHSD values consistent with those of the relevant real topographies when these real mean topographies had low MPHSD values. This is because parameters governing the misalignments between single and mean topographies are difficult to measure. Despite this approach, the simulation is unable to consistently simulate mean topography MPHSD values observed in real mean topographies across the full range of values. Specifically, variability tended to be less well reproduced within mean topographies with higher MPHSD. This serves as a motivation for the further development and testing of more sophisticated

means of simulating the image acquisition and computation processes involved in mean topography generation by the HRT device and software.

MPHSD is a simple and easy to appreciate metric which gives a measure of the global difference between three single topographies composing a mean topography. However it is not an accurate estimate of misalignment between single topographies and is affected by the underlying anatomical features in the image. The provision of more specific metrics such as the level of 'success' of topography alignment and the optical quality of images in the CSLO stack, both independent of the underlying morphology, would improve the acquisition and post-hoc analysis of mean topography images. A robust segmentation to automatically identify the anatomical features and separate them from high frequency noise in the topography would help to establish the optical quality of the imaging system. These would provide information as to how close the variability of a mean topography is to its optimal lower limit given fixed scanning conditions i.e. with minimal eye movement, spherical and cylindrical correction and adequate illumination. For example, at acquisition, an identification of poor alignment could help with deciding to scan again attempting to minimise eye movement, whereas an identification of poor optical quality would help to conclude that inferences from imaging data may be limited from the outset.

This analysis aims to provide a better understanding of the factors which influence MPHSD, the most widely used and the only software-supplied measure of within mean topography variability. It leads to the conclusion that additional metrics could give a clearer message to the clinician or scientist when deciding how to appropriately interpret and use data from HRT images.

5. Simulation of Series of Optic Nerve Head Images

As seen in Chapter 2 and in previous literature, when stereoscopic disc photograph assessment or visual field progression are used as the reference standard for progression/stability, there is poor discrimination between stable and progressing eyes by HRT progression algorithms. This apparent poor discrimination may be due partly to poor performance of the algorithms and partly to inaccuracies in the reference standard itself. Furthermore, poor between-expert agreement in identifying progression based on stereoscopic photographs in previous studies (Coleman et al., 1996, Azuara-Blanco et al., 2003, Jampel et al., 2009) (and also exhibited by the frequency of eyes requiring adjudication in Chapter 2) illustrates the short-comings of stereophotography as a reference standard. For this reason, the concept of a simulation mimicking stable serial images is a hugely useful one. Progression algorithms can be applied to the simulated stable series and criteria selected to obtain low false positive rates and the false positive rates of different progression algorithms can be compared with each other, knowing that the image series are truly stable.

Physical eye models have been created from synthetic materials to investigate the accuracy of the CSLT to image certain structures (Dreher and Weinreb, 1991) and in-vitro fixed model eyes can helpfully examine the characteristics of reflected laser light from retinal tissue (Rakebrandt et al., 2003). Insights on potential artefacts in the topographic fitting procedure for CSLT have been provided in other applications of this model eye approach (Bartsch and Freeman, 1993). Realistic and

sophisticated computer simulation is common in other areas of medical imaging, in particular neuro-imaging (Collins et al., 1998, Kwan et al., 1999). In these fields it is widely considered the best way to validate automated post-processing algorithms, such as assessing the performance of the alignment of non-rigid images and volumes over time (Crum et al., 2004, Holden et al., 2002). However, with regard to the detection of glaucomatous progression, the practice of realistically simulating longitudinal image series has been neglected, with some exceptions in SLP (Vermeer et al., 2006) and elementary work in CSLT (Patterson et al., 2005) similar to the topography simulation in Chapter 4. The predominant focus in glaucoma has been on simulation of visual field series (Gardiner and Crabb, 2002b, Spry et al., 2000, Vesti et al., 2003, Gardiner and Crabb, 2002a, Jansonius, 2007). The key advantage of this approach in CSLT imaging is having exact knowledge that the morphology of the original structure is not changing, because all images are derived from the same baseline scan. This simulation can then provide a test-bed for comparing false positive rates of HRT change detection methods.

In this chapter, a virtual platform is developed in which components of the optic disc topographic image formation process of the HRT are simulated computationally, taking into account features relevant to topographic variability such as eye movements and other sources of noise during and between HRT examinations. This aim is implemented by using empirical and theoretical parameters of these noise sources to propagate HRT series from real baseline scans. The resulting simulated HRT images will be analogous to the real HRT images obtained in clinics with variability similar to that inherent in real 'stable' series.

The work in this chapter has formed a paper published in Investigative Ophthalmology and Visual Science (O'Leary et al., 2010a). Parts of this work were

also presented at the Image Morphometry and Glaucoma in Europe Meeting, Leuven, Belgium February 12-13, 2009 and Rome, Italy February 4-5, 2010 and also at the Association for Research in Vision and Ophthalmology Meeting, Fort Lauderdale on May 3-7 2009 and May 2-6, 2010.

5.1 Methods

5.1.1 Data

HRT II scans previously acquired for a study on test re-test measurement variability are used; the data are fully reported on elsewhere (Strouthidis et al., 2005b) and in Chapters 3 and 4. In summary, they consisted of the images of both eyes from 74 patients (43 OHT, 31 POAG) having 5 HRT II examinations within 6 weeks. All HRT II data were processed in the latest HRT III software viewer version (ONH Viewer Module 3.1.2.15). From 148 eyes of 74 patients, 127 eyes of 66 patients were selected on the basis of available raw data (having 5 computable mean topographies and having a baseline scan with original confocal image stacks).

5.1.2 Simulation Development

The HRT II acquires a confocal image stack comprised of 'optical sections' taken through a sequence of focal planes 62.5μm apart in a horizontal scanning angle of 17.5° and vertical scanning angle of 15° around the ONH surface. These are aligned to compensate for eye movements occurring during scan acquisition. Each optical section image is sampled as an array of 384 rastered horizontal line scans each sampling 448 pixels. The intensity of light reflected in each optical section for a location on the ONH/retinal surface is used to calculate the surface height at that location (approximately the depth position of the optical section with the highest reflectance intensity at that location). The matrix of surface height values of the central (384 x 384) sampled locations forms the topography image with the information from the 64 horizontally peripheral pixels used only prior to topography formation.

The simulation uses the unprocessed (unaligned) confocal image stack as the basis for simulation. For the purposes of simplicity and clarity, a *scan triplet* is used to refer to the three single image stacks (referred to as *single scans*) obtained during each acquisition or *examination*. The patient is repositioned at the HRT between image acquisitions. It is from this scan triplet that three *single topographies* are derived; these are aligned and averaged to form the *mean topography*.

One baseline single scan for each eye is chosen as the 'seed' for the generation of the simulated series. Each optical section is aligned with the section above and below using a cross correlation algorithm, to reduce eye and head movements already potentially present in the seed data. Within each seed stack, various sources of noise, present during image acquisition, that potentially result in variability between the generated topography (single and mean) images is added in the following order: (1) within examination eye movement, (2) within examination head movement, (3) between examination head movement and (4) device noise consisting of quantum effect noise (shot/photon counting) and electronic noise (Johnson-Nyquist/thermal). Simulated single topographies are formed from resultant image stacks using the HRT III software; the simulated mean topographies are calculated from the simulated single topographies. The input and sequence of the addition of these sources of noise are shown in Figure 5.1.
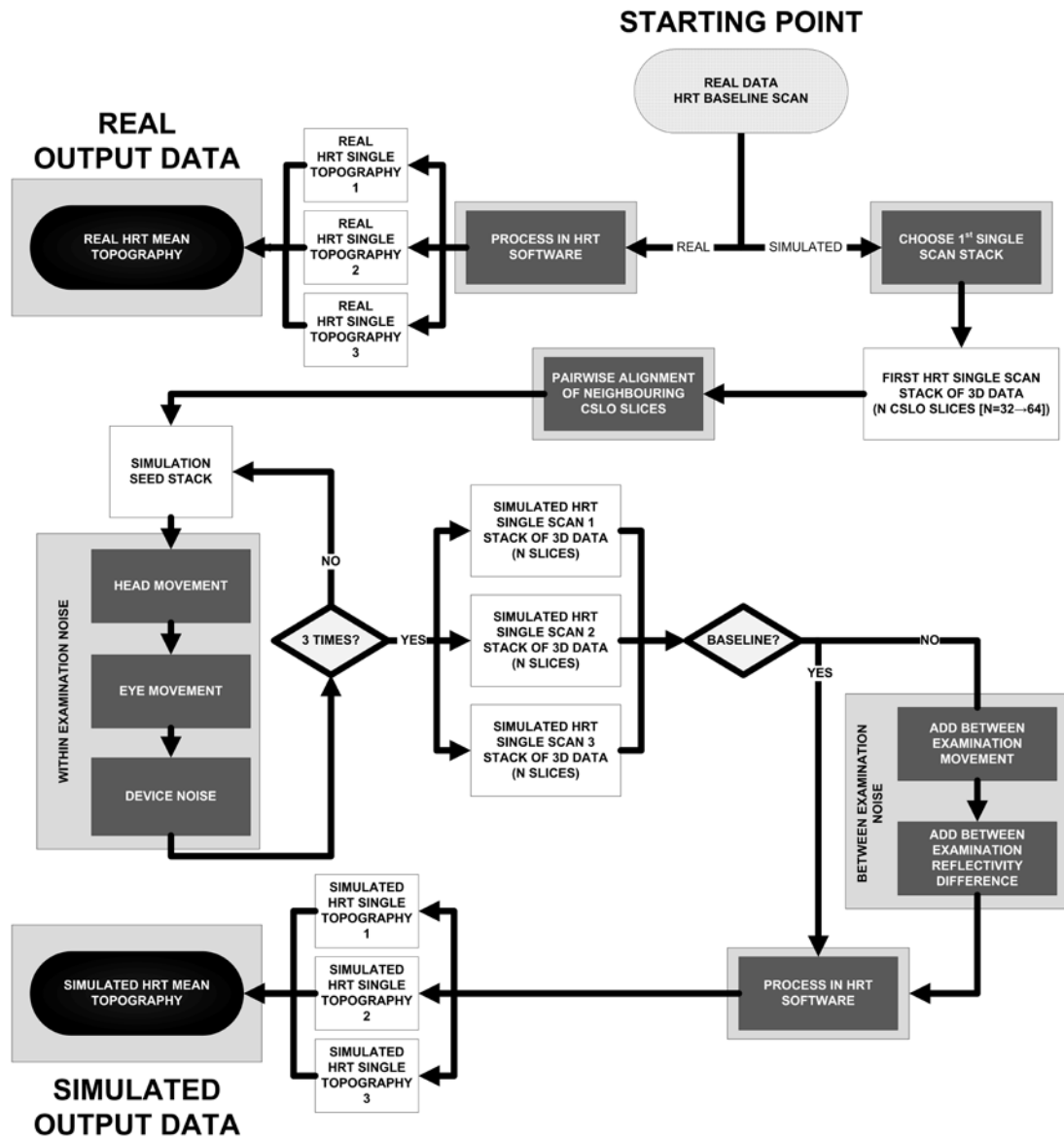
Figure 5.1 Schematic of simulation: HRT single and mean topography formation formed from a single scan volume of CSLO optical sections. Processes are represented by grey boxes with intermediate data states by white boxes and initial and final data states by rounded boxes.

5.1.2.1 Within Examination Eye Movements

In HRT image acquisition, the patient is asked to fixate on a target before and during the acquisition. Thus the eye movement model consists of the three main components of normal fixational eye movements: ocular micro-tremor, ocular drift and microsaccades. Ocular micro-tremor is assumed to have an amplitude range of 0-2 arcmin (median 17.5 arcsec) (Ratliff and Riggs, 1950) with a frequency power spectrum from 0 Hz to 150 Hz (Spauschus et al., 1999). Ocular drift is assumed to have linear speed an amplitude range of 3-12 arcsec with durations spanning the intervals between microsaccades (Riggs et al., 1954). Microsaccades are assumed to have mean amplitude of 30 arcmin and mean period of 30ms (Schulz, 1984). Mean speeds of microsaccades are calculated from the 'main sequence' relationship (Zuber and Stark, 1965) and are parametrically related to the amplitudes of microsaccades. Each microsaccade speed is non-linear and is approximated to that of a half-Gaussian function. The mean frequency of microsaccades is assumed to be 1.5 per second (Engbert and Mergenthaler, 2006).

Certain assumed rules are enforced in the eye movement model: drift and micro-tremor take place simultaneously; neither drift nor micro-tremor can occur during microsaccades; no subsequent ocular drift can be in the same direction ($\pm 90^{\circ}$) as the previous microsaccade. This approximates the directional frequencies eye movements observed in previous studies (Schulz, 1984). No initial directional preference is chosen for eye movements though results have shown that horizontal and vertical eye movements do display different characteristics (Liang et al., 2005).

From these parameters and rules, a retinal-trace (x, y, t) (the path of the retina in transverse axes over time) is derived for the position of the retina. The sampling period of each optical section is approximately 24ms (frequency 42 Hz) and within

each section the rastered horizontal line sampling period is approximately 62 μs

(frequency 16 kHz). As the latter frequency is many orders of magnitude above

maximum frequencies recorded for eye movements, the horizontal line scan time is

considered a sufficient sampling frequency for the eye movement model (i.e.

movement occurs between line scans and between optical sections). The transverse

(x and y) spatial sampling for each HRT pixel is approximately 2 arcmin in an

emmetropic eye.

5.1.2.2 Within Examination Head Movements

These are represented by scaling ('magnification') and the translational and

rotational movements of the retina to correspond with movements of the head.

There is little research on head movement parameters during positioning on chin

and head rests but studies have shown that a mean of about 2.5% of head motion

remains uncorrected by translational compensatory eye movements (Ferman et al.,

1987). As a consequence only translations along the 'z' axis (in depth) of scanning

are included in the head movement model. This model is implemented by applying

cumulative, random, axial translation (with appropriate scaling) and rotation to

each sequentially scanned optical section throughout the scan triplet.

5.1.2.3 Between Examination Movement

 Global uniform transformation and change in overall reflectivity of the confocal

stack mimics variability resulting from placement of the head anew on the head and

chin rests. A standard deviation of 10 pixels is chosen (by assumption) for the

normal distribution from which lateral translations of the follow-up stack relative to

baseline can be chosen as an estimate for the operator uncertainty in centring the

ONH upon scanning. Changes in the autofocus settings of the machine (correct axial

placement of central CSLO section) are modelled by random small scaling and depth translations. Differences in initial head angle placement in each examination are mimicked by rotations of the scan triplet about each axis with relation to the baseline.

For all eye and head movements, implementation of deriving new intensity values at each new simulated pixel involves the defining of new coordinates for every pixel in the CLSO section stack under a given transformation. "Filling-in" of unknown data between known intensity values at fixed pixel coordinates requires interpolation. As a standard approach in many similar problems, bilinear or trilinear interpolation is chosen for this. It applies products of linear functions to the data of the neighbouring 4 or 8 pixels in two or three-dimensional space to obtain intensity values of new coordinates which do not coincide exactly with the coordinates of any pixel in the seed stack. If the transformation only involves transverse components, then bilinear interpolation is only performed using the values of the new coordinate's transverse neighbours. If axial components are involved in the transformation, then trilinear interpolation is performed using the axial neighbouring pixel information in addition.

In the rare cases where there is no valid measurement from the reflectance signal from the focussed spot, these areas remain 'invalid' under the new coordinate system. Bilinear/trilinear interpolation will render neighbouring values close enough also 'invalid' under the coordinate transform. The effect of movement which causes the re-sampled pixels to fall outside the boundaries of the original optical section, known as "windowing", is an important technical point to consider. Null values are used and the topography formation and alignment algorithms will ignore information at these pixels. This effect occurs mostly to the edges of the

optical section images. As previously stated, there are 64 extra horizontally peripheral pixels available for use in each optical section before topography formation. The simulation uses the information of these extra pixels to remove the effects of windowing from horizontal axis movement.

As the dwell time of the scanning beam at each sampled point is $<0.5 \times 10^{-6}$ s the effect of optical blur or 'smearing' of the optical point spread function (PSF) due to eye movements is considered negligible for this model. Even at the maximum simulated eye speed, the maximum displacement during this dwell time would be $<2.5 \times 10^{-4}$ of the transverse spatial sampling interval between pixels (one pixel width). One pixel width is approximately equal to a typical full width at half maximum of the PSF in the HRT scanning system as referred to in the imaging systems lateral resolution specification (Heidelberg Engineering, 2010).

5.1.2.4 Device Noise

The operating principles of the HRT are such that retinal reflected light is gathered point by point and focussed onto a detector. A single spot detector - an avalanche photodiode (APD) is used for sequentially measuring the incident reflectance intensity values. The principal sources of device noise in HRT image acquisition are assumed to be intrinsic noise (Pawley, 1994) in the form of quantum or photon-counting (shot) noise (Goodman, 1985) along with electronic noise which is primarily thermal noise (Johnson/Nyquist) and APD noise (Vasilescu, 2005). Photon counting noise can be modelled as a Poisson process where the intensity measured at the detector (proportional to number of photons striking the detector) for a sampled point can be chosen from a Poisson distribution with a mean intensity of λ. In this simulation λ is given by the intensity of the pixel of the seed optical section after eye and head movements have been added. Thermal and APD noise which are

intrinsic noise from the properties of electronic circuitry in general and semiconductor diodes respectively can be combined and approximated to white noise with given amplitude. Each pixel in each optical section has added spatially independent noise sampled from a normal distribution with a mean of zero and with the amplitude of the noise determined by the standard deviation of the distribution. In this simulation, the standard deviation is estimated at 2% of the maximum 256 stored intensity grey-level values in an 8-bit analogue-to-digital converter. After noise is introduced, each pixel intensity value is rounded to the nearest 8-bit integer to represent the digitization of the signal in the HRT optical section.

5.1.2.5 Noise Follow-up Time Dependence

The difference between one scan triplet and another is simply modelled by the change in initial head and eye position corresponding to a repositioning of the head onto the head and chin rest and a resetting of the acquisition centration on the ONH. The within scan triplet noise parameters do not change with time nor do the differences between examinations. These assumptions are supported by the finding of no difference in variability between examinations with a range of intervals (1 hour to 4 weeks) too short for age related changes to have occurred (Chauhan and MacDonald, 1995).

5.1.2.6 Implementation

This simulation model has been implemented in MATLAB® (2009a The MathWorks Inc. Natick, Massachusetts); it contains the full technical specifications and is freely available upon request from the authors. As indicated in Figure 5.1, all simulated

optical stack data are imported into the HRT III software for topography calculation and alignment. This processing is identical to that of real data.

5.1.3 Simulation Testing

The appropriateness of the simulated images is assessed quantitatively by comparing the following measures between the real and simulated topographies for each eye.

5.1.3.1 Local within examination variability

This is a map of *within* examination pixel height standard deviations ($PHSD_w$). Each pixel of this map $PHSD_w(x, y)$ is the sample standard deviation at that pixel across the three aligned single topographies comprising a mean topography.

5.1.3.2 Local between examination variability

This is a map of *between* examination pixel height standard deviations ($PHSD_b$). Each pixel of this map $PHSD_b(x, y)$ is the sample standard deviation at that pixel across all mean topographies in the series.

5.1.3.3 Global within examination variability

This is the mean *within* examination pixel height standard deviation ($MPHSD_w$). $MPHSD_w$ is used as an overall measure of within examination quality and is provided in the HRT III software. It is the geometric mean of all pixels in the $PHSD_w$ map of a mean topography.

5.1.2.4 Global between examination variability

This is the mean of *between* examination pixel height standard deviation ($MPHSD_b$). $MPHSD_b$ is used in this study as a global measure of the variability between mean

topographies in a series. It is the geometric mean of all pixels in the PHSD$_b$ map for each series.

5.1.2.5 Clinical measure of variability: Neuroretinal rim area variability of mean topographies

As with previous chapters, RA is considered to present the best option from all the HRT stereometric parameters for detecting and tracking glaucomatous changes in the ONH. In addition its variability has been well characterised (Owen et al., 2006, Tan et al., 2003). Thus reproducing longitudinal RA variability will present a major validation of any realistic simulation of longitudinal HRT series. In this study the Moorfields fixed, standard, reference plane is used to calculate RA (Poli et al., 2008).

RA coefficient of variation (CV) is calculated as the ratio of the standard deviation to the mean of the RA across mean topographies in a series. The RA CV is compared between real and corresponding simulated mean topography series to examine the performance of the simulation in mimicking the variability of a clinically meaningful parameter.

Single value metrics of variability (MPHSD$_w$, MPHSD$_b$, RA CV) are compared by plotting the paired values (or mean value where appropriate) of simulated and real series and examining the mean difference. Mean differences (bias) and the 95% limits of agreement between real and simulated series are calculated for these metrics. Analysis of proportional bias and agreement is also performed i.e. any change in the bias or agreement with the measurement value itself was investigated.

5.1.2.6 Correlation in the spatial distribution of topographic variability between real and simulated series

The maximal value of the NCC, a standard image processing metric for similarity of two images, (Eskicioglu and Fisher, 1995) (as used in Chapter 4) is used to compare the local variability measures ($PHSD_w$, $PHSD_b$ maps) of real and simulated topographies. The term maximal is used as $PHSD_w$ and $PHSD_b$ maps can be shifted along x and y coordinates (with an upper limit of 20 pixels) to find the maximal NCC. This is a logical approach as the real and simulated series will have different baselines and as such PHSD maps will need to be approximately aligned to each other. This metric captures the extent of mutual spatial information in the real and simulated variability maps and thus how well the location of topographic variability is reproduced by the simulation. NCC ranges from a minimum possible value of -1, representing perfectly anti-correlated image data, to a value of +1, representing perfectly correlated image data, where a value of 0 represents no correlation whatsoever.

NCC values from comparing real and simulated mean topography $PHSD_w$ variability maps are benchmarked against NCC values from comparisons of the real mean topography $PHSD_w$ variability maps with each other. The NCC for a real/simulated series comparison is the mean of the 25 possible pairs of comparisons. The NCC for the real/real series comparison is the mean of 10 possible pairs of comparisons.

5.2 Results

Of the selected 127 eyes from 66 patients 63 were left eyes and 64 were right eyes. The median patient age at baseline examination was 70 years (inter-quartile range: 63– 75 years).

Mean differences (bias) and 95% limits of agreement between real and simulated data for $MPHSD_w$, $MPHSD_b$, RA CV and the NCC values for $PHSD_w$ maps are displayed in table 5.1. Results of analysis of proportional effects on agreement between real and simulated series are also included in table 5.1 with statistical significance of linear relationships between biases and average measurements and statistical significance of linear relationships between 95% limits of agreement and average measurements. In addition, a log transformation is used on mean values and before differences are calculated between real and simulated measures, an approach designed to ensure robustness against the effect of the heteroscedasticity and potential outliers on any measured proportional effects,(Bland and Altman, 1986) These characteristics are clear in the Bland-Altman plots of Figures 2 and 5. Accordingly log-transformed significance of proportionally dependent bias and 95% limits of agreement are included in table 5.1.

Table 5.1: Summary of differences (bias and 95% limits of agreement (LoA)) between the simulated and real image series for global, regional and local measures of variability: mean of *within* examination pixel height standard deviation (MPHSD$_w$), mean of *between* examination pixel height standard deviation (MPHSD$_b$), RA CV and NCC of PHSD$_w$. maps.

		Measures of Difference					
		Uniform		Significance of Proportional Dependence			
				Linear		Log-transformed	
		Bias	95% LoA	Bias	95% LoA	Bias	95% LoA
Measure of Variability	MPHSD$_w$	3.5μm	-20.9μm to 28.8μm	p=0.10	*p<0.01*	p=0.61	*p<0.01*
	MPHSD$_b$	2.0μm	-5.4μm to 9.3μm	*p<0.01*	*p<0.01*	p=0.35	p=0.27
	RA CV	-2.1%	-17.6% to -13.4%	p=0.09	p=0.08	p=0.40	p=0.07
	NCC PHSD$_w$	0.052	0.039 to 0.065	*p<0.01*	p=0.25	*p<0.01*	p=0.87

Bland-Altman plots in Figures 5.2 (a) and 5.2 (b) show the agreement between paired real and simulated eye series for MPHSD$_w$ and MPHSD$_b$ respectively. Agreement between paired real and simulated topography series for RA CV values for global RA is shown in the Bland-Altman plot in Figure 5.3. Linear proportional biases and 95% limits of agreement found to have statistical significance in either linear or log-transformed domains (p<0.05) are displayed on all Bland-Altman plots.
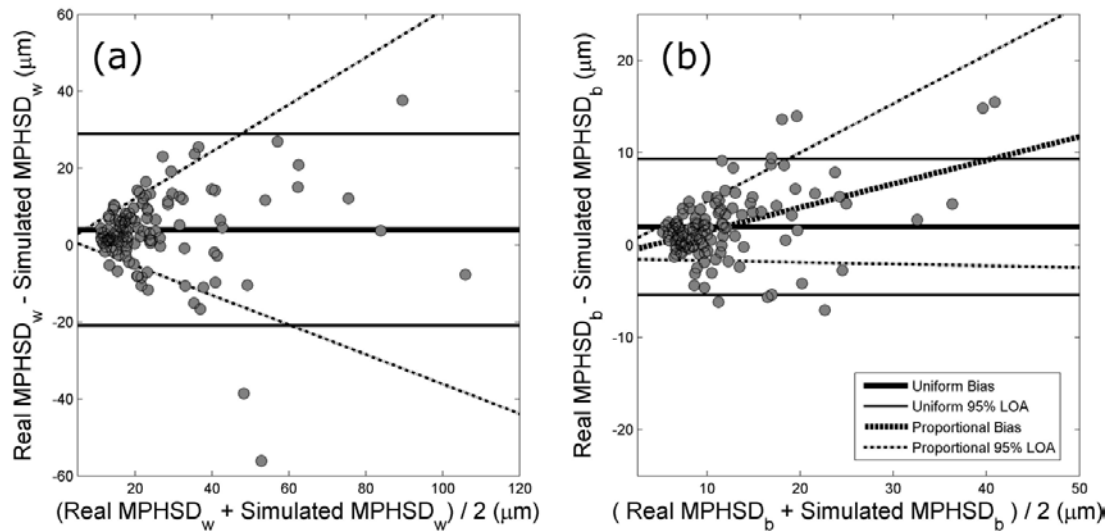
Figure 5.2 Bland-Altman plots showing series-wise, agreements between (a) average within examination MPHSD (MPHSD$_w$) for real and simulated mean topography data and (b) agreement of between examination (MPHSD$_b$) for real and simulated mean topography data. The mean difference (bias) of average MPHSD$_w$ is 3.5μm (95% limits of agreement: -20.9μm to 28.8μm). The mean difference of MPHSD$_b$ is 2.0μm (95% limits of agreement (LoA): -5.4μm to 9.3μm). Uniform 95% LoA illustrate only approximate limits of agreement as heteroscedasticity of this data is apparent. Dashed lines indicate statistically significant linear proportional bias for MPHSD$_b$ and the significant linear proportional increase of the widths of the 95% LoA for MPHSD$_w$ and for MPHSD$_b$.
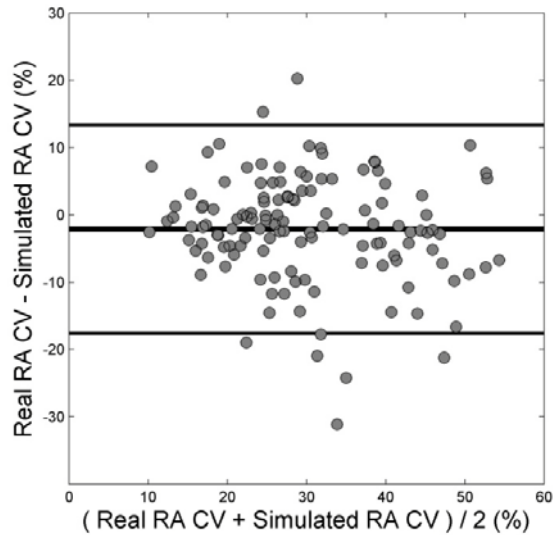
Figure 5.3 Bland-Altman plots showing series-wise agreement between RA CV for real and simulated data (%). Mean difference between values for real and simulated data is -2.1% (95% limits of agreement (LoA): -17.6% to 13.4%). No proportional effects were found on the bias or LoA.

Local variability correlation between real and simulated data is shown in Figure 5.4. The average, series-wise, maximal, NCC of PHSD$_w$ distribution between simulated and real data is shown in Figure 5.4(a) with a mean of 0.58 (standard deviation 0.12). The distribution of series-wise, maximal NCC of PHSD$_b$ maps between simulated and real data is shown in Figure 5.4(b) with a mean of 0.54 (standard deviation 0.10). The distribution of average, series-wise, maximal NCC between real PHSD$_w$ maps and other real PHSD$_w$ maps in the same series is shown in Figure 5.4(c) with a mean of 0.64 (standard deviation 0.14).
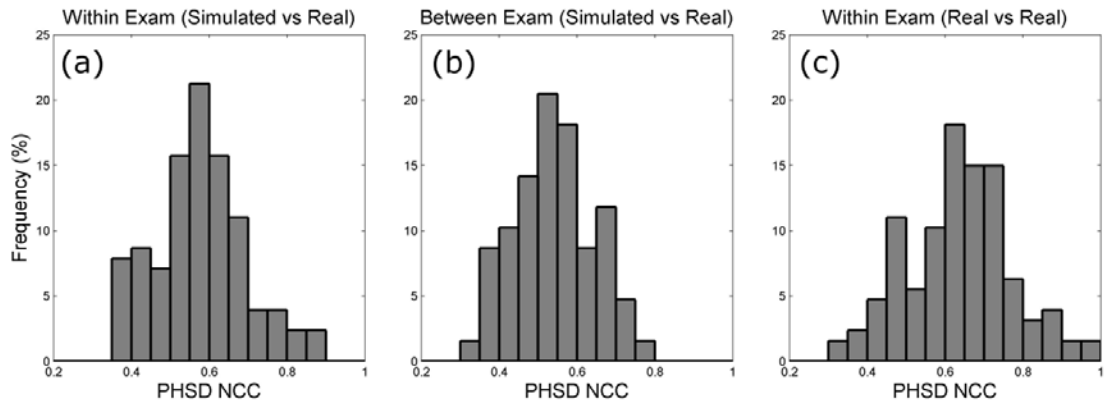
Figure 5.4 Distributions of maximal NCC of pixel standard deviation maps between real and simulated data (a) within examination (PHSD$_w$) averaged over all pair-wise comparisons and (b) between examination (PHSD$_b$). (c) The series average NCC values of all pair-wise pixel standard deviation maps between mean topographies in the same series. Value extremes are interpreted as follows: -1: perfectly negatively correlated, 0: uncorrelated, 1: perfectly positively correlated.

Agreement of average NCC of real/real PHSD$_w$ maps against average NCC of real/simulated PHSD$_w$ maps is shown by the Bland-Altman plot in Figure 5.5. The mean difference is 0.052 and 95% limits of agreement are 0.039 and 0.065.
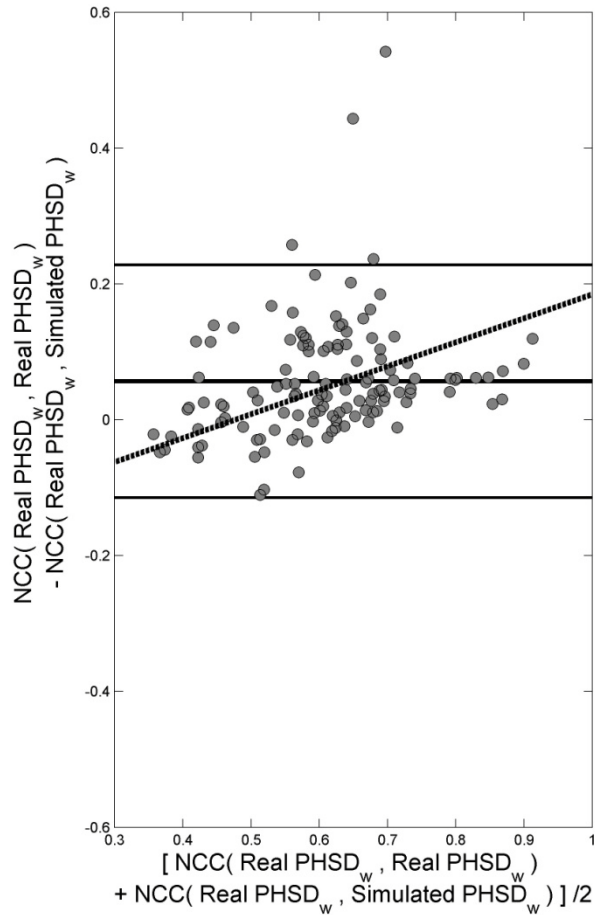
Figure 5.5 Benchmarking of PHSD_w NCC values between real and simulated data: Bland-Altman plot showing agreement of average NCC for real/real PHSD_w map comparisons and average NCC for real/simulated PHSD_w map comparisons. Solid lines represent a uniform bias of 0.052 and 95% limits of agreement of 0.039 to 0.065. The dashed line represents the statistically significant linear proportional bias.

Examples of mean reflectance images, mean topographies and corresponding PHSD_w maps for real and simulated data are shown in Figures 5.6 and 5.7. Examples of series average mean reflectance images, series average mean topographies and corresponding PHSD_b maps for real and simulated data are shown in Figures 5.8 and 5.9.
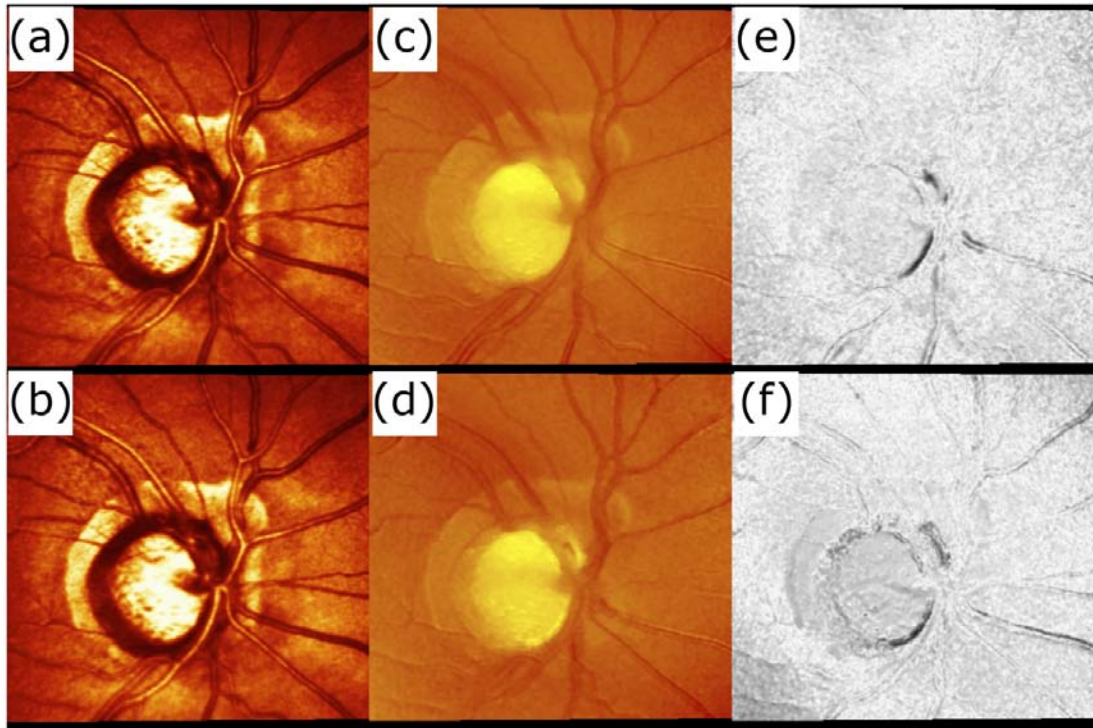
Figure 5.6 Qualitative display of within examination, local variability for real and simulated pairs. (a) Real mean reflectance image. (b) Corresponding simulated mean reflectance image. (c) Real mean topography (mean of within examination pixel height standard deviation (MPHSD$_w$) 20μm). (d) Corresponding simulated mean topography (MPHSD$_w$ 22μm). (e) Log of pixel height standard deviation (PHSD$_w$) maps of real mean topography – darker areas represent areas of higher variability. (f) Log of PHSD$_w$ maps of corresponding simulated mean topography. Maximal normalised cross correlation of these two maps (e) and (f) is 0.55.

Figure 5.7 Qualitative display of within examination, local variability for real and simulated pairs. (a) Real mean reflectance image. (b) Corresponding simulated mean reflectance image. (c) Real mean topography (mean of within examination pixel height standard deviation (MPHSD$_w$) 17μm). (d) Corresponding simulated mean topography (MPHSD$_w$ 16μm). (e) Log of PHSD$_w$ maps of real mean topography - darker areas represent areas of higher variability. (f) Log of PHSD$_w$ maps of corresponding simulated mean topography. Maximal normalised cross correlation of these two maps (e) and (f) is 0.37.

Figure 5.8 Qualitative display of between examination, local variability for real and simulated pairs. (a) Real series-average mean reflectance image. (b) Corresponding simulated series-average mean reflectance image. (c) Real series-average mean topography (mean of between examination pixel height standard deviation (MPHSD$_b$) 39μm). (d) Corresponding simulated series-average mean topography (MPHSD$_b$ 38μm). (e) Log of PHSD$_b$ maps of real mean topography series - darker areas represent areas of higher variability. (f) Log of PHSD$_b$ maps of corresponding simulated mean topography series. Maximal normalised cross correlation of these two maps (e) and (f) is 0.51.

Figure 5.9 Qualitative display of between examination, local variability for real and simulated pairs. (a) Real series-average mean reflectance image. (b) Corresponding simulated series-average mean reflectance image. (c) Real series-average mean topography (mean of between examination pixel height standard deviation (MPHSD$_b$) 9μm). (d) Corresponding simulated series-average mean topography (MPHSD$_b$ 9μm). (e) Log of PHSD$_b$ maps of real mean topography series - darker areas represent areas of higher variability. (f) Log of PHSD$_b$ maps of corresponding simulated mean topography series. Maximal normalised cross correlation of these two maps (e) and (f) is 0.73.

5.3 Discussion

There is no gold-standard method for detecting structural changes in the glaucomatous eye. One solution might be afforded by simulating series of ONH measurements with well estimated measurement variability characteristics. This study proposes a blueprint for such an approach, using images from CSLO technology. This simulation is based on empirically derived parameters from previous studies and HRT specifications from published reports. The usefulness of a simulation is critically dependent on the degree to which it reproduces the characteristics of real data. The simulation presented in this study reproduces well both the spatial characteristics, and the magnitude, of variability in short-time series of HRT data both within examination acquisition and between examinations. Image correlation of real and simulated spatial variability maps tend to be slightly less than the correlation of real spatial variability maps with each other, but the quantitative and qualitative differences are very small. The simulation also generates realistic values for RA which is a clinically useful parameter for monitoring change. The contribution simulation can have, independent of, and before the collection of real data for scientific studies or clinical trials which aim to assess the validity of change detection measures, cannot be overstated. Given any number of baseline patient scans, it enables unlimited modification to the length of series, frequency of testing and variability over time of the data. It also may reduce potentially wasted cost and time involved in data collection given that study design may be optimised in advance by testing preliminary hypotheses on simulated test-bed data.

The simulations were bench-marked against real short-time series. Clinically significant change is highly unlikely to have taken place during the short study

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period and age related effects will also be almost non-existent; the only changes in the series over time will be real fluctuations in the tissue morphology and variability in the measuring device and settings. This study has used parameters from previous studies on the movement of the eye (and, as a result, the movement of the ONH) during fixation from previous studies, along with the reasonable estimates of operator variability in initial ONH centration and the variability inherent in the device hardware in an attempt to make these assumptions as realistic as possible. Errors in estimating these parameters may result in inaccuracies in the simulation. Other potential sources of variability, such as the ocular pulse and IOP changes, have not been introduced as they have not been sufficiently well characterised. The ocular pulse has been shown to contribute to changes in vessel calibre (Chen et al., 1994, Knudtson et al., 2004) and changes in the backward and forward movements of the retinal surface in vessel free areas (Chauhan and McCormick, 1995). Long-term and short-term IOP changes can also cause changes in the vessel calibre (Shin et al., 1991) along with changes in optic disc cupping (Parrow et al., 1992) and size (Poostchi et al., 2010). The apparent random parabolic deformation of topographies over time due to random changes in the distance of the eye from the device between examinations (known as parabolic error) has been observed to be significant (Balasubramanian et al., 2011). The simulation does not account for these effects but it might be possible to do so using appropriate parameters. Despite these potential shortcomings, the simulations appear to realistically reproduce observed topographic variability.

Previous attempts to simulate series of ONH or retinal images are few and far between. One approach involved simulating retardance image series obtained from SLP baseline images (Vermeer et al., 2006). SLP differs from CSLT in that it measures relevant anatomy in a different manner, and has different sources of

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variability. Another simulation methodology, specific to HRT data, has used small misalignments of identical finalised or post-processed single topographies to generate longitudinal series (Patterson et al., 2005). This approach formed the basis of the simulation used in Chapter 4 to investigate MPHSD. Although it reproduces some of the global variability inherent in no-change patient HRT data, by design it cannot reproduces the spatial and feature dependent variability involved in topography fitting and topography alignment. It is also unsatisfactory from a theoretical point of view as it is difficult to estimate the relationship of acquisition noise factors and the variability of post-processed topographies. The methodology presented in this study contrasts by using all the three-dimensional information acquired in the HRT scan. Further, a key component of the simulation described in the current study is that the simulated scans are re-imported into the HRT III software. Thus any misalignments added both within examination and between examination will be subject to partial correction by the HRT III alignment algorithms. This is consistent with the procedure for real patient data.

Although within examination single topographies are more closely related than between session topographies, because of the effects of between examination repositioning of the patient and re-alignment of the scanner to the eye/ONH, the simulation assumes no auto-correlation in time (knowing one value does not provide you with any information of another value) between examinations. Also, the within examination noise parameters do not change with time nor do the differences between examinations. These assumptions are justified on the basis of study results showing no difference in variability between scans with intervals varying from 1 hour to 4 weeks (Chauhan and MacDonald, 1995).

There are a few cases of variability being poorly reproduced in simulated series as can be seen in Figure 5.2(a). Here, the largest anomalies are above the line of agreement showing that, in these cases, the average, global within mean topography variability is higher in the simulation than in real series. This may indicate that the optical section stack used to seed the simulation has more motion noise or lower image quality than those in the rest of the real series. Although the simulation model presented here will allow some alignment before noise addition, it is never possible to attain a motion-free, noise-free image stack. Thus the simulation depends on the optical section stack selected to seed each simulated series. Apart from random differences in a small sample of 5 mean topographies, there may be other potential causes for discrepancies in reproducing the variability of real data, for example, some differences in RA variability may be due to small differences in contour line placement between the real and simulated baseline topographies.

Windowing, as stated in the methodology may have small systematic effects on differences in the calculations of variability between real and simulated data due to the potential null values on optical section and topography image edges. However, windowing caused by axial shifts should not affect the topography formation process as any missing data will be in the low intensity "tails" of each axial intensity profile. Topographic height information is mostly derived from the central maximum of each axial intensity profile. Vertical axis shifts have the potential to most affect the topography formation. Despite this, between examination, vertical translations of scan triplets greater than 20 pixels between scans resulting in the loss of more than 20 pixels at the top or bottom of a mean topography will occur in approximately only 5% of simulated follow-up examinations and furthermore have only a small effect on the alignment algorithms of the HRT and the calculation of variability values.

The simulation makes the simplifying assumption that eye movement and measurement noise parameters are the same for all patients. This assumption seemed sufficient in reproducing many of the characteristics of real data. Furthermore, it is important to note that in real patient data, patients with varying degrees of lens opacity were scanned. This allowed the effects of a wide range of image quality to be modelled, with some eyes having high within and between examination variability. That this is also observed in the simulated data suggests that much of the observed range of image variability is a consequence of the range in the quality of ocular media and the structure of the ONHs themselves.

Future work could usefully consider the formation process in the CSLO pixels (Gruppetta, 2009), allowing the modelling of other processes which occur before the summation of light at the detector for each pixel by modifying the PSF during and between acquisition sessions. These include changes in pupil diameter, media opacities and changes in uncorrected lower order aberrations (defocus and astigmatism). This simulation provides a model to test progression algorithm specificity appropriate to the baseline image quality, but cannot mimic systematically changing image quality over time. The long-term changes in the optical quality of the eye over time (increased scatter and absorption) resulting in a reduced and noisier light signal reflected to the device detector will need to be addressed in order to mimic this feature in some topographic series over time.

The introduction in the simulations of topographic changes associated with focal and diffuse glaucomatous damage in the ONH need to be investigated. By adding change to a series of replicate HRT scans, and then overlaying measurement noise from this simulation, the sensitivity of change detection algorithms may be assessed

(as the underlying location, temporal sequence and magnitude of the superimposed change is known).

A stable series of measurements can be generated by simply adding random noise. Such series may also be used to test specificity of statistical change detection algorithms developed for detecting progression. However, in reality, there are many other sources of variability in ONH scans such as eye movements, head movements and device noise, the effects of which may be falsely detected as true morphological change by these techniques. Such variability is manifestly more complex than simple, additive noise and therefore a more realistic stable series simulation is needed to more accurately test the specificity of these algorithms. Stability in any clinical series of data is uncertain, whereas in a modelled series it is certain.

So, in conclusion, the methodology outlined in this work has definite use in producing benchmark data on which the false positive rates of statistical methods for detecting HRT progression can be examined. This could also improve the confidence of change detected by these techniques in clinical studies. Simulating series of progressive change in HRT series awaits further investigation. Still, the results of this study are presented as a proof of principle for a solution towards the seemingly intractable problem of developing reliable methods for detecting disease progression in glaucoma.

6. Conclusion and Further Work

As continued research expands, modern knowledge of the various factors characterising glaucomatous ONH progression will undoubtedly change. The objectives of this thesis are to assess progression detection algorithms in ONH data from CLST technology, specifically the HRT. The key findings in the sections of this thesis are as follows.

Chapter 2 compares optic disc changes using automated analyses of HRT images with assessments, by glaucoma specialists, of change in stereoscopic photographs. Different statistical methods for detecting structural changes in HRT images exhibit only moderate agreement with each other and have poor agreement with expert-assessed change in optic disc stereophotographs. Because it is undermined by limitations in the specificity and between-observer agreement, the use of optic disc photograph assessment alone as a reference standard is not helpful in determining the optimal automated change detection algorithm in series of HRT images.

Chapter 3 examines the effects of measurement variability across a range of characteristics on the performance of some basic univariate statistical techniques to detect underlying trends. This work demonstrates that serial autocorrelation of measurement error can have large detrimental effects on the statistical power to detect change in these methods even when the measurement noise is low. Of the methods investigated, similar powers are observed on average across a range of measurement noise characteristics – but non-parametric, rank-based methods offer the advantage of not depending on linear change over time and may be preferable for progression detection.

Chapter 4 finds quality metrics of HRT images to be highly predictive of within examination pixel-wise measurement variability – measured by the MPHSD. A simple simulation is used to demonstrate that uniform levels of misalignment noise applied to different ONH images before image averaging can produce a wide range of MPHSD values. This indicates the dependence of MPHSD on the ONH morphology itself. Subjective assessment of image quality of HRT data exhibits a low between-observer agreement but averaged panel scores have good agreement with MPHSD values. The average MPHSD across a series of HRT images variability is a strong predictor of series RA variability but for an individual examination MPHSD was not predictive of the reliability of the RA measurement. This indicates that MPHSD may not be a useful way to weight individual examination information.

Chapter 5 describes how a novel simulation propagates HRT pseudo-longitudinal series from the three-dimensional data of a single baseline scan. The simulation is implemented using empirical and theoretical measurement noise parameters. This approach is validated by its ability to reproduce the variability of CLST data both on a local and global scale of the ONH. This means it can provide a test-bed to evaluate the specificity and sensitivity of CSLT progression detection algorithms.

In summary, the new contributions to the field of imaging in glaucoma are:

- establishing that methods such as using expert assessed ONH stereophotographs as reference standards for glaucomatous change do not provide useful insights on how well or badly HRT glaucoma progression algorithms perform

- demonstrating considerable disagreement between current HRT progression detection algorithms motivating a continued search for an optimum technique

- characterising the strong relationships of image quality and morphological characteristics with HRT measurement variability

- emphasising the detrimental effects of measurement autocorrelation on the ability to detect statistically significant trends in RA data

- the development of a novel and empirically based simulation which can reproduce virtual HRT data with variability similar in magnitudes and spatial characteristics to real data and thus address these issues

Following these findings, future work could usefully consider:

- discovering predictors of the noisy measurements in HRT imaging and in other imaging modalities

- modelling change in the developed simulation of longitudinal HRT image series

- application of the simulation methodology developed in this thesis to other imaging modalities

- investigate the advantages of rank-based over parametric-based trend detection techniques for non-linear models of change

- devising change detection algorithms for HRT and newer imaging modalities based on these conclusions and testing these on simulated and real data

APPENDIX A: Linear Regression Permuted Test Statistics

The univariate OLS linear regression solution considers the problem of fitting n observed data points (x_i, y_i) for independent variable x and dependent variable y to a straight-line model:

$$y(x) \;=\; y(x;\,\alpha,\beta) \;=\; \alpha \;+\; \beta x + \varepsilon$$

where α is the predicted value of y when $x=0$ and β describes the estimated linear trend of y with changing x. The measurement errors ε_i at each point are denoted by ε. OLS linear regression derives a solution to this model by searching for the parameters α and β which minimise the measurement errors ε_i. From this solution, a test statistic can be derived from which the statistical significance of the slope of fitted line β can be inferred. The following analysis examines the effect of reordering while using a permutation test on this test statistic. The estimate β and its standard error S_β are defined by

$$\beta = \left(\frac{nS_{xy} - S_x S_y}{nS_{xx} - S_x{}^2} \right),$$

$$s_\beta = \sqrt{\frac{nS_\varepsilon}{nS_{xx} - S_x{}^2}},$$

where

$$S_\varepsilon = nS_{yy} - S_y{}^2 - \beta^2\left(nS_{xx} - S_x{}^2\right)$$

and $S_x = \sum_{i=1}^n x_i, S_y = \sum_{i=1}^n y_i, S_{xx} = \sum_{i=1}^n x_i{}^2, S_{yy} = \sum_{i=1}^n y_i{}^2, S_{xy} = \sum_{i=1}^n x_i y_i$ are the OLS linear regression coefficients.

In the paradigm of a permutation test, x_i are randomly re-ordered while fixing the order of y_i. Thus during a permutation test, $n, S_x, S_y, S_{xx}, S_{yy}$ are fixed. It is possible to compare the ranking effects from re-ordering on β and s_β by treating these as constants during permutation. The slope test statistic from OLS linear regression is then:

$$\frac{\beta}{S_\beta} = \frac{\beta}{\sqrt{\frac{nS_\varepsilon^2}{\left(nS_{xx} - S_x{}^2\right)}}}$$

$$= \frac{\beta}{\sqrt{\frac{n\left\{\frac{nS_{yy} - S_y{}^2 - \beta\left(nS_{xx} - S_x{}^2\right)}{n-2}\right\}}{\left(nS_{xx} - S_x{}^2\right)}}}$$

$$= \frac{\beta}{\sqrt{C_1 - \beta C_2}}$$

$$= f(\beta)$$

where:

$$C_1 = \frac{\left(n^2 S_{yy} - nS_y{}^2\right)}{(n-2)\left(nS_{xx} - S_x{}^2\right)},$$

$$C_2 = \frac{\left(n^2 S_{xx} - nS_x{}^2\right)}{(n-2)\left(nS_{xx} - S_x{}^2\right)}$$

are invariant throughout permutation. For a real, meaningful ($n > 2$) solution of the test-statistic $\frac{\beta}{S_\beta}$, the denominator must yield:

$$C_1 - \beta C_2 > 0.$$

Thus during a permutation test the test statistic $f(\beta)$ is a monotonic (order-preserving) function of β. Stated formally, this requires for any j^{th} and k^{th} permutations given

$$\beta_k > \beta_j$$

that

$$f(\beta_k) > f(\beta_j).$$

This means that the order the resulting test-statistic from each permutation $\frac{\beta}{S_\beta}$ will be unchanged relative to the respective β for that permutation.

APPENDIX B – Image Transformation Details

The affine transformation (a linear transformation with translation) of a set of i points in three-dimensional space with coordinates (x_i, y_i, z_i) to a point with new coordinates (x_i', y_i', z_i') can be represented by matrix algebra. When considering only rotation, shear, scaling and translation or shift, each can be given by the following affine transformation matrices using homogeneous coordinates:

Translation along x, y, z axes by displacements t_x, t_y and t_z:

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ t_x & t_y & t_z & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{T}\boldsymbol{p}$$

Rotation about z axis by angle θ:

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} \cos\theta & -\sin\theta & 0 & 0 \\ \sin\theta & \cos\theta & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{R}_z\boldsymbol{p}$$

Rotation about x axis by angle $\emptyset$:

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos\emptyset & -\sin\emptyset & 0 \\ 0 & \sin\emptyset & \cos\emptyset & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{R}_x\boldsymbol{p}$$

Rotation about y axis by angle ω:

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} \cos\rho & 0 & \sin\rho & 0 \\ 0 & 1 & 0 & 0 \\ -\sin\rho & 0 & \cos\rho & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{R}_y \boldsymbol{p}$$

Scaling in x, y, z axes by scale factors s_x, s_y and s_z:

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} s_x & 0 & 0 & 0 \\ 0 & s_y & 0 & 0 \\ 0 & 0 & s_z & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{S}\boldsymbol{p}$$

Shearing in the xy, xz, yz planes by factors e_{xy}, e_{yx}, e_{xz}, e_{zx}, e_{yz} and e_{zy}:

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & e_{xy} & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{E}_{xy}\boldsymbol{p}$$

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & e_{yz} & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{E}_{yx}\boldsymbol{p}$$

$$\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & e_{xz} & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = \mathrm{E}_{xz}\boldsymbol{p}$$

$$
\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ e_{zx} & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = E_{zx}\boldsymbol{p}
$$

$$
\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & e_{yz} & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = E_{yz}\boldsymbol{p}
$$

$$
\begin{bmatrix} x_i' \\ y_i' \\ z_i' \\ 1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & e_{zy} & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ z_i \\ 1 \end{bmatrix}, \qquad \boldsymbol{p}' = E_{zy}\boldsymbol{p}
$$

Finally all transformations can be combined with the new coordinates $\boldsymbol{p}'$ depending on the ordering of each transformation. An example below would represent image coordinates being transformed first, then rotated about the z, x, y axes then scaled and finally sheared:

$$
\boldsymbol{p}' = E_{xy}E_{yx}E_{xz}E_{zx}E_{yz}E_{zy}\ S\ R_y R_x R_z T\boldsymbol{p}
$$

In designing the simulation for HRT mean topography generation, the probability distributions for all the required parameters are chosen from the following random distributions:

$$
t_x \sim N\left(0, \frac{W}{256}p\right), \qquad t_y \sim N\left(0, \frac{W}{256}p\right), \qquad t_z \sim N(0, 10\mu m)
$$

$$
\theta \sim N(0°, 0.25°), \qquad \varnothing \sim N(0°, 0.1°), \qquad \rho \sim N(0°, 0.1°)
$$

$$
s_x \sim N\left(0, \frac{W}{10}\right), \qquad s_y \sim N\left(0, \frac{W}{10}\right), \qquad s_z \sim N\left(0, \frac{W}{10}\right)
$$

$$e_{xy} \sim N(0,10), \qquad e_{xy} \sim N(0,10)$$

$$e_{xy} \sim N(0,10), \qquad e_{xy} \sim N(0,10)$$

$$e_{xy} \sim N(0,10), \qquad e_{xy} \sim N(0,10)$$

where W is the width and height of the square image in pixels and p is the unit of one pixel width. Where no units are indicated, parameters are dimensionless ratios.

Transformations are applied at a sub-pixel level, using a bilinear interpolation algorithm.

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List of Publications

Manuscripts

N. O'Leary, D.P. Crabb, S.L. Mansberger, B. Fortune, M.D. Twa, M.J. Lloyd, A. Kotecha, D.F. Garway-Heath, G.A. Cioffi, C.A. Johnson (2010). "**Glaucomatous progression in series of stereoscopic photographs and Heidelberg Retina Tomograph images.**" Archives of Ophthalmology; 128; 5; 560-568.

N. O'Leary, D.P. Crabb, D.F. Garway-Heath (2010). "**An in silico model of scanning laser tomography image series: an alternative benchmark for the specificity of progression algorithms.**" Investigative Ophthalmology and Visual Science; 51; 12; 6472-6482.

Conference Presentations: Published Abstracts

N. O'Leary, A.J. Patterson, D.F. Garway–Heath, and D.P. Crabb. "**Investigating Mean Pixel Height Standard Deviation: An Image Quality Metric Used in Scanning Laser Tomography.**" Applied Research in Vision and Ophthalmology Meeting, May 2006, Fort Lauderdale, Florida, USA
Investigative Ophthalmology and Visual Science 2006 47: E-Abstract 3353.

N. O'Leary, S. L. Mansberger, M. D. Twa, B. A. Fortune, M. J. Lloyd, G. A. Cioffi, C. A. Johnson, D. F. Garway-Heath, and D. P. Crabb. "**Glaucomatous Progression in Series of Stereo-Paired Photographs and Heidelberg Retinal Tomography Images.**" Applied Research in Vision and Ophthalmology Meeting, May 2008, Fort Lauderdale, Florida, USA. Investigative Ophthalmology and Visual Science 2008 49: E-Abstract 5431.

N. O'Leary, M. Balasubramanian, D. F. Garway-Heath, and D. P. Crabb. "**Developing and Testing a Heidelberg Retina Tomograph Image Simulation Method for Application to Optimise Glaucoma Progression Algorithms.**" Applied Research in Vision and Ophthalmology Meeting, May 2009, Fort Lauderdale, Florida, USA. Investigative Ophthalmology and Visual Science 2009 50: E-Abstract 2249.

N. O'Leary, D. P. Crabb, and D. F. Garway-Heath. "**An alternative reference standard for structural progression in glaucoma: an *in silico* model of scanning laser tomography image series.**" Applied Research in Vision and Ophthalmology Meeting, May 2010, Fort Lauderdale, Florida, USA. Investigative Ophthalmology and Visual Science 2010 51: E-Abstract 4010.

Other Presentations

N. O'Leary, A.J. Patterson, D.F. Garway–Heath, and D.P. Crabb. "**Investigating Mean Pixel Height Standard Deviation: An Image Quality Metric Used in Scanning Laser Tomography.**" Image Morphometry and Glaucoma in Europe Meeting, January 2006, Mannheim, Germany.

N. O'Leary, S. L. Mansberger, M. D. Twa, B. A. Fortune, M. J. Lloyd, G. A. Cioffi, C. A. Johnson, D. F. Garway-Heath, and D. P. Crabb. "**Glaucomatous Progression in Series of Stereo-Paired Photographs and Heidelberg Retinal Tomography Images.**" Image Morphometry and Glaucoma in Europe Meeting, February 2008, Rotterdam, The Netherlands.

N. O'Leary, D. F. Garway-Heath, and D. P. Crabb. "**Developing and Testing a Heidelberg Retina Tomograph Image Simulation Method for Application to Optimise Glaucoma Progression Algorithms.**"
Image Morphometry and Glaucoma in Europe Meeting, February 2009, Leuven, Belgium.

N. O'Leary, D. F. Garway-Heath, and D. P. Crabb. "**An alternative reference standard for structural progression in glaucoma: an *in silico* model of scanning laser tomography image series.**"
Image Morphometry and Glaucoma in Europe Meeting, February 2010, Rome, Italy.