GD_x-MM: AN IMAGING MUELLER MATRIX RETINAL POLARIMETER

by

Karen Marie Twietmeyer

Copyright © Karen Marie Twietmeyer 2007

A Dissertation Submitted to the Faculty of the

COLLEGE OF OPTICAL SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2007

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Karen Twietmeyer entitled GDx-MM: An Imaging Mueller Matrix Retinal Polarimeter and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

Russell A. Chipman Date: Oct. 23, 2007

Stanley Pau Date: Oct. 23, 2007

James Schwiegerling Date: Oct. 23, 2007

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.
I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director: Russell Chipman Date: 10/30/2007

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the copyright holder.

SIGNED: Karen Twietmeyer

ACKNOWLEDGEMENTS

I would like to acknowledge my sources of funding: NIH grant EY007624 through Dr. Ann Elsner at Indiana University; the ARCS Foundation; the Boyd Foundation; University of Arizona TRIF Photonics Graduate Student Support; and the University of Arizona Imaging Scholarship.

Special appreciation to my colleagues in the Polarization Lab and Optics Shop at the University of Arizona College of Optical Sciences: Karlton Crabtree, Brian Daugherty, Phillip McCulloch, Noah Gilbert, Tiffany Lam, Steve McClain, Anna-Britt Mahler, Greg Smith, Charles Burkhart and Tom Zobrist.

Special appreciation to my colleagues at Indiana University School of Optometry: Ann Elsner, Steve Burns, Yanming Zhao, Bryan Haggerty, and Dean VanNasdale.

Extra special appreciation to Paula Smith: thank you so much for your constant assistance, kindness, friendship, and moral support.

DEDICATION

This dissertation is dedicated with all my heart to my husband Eric. Without his love, faith, and support I could never have come this far.

TABLE OF CONTENTS

LIST OF FIGURES.....	9
LIST OF TABLES.....	14
ABSTRACT.....	15
1. INTRODUCTION.....	17
2. EYE STRUCTURE, DISEASE, AND POLARIZATION PROPERTIES.....	22
2.1 Introduction.....	22
2.2 Structure of the eye and retina.....	22
2.3 Models for interaction of polarized light with ocular structures.....	30
2.4 Experimental methods for ocular polarization interactions.....	37
2.5 Polarization properties of ocular structures.....	46
2.6 Retinal diseases.....	58
2.7 Polarization methods for disease diagnostic.....	64
2.8 Advantages of GDx-MM over other technologies.....	68
2.9 Conclusions.....	71
3. POLARIZATION AND POLARIMETRY.....	72
3.1 Introduction.....	72
3.2 Polarization.....	72
3.3 The Mueller calculus.....	74
3.4 Extraction of polarization parameters.....	79
3.5 The Poincaré Sphere.....	86
3.6 Polarimetry.....	89
3.7 Physically realizable Mueller matrices.....	96
3.8 Conclusions.....	102
4. OPTIMIZATION OF MUELLER MATRIX POLARIMETERS.....	103
4.1 Introduction.....	103
4.2 Background.....	103
4.3 Rank, range, and nullspace.....	105
4.4 Stability in the presence of error.....	106
4.5 Singular Value Decomposition.....	109
4.6 Geometrical interpretation.....	112
4.7 Error analysis.....	114
4.8 Forming a minimizable error metric.....	118
4.9 Illustrative examples.....	122
4.10 Conclusions.....	133

TABLE OF CONTENTS – Continued

5. THE LASER DIAGNOSTICS GDx.....	134
5.1 Introduction.....	134
5.2 Background and basic operation.....	134
5.3 Limitations of the GDx.....	138
5.4 Optical paths.....	142
5.5 Radiometry.....	145
5.6 Polarization path model and analysis.....	151
5.7 Electronics function.....	164
5.8 Reverse engineering.....	165
5.9 Conclusions.....	167
6. GDx-MM DESIGN AND DEVELOPMENT.....	168
6.1 Introduction.....	168
6.2 Polarization analysis.....	168
6.3 Optical path.....	178
6.4 Radiometry.....	183
6.5 System timing.....	185
6.6 Electronics.....	188
6.7 Conclusions.....	198
7. COMPONENT ANALYSIS.....	200
7.1 Introduction.....	200
7.2 Liquid crystal retarders.....	200
7.3 Laser source.....	210
7.4 Scanners and scan mirrors.....	211
7.5 Avalanche photodiode detector.....	214
7.6 Rotating waveplate.....	220
7.7 Conclusions.....	221
8. CALIBRATION.....	222
8.1 Introduction.....	222
8.2 Overview of calibration methods.....	222
8.3 Generator calibration.....	224
8.4 Analyzer calibration.....	228
8.5 Creation of calibration dataset.....	232
8.6 Conclusions.....	234
9. ERROR ANALYSIS.....	236
9.1 Introduction.....	236
9.2 Polarimeter-based error sources.....	236
9.3 Simulation results.....	239
9.4 Calibration error.....	245

TABLE OF CONTENTS – Continued

9.5 Conclusions.....	250
10. USE MODEL AND DATA PROCESSING.....	251
10.1 Introduction.....	251
10.2 Use with human subjects.....	251
10.3 From raw data to image stack.....	254
10.4 From image stack to polarization images.....	258
10.5 Conclusions.....	261
11. INSTRUMENT VALIDATION.....	262
11.1 Introduction.....	262
11.2 Validation hardware.....	262
11.3 Validation measurements.....	265
11.4 Conclusions.....	277
12. HUMAN SUBJECT USE AND DATA ANALYSIS.....	278
12.1 Introduction.....	278
12.2 Human subject interface issues.....	279
12.3 Creation of Mueller matrix images.....	280
12.4 Anterior segment compensation.....	287
12.5 Image gradients and noise analysis.....	298
12.6 Results for Indiana University subjects.....	302
12.7 Discussion of results.....	308
12.8 Comparison to GDx.....	311
12.9 Utility of polarization parameter plots.....	312
12.10 Other polarization parameters.....	314
12.11 Retina mosaics.....	316
12.12 Conclusions.....	320
13. CONCLUSIONS AND FUTURE WORK.....	321
13.1 Introduction.....	321
13.2 Review of accomplishments.....	321
13.3 Discussion and future work.....	324
13.4 Overall conclusions.....	327
APPENDIX A: ACRONYMS.....	329
APPENDIX B: SYSTEM SPECIFICATIONS.....	330
REFERENCES.....	331

LIST OF FIGURES

Figure 2.1:	A section through the human eye showing ocular structures.....	23
Figure 2.2:	A normal human retina as seen through an ophthalmoscope.....	25
Figure 2.3:	The layers of the central human retina.....	26
Figure 2.4:	Ocular quadrants, axes, and retinal landmarks for right and left eyes.....	29
Figure 2.5:	Cylindrical structure of the RNFL.....	33
Figure 2.6:	Kemp model of form birefringence and biattenuance.....	35
Figure 2.7:	Schematic diagram of a polariscope instrument used to view the fourth Purkinje image.....	38
Figure 2.8:	Schematic diagram of a Mueller matrix ellipsometer.....	39
Figure 2.9:	Schematic of a camera-based incomplete polarimeter.....	40
Figure 2.10:	Schematic of an incomplete scanning laser polarimeter.....	41
Figure 2.11:	Schematic diagram of a PS-OCT setup.....	43
Figure 2.12:	Structural detail of retinal layers obtained using OCT.....	43
Figure 2.13:	Schematic of a complete Mueller matrix imaging polarimeter.....	45
Figure 2.14:	Schematic of the polarimeter designed by Lara and Dainty.....	46
Figure 2.15:	Corneal retardance distribution as measured using PS-OCT.....	49
Figure 2.16:	Retinal birefringence distribution as a function of depth.....	51
Figure 2.17:	Retinal data obtained with a complete Mueller matrix imaging polarimeter.....	54
Figure 2.18:	Ocular components which influence polarization.....	57
Figure 2.19:	Fundus photographs of retinas for comparison of disease process.....	64
Figure 2.20:	Algorithm used to calculate a depolarization image from GDx data.....	67
Figure 2.21:	A comparison of various types of fundus images.....	67
Figure 3.1:	The Poincaré Sphere, showing the precession of polarization states.....	88
Figure 3.2:	Basic light measuring polarimeter.....	91
Figure 3.3:	Basic sample measuring polarimeter.....	94
Figure 4.1:	The trajectory of an optimized rotating retarder Stokes polarimeter plotted on the Poincaré sphere.....	113
Figure 4.2:	Schematic of Dual Rotating Retarder (DRR) polarimeter.....	124
Figure 4.3:	Schematic of Dual Variable Retarder (DVR) polarimeter.....	125
Figure 4.4:	Sample trajectories on the Poincaré sphere.....	126
Figure 4.5:	Condition number as a function of waveplate retardance for the DRR polarimeter.....	127
Figure 4.6:	Condition number as a function of rotating retarder increments for the DRR polarimeter.....	128
Figure 4.7:	Condition number as a function of polarimeter variables for the DVR polarimeter.....	129
Figure 4.8:	Singular Values for the example polarimeter configurations.....	130
Figure 4.9:	Calculated mean and SD error for the example polarimeter configurations.....	131
Figure 4.10:	EM metric to optimize retardance for the DRR polarimeter.....	132

LIST OF FIGURES – Continued

Figure 5.1:	Laser Diagnostics GDx Retinal Nerve Fiber Analyzer.....	135
Figure 5.2:	Output data generated by the GDx.....	137
Figure 5.3:	Diagram of GDx scanning path	144
Figure 5.4:	Diagram of GDx imaging path for the outgoing and return light.....	145
Figure 5.5:	GDx polarization path.....	153
Figure 5.6:	Singular Values for GDx polarimeter.....	157
Figure 5.7:	Simulated error in measurement of linear retardance as a function of degree of polarization.....	160
Figure 5.8:	Simple model for GDx measurement of retinal retardance.....	161
Figure 5.9:	Theoretical intensity measurement of a linear retarder using the GDx polarimeter.....	163
Figure 6.1:	GDx-MM polarization component layout.....	170
Figure 6.2:	Accessible portion of Poincaré sphere for different orientations of variable retarders.....	172
Figure 6.3:	Trajectories on Poincaré sphere for LCR retardances and total rotations of the rotating waveplate.....	173
Figure 6.4:	Log condition number as a function of rotating waveplate retardance.....	176
Figure 6.5:	Optical and polarization component imaging path in the GDx-MM.....	181
Figure 6.6:	Code V model of the GDx-MM outgoing imaging path, for scanners in the central position.....	182
Figure 6.7:	Code V model of the GDx-MM outgoing imaging path, for scanners giving maximum field of view on the retina.....	182
Figure 6.8:	Code V model for the GDx-MM incoming path, for scanners in the central position.....	183
Figure 6.9:	Diagram of the GDx-MM timing structure.....	188
Figure 6.10:	Electronics system architecture for the GDx-MM.....	193
Figure 6.11:	The modified scan head, viewed from both sides.....	193
Figure 6.12:	The GDx-MM system.....	194
Figure 6.13:	Start button and motor homing circuit.....	195
Figure 6.14:	Motor double pulsing circuit.....	196
Figure 6.15:	Detector manual control circuit.....	197
Figure 6.16:	Custom electronics circuit box.....	198
Figure 7.1:	LCR composition and liquid crystal orientation as a function of applied voltage.....	203
Figure 7.2:	Typical retardance vs. voltage curve for liquid crystal variable retarders.....	206
Figure 7.3:	Examples of nonuniformity in retardance magnitude across clear aperture of two representative liquid crystal variable retarders.....	207
Figure 7.4:	Change in retardance as a function of angle of incidence over a 10° cone for a liquid crystal variable retarder.....	208

LIST OF FIGURES – Continued

Figure 7.5:	Typical switching profiles for liquid crystal variable retarders.....	209
Figure 7.6:	Scan of graph paper showing slight scan distortion over the field of view.....	213
Figure 7.7:	Measured linearity of the GDx-MM detectors.....	218
Figure 7.8:	Fast Fourier transform of the GDx-MM scan of a graph paper target.....	219
Figure 8.1:	Generator calibration setup, showing the scan head and external Stokes polarimeter mounted on an optical bench.....	225
Figure 8.2:	Degree of Polarization of measured generator states.....	227
Figure 8.3:	A comparison of measured generator states and ideal model plotted on the Poincaré sphere.....	227
Figure 8.4:	Analyzer calibration setup, showing the scan head and external Stokes polarization state generator mounted on an optical bench.....	229
Figure 8.5:	Degree of Polarization of measured analyzer states.....	232
Figure 8.6:	A comparison of measured analyzer states and ideal model plotted on the Poincaré sphere.....	232
Figure 9.1:	Effect of polarimeter error sources on reconstruction of the sample Mueller matrix and extracted polarization parameters.....	242
Figure 9.2:	Individual error source contributions to the error in reconstruction.....	244
Figure 9.3:	Evidence of detector gain drift in the calibration dataset.....	248
Figure 9.4:	Drift in output Stokes vector over one hour.....	249
Figure 10.1:	Use of the GDx-MM by an operator, and correct positioning of the subject on the chin rest.....	252
Figure 10.2:	Main control panel for the GDx-MM.....	254
Figure 10.3:	Labview application for converting raw data stream of 32 Mbytes to an image stack of 72 images.....	256
Figure 10.4:	Labview application for registration of the 72 images in the dataset.....	257
Figure 10.5:	Example Mueller matrix image.....	259
Figure 10.6:	Example polarization parameter image.....	260
Figure 10.7:	Summary of the process of converting the 72-image stack into a polarization parameter image.....	260
Figure 11.1:	Code V model of the GDx-MM outgoing path including two-lens telecentric system.....	263
Figure 11.2:	The validation rail mounted on the scan head.....	264
Figure 11.3:	Intensity profiles over the 72 polarimeter states for a rotating linear polarizer target.....	268
Figure 11.4:	Comparison of ideal and measured intensity profiles for polarizer target at 15° orientation.....	268
Figure 11.5:	Error in polarization parameters for a rotating linear polarizer target, as a function of polarizer orientation.....	269

LIST OF FIGURES – Continued

Figure 11.6: Intensity profiles over the 72 polarimeter states for a rotating quarter wave plate target.....	271
Figure 11.7: Comparison of ideal and measured intensity profiles for quarter wave plate target at 15° orientation.....	271
Figure 11.8: Error in polarization parameters for quarter wave plate target as a function of waveplate orientation.....	272
Figure 11.9: Error in polarization parameters for variable wave plate target, as a function of waveplate retardance.....	273
Figure 11.10: Air profile, showing a small variation in intensity due to normal incidence back-reflections from the GDx optics.....	274
Figure 11.11: Spectralon profile, showing an up to 20% variation in intensity with polarization state.....	275
Figure 11.12: Measured intensity profiles over the 72 polarization states for a linear polarizer oriented at 45°.....	276
Figure 12.1: Raw data sequence for ONH.....	282
Figure 12.2: Raw data sequence for macula.....	283
Figure 12.3: Mean intensity images for ONH and macula.....	284
Figure 12.4: Example Mueller matrix images for Subject 12.....	285
Figure 12.5: Typical Frobenius error images.....	286
Figure 12.6: Example of a bowtie model simulation, based on measured parameters from Subject 3.....	289
Figure 12.7: General bowtie method, using macula linear retardance image.....	290
Figure 12.8: Various methods for anterior segment compensation, demonstrating significant differences in compensation results.....	296
Figure 12.9: Varying δ_{AS} and θ_{AS} within $\pm 8^\circ$ of the values found using the screen method demonstrating the wide range of compensation.....	297
Figure 12.10: Random noise analysis method for Spectralon and macula	298
Figure 12.11: Comparison of images for different registration methods.....	301
Figure 12.12: Linear Retardance plots for subjects 1, 3, 7, 8, 11, and 12.....	303
Figure 12.13: Retardance Orientation plots for subjects 1, 3, 7, 8, 11, and 12.....	304
Figure 12.14: Diattenuation Magnitude plots for subjects 1, 3, 7, 8, 11, and 12.....	305
Figure 12.15: Diattenuation Orientation plots for subjects 1, 3, 7, 8, 11, and 12.....	306
Figure 12.16: Depolarization Index plots for subjects 1, 3, 7, 8, 11, and 12.....	307
Figure 12.17: Comparison between GDx-MM and GDx uncompensated linear retardance data for the maculas of three subjects.....	311
Figure 12.18: Comparison between GDx-MM and GDx uncompensated linear retardance data for the ONHs of three subjects.....	312
Figure 12.19: Other polarization parameter images for macula region of Subject 12.....	315
Figure 12.20: Other polarization parameter images for ONH of Subject 12.....	315

LIST OF FIGURES – Continued

Figure 12.21: Subject A1 left eye mosaic.....	317
Figure 12.22: Subject A2 right eye mosaic.....	318
Figure 12.23: Subject A2 left eye mosaic.....	319
Figure 13.1: Final delivery of GDx-MM to Indiana University.....	328

LIST OF TABLES

Table 2.1: Overview of corneal retardance measured by various researchers.....	48
Table 2.2: Overview of retinal birefringence and retardance measured by various researchers.....	52
Table 2.3: Overview of retinal diattenuation and degree of polarization measured by various researchers.....	53
Table 4.1: Summary of polarimeter polarization components.....	123
Table 4.2: Summary of polarimeter error sources.....	123
Table 5.1: Values of parameters for calculating GDx radiometry.....	149
Table 5.2: Calculated radiometric quantities.....	150
Table 5.3: Values of detector parameters.....	151
Table 6.1: Polarimeter rank and condition number vs. retardance and orientation of rotating waveplate	177
Table 6.2: Variables which modify the radiometry for GDx-MM.....	185
Table 9.1: Systematic error sources.....	237
Table 9.2: Zero mean random error sources.....	237
Table 9.3: Worst case systematic error in polarization parameters.....	240
Table 9.4: Standard deviation error in polarization parameters.....	241
Table 9.5: Drift type error sources during the duration of calibration.....	246
Table 11.1: Validation measurement results.....	277
Table 12.1: Comparison of results for AS compensation methods.....	293
Table 12.2: Statistical polarization parameter values for Indiana University subjects.....	308

ABSTRACT

Retinal diseases are a major cause of blindness worldwide. Although widely studied, disease mechanisms are not completely understood, and diagnostic tests may not detect disease early enough for timely intervention. The goal of this research is to contribute to research for more sensitive diagnostic tests that might use the interaction of polarized light with retinal tissue to detect subtle changes in the microstructure. This dissertation describes the GDx-MM, a scanning laser polarimeter which measures a complete 16-element Mueller matrix image of the retina. This full polarization signature may provide new comparative information on the structure of healthy and diseased retinal tissue by highlighting depolarizing structures as well as structures with varying magnitudes and orientations of retardance and diattenuation.

The three major components of this dissertation are: 1. Development of methods for polarimeter optimization and error analysis; 2. Design, optimization, assembly, calibration, and validation of the GDx-MM polarimeter; and 3. Analysis of data for several human subjects. Development involved modifications to a Laser Diagnostics GDx, a commercially available scanning laser ophthalmoscope with incomplete polarization capability. Modifications included installation of polarization components, development of a data acquisition system, and implementation of algorithms to convert raw data into polarization parameter images. Optimization involved visualization of polarimeter state trajectories on the Poincaré sphere and a condition number analysis of the instrument matrix. Retinal images are collected non-invasively at 20 μm resolution

over a 15° visual field in four seconds. Validation of the polarimeter demonstrates a polarimetric measurement accuracy of approximately $\pm 5\%$.

Retinal polarization data was collected on normal human subjects at the University of Arizona and at Indiana University School of Optometry. Calculated polarization parameter images reveal properties of the tissue microstructure. For example, retardance images indicate nerve fiber layer thickness and orientation, and depolarization images (uniform for these normal subjects), are predicted to indicate regions of disease-related tissue disruption. This research demonstrates a method for obtaining a full polarization signature of the retina in one measurement using a polarimetrically optimized instrument, and provides a step toward the use of complete retinal imaging polarimetry in the diagnosis and monitoring of retinal disease.

CHAPTER 1: INTRODUCTION

Light is a transverse wave with specific statistical magnitude and phase relations between orthogonal field components. Active polarimetry discerns information about samples – for example a cell, a crystal, the retina, or the sky – by the changes in magnitude and phase after an interaction with polarized light. Active polarimeters have been utilized for at least 200 years to generate the incident polarized light and/or analyze the change in magnitude and phase after interaction. Polarimetry information complements the information obtained with other probing methods such as microscopy, spectroscopy, and interferometry. There is a wide range of applications where accurate polarimetry could provide valuable information but where its application has not been widely explored due to the complexity of polarized light interactions and the corresponding complexity of the instruments. Only in the last 20 years has polarimetry been developed as an active field within the optical sciences.

There are many instances where active polarimeters might be useful in biomedical research. Individual cells and cell layers tend to have a regular structure which yields measurable differences in the magnitude and phase of polarized light upon interaction. Thus polarized light might for example be used to explore the normal structure of a tissue layer or to diagnose when that layer has been damaged due to a disease process. Many biomedical researchers have recognized the value of diagnostic techniques utilizing polarized light, but these techniques have not entered the mainstream of health care as

approved and accepted diagnostic instruments. There is thus a large under-explored market for polarization-based instruments which might be utilized in a hospital, clinic, or physician's office to assist in the diagnosis and monitoring of disease.

Biomedical applications for polarized light include probing accessible tissue structures where disease processes commonly occur. One example is the retina, which is subject to a number of common diseases such as glaucoma, age-related macular degeneration, and diabetic retinopathy, all of which have improved prognosis when detected early. Many researchers have utilized polarized light to investigate the structure of the retina, and a smaller number have explored the use of polarized light as a disease diagnostic. For example, retardance measurement has been used to determine when the nerve fiber layer is thinner than normal [1]. An increase in depolarization has been used to visualize tissue disruptions [2]. One commercially available instrument, the Laser Diagnostics GDx, utilizes polarized light to assist in the detection and monitoring of glaucoma. This dissertation will show that the GDx is not well optimized polarimetrically and cannot measure a full range of polarization parameters.

In this dissertation a retinal imaging polarimeter, the GDx-MM, is described. The GDx-MM is optimized to accurately measure a full range of polarization parameters, and has an architecture which may be adapted to clinical use. This work received major funding from Subaward #EP-17093 of National Institutes of Health National Eye Institute grant 5R01EY007624-17 entitled "The Extent of Retinal Damage". This grant was awarded to

principal investigator Dr. Ann Elsner at Indiana University School of Optometry, with Dr. Russell Chipman at the University of Arizona as a co-investigator. The specific aim of this grant is to determine the spatial extent of retinal damage using noninvasive, quantitative mapping of visual function and structure. Development of the GDx-MM contributes to the goal of developing noninvasive technologies to better understand retinal function and change due to disease, with particular emphasis on improving early diagnosis of age-related macular degeneration.

To develop the GDx-MM, extensive modifications (polarimetric, optical, and electronic) were made to a Laser Diagnostics GDx. This platform provided resources including an eye-safe retinal laser scanner and a comfortable patient interface with methods to align the eye to the instrument. The original GDx instrument was provided to the University of Arizona by Dr. Elsner, and then returned to Indiana University upon completion of the modifications. In the collaboration between the two universities, the University of Arizona provided expertise in polarimetry, optical design, and electronic design, and Indiana University provided invaluable insight into ocular structure and polarization properties to guide the design. The individual contributions of the two universities are outlined in the following paragraphs.

Polarimetry design for the GDx-MM was performed under the guidance of Dr. Chipman at the University of Arizona. While polarimeter optimization has been reasonably (though not extensively) discussed in the literature, the optimization of sample measuring

polarimeters which measure a 16 dimensional Mueller matrix (as opposed to simpler light measuring polarimeters which measure a 4 dimensional Stokes vector) has not been fully developed. In a major portion of this dissertation, polarimeter optimization theory has been developed for Mueller matrix polarimeters, and an error analysis method has been developed to predict inaccuracy due to polarimeter and measurement error. The optimization theory and error analysis method were applied to optimize the GDx-MM polarimeter design and predict the accuracy given knowledge of the error sources. Optimization was performed within the limits imposed by using the GDx as a starting point, i.e. that the existing polarization components could be removed or replaced with components of similar size and optical properties, and the available mounting space for additional components in the optical path was limited.

To be able to measure a full range of polarization parameters, two new polarization components (liquid crystal variable retarders) were inserted, one each in the outgoing and incoming paths, and the original rotating half wave plate was replaced with a waveplate having lower retardance. The optimization process dictated the retardances and orientations of these new elements. Appropriate optical and mechanical design allowed the new polarization elements to be incorporated into the optical path while maintaining imaging performance. An external computer-controlled data acquisition system was implemented to support the new requirements, including collecting a larger number of images and controlling the polarization elements. The polarimeter was then calibrated to provide an accurate description of the polarimeter function. Programs were written to

perform the several processing steps necessary to convert the raw data to polarization parameter images. Finally, a validation was performed to quantify the measurement accuracy. This work was performed at the University of Arizona.

To demonstrate proof of concept, the GDx-MM was used on several human subjects to collect retinal polarization data. Data was collected over a wide retinal field of view for both eyes of two normal subjects at the University of Arizona, and later on the optic nerve head and macula regions for the right eyes of 15 normal subjects at Indiana University. In working with human subjects it was sometimes difficult to achieve good alignment and focus without pupil vignetting. Saccadic eye movement and blinking during the measurement were common. However, several good quality datasets were obtained. To facilitate data interpretation, guidance on the character and polarization properties of retinal tissue was provided by Dr. Elsner. The calculated polarization parameter images clearly reveal properties of the retinal tissue structure, thus demonstrating the potential of Mueller matrix imaging polarimetry. This research points toward design improvements in later generation devices, which may be used to perform larger studies including diseased patients to obtain a more thorough understanding of the value of the measured polarization parameters in diagnosing and monitoring retinal disease.

CHAPTER 2: EYE STRUCTURE, DISEASE, AND POLARIZATION PROPERTIES

2.1: Introduction

To better understand the general topic of polarization interactions in the eye, it is useful to outline the basic light-encountering structures in the eye and describe their microstructure. Once the structural components are understood, polarization properties can be predicted based on the tissue microstructure. In this chapter various models which have been developed for the interaction of polarized light with tissue will be discussed. Retinal diseases modify many tissue properties, thus changing the interaction with polarized light. This chapter also presents a review of the most prevalent retinal diseases, and describes research in the area of using polarization to measure ocular polarization properties and to diagnose retinal diseases. Finally, the basic advantages of the GDx-MM over existing techniques for retinal polarization imaging and disease diagnosis are presented and discussed.

2.2: Structure of the Eye and Retina

The human eye [3], Figure 2.1, can be divided into outer and inner regions. The outer region of the eye consists of two parts: the anterior cornea, a transparent layer with optical power through which light enters the eye; and the posterior sclera, a dense, white, opaque layer providing a boundary at the rear of the eye. Major structures within the inner eye include the iris, a ring of tissue which forms the aperture stop of the eye; the lens, providing additional optical power; the ciliary body, a ring of tissue surrounding the lens; and the retina, a layered tissue structure where an optical image of the external

world is formed and sensed. The inner region of the eye consists of three parts: the anterior chamber, lying between the cornea and iris and containing aqueous fluid; the posterior chamber, framed by the lens, iris, and ciliary body and containing aqueous fluid; and the vitreous chamber, lying between the lens and retina and containing a gelatinous mass called the vitreous humour. Between the retina and sclera is a blood-rich layer, the choroid, which supports the metabolic processes in the retina. The path of image-forming light through the eye is: cornea, aqueous fluid, lens, vitreous humour, and retina.

Figure 2.1. A section through the human eye showing ocular structures and a schematic enlargement of retinal structure. Reprinted with permission from [4].

The majority of the eye's refracting power is provided by the cornea [5]. The layers comprising the cornea are the epithelium, Bowman's membrane, stroma, Descemet's

membrane, and endothelium. The typical thickness of the cornea is about 0.6 mm; the stroma is the thickest layer at approximately 0.5 mm. The stroma is composed of 200-300 stacked collagen layers termed lamellae, about 1.5-2.5 μm thick, that run the length of the cornea. Each lamella contains cylindrical shaped collagen fibrils which are parallel to each other. Fibrils have diameters of 32-36 nm and are separated by 20-50 nm, substantially smaller than the wavelength of visible light. The angular orientation of fibrils is uniform within a given layer but random with potentially large angle between adjacent layers. The refractive index of the fibrils, about 1.47, is larger than that of the surrounding medium, about 1.354. [5]

The lens [5] provides additional optical power which is varied as the ciliary muscle within the ciliary body is contracted, changing the shape to provide focal accommodation. The bulk, about 3.6 mm thick, is comprised of cellular tissue with non-uniform refractive index. The tissue is comprised of 8 μm diameter fibres oriented radially. The fibers exhibit localized structural order.

The aqueous fluid is a watery fluid generated by the ciliary body to support the metabolic processes of the cornea and lens and maintain corneal shape. The composition is 99% water [6], but also contains small amounts of glucose, albumen, proteins, amino acids, and lactate. The vitreous humour is a tough gelatinous substance considerably more viscous than water, though the water content is also about 99%. In addition to salts, sugars, and acids such as hyaluronic acid, the vitreous humour contains a network of long,

thin, separated collagen fibrils, distributed throughout the posterior chamber in loose bundles which are cross-linked by fibrils that extend between bundles [7].

The retina [5], of primary importance to this research, is the light-sensitive surface where the optical image is formed. Distinct features (Figure 2.2) include the macula, the region of highest visual acuity; the fovea, the central part of the macula which is free of blood vessels; and the optic disc or optical nerve head (ONH), the entry point of the optic nerve and the region of lowest visual acuity.

Figure 2.2: A normal human retina as seen through an ophthalmoscope, showing the major retinal features and vasculature. Reprinted with permission from [4].

The retina is comprised of a number of distinct layers (Figure 2.3), each with unique structure. The overall thickness ranges from 50 μm at the foveal pit, to 600 μm at the ONH. The structure contains three layers of nerve cells sandwiching two layers of synapses, as well as a photosensitive layer.

Figure 2.3: The layers of the central human retina. (a): schematic view: (b): light micrograph. Reprinted with permission from [4].

From outer surface (adjacent to the vitreous humour) to inner surface (adjacent to the sclera), the layers of the retina are as described below:

Inner limiting membrane (ILM): thin layer which provides a boundary between the vitreous chamber and retinal layers.

Retinal nerve fiber layer (RNFL): A 30-120 μm thick layer composed of unmyelinated axons of retinal ganglion cells, gathered into bundles. These cells consist in part of microtubules, collagen filaments, and actin-myosin complexes, all of which are effectively parallel oriented cylinders, with size comparable to the wavelength of light,

and refractive index slightly larger than that of the surrounding medium. The fibers are radially oriented around the optic disc. The RNFL is thicker in superior and inferior regions, and thinner in temporal and nasal regions.

Layer of ganglion cells (GCL) – A layer of neurons which transmit information to the optic nerve head. The neurons consist of large ganglion cells, which are flask-shaped cells with variable size.

Inner plexiform layer (IPL) – A synapse layer which connects the INL neurons to the layer of ganglion cells. It is composed of a dense assembly of small fibrils.

Inner nuclear layer (INL) – A layer of neurons consisting of bipolar (vertical, roundish), horizontal (roundish flattened), amacrine cells (roundish) cells which are close packed.

Outer plexiform layer (OPL) – A very thin synapse layer which connects the ONL neurons to the INL neurons.

Outer nuclear layer (ONL) – A layer of oval-shaped neurons connecting to the rods and cones.

Outer limiting membrane (OLM) – A thin layer separating the retinal neural layers from the photoreceptors.

Photoreceptor layer (rods and cones) – Vertically oriented light sensitive cells of two types: the cylindrical rods, useful for low-light and grayscale vision; and the cone-shaped cones, somewhat larger than rods, which are useful for high-light and color vision.

Retinal pigment epithelium (RPE) – A pigmented (grey and black) absorbing and scattering cell layer which nourishes retinal cells, drawing from the choroid. It consists of a single layer of hexagonal cells that are densely packed with pigment granules.

Bruch's membrane – The innermost layer of the choroid, having 5 layers consisting of elastic and collagenous fibers. It is quite thin (50 μm) and separates the choroid from the RPE.

The structure of the retina in the region of the fovea is slightly different. Here cone receptors are at maximum packing density. At the center of the fovea is the foveal pit, a very thin region where most of the retinal layers have been displaced concentrically, leaving only a very thin section of cone cells with a thin overlying section of Outer Plexiform Layer called Henle's fiber layer.

Henle fiber layer (in macula): A layer of elongated photoreceptor axons oriented radially about the fovea. This layer has similar structure to the RNFL, but is even more uniform.

For reference the retina is divided into quadrants. The upper quadrants are labeled “superior” and the lower are labeled “inferior”. The quadrants towards the outer portion of the eye are labeled “temporal” and those towards the nose are labeled “nasal”. A horizontal axis centered on the eye can be used to identify the alignment of certain structures. To an outside observer facing the cornea of the right (left) eye, a clockwise (counter-clockwise) rotation of the axis is referred to as “nasally downward” or ND, and a counter-clockwise (clockwise) rotation is termed “nasally upward” or NU. In both eyes the macula is temporal to the ONH. Ocular quadrants and retinal landmarks are summarized in Figure 2.4.

Figure 2.4: Ocular quadrants (superior, inferior, temporal, nasal); axes (NU = nasally upward, ND = nasally downward); and retinal landmarks (optic nerve head and macula), for right and left eyes.

2.3: Models for interaction of polarized light with ocular structures

The human visual system has limited ability to distinguish polarized light, and is in effect polarization-blind. Polarization vision is present in certain insects and animals such as the octopus, where the ability to detect polarization is due to a regular ordering of the light-sensitive structures. For example, in insects and octopus the rhodopsin (visual pigment) molecules are aligned parallel to the regularly arranged microvilli tubes, and these molecules preferentially absorb light that oscillates along their dipole axis. In insects the microvilli are arranged in a radiating star pattern [8]; in octopus adjacent photoreceptors have orthogonal orientations [9]. The photoreceptor structures in the human eye, by comparison, are randomly oriented and so there is no overall polarization sensitivity. However, an ocular polarization phenomenon termed Haidinger's brush, first described by von Haidinger in 1844 [10], manifests as a yellow/blue bowtie pattern which is faintly visible to many (though not all) people. This pattern is due to the composition and arrangement of pigment molecules in the macular region. Since the human visual system is not designed for visualization of polarized light, it is necessary to use an external means to study the interaction of polarized light with ocular structures. This is typically done by delivering polarized light to the eye, then observing the polarization properties of the light that is returned from the eye (i.e. by backscatter or reflection). For polarized light to reveal information about ocular and in particular retinal tissue, several requirements must be met:

- There must be a mechanism to return light back in the direction of the probing beam.

This might be a reflecting or scattering event, from the tissue bulk or from interfaces between tissues.

- The returned light must be able to exit the eye and be collected. This might not be the case if the returned light is generated deep within the retinal structure and is absorbed and/or scattered away from the collection optics, or if the return light is vignetted by the pupil.

- For the presence of non-depolarizing properties (diattenuation and retardance, as discussed in Chapter 3), the tissue must have a regular structure over a sufficiently large volume, so as to build up a measurable change in the magnitude and/or phase of the incident polarized light. This change will be specific to the structure of the tissue.

- For the presence of depolarizing properties (discussed in Chapter 3), the tissue must have a structure such as to measurably scramble the magnitude and/or phase of the incident polarized light. Depolarization may for example be the result of spatial variation or scattering within the tissue structure.

- If there are multiple tissue layers in the light path with multiple polarization effects, then they must all be considered when analyzing the polarization state of the return light.

There is value in analyzing the known geometrical properties of the tissues present in the eye, to be able to predict and model their polarization effect. From tissue sectioning, polarized light microscopy [11], electron microscopy [12], and other techniques, it is known that those ocular tissues with most regular structure are the stroma in the cornea, the interior of the lens, the nerve fiber layer in the retina, and Henle's layer in the retina. These anisotropic structures all contain long thin parallel-oriented cylinders (such as collagen fibrils or microtubules), uniformly distributed within the surrounding medium, and with dimension smaller than the wavelength of visible light (Figure 2.5). Modeling efforts have thus focused on analysis of regular arrays of small cylindrical structures embedded within a medium of slightly lower refractive index. It was first shown by Wiener (1912) [13] in his theory of mixed dielectrics that this type of structure, solely due to the geometry and the small difference in refractive index between cylinders and medium, presents different refractive index to light oscillating along the cylinder axes and light oscillating perpendicular to the cylinder axes. The two light components propagate at different phase velocity, just as if the structure were a uniaxial crystal with optic axis parallel to the cylinders, and the phase retardance between light components increases linearly as a function of propagation distance. This property is termed "form birefringence" [14]. Hemenger [15] further deduced that the same structure leads to a higher absorption for light oscillating parallel to the cylinders, than for light oscillating perpendicular to the cylinders (similar to a wire grid polarizer), so that diattenuation increases with propagation distance. This property is termed "form dichroism".

Figure 2.5: Cylindrical structure of the RNFL. Reprinted with permission from [12].

Though the basic model is relatively straightforward, the simple assumptions are not sufficiently accurate to give reliable estimates for polarization properties such as birefringence and diattenuation in tissue, a rather complicated medium. Several researchers have customized the theory for specific ocular tissues of interest. Bragg and Pippard [16] expanded on Wiener's theory for the case of aligned ellipsoidal particles. Oldenbourg [17] reexamined Wiener's theory and applied an improved model to compute form birefringence of macromolecules. Zhou and Knighton [12] considered only the RNFL, and developed a model to predict light scattering and birefringence resulting from light interaction with four cylindrical tissue constituents: cell membranes around nerve axons (outer diameter 10 nm), microtubules (outer diameter 25 nm, length 10-25 μm), neurofilaments (outer diameter 10 nm), and mitochondria within nerve axons (100-200 nm outer diameter, 1-2 μm length). By vector summation of the electric fields induced by the incident radiation at each cylinder, and assuming that there is no interaction between cylinder fields, an expression is found for form birefringence which has strong dependence on the ratio of refractive index in cylinders and medium, the volume fraction

of cylinders in the medium, and the index of the cylinders. It is remarked that deviations from parallelism and uniform packing significantly affect the theory predictions. This theory was later extended by Huang and Knighton [18] to incorporate interactions between RNFL scattering and birefringence; this model predicts that the RNFL reflectance has weak intrinsic diattenuation and preserves the degree of polarization, but these conclusions are highly dependent on refractive index ratio.

Kemp et al. [19] developed complementary expressions for form birefringence and a newly defined quantity, form biattenuance, which describes the differential attenuation of light components by either absorption (dichroism) or scattering, and which can be expressed on a per-unit-depth basis. The theory is developed by considering material composed of alternating anisotropic layers containing a single layer of embedded cylinders and isotropic layers containing water. The Fresnel relations are used to find expressions for form birefringence and form biattenuance; the important distinction between the two is that retardance derived from birefringence is wavelength independent and linearly dependent on thickness, whereas diattenuation derived from biattenuance is wavelength dependent and nonlinearly dependent on thickness. As the work of Kemp et al. is most recent and presents a general theory for the development of form-based retardance and diattenuation which is applicable to ocular tissues, this work is summarized here as representative of the theory in general. The model parameters are summarized in Figure 2.6.

Figure 2.6: Kemp model of form birefringence and biattenuance, consisting of alternating anisotropic and isotropic layers. The anisotropic layers contain embedded cylinders with different refractive index from the surrounding medium, and the isotropic layers contain water. Reprinted with permission from [19].

Consider the complementary terms birefringence Δn (differential refractive index for two polarization eigenstates) and biattenuance $\Delta\chi$ (differential transmittance for two polarization eigenstates), and the complimentary terms phase retardance δ (differential phase difference for two polarization eigenstates) and relative attenuation ε (differential magnitude change for two polarization eigenstates). The relationship among these terms is

$$\frac{\delta}{\Delta z} = \frac{2\pi}{\lambda_0} \Delta n, \quad \frac{\varepsilon}{\Delta z} = \frac{2\pi}{\lambda_0} \Delta\chi, \quad (2.1)$$

where λ_0 is the center wavelength, and both phase retardance and relative attenuation have been expressed in terms of the layer thickness Δz . Diattenuation D is related to relative attenuation by

$$D = \tanh \varepsilon \approx \varepsilon \quad \text{for small } \varepsilon. \quad (2.2)$$

Let the anisotropic layers have thickness h_1 and the embedded cylinders have index n_w , center-to-center spacing a ($h_1 \leq a \ll \lambda_0$) and be imbedded in water with index n_w . Let the isotropic layer have thickness h_2 and index n_w . Then the effective refractive indices parallel (n_p) and perpendicular (n_s) to the fibers are [17]

$$n_p^2 = \left(\frac{h_1}{a}\right)n_f^2 + \left(1 - \frac{h_1}{a}\right)n_w^2, \quad n_s^2 = n_w^2 + \frac{(h_1/a)(n_f^2 - n_w^2)}{1 + \frac{1}{2}\left(1 - \frac{h_1}{a}\right)\left(\frac{n_f^2 - n_w^2}{n_w^2}\right)}. \quad (2.3)$$

Then Δn and $\Delta \chi$ can be expressed very compactly as

$$\Delta n = \frac{h_1}{h_1 + h_2}(n_p - n_s), \quad \Delta \chi = -\frac{\lambda_0}{2\pi(h_1 + h_2)} \ln \left[\frac{n_p(n_s + n_w)^2}{n_s(n_p + n_w)^2} \right]. \quad (2.4)$$

Kemp et al. point out that the ratio $\Delta \chi / \Delta n$ is dependent on the structural dimensions of the cylindrical structures in the material, and that Δn and $\Delta \chi$ are closely related but

independent parameters which convey different information about tissue microstructure. Though models of this type are useful for predicting the polarization effects found in ocular tissues, they have limited application in quantifying these effects due to strong dependencies on variables which are difficult to specify accurately, such as indices of refraction, cylinder size, and packing density [20]. Artifacts in measurement techniques such as multiply scattered light and speckle noise, variations in cellular structure over sample thickness, and variations in the general condition of the sample complicate the ability to directly compare model predictions to measured results [19].

2.4: Experimental methods for ocular polarization interactions

A wide range of techniques has been employed to study the polarization effects in the eye. A summary of the most important and relevant techniques which have been utilized in the past 20 years is presented here. Earlier studies utilized a human observer or fundus camera together with a polariscope (combinations of manually rotated polarizers and quarter wave plates) to estimate the extinction ratio and hence the ellipticity of the reflected light [21-22].

To determine polarization properties of the cornea a polariscope (Figure 2.7) has often been used to view the reflection of the source from the posterior surface of the lens, a reflection termed the fourth Purkinje image [23-25]. This measurement determined that corneal retardance is significant. With the polariscope method retardance magnitude and axis could be quantified, but quantification of less significant polarization properties such

as diattenuation and depolarization was not reliable. A substantial improvement was the Mueller matrix ellipsometry technique reported in 1985 by van Blokland and Klein Brink [26-29]. This was the first method to use a complete Mueller matrix polarimeter together with analysis methods based on the Mueller calculus and Poincaré sphere trajectories, which set the stage for later research efforts which have provided quantitative and accurate measurements of retinal polarization properties. This nonimaging technique utilized a dual rotating retarder polarimeter configuration with a single photomultiplier detector (Figure 2.8). Polarization properties of the cornea and retina were calculated from measured intensity vectors by decomposition into Fourier components. This approach provided a full Mueller matrix of the eye which showed significant retinal depolarization. However, published Mueller matrix algorithms available at that time did not provide accurate decompositions of polarization properties for depolarizing Mueller matrices (the Lu-Chipman algorithm [30] published in 1996 provided an appropriate decomposition method).

Figure 2.7: Schematic diagram of a polariscope instrument used to view the fourth Purkinje image. P,A: linear polarizers; BVR: variable retarder; LED: light-emitting diode; P_{IV} : fourth Purkinje image; L_1 , L_2 : collimating lenses; L_3 : eyepiece. Reprinted with permission from [23].

Figure 2.8: Schematic diagram of a Mueller matrix ellipsometer. PSG: polarization state generator; PSD: polarization state detector; Ar-Kr: laser source; LP: linear polarizer; RP: retardation plate; FS: field stop; AS: aperture stop; PM: photomultiplier. Reprinted with permission from [29].

To quickly measure polarization properties over a wide ocular field of view, one of two polarimetry methods is now generally used. In camera-based polarimetry methods, polarized light is delivered over the desired ocular region, and the return light from that region is analyzed and imaged by a camera (Figure 2.9). A common configuration is a fixed polarizer in the generator and a rotating retarder and fixed polarizer in the analyzer [20,31-33]. Related configurations include a rotating retarder in the generator [34] or use of a liquid crystal variable retarder [35]. A limitation is that the generator and analyzer must be uniform or at least carefully calibrated over a large region.

Figure 2.9: Schematic of a camera-based incomplete polarimeter. P1 and P2: polarizers; $\lambda/4$: quarter wave plate; M1, M2, and M3: mirrors; L1 and L2: lenses; BS: beamsplitter AP: pupil; OB: objective; DP-CCD: camera. Reprinted with permission from [31].

A second technique, scanning laser polarimetry (SLP), has been used by many researchers to measure ocular polarization properties [36-38] (Figure 2.10). The majority of these studies have used the GDx, a commercial retinal SLP commercialized by Laser Diagnostics Inc. (recently acquired by Carl Zeiss Meditec). The SLP technique is based on a scanning laser ophthalmoscope, a device which uses scanning mirrors to deliver a two-dimensional laser scan pattern to the retina, and simultaneously descans the return light to a detector. Intensity images of the retina are assembled through appropriate reconstruction of the detector signal. The addition of polarization elements in the outgoing path (generator) and incoming path (analyzer) give the SLP its polarimetric capability. Because retardance is known to be the largest polarization effect in the eye, incomplete camera-based and SLP configurations have been designed to measurement

only linear retardance. For example, the GDx instrument can measure linear retardance but not diattenuation or depolarization. The GDx has been used widely in clinical studies to measure retinal nerve fiber layer retardance, with results reported by hundreds of researchers [1,39,40]. However, measurements are known to be inaccurate in the presence of ocular depolarization and diattenuation.

Figure 2.10: Schematic of an incomplete scanning laser polarimeter. LD: laser diode; P1 and P2: polarizers; M: mirror; QWP: quarter wave plate; I: iris; GS: galvonometric scanner; BS: beamsplitter; P: pinhole; PMT: photomultiplier tube. Reprinted with permission from [38].

In the past 15 years polarization capability has been added to optical coherence tomography (OCT) instruments to provide polarization profiles of ocular tissues with depth resolution of a few micrometers. This technology, first reported by Hee [41], is termed polarization sensitive OCT (PS-OCT, Figure 2.11). In basic OCT technology, a low-coherence source is used in a Michelson or Mach-Zehnder type interferometer. With its short coherence length interferograms are produced which accurately identify reflecting or back-scattering layers located where the sample and measurement arms are of equal length. Interpretation of the interferograms gives optical path lengths, which are converted to distances by assuming refractive indices. Very high resolution three-

dimensional structural information about tissues can be assembled (Figure 2.12). To obtain polarization information, the source is generally linearly polarized at 45° or circularly polarized to ensure that horizontal and vertical components are equal in intensity. Two channels are required through the sample and reference arms (for horizontal and vertical light), and the phase relationship between them must be recorded after passing through a polarization analyzer such as a fixed or variable retarder. Mueller matrix methods are often used to reconstruct polarization parameters [42,43]. Depending on generator and analyzer configuration, PS-OCT can be used for simultaneous depth-resolved determination of reflectivity, retardation, slow axis orientation, and relative attenuation [44-48]. Problems with this approach include speckle noise, inaccuracy in refractive index estimates, and high cost. PS-OCT has so far been limited since multiply scattered light that has lost coherence is rejected; thus depolarization cannot be measured and a full Mueller matrix cannot be obtained.

Figure 2.11: Schematic diagram of a PS-OCT setup. PBS1 and PBS2: polarizing beam splitters; QW1 and QW2: quarter wave plates; DET1 and DET2: photodetectors; SLD: superluminescent diode; PZT: piezoelectric transducer; A/D: analog to digital converter. Reprinted with permission from [41].

Figure 2.12: Structural detail of retinal layers obtained using OCT. RNFL: retinal nerve fiber layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; IPR: interface between inner and outer segments of photoreceptor layer; RPE: retinal pigment epithelium; C/C: choriocapillaris and choroid. Reprinted with permission from [49].

Mueller matrix imaging polarimetry (the technology utilized in this dissertation) is an extension of the camera-based and scanning methods described above, using a polarimeter that is complete so that a full Mueller matrix at every point in a retinal image may be measured. Full Mueller matrix imaging polarimetry of ocular structures has been reported by two groups: the first, a collaboration between Juan Bueno at Universidad de Murcia and Melanie Campbell at the University of Waterloo, and the second, Chris Dainty and David Lara at the National University of Ireland. Bueno and Campbell have reported on three different configurations: a camera-based type with polarizer, variable liquid crystal retarder, and removable quarter wave plate in both generator and analyzer [50-52]; a camera-based type with polarizer and rotating quarter wave plate in generator and analyzer [53,54]; and a scanning type with polarizer and rotating quarter wave plate in generator and analyzer mounted in a confocal scanning laser microscope / ophthalmoscope [55,56]. A representative system layout is shown in Figure 2.13. Exactly 16 measurements are made (pairs of four combinations each of generator and analyzer) to produce Mueller matrix images. The camera-type polarimeters have been used to quantitatively measure degree of polarization, diattenuation, polarizance, retardance, retardance axis, and ellipticity in the in vitro cornea [53] and lens [54], and have provided a small amount of data on the in vivo eye [51]. The scanning-type polarimeters have been applied to image quality and feature visualization improvement by use of the top row of the Mueller matrix image to compute images with higher signal to noise ratio or entropy [56, 57].

Figure 2.13: Schematic of a complete Mueller matrix imaging polarimeter. P1 and P2: polarizers; QWP1 and QWP2: quarter wave plates; BS: beamsplitter. Reprinted with permission from [55].

Lara and Dainty have reported a polarimetric configuration which, though designed to probe biological samples and in particular the human eye, is at an early stage of development and no data has yet been presented [58,59]. Their polarimeter design (Figure 2.14) is based on a confocal microscope. At present imaging is performed by manual lateral and axial translation of the sample. However, the polarization sectioning process differs significantly from other methods used to perform ocular studies and is demonstrated to be highly stable and well calibrated in the presence of error sources. In the generator section is a polarizer followed by two Pockels cells, electro-optic modulators which function as variable linear retarders. By appropriate choice of orientations and retardances polarization states over the entire Poincaré sphere can be accessed. The analyzer section is a division of amplitude polarimeter design in which the

beam is split into four components (horizontal polarized, vertical polarized, 45° polarized, and right circular polarized) and simultaneously directed to four photodetectors. This arrangement allows for the complete measurement of the Stokes vector from the sample. A total of 256 polarization states are generated and analyzed, and Mueller matrices are computed from the appropriate 24 Fourier components of the intensity modulations measured at the detectors. There are no moving parts, and thus Mueller matrix data at each point can be obtained at high speed.

Figure 2.14: Schematic of the polarimeter designed by Lara and Dainty. ND: neutral density; Glan-Taylor: polarizing prism; Pockels 1 and 2: Pockels cell variable retarders; M1: mirror; L1: collimating lens; Bs1, Bs2, Bs3, and Bs4: non-polarizing beamsplitters; PBs4: polarizing beamsplitter; Obj1, Obj2, and Obj3: objective lenses; Ph: pinhole; Qwp: quarter wave plate; P0 and P45: linear polarizers; D1-D4: photodetectors. Reprinted with permission from [59].

2.5: Polarization properties of ocular structures

The published findings on the polarization properties of ocular structures are not always consistent, due to differences in technique, the use of various eye sources (pig, sheep,

cow, rat, human), and tissue quality differences between in vitro and in vivo measurements. The following is an overview of the current state of knowledge.

Cornea

The transparency of the cornea and lens implies a level of structural organization [60-62], which in turn suggests the presence of measurable polarization effects. In the case of the cornea, polarization effects are due to the sheets of stacked collagen fibril lamellae of different orientations which are present in the stroma. The cylindrical fibers, stacked in an interfiber medium of lower refractive index, give rise to both linear form birefringence (retardance) [53] and form dichroism (diattenuation) [63]. van Blokland and Verhelst found that the cornea behaves as a biaxial crystal with the fast axis normal to the surface and the slow axis pointing nasally downward [28]. This may imply that the fiber layers are not oriented completely at random, but instead have a preferential direction. It has also been suggested that the birefringence is due to stress and the resulting distortion as the fibrils are held at regular intervals by proteoglycan molecule components of the extracellular matrix [64].

A summary of various researcher's measurements of corneal retardance magnitude and orientation is presented in Table 2.1. Some results are not spatially resolved and may represent an average over a substantial corneal area. Goetzinger, Pircher, and their colleagues [44, 65] produced spatially-resolved retardance magnitude and orientation maps using PS-OCT, and found lowest single-pass retardance in the center of the cornea for

beams normal to the surface, increasing in all radial directions (with axial symmetry) and for oblique angles (Figure 2.15). The slow axis was found to vary approximately linearly with azimuth angle, constant with depth. These results are consistent with the presence of lamellae sheets having two preferential, orthogonal directions interspersed in a background of lamellae with random orientation. The several methods used to measure corneal retardance use incomplete polarimeters, and may not measure retardance accurately in the presence of diattenuation and depolarization. However, it can be concluded from the published results that corneal retardance lies in the approximate range of $0^\circ - 120^\circ$, and the axis ranging from slightly nasally downward to strongly nasally upward.

Table 2.1: Overview of corneal retardance measured by various researchers

Reference	Conditions	Retardance	Axis
36	SLP 780 nm in vivo human corneas (106)	14 nm - 182 nm ($6.5^\circ - 84^\circ$) double pass	13° NU - 73° ND
23	Polariscope 585 nm in vivo human corneas (73)	0 nm-190 nm ($0^\circ - 117^\circ$) double pass	13° NU - 72° ND
44	PS-OCT 828 nm in vitro human corneas	$13^\circ - 30^\circ$ single pass	Linear variation w/ azimuth angle
34	Camera-based polarimeter 633nm in vitro human corneas (12)	$9^\circ - 43^\circ$ single pass	Constant in center cornea

Figure 2.15: Corneal retardance distribution as measured using PS-OCT. (a): retardance, blue = 0° and red = 90° . (b): fast axis orientation, blue = -90° and red = $+90^\circ$. Reprinted with permission from [65].

Diattenuation in the cornea is a much smaller effect, due to dichroism [63] and anisotropic light scattering by the collagen fibers [47]. Diattenuation was measured by Bueno and Jaronski using a complete Mueller matrix imaging polarimeter on excised bovine and porcine corneas [53] and found to be in the range 0.03 ± 0.01 single pass. They also reported a high degree of polarization of 0.9 ± 0.1 single pass. Measurements by Louis-Dorr et al. [63] using an incomplete polarimeter on excised sheep corneas showed that corneal dichroism is extremely weak.

Studies of the crystalline lens indicate that it has an intrinsic birefringence induced by a regular arrangement of cellular structures, as well as form birefringence due to the long-range structure of fiber membranes and interstitial cytoplasm [29,54]. Studies by Bettelheim [66] and Weale [67] indicate that the intrinsic and form birefringence are of opposite sign and similar magnitude so that the measurable retardance is small. Using nine older eyes from an eye bank, Bueno and Campbell [54] found lens retardance of

$7^\circ \pm 2^\circ$ which was axially symmetric about the center of the lens, and decreasing from center to edge. The orientation of the slow axis varied between individuals. They also measured diattenuation of 0.05 ± 0.03 and degree of polarization of 0.64 ± 0.04 . In general, the polarization properties of the lens are much less significant than those of the cornea, and are often neglected.

In addition to polarization effects in the cornea and lens, small effects have been found in the aqueous humor [68,69]. Primarily due to the presence of glucose, albumin, and ascorbic acid, but also due to the presence of at least 20 other compounds, the aqueous humor exhibits optical activity. The optical activity arises because compounds are chiral (have a “handedness”); as a result the plane of polarization of linear polarized light is rotated as it traverses the aqueous solution. Baba has measured the rotation to be less than 14 millidegrees for a 1 cm pathlength [69]. This effect is extremely small and has never been considered in any retinal polarization studies.

Retina

Only the retinal nerve fiber layer (RNFL) and Henle’s fiber layer have been demonstrated to exhibit substantial polarization properties, though the photoreceptor layer and Bruch’s membrane also contribute to polarization. RNFL birefringence has been studied by several researchers, and is known to vary with position around the optic nerve head (ONH), being highest in superior and inferior locations, and lowest in nasal and temporal regions, probably due to lower neurotubule density [47,49] (Figure 2.16). The

birefringence profile is approximately constant along circles centered on the ONH, and given that bundles are radially oriented this suggests that birefringence is constant along the bundles [37]. Total retardance depends on both birefringence and thickness; RNFL thickness is greatest in the inferior and superior regions, and thins with distance from the ONH [49]. Birefringence and retardance measurements obtained by various researchers are summarized in Table 2.2. Birefringence values are calculated using retardance and thickness measurements, which may be obtained using the same or different instruments. As the tissue characteristics vary within and between samples, retardance and thickness measurements also vary, but the calculated birefringence should be directly comparable. In some cases the researchers did not explicitly state the measured retardance values and so approximate ranges are inferred from graphs.

Figure 2.16: Retinal birefringence distribution (solid line) as a function of depth as measured using PS-OCT. The vertical line indicates the boundary of the RNFL. The dashed line indicates the slope of double pass phase retardation per unit depth. (a): region temporal to the ONH. (b): region superior to the ONH. Reprinted with permission from [49].

Table 2.2: Overview of retinal birefringence and retardance measured by researchers

Reference	Conditions	Birefringence	Retardance
70	Ellipsometer + histology 514 nm in vitro monkey retina	0.19 nm/ μm (0.13 $^{\circ}/\mu\text{m}$)	0.9 $^{\circ}$ – 23.7 $^{\circ}$ double pass
33	Imaging polarimeter + histology 440 – 830 nm in vitro rat retina nerve fibers	0.23 nm/ μm (0.1 – 0.19 $^{\circ}/\mu\text{m}$)	3.17 +/- 0.13 nm (1.4 $^{\circ}$ – 2.6 $^{\circ}$) single pass
45	PS-OCT 850 nm in vivo human retina	0.29 $^{\circ}/\mu\text{m}$	~ 50 $^{\circ}$ double pass
49	PS-OCT 840 nm in vivo human retinas (2)	0.10 – 0.35 $^{\circ}/\mu\text{m}$	~ 15 $^{\circ}$ – 75 $^{\circ}$ double pass
37	SLP + OCT 780 nm / 800 nm in vivo human retinas (26)	0.32 +/- 0.03 nm/ μm (0.15 +/- 0.01 $^{\circ}/\mu\text{m}$)	~ 10 $^{\circ}$ - 50 $^{\circ}$ double pass
47	PS-OCT 830 nm in vivo monkey retina	0.076 – 0.173 $^{\circ}/\mu\text{m}$	3.9 $^{\circ}$ – 29.5 $^{\circ}$ double pass

It can be concluded that RNFL birefringence lies in the approximate range of 0.1 – 0.35 $^{\circ}/\mu\text{m}$, and in vivo human retinal retardance lies in the approximate range of 15 $^{\circ}$ - 75 $^{\circ}$, comparable to that of the cornea.

Several studies, summarized in Table 2.3, suggest that the RNFL has weak diattenuation and high degree of polarization. Since measured values depend on the RNFL thickness there is some variation among the results. In addition to the quantified results, Campbell and Bueno 2005 [56] found using complete Mueller matrix polarimetry that the RNFL has a high degree of polarization, polarizance, and diattenuation, particularly in the regions of

the lamina cribosa and neural retinal rim (Figure 2.17). Polarization images obtained using this instrument have clear artifacts such as a vertical line and shadows next to vessels (due to imperfect image registration) which partly obscure the polarization content.

Table 2.3: Overview of retinal diattenuation and Degree of Polarization (DoP) measured by various researchers

Reference	Conditions	Diattenuation	DoP
71	Dichrograph Xenon broadband source in vitro sheep retina	0.001 single pass	
20	Imaging micropolarimeter 440 – 830 nm in vitro rat retina nerve fibers	0.3 double pass	1 along bundles, 0.8 – 0.87 between bundles
72	PS-OCT ~850 nm (presumed) in vivo human retina	0.063 double pass	
19	PS-OCT 830 nm in vivo primate retina	0.023 double pass	
73	Complete imaging polarimeter 633 nm in vivo human retinas (3)	0.1 double pass	
26	Mueller matrix ellipsometer 514 nm in vivo human retinas (2)		0.85 – 0.89 double pass
74	Mueller matrix ellipsometer 488 - 647 nm in vivo human retina		0.8 – 0.9 double pass
31	Incomplete imaging polarimeter 780 nm in vivo human retinas (7)		0.67 – 0.92 double pass
75	Complete imaging polarimeter 633 nm in vivo human retinas (5)		0.05 – 0.75 double pass

Figure 2.17: Retinal data obtained with a complete Mueller matrix imaging polarimeter. The four images represent the top row of the Mueller matrix calculated on a pixel by pixel basis. The scale is for the second, third, and fourth elements; in the first image, black corresponds to zero and white corresponds to one. Reprinted from [56].

As the RNFL is an outer layer with high reflectivity and scattering and significant retardance, its presence obscures the polarizing properties of the underlying, deeper retinal layers. However, polarization-sensitive optical coherence tomography (PS-OCT) has enabled study of these lower layers. One study found a lack of birefringence in the layers between the Outer Plexiform Layer and the RNFL [76], and another study found a lack of birefringence in all layers between the RPE and RNFL [49]. The Henle fiber layer, consisting of elongated photoreceptor fibers extending radially from the fovea, exhibits form birefringence and form diattenuation properties which are similar to those of the RNFL [26,77]. Single-pass retardance is approximately 10° at visible wavelengths [26]. Diattenuation in the macular area is also due to the alignment of pigment molecules within the fiber cell membranes, and is the source of the Haidinger's brush polarization pattern [77].

At the central fovea, an hourglass or bowtie shaped retardance pattern has been observed by several researchers. It has been proposed that this pattern is due to the overlap of two ocular structures which can be modeled as linear retarders: the corneal stroma with fixed retardance magnitude and orientation, and the Henle fibers in the retina with fixed retardance magnitude and radial slow axis orientation [26]. This combination forms a composite linear retarder having axis orientation which varies linearly with azimuth angle. Where the two axes are aligned the retardances add, and where orthogonal they subtract. Pircher [76] has confirmed using PS-OCT that the retinal retarder is localized in the region of the Henle layer. van Blockland [26] and Zhou and Weinreb [78] discuss the use of the bowtie pattern to infer the magnitudes of the two retarders and the orientation of corneal retardance. This topic is of particular importance in scanning laser polarimetry and will be further discussed in Chapters 5 and 12.

The cone outer segments have been reported to exhibit negative form birefringence because of the stratified structure. The optic axis is directed along the cone segment, and very small birefringence of -0.009 has been reported [79]. It was demonstrated by van Blockland et al. [74] that light guided by photoreceptors retains polarization; this effect was also observed by Burns et al. [80]. The RPE [81] consists of a single layer of hexagonal cells densely packed with pigment granules. Membranes separate the cell layer from the photoreceptor segments above and from Bruch's membrane and the lower choroid layer below. This layer exhibits strong absorption and backscatter. Polarization studies of this region using PS-OCT have detected two distinct layers, the upper

polarization maintaining, and the lower having strong depolarized back-scatter [76].

However, the incident polarized light which traverses Bruch's membrane to reach the underlying choroid is apparently highly polarized. The choroid itself has been observed to preserve polarization [49,76], but this observation may be influenced by the presence of strongly birefringent collagen in the underlying sclera, or high absorption due to blood vessels in the choroid [49].

Given the large number and varying composition of ocular structures it is important to identify the main sources of the return light on which the polarization analysis is based, and to classify these sources in terms of their expected polarization effect and overall contribution to the return flux [82]. The tissues within the eye producing the highest magnitude polarization-preserving back-reflections are the vitreous-retinal interface and the RNFL-ganglion cell layer interface. Tissue layers which have a regular structure and thus preserve polarization include the cornea, lens, RNFL, Henle fiber layer, cone and rod photoreceptors, Bruch's membrane, and the underlying sclera. The superficial layers above highly reflecting interfaces (cornea, RNFL, and Henle layer) are the principal sources of birefringence and diattenuation (the exception being in areas such as the scleral crescent region temporal to the optic nerve head, where photoreceptors and RPE are absent). Tissue layers which have an irregular structure and would be expected to scramble polarization include the neural layers, cone cell bodies, RPE, and choroid. As the RPE and choroid are the strongest scatterers these deeper structures are emphasized

by the multiply scattered depolarized return light. A schematic of ocular structures which influence polarization is shown in Figure 2.18.

Figure 2.18: Ocular components which influence polarization. Tissues retaining polarization content are labeled in black; those which scramble polarization content are labeled in gray. Reprinted with permission from [82].

In summary, measurable polarization properties such as retardance, diattenuation, and optical activity are present in most ocular structures, but only a few structures present strong polarization properties. Among all ocular structures, the largest effect is linear form-based retardance within the cornea, and substantial variation exists between individuals in magnitude and orientation. Also significant is linear form-based retardance in the RNFL and Henle layers of the retina. In this case the variation in magnitude and orientation around the perimeter of the optic nerve head and macula is larger than the

variation between individuals. Minor amounts of retardance are found in the lens and other layers of the retina, and minor amounts of diattenuation are found in the cornea, lens, and retina. Degree of polarization is on the order of 0.9 for a young healthy eye, but decreases substantially with age, on the order of 0.6 at age 70 [31].

2.6: Retinal Diseases

The most prevalent retinal diseases are glaucoma, age-related macular degeneration (AMD), and diabetic retinopathy (DR). Together these diseases account for approximately 25% of blindness worldwide, and are the leading causes of blindness in developed nations [83] (the leading cause of blindness worldwide, particularly in undeveloped nations, is cataracts). These diseases affect retinal tissue in characteristic ways, leaving footprints which may be utilized to diagnose and monitor disease progression. However, the root causes of these diseases are poorly understood, and current methods often cannot detect the earliest signs and symptoms. In this section, each of the most prevalent retinal diseases is discussed, in terms of its effects on retinal tissue, and also in terms of current diagnostic methods, demonstrating why a polarization-based early diagnosis might significantly improve prognosis for disease sufferers.

Glaucoma [84] is the second leading cause of blindness world-wide, affecting over 60 million persons, and is the leading cause in developed nations [83]. It is related to the failure of the aqueous humor to adequately drain through the trabecular meshwork. As such it is often, though not always, associated with an increase in intra-ocular pressure.

Due to the increased pressure and/or other mechanisms, the retinal ganglion cells are deprived of neurotrophins, primarily BDNF, which is blocked at the lamina cribrosa. The resulting release of excitotoxins such as glutamate overstimulate receptors at the cell surface, allowing excess calcium to enter the cell and causing a chemical chain reaction which stimulates the production of free radicals and reactive oxygen. This process eventually leads to the progressive apoptosis of retinal ganglion cells and axons of the RNFL. The RNFL thickness decreases as the disease progresses and is a primary diagnostic indicator. Researchers have also reported on ischemia and the failure of autoregulation mechanisms as causative factors [85,86]. The most common form of glaucoma is primary open-angle glaucoma, in which the drainage system remains open but there is a slow but progressive loss of optic nerve fibers, leading to excavation of the optic disc causing tunnel vision and eventual total blindness. A typical fundus image for a glaucomatous eye is shown in Figure 2.19b.

The traditional diagnostic tests for glaucoma include optic disc assessment, measurement of intraocular pressure, and visual field test. However, several studies have demonstrated that a large percentage of patients experience structural damage to the RNFL several years before the onset of measurable visual field loss, indicating the need for improved diagnostic testing [87-89]. A recent study indicated that as many as 50% of primary open-angle glaucoma patients were unaware of their disease at the time of diagnosis, with symptoms appearing only after significant visual loss had occurred. One of the principal indicators of glaucoma, elevated intraocular pressure, occurs in only half of primary

open-angle glaucoma cases [90,91], and is commonly present in patients with ocular hypertension, who do not develop glaucoma [92]. The need for improved early diagnostics has led to the recent introduction of methods for directly probing the RNFL. These include scanning laser ophthalmoscopy or SLO (e.g. Talia Technology RTA) which offers high resolution fundus imaging; scanning laser polarimetry (e.g. Laser Diagnostic Technologies GDx), which adds polarization capabilities to an SLO to calculate RNFL thickness from a measurement of retinal retardance; confocal scanning laser microscopy (e.g. Heidelberg Retina Tomograph), used to obtain three-dimensional images of retinal topography; optical coherence tomography or OCT (e.g. Carl Zeiss Meditec Stratus OCT), a low coherence interferometry technique used to provide a cross-sectional view of retinal structure; and laser Doppler blood flow measurement (e.g. Heidelberg Retina Flowmeter) which provides mapping of retinal micro-circulation. It is important that diagnostic instruments such as the GDx and OCT are able to quantify the extent and progression of glaucoma, thus streamlining the diagnosis process and simplifying the evaluation of new drugs during the drug trial process. No such quantifying diagnostic instruments are currently available for AMD or DR.

Treatment of glaucoma has traditionally focused on methods to reduce intraocular pressure, including medication, laser trabeculoplasty, and surgical trabeculectomy. A wide range of studies have proved the efficacy of these methods, and demonstrated that treatment given before symptoms develop is highly beneficial in improving eventual outcome [93-95].

AMD [96] is the third-leading cause of blindness world-wide, and the second leading cause in developed nations [83]. This disease affects the macular region of the retina, and is initiated as abnormalities develop in the enzymatic activity of RPE cells, causing them to lose their ability to pump essential nutrients to the retina and to move waste products down to the underlying choroidal blood vessels. As a byproduct of this process, extracellular excretions of waste products form lipofuscin deposits (drusen) between the basement membrane of the RPE and the collagenous zone of Bruch's membrane. The drusen are initially small and tend to be clustered mainly in the macular area, but can vary in size, shape, color, distribution, amount, and consistency. As AMD progresses the drusen proliferate and increase in size. The number and appearance of drusen is an important diagnostic factor for AMD. Eventually the RPE cells become engorged and die, leading to death of the overlying photoreceptors, which causes an overall thinning of macular tissue. In the advanced atrophic stage, areas of pigment clumps and isolated patches of bare choroid coalesce and spread from the macula to the fovea, resulting in complete loss of central field vision. A typical fundus image for an eye affected by AMD is shown in Figure 2.19c.

Standard diagnostic methods for AMD include visual field test, color fundus imaging, ocular pressure test, and fluorescein angiogram to detect drusen. As many of the retinal changes occur in retinal layers which are below the highly reflective neural layers, traditional methods do not have good contrast in detecting these changes. Scanning laser ophthalmoscopy, infrared confocal imaging, and optical coherence tomography are more

recent techniques which may be useful for detecting drusen and fluid deposits. As these structures are highly scattering, it is preferable to reject the directly backscattered light and collect multiply scattered light. In the early stages the disease progresses gradually, and widespread retinal changes have been shown to occur long before the disease is apparent [97,98]. Before the disease becomes advanced it may be treated with laser surgery, injected drugs, or photodynamic therapy, but lost vision cannot be restored.

DR [99] is the third leading cause of blindness in developed nations [83], and the leading cause of new blindness for individuals aged 35-65. Abnormal glucose metabolism causes small abnormalities in capillary walls. Microaneurysms, or small weakness in the capillary wall, leak serum or rupture, usually in the inner nuclear layer (INL). In the macular region this effect is termed macular edema. Other types of haemorrhages with distinct appearance may develop in the outer and inner plexiform layers (OPL and IPL), and semi-solid hard exudates consisting of accumulated and condensed plasma may form. Eventually there is a substantial blockage of retinal blood flow, leading to ischaemia, hypoxia, beading/looping of vessels, and infraction of the NFL. Damage occurs to the retinal autoregulation mechanism, which stimulates neovascularization (growth of immature fragile blood vessels) along the surface of the retina; however, the new vessels are highly permeable and leak blood into the retina. Vision loss occurs in well-defined areas in any portion of the visual field. A typical fundus image for an eye affected by DR is shown in Figure 2.19d.

Standard diagnostic methods include a visual acuity test, an ophthalmoscopic exam, and intraocular pressure test. The most effective diagnostic is grading of severity of aneurisms and neovascularization using stereoscopic fundus photography or fluorescein angiography. Unfortunately, DR can cause irreversible damage before visual loss is noticed [100]. Effective glycemic control once the diabetes is diagnosed is very effective at controlling DR [101-103]. After substantial damage has occurred laser photocoagulation can be used to seal leaking vessels and prevent further vision loss, but normal vision cannot be restored [104,105]. Advanced cases are sometimes treated with vitrectomy. Optical methods for measurement of blood-oxygen saturation in retinal vessels (retinal oximetry) to detect compromised autoregulation and/or hypoxia show promise but are still in the experimental stage [106,107]. Early detection of retinal abnormalities is considered to be essential in preventing loss of vision [108], and the benefits of early management have been seen to persist for years [101].

Figure 2.19: Fundus photographs of retinas for comparison of disease process. (a): normal fundus; (b): retina showing widespread cell death and excavation of the optic disc due to advanced glaucoma; (c): retina showing drusen (white spots) due to age-related macula degeneration; (d): retina showing neovascularization (proliferation of immature vessels), as indicated by arrows, due to diabetic retinopathy. Reprinted with permission from [4].

2.7: Polarization methods for disease diagnostic

The primary effects of retinal diseases on tissue microstructure are thinning and disordering of specific layers. Thinning of a birefringent layer would reduce retardance; this is the case for the RNFL in glaucoma. Disordering of a layer would reduce birefringence and differential attenuation as these properties are dependent on the form-based nature of the structure; thus retardance and diattenuation would be lower. Disorder would also lead to higher scattering; thus depolarization would be higher than the normal level due primarily to scattering from the RPE. It is expected, therefore, that polarized light is able to detect the breakdown of regular microstructure (for example by cell death and neovascularization) as well as the presence of disease-indicating scattering structures such as drusen and edema.

The most common use of a polarization property for disease diagnostic is the measurement of RNFL retardance (and by extension thickness), which can serve as an indicator for the early beginnings and later progression of glaucoma. Hundreds of researchers have reported on the efficacy of the GDx for this purpose, and many have reported a high discriminating ability for separating normal and glaucomatous eyes [109-111]. An excellent overview is provided by Bagga [112]. However, the accuracy and efficacy of the GDx is limited. Limiting factors include overlap between thickness ranges for normal and glaucomatous eyes; the incorrect assumption of constant retinal birefringence; incomplete compensation of anterior segment birefringence; and inaccuracy in the presence of depolarization, particularly in older eyes and those with scattering conditions such as cataracts. A more detailed discussion of the GDx and its use and limitations in retinal disease diagnosis is provided in Chapter 5.

The GDx has been modified for use in other types of polarization based diagnosis. Dr. Ann Elsner at Indiana University and her research associates have modified a GDx to detect and quantify changes associated with AMD and other maculopathies [2,113,114]. They developed an algorithm (Figure 2.20) to produce depolarized and average intensity images (Figure 2.21) from the minimum value of light detected at the cross detector for all input polarization angles, which presumably is representative of the multiply scattered light originating at larger depths within the retinal tissue. Burns et al. [2] report a higher contrast for pools of fluid, drusen, retinal vascular changes, and other subretinal changes using this method. The depolarized images show higher intensity for maculopathy

patients due to increased multiply-scattered light [115]. Using a similar technique, Elsner computed several images including birefringence, crossed polarized, parallel polarized, depolarized, and average intensity [116]. Different images were found to emphasize different features in diabetic retinopathy; for example, images with higher degree of polarization emphasized edema, and depolarized images had high contrast for vessel abnormalities. An analysis of regions of peripapillary hyperpigmentation and exudative lesions in glaucoma and AMD demonstrated improved visualization of pigmentary changes and fluid boundaries [117-120]. Leakage points and areas of fluid in central serous chorioretinopathy (a retinal disease displaying retinal detachment and leakage at the RPE) could also be readily localized in the depolarized light images [121]. In another study, lack of the Henle-fiber bowtie pattern in patients with AMD was associated with serious pathological retinal changes [122]. Most recently the technique was extended by use of a pseudo-color scale based on cardinal directions of color to display relative phases and magnitudes in the birefringence images [82]. It was found that many standard features of AMD such as pools of fluid and abnormal vasculature were readily visible in the polarization images. The depolarized light image particularly emphasized the borders of neovascular membranes and tissue disruptions. The parallel polarized light image emphasized superficial fluid pools, and birefringence fringes were seen surrounding a pigment epithelial detachment.

Figure 2.20: Algorithm used to calculate a depolarization image from raw GDx data: top left (blue) parallel detector; bottom left (red) cross detector. Graphs show intensity variation at a single pixel over the 20 GDx polarization states. A fast Fourier transform is performed to generate the smooth filtered curves from the sampled data. The minimum of the crossed detector curve at each pixel is used to compute the depolarization image; the average of the two detector curves at each pixel is used to compute an average intensity image. Reprinted with permission from [123].

Figure 2.21: A comparison of various types of fundus images. A: Standard fundus photograph; B: GDx raw intensity image; C: RNFL thickness map produced by GDx instrument; D: Calculated depolarized light image; E: Calculated average image; F: Calculated polarization modulation image. Reprinted with permission from [2].

2.8: Advantage of GDx-MM over other technologies

Of the polarization-based methods described for ocular investigation, only the complete Mueller matrix imaging polarimeters are capable of providing a full polarization signature over a substantial portion of the retina. Incomplete polarimeters are unable to fully separate out the 16 parameters of the Mueller matrix, leading to mixing of polarization parameters. The complete polarimeters described in the literature have disadvantages which arise from the need to acquire at least 16 images at different polarization states. For example, the generator and analyzer polarization components must be able to be varied accurately; the detector must have a large dynamic range; a longer measurement time and more processing power are needed to collect the images; and the images must be accurately co-registered. The configurations described by Bueno and Campbell [50-56] utilize polarization elements in a polarimetrically optimal configuration. However, as rotation increments are unequal the elements are manually translated, and collection of a full dataset is time consuming. The camera type systems require up to 500 ms to record a single image [54], and the systems with a single pair of liquid crystal variable retarders must have quarter wave plates manually introduced into the beam path for some measurements. The noise in the camera systems is pronounced; error sources include intensity fluctuations in the source, irregularities due to noisy pixels, and saturated central regions due to corneal reflex and refocusing of the return beam by the lens. The 256x256 pixel images are downsampled to 64x64 or 32x32 to reduce the effect of noise, and so resolution is poor. The beam footprint on the cornea (5 mm) is large enough that there is significant variation in corneal birefringence,

producing an erroneously high depolarization measurement. Subjects must be well trained to maintain fixation and assist in focusing the system, have their lens accommodation paralyzed and pupils dilated, and have their heads rigidly fixated, making these systems impractical for use in a clinical setting. Bueno and Campbell's scanning-type polarimeter [56] improves on the camera-based polarimeters, in that it is faster, utilizes a confocal pinhole for higher resolution, and has a smaller beam footprint on the cornea (2.5 mm). However, no quantitative data has been reported on the polarization properties of eyes. With the Lara/Dainty system [58,59] scanning is performed manually using precision translation stages, making it unsuitable for clinical use.

All reported Mueller matrix imaging polarimeters capture exactly 16 images, though signal-to-noise improvement could be achieved with more measurements (at the expense of a longer measurement time). Many of the measured Mueller matrices are almost certainly not physically realizable (i.e. degree of polarization is not in the range 0-1) due to the presence of noise. No mention has been made of analyzing the accuracy of the measurements through calculating the distance of the measured Mueller matrix from the nearest physically realizable matrix (discussed in Chapter 3), or of improving the accuracy of polarization parameter extraction through use of this nearest matrix.

The GDx-MM polarimeter presents a substantial improvement over the above described imaging Mueller Matrix polarimeters. It is completely automated and records 72 polarization images over a 15° visual field with 20 μm resolution in 4 seconds, fast

enough for use without lens paralysis or pupil dilation. The signal to noise ratio is moderate over a wide dynamic range and down-sampling of images is not required. The beam footprint at the cornea is small (about 2.5 mm), so that the depolarization due to corneal birefringence variation is minor. The polarimeter design has been optimized for good performance in the presence of error sources, and the data reduction is more sophisticated than that used by Bueno and Campbell and includes the conversion to physically realizable Mueller matrices.

An accuracy comparison among the various instruments is problematic as they were calibrated and validated using different methods. The instruments of Bueno and Campbell utilized ideal Mueller matrix models of the generator and analyzer (rather than a measurement-based calibration process) to describe the instrument, and validation was performed by measuring an empty chamber and a quarter wave plate at a few orientation angles. Errors in Mueller matrix elements are 1-3%, resulting in waveplate retardance and orientation errors of up to $\pm 1.5^\circ$ and degree of polarization error of 0.05. The Lara / Dainty instrument is calibrated by direct measurement of four polarization targets using the eigenvalue calculation method [124], and validation was performed by repeated measurement of these targets plus a fixed-orientation polarizer and a fixed-orientation quarter wave plate. Errors in Mueller matrix elements are in the range 0.2 – 7.2%, but no information is provided on errors in polarization parameters. As will be discussed in Chapters 9 and 11, calibration and validation of the GDx-MM provide a more comprehensive picture of the GDx-MM accuracy and repeatability. The accuracy is

comparable to the values listed above. Given these advantages the GDx-MM is a more practical instrument for general purpose investigation of ocular polarization, as well as a solid platform for future development.

2.9: Conclusions

The eye is a complex assembly of differently structured tissues having microstructure which may alter the state of incident polarized light. When retinal disease occurs there is a change in microstructure which may be detected using polarimetry. The high sensitivity of this technique may provide an earlier diagnosis than is currently available, enabling valuable early intervention. In this chapter the basic structures of the normal eye have been described, as well as models and measured data describing the normal ocular polarization interaction. Diseases of the retina and their effect on the polarization interaction were reviewed. Many different instruments have been used to quantify the polarization interaction, and a few have been used to diagnose retinal disease. The advantages of the GDx-MM over similar instruments should make this polarimeter more effective in detecting changes to the retinal microstructure.

CHAPTER 3: POLARIZATION AND POLARIMETRY

3.1: Introduction

In this chapter, basic polarization theory and polarimetry concepts are summarized, providing a basis for the development of the GDx-MM polarimeter. The Mueller calculus and Poincaré sphere are introduced as useful tools, and the Mueller calculus is used to derive the basic defining equations for the design of a polarimeter which may be used to measure the Mueller matrix of a sample. Finally, a technique is shown for converting the measured Mueller matrix to a physically realizable Mueller matrix, an important step in the analysis of measured data which may contain error.

3.2: Polarization

Light is a transverse electromagnetic wave, with associated electric and magnetic fields which oscillate in phase 90° apart in the plane perpendicular to propagation. The character of the oscillations, as determined by amplitude and phase parameters in the x and y directions, establishes the polarization of the light. By convention the electric field is used to define the polarization state. The electric field of a monochromatic plane wave with propagation along the z axis is represented as the following vector, with magnitude and direction changing as a function of position and/or time:

$$\vec{E}(\vec{r}, t) = \text{Re}[\hat{x} A_x e^{i(kz - \omega t + \phi_x)} + \hat{y} A_y e^{i(kz - \omega t + \phi_y)}]. \quad (3.1)$$

In the general case, the electric field vector precesses in an elliptical pattern, tracing an ellipse once per wavelength (as a function of position), or per inverse frequency (as a function of time). This ellipse embodies the polarization state. If the x and y phases are equal, the ellipse collapses to a line, and this is termed linear polarization. If the x and y amplitudes are equal and the relative phase difference is $\pm 90^\circ$, the ellipse expands to a circle, and this is termed circular polarization. The sign of the phase difference determines the handedness of the precession, which is right-handed for positive angles and left-handed for negative angles. The electric field as shown above can be decomposed into pairs of orthogonal components, such as horizontal and vertical, 45° and 135° , and left handed and right handed. Evaluation of these pairs leads to simplified polarization analysis as discussed in the following section. A general reference on the basis of polarization in electromagnetic theory is [125].

The above discussion assumes that the light beam is monochromatic and thus coherent. In any real physical situation, a light beam is not truly monochromatic, and thus has some degree of incoherence and in fact may be essentially incoherent. Polarized partly or fully incoherent light beams may be combined to form fully polarized, partially polarized, or unpolarized light. The addition of polarized coherent light beams can result in partially polarized or unpolarized light if the beams have different wavelengths. In general, partially polarized light has a randomly changing polarization state but with a preference towards a particular state, and unpolarized light has a truly randomly distributed polarization state as a function of time and position.

When a light beam interacts with a sample, the polarization state of any emergent beam (transmitted, reflected, diffracted, scattered) is altered by the sample in ways that depend on the microstructure of the sample. For example, when a beam of any polarization state (including unpolarized) is incident on a polarizer material such as Polaroid, one component of the electric field is fully transmitted, while the orthogonal component is fully absorbed. The emergent beam is thus linearly polarized. The material is polarizing due to the dichroic nature of its thin layer of parallel needle-like crystals. When a polarized beam is incident on a retarder material such as a birefringent crystal, one component is delayed in phase with respect to the orthogonal component. This is due to the crystalline structural arrangement, in which the index of refraction and thus the optical thickness differ for different polarization orientations. For these example materials, knowledge of the polarization state of the incident beam, together with a measurement of the polarization state of the emergent beam, allow for further insight into the microstructure of the sample. The basis of the polarimetric measurement as discussed in Section 3.6 is to probe a sample with multiple polarization states and measure the emergent states, in an effort to better understand a sample's microstructure.

3.3: The Mueller calculus

The Mueller calculus, consisting of the Stokes vector and Mueller Matrix formalisms [126], is a principal system for calculation of polarization properties in the field of optics. This system allows for integrated calculation of all polarization properties of a sample: diattenuation, polarizance, retardance, and depolarization. As such it is an extremely

important tool in the design of polarimeters and the analysis of their output. The Mueller calculus is easily extensible to complex problems and provides an intuitive understanding of the polarization properties of a sample.

The Stokes formalism consists of a 4-element Stokes vector S , which characterizes the polarization properties of an incoherent light beam with respect to its intensity. The four real-valued components of S (S_0, S_1, S_2, S_3) have units of Watts per meter squared and describe respectively the irradiance of the beam; the preference for horizontal or vertical linear polarization; the preference for 45° or 135° linear polarization; and the preference for right or left handed rotation. In matrix form,

$$S = \begin{pmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{pmatrix} = \begin{pmatrix} P_H + P_V \\ P_H - P_V \\ P_{45} - P_{135} \\ P_R - P_L \end{pmatrix}, \quad (3.2)$$

where P_H , P_V , P_{45} , P_{135} , P_R , and P_L are the measured flux in the horizontal, vertical, 45° , 135° , right, and left polarization states respectively, as measured with ideal polarizers in front of a radiometer [127]. Stokes vectors are often scaled to the irradiance (S_0 component) to emphasize the polarization content:

$$S_{norm} = \begin{pmatrix} 1 \\ S_1/S_0 \\ S_2/S_0 \\ S_3/S_0 \end{pmatrix}. \quad (3.3)$$

The measured Stokes vector may include an average over wavelength, solid angle, and beam extent, which should be considered in analysis of the result. A coordinate system must be established to provide consistency in measurement; the standard definition is for the Stokes vector to be defined relative to a right handed x - y coordinate system that is perpendicular to the direction of light propagation [128].

Properties of interest for a Stokes vector include the degree of polarization DoP , degree of linear polarization $DoLP$, and degree of circular polarization $DoCP$. These are defined as follows [126]:

$$DoP = \frac{\sqrt{S_1^2 + S_2^2 + S_3^2}}{S_0}, \quad DoLP = \frac{\sqrt{S_1^2 + S_2^2}}{S_0}, \quad DoCP = \frac{S_3}{S_0}. \quad (3.4)$$

The Mueller matrix formalism consists of a 4x4 real-valued dimensionless matrix M which relates any Stokes vector S which is incident on a sample, to a Stokes vector S' which is emergent from a sample. The sample polarization properties are fully described by Mueller matrix M via

$$MS = S' = \begin{pmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{pmatrix} \begin{pmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{pmatrix} = \begin{pmatrix} S'_0 \\ S'_1 \\ S'_2 \\ S'_3 \end{pmatrix}. \quad (3.5)$$

The Mueller matrix formalism is convenient when working with a cascade of polarization elements; in this case the Mueller matrix representing the system is given by the right-to-left product of the matrices of the individual elements:

$$M_{total} = M_n M_{n-1} \dots M_2 M_1. \quad (3.6)$$

Light/matter interactions may include transmission, reflection, diffraction, and/or scattering events. The polarization properties of each event is represented by a Mueller matrix for that process. In addition, as with the Stokes vector, the Mueller matrix is dependent on other factors, such as wavelength, position, solid angle, angle of incidence, and angle of scatter. As the aperture over which a Mueller matrix is measured has finite extent, there is an averaging,

$$M = \frac{1}{i} \sum_i M_i = \frac{\int M(\vec{r}) d\vec{r}}{\int d\vec{r}}, \quad (3.7)$$

where the first expression is a discrete sum over a pixelated aperture with i pixels, and the second expression is an integration over a continuous aperture with radius r . The

coordinate systems of the incident Stokes vector, Mueller matrix, and emergent Stokes vector must be defined and carried through the calculations consistently so that the polarization characteristics of the sample are correctly identified.

The 16 degrees of freedom (DOF) in the sample polarization signature is encoded in the 16 Mueller matrix elements as follows: intensity (1 DOF); diattenuation, or the unequal attenuation of orthogonal polarization states (3 DOF); retardance, or the difference in phase between orthogonal polarization states (3 DOF); and depolarization, or the coupling of polarized into unpolarized light (9 DOF). An advantage of the Mueller matrix is that it can be decomposed into sections which give “at-a-glance” insight into the polarization properties of the sample. For example:

The m_{00} element is the intensity change on interaction. Often the entire Mueller matrix is normalized to m_{00} to isolate the polarization effect.

The first row $\{m_{01}, m_{02}, m_{03}\}$ is the diattenuation vector, which gives insight into the variation of intensity transmittance with incident polarization state.

The first column $\{m_{10}, m_{20}, m_{30}\}$ is the polarizance vector, which gives insight into the extent to which the sample converts unpolarized light into polarized light.

The elements $\{m_{12}, m_{13}, m_{23}, m_{21}, m_{31}, m_{32}\}$ give insight into the retardance, or phase difference.

The elements $\{m_{11}, m_{22}, m_{33}\}$ give most insight into the magnitude of depolarization present, though depolarization is spread through other elements as well.

3.4: Extraction of polarization parameters

While these “at-a-glance” elements provide quick insight, it is also possible and illuminating (though certainly more computationally intensive) to decompose the Mueller matrix into component diattenuation, retardance, and depolarization matrices, so that individual polarization effects of the sample can be studied:

$$M = M_{depol} M_{ret} M_{diatt} , \quad (3.8)$$

where M_{depol} , M_{ret} , and M_{diatt} are the component Mueller matrices associated with depolarization, retardance, and diattenuation, respectively. An algorithm for this task has been described by Lu and Chipman [129]. Once the component matrices have been calculated, scalar metrics may be derived for particular polarization properties of the sample [128]. These metrics give an overview of the polarization nature of the sample.

For any sample, there is an incident polarization state which produces an emergent state with maximum intensity, and another incident state which produces an emergent state

with minimum intensity. Diattenuation describes the relative attenuation of these two emergent states. The Mueller matrix of a linear diattenuator with eigenvalues q and r and orientation θ is given by

$$M_{diatt,L} = \frac{1}{2} \begin{pmatrix} q+r & (q-r)\cos 2\theta & (q-r)\sin 2\theta & 0 \\ (q-r)\cos 2\theta & (q+r)\cos^2 2\theta + 2\sqrt{qr}\sin^2 2\theta & (q+r-2\sqrt{qr})\cos 2\theta \sin 2\theta & 0 \\ (q-r)\sin 2\theta & (q+r-2\sqrt{qr})\cos 2\theta \sin 2\theta & 2\sqrt{qr}\cos^2 2\theta + (q+r)\sin^2 2\theta & 0 \\ 0 & 0 & 0 & 2\sqrt{qr} \end{pmatrix}, \quad (3.9)$$

and that of a circular diattenuator with eigenvalues q and r is given by

$$M_{diatt,C} = \frac{1}{2} \begin{pmatrix} q+r & 0 & 0 & q-r \\ 0 & 2\sqrt{qr} & 0 & 0 \\ 0 & 0 & 2\sqrt{qr} & 0 \\ q-r & 0 & 0 & q+r \end{pmatrix}. \quad (3.10)$$

Four standard metrics can be calculated based on the Mueller matrix for a general diattenuator:

Diattenuation magnitude D (considers all incident polarization states)

$$D = \frac{\sqrt{m_{01}^2 + m_{02}^2 + m_{03}^2}}{m_{00}}, \quad (3.11)$$

linear diattenuation magnitude LD (considers only linear incident polarization states)

$$LD = \frac{\sqrt{m_{01}^2 + m_{02}^2}}{m_{00}}, \quad (3.12)$$

circular diattenuation magnitude CD (considers only circular incident polarization states)

$$CD = \frac{m_{03}}{m_{00}}, \quad (3.13)$$

and diattenuation orientation DO (angular orientation of emergent state with highest intensity)

$$DO = \frac{1}{2} \tan^{-1} \frac{m_{02}}{m_{01}}. \quad (3.14)$$

The above magnitude metrics range from 0 to 1, where 0 indicates no relative attenuation, and 1 indicates complete attenuation of one emergent state. The orientation ranges from $-\pi$ to $+\pi$.

Polarizance gives the degree of polarization of an emergent state when unpolarized light is incident. Four standard metrics can be defined based on Polarizance:

Polarization magnitude P (considers all emergent polarized states)

$$P = \frac{\sqrt{m_{10}^2 + m_{20}^2 + m_{30}^2}}{m_{00}}, \quad (3.15)$$

linear polarizance LP (considers only emergent linear polarized states)

$$LP = \frac{\sqrt{m_{10}^2 + m_{20}^2}}{m_{00}}, \quad (3.16)$$

circular polarizance CP (considers only emergent circular polarized states)

$$CP = \frac{m_{30}}{m_{00}}, \quad (3.17)$$

and polarizance orientation PO (angular orientation of emergent state with highest intensity)

$$PO = \frac{1}{2} \tan^{-1} \frac{m_{20}}{m_{10}}. \quad (3.18)$$

The magnitude and orientation ranges are the same as for diattenuation.

For any sample, there are two incident polarization states which are unchanged after interaction with the sample. These are known as the eigenpolarizations of the sample. Retardance describes the relative phase delay introduced between the eigenpolarizations. Often the two eigenpolarizations are orthogonal; in this case the axis of the polarization with leading phase is termed the fast axis, and the orthogonal axis is termed the slow axis. The Mueller matrix of a linear retarder with retardance δ and fast axis orientation θ is given by

$$M_{ret,L} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos^2 2\theta + \cos\delta \sin^2 2\theta & (1 - \cos\delta)\cos 2\theta \sin 2\theta & -\sin\delta \sin 2\theta \\ 0 & (1 - \cos\delta)\cos 2\theta \sin 2\theta & \sin^2 2\theta + \cos\delta \cos^2 2\theta & \cos 2\theta \sin\delta \\ 0 & \sin\delta \sin\theta & -\cos 2\theta \sin\delta & \cos\delta \end{pmatrix}, \quad (3.19)$$

and that of a circular retarder with retardance δ is

$$M_{ret,C} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos\delta & \sin\delta & 0 \\ 0 & -\sin\delta & \cos\delta & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}. \quad (3.20)$$

Four standard metrics can be calculated based on the Mueller matrix of a pure retarder:

retardance magnitude R (considers all incident polarization states)

$$R = \frac{m_{11} + m_{22} + m_{33} - 1}{2}, \quad (3.21)$$

linear retardance magnitude LR (considers only linear incident polarization states)

$$LR = \begin{cases} \frac{R\sqrt{m_{11}^2 + m_{22}^2}}{2\sin R} & R > \pi \\ \frac{R\sqrt{(m_{23} - m_{32})^2 + (m_{31} - m_{13})^2}}{2\sin R} & R \leq \pi \end{cases}, \quad (3.22)$$

circular retardance magnitude CR (considers only circular incident polarization states)

$$CR = \begin{cases} \frac{Rm_{33}}{2\sin R} & R > \pi \\ \frac{R(m_{12} - m_{21})}{2\sin R} & R \leq \pi \end{cases}, \quad (3.23)$$

and retardance orientation RO (angular orientation of the fast axis)

$$RO = \begin{cases} \frac{1}{2} \tan^{-1} \frac{m_{22}}{m_{11}} & R > \pi \\ \frac{1}{2} \tan^{-1} \frac{(m_{31} - m_{13})}{(m_{23} - m_{32})} & R \leq \pi \end{cases}. \quad (3.24)$$

The magnitude metrics range in value from 0 to 2π , and the orientation metric ranges from $-\pi$ to $+\pi$.

Depolarization describes the transformation of polarized light into unpolarized light.

While this concept is simple, the large number of DOF involved are an indication that this is a complex concept which is difficult to quantify. The Mueller matrix of a pure depolarizer is given by

$$M_{dep,Pure} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad (3.25)$$

and that of a partial depolarizer with exiting degree of polarization dependent on the incident polarization state is

$$M_{dep,Part} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & a & 0 & 0 \\ 0 & 0 & b & 0 \\ 0 & 0 & 0 & c \end{pmatrix}. \quad (3.26)$$

Various metrics have been developed to quantify depolarization in Mueller matrices [130]. The Degree of Polarization DoP_M is defined as the mean value of the DoP of the Stokes vectors emergent from a sample, over all possible incident Stokes vectors:

$$DoP_M = \frac{1}{4\pi} \int_0^\pi \int_{-\pi/2}^{\pi/2} DoP[MS(\theta, \phi)] \cos \phi \, d\theta \, d\phi . \quad (3.27)$$

DoP_M ranges from 0 to 1, with 0 indicating a completely depolarizing Mueller matrix, and 1 indicating a completely nondepolarizing Mueller matrix. An alternate metric, the Depolarization Index DPI , is more straightforward to calculate:

$$DPI = 1 - \frac{1}{\sqrt{3}} \sqrt{\sum_{i,j} \left(\frac{m_{ij}}{m_{00}} \right)^2} - 1 . \quad (3.28)$$

The range is the same as for DoP_M . DoP_M and DPI produce nearly identical results in most cases, though the results do diverge for certain classes of Mueller matrices [130]. For this work DPI is used exclusively.

3.5: The Poincaré Sphere

While calculating metrics for diattenuation, polarizance, retardance, and depolarization is useful in understanding the static properties of a sample, it is also helpful to utilize a graphical tool to visualize relative polarization state positions, and to track state trajectories. The Poincaré Sphere [128] is a standard tool for this purpose, and is utilized extensively in this dissertation.

The Poincaré Sphere (Figure 3.1) is constructed from the $\{S_1, S_2, S_3\}$ components of the Stokes vector, and contains no information on intensity. Each valid (physically realizable) combination of these three components defines a polarization ellipse which is mapped to a point on the sphere according to:

$$S_1 = \cos 2\varepsilon \cos 2\theta, \quad S_2 = \cos 2\varepsilon \sin 2\theta, \quad S_3 = \sin 2\varepsilon, \quad (3.29)$$

where ε is the ellipticity of the polarization ellipse and θ is the azimuth. In the Poincaré sphere construction, 2ε is the latitude on the sphere (ranging from $-\pi/2$ to $\pi/2$), and 2θ is the longitude (ranging from 0 to 2π). Completely polarized states lie on the surface of the sphere with radius 1; as the degree of polarization declines, the point moves along a radial trajectory towards the center of the sphere, with radial distance given by the *DoP* of the Stokes vector.

Figure 3.1: The Poincaré Sphere, showing the precession of polarization states with latitude and longitude.

Several simple polarization representations can be identified on the Poincaré Sphere.

Linearly polarized states lie on the equator with $2\varepsilon = 0$, with horizontal linear at $2\theta = 0$ and vertical linear diametrically opposite at $2\theta = \pi$. The north hemisphere is right elliptically polarized states, with right circular at the north pole at $2\varepsilon = \pi$. The south hemisphere is left elliptically polarized states, with left circular at the south pole at $2\varepsilon = -\pi$. Points along a given latitude line (ε fixed) have the same ellipticity, but varying orientation. Points along a given longitude line (θ fixed) have the same orientation, but varying ellipticity. Orthogonal polarization states (those that add to give unpolarized light) are located on diametrically opposite sides of the sphere.

Given an incident polarization state, it is straightforward to use the Poincaré sphere to predict the polarization state of the state emergent from an ideal polarization element or chain of elements. An ideal polarizer oriented at angle 2θ shifts any polarization state to the sphere's equator at angle 2θ . An ideal retarder, with retardance δ and oriented at angle 2θ , produces a left-handed rotation on the sphere of magnitude δ about an axis passing through 2θ and $2\theta + \pi$. These techniques are helpful in predicting the effect of polarization elements on a light beam with known polarization, in selecting appropriate polarization elements to transform the polarized beam in a desired way, and in selecting appropriate components to measure the polarization of a beam with unknown polarization state. The Poincaré sphere is a valuable tool in polarization design, in which polarization components must be selected which effectively span the polarization space.

The Poincaré sphere has other important uses, several of which are utilized in this dissertation. The change in polarization state with respect to some parameter, such as time or temperature, can be tracked on the sphere. In addition, ideal polarization data, for example from a model, can be compared to measured data by plotting both on the same sphere.

3.6: Polarimetry

A polarimeter is an optical instrument used to determine the polarization properties of a light beam or sample [126]. A light-measuring polarimeter measures the polarization properties of a light beam, and consists of a variable polarization element or chain of

elements placed in front of a radiometer (for example, the variable element might be a rotatable polarizer). The polarization element(s) are referred to as the Polarization State Analyzer (PSA). Multiple configurations of the PSA are utilized to obtain several radiometric measurements, which are then combined via a data reduction algorithm to determine the polarization state of the beam. A sample-measuring polarimeter measures the relationship between polarized light beams incident on and exiting from a sample, and characterizes the polarization effect of the sample. The exiting beam may be transmitted, reflected, diffracted, or scattered. For the sample measuring polarimeter, a polarization element or chain of elements termed the Polarization State Generator (PSG) delivers a polarization state to the sample, and a PSA analyzes the light returned from the sample. Radiometric measurements are obtained at multiple configurations of the PSG and PSA and then combined to determine the polarization effect of the sample.

The polarization elements for the PSG and PSA should be carefully chosen to accurately measure the polarization properties of the light beam or sample of interest. The PSG and PSA must be variable (for example, an element might rotate to various orientations). In some cases it is desirable to minimize the number of configurations to reduce measurement time, or to increase the number of configurations to increase redundancy and reduce sensitivity to error. Given that there are 16 DOF in polarization space, polarimeter design is often complicated. A polarimeter which is capable of measuring any Stokes vector or Mueller matrix within the full polarization space is termed “complete”. In some cases a polarimeter may be designed to measure a subset of the

polarization space, particularly when there is prior knowledge about the sample. For example, a polarimeter might be designed to measure only linearly polarized states. Such a polarimeter is termed “incomplete”. This is an important distinction; as discussed in Chapter 4, measurements performed by an incomplete polarimeter will be inaccurate if some properties of the sample lie within the inaccessible region of the polarization space.

Polarimeter design may be facilitated through use of the Mueller calculus [126]. The chains of polarization elements comprising the PSG and PSA can be represented by Mueller matrices to provide a mathematical description of the polarimeter. This is then used to optimize the polarimeter and estimate its performance. First consider a light-measuring polarimeter with a PSA having $i = 1..N$ configuration states, used to measure an unknown light beam described by Stokes vector S (Figure 3.2).

Figure 3.2: Basic light measuring polarimeter with $i = 1..N$ states, consisting of an analyzer A , and detector with measured intensity P .

Each PSA configuration can be described by a Mueller matrix A_i , which on interaction with S gives a new Stokes vector S'_i :

$$A_i S = S'_i = \begin{pmatrix} a_{00i} & a_{01i} & a_{02i} & a_{03i} \\ a_{10i} & a_{11i} & a_{12i} & a_{13i} \\ a_{20i} & a_{21i} & a_{22i} & a_{23i} \\ a_{30i} & a_{31i} & a_{32i} & a_{33i} \end{pmatrix} \begin{pmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{pmatrix} = \begin{pmatrix} S'_{0i} \\ S'_{1i} \\ S'_{2i} \\ S'_{3i} \end{pmatrix}. \quad (3.30)$$

However, the radiometer measures only the intensity (i.e. first element) of S'_i . Therefore only the first row of A_i is involved and the equation reduces to

$$A_i S = S'_i = \begin{pmatrix} a_{00i} & a_{01i} & a_{02i} & a_{03i} \end{pmatrix} \begin{pmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{pmatrix} = S'_{0i}. \quad (3.31)$$

All N configurations can be incorporated into one matrix equation,

$$W S = \begin{pmatrix} a_{00,1} & a_{01,1} & a_{02,1} & a_{03,1} \\ a_{00,2} & a_{01,2} & a_{02,2} & a_{03,2} \\ \vdots & \vdots & \vdots & \vdots \\ a_{00,N} & a_{01,N} & a_{02,N} & a_{03,N} \end{pmatrix} \begin{pmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{pmatrix} = \begin{pmatrix} S'_{0,1} \\ S'_{0,2} \\ \vdots \\ S'_{0,N} \end{pmatrix} = \begin{pmatrix} P_1 \\ P_2 \\ \vdots \\ P_N \end{pmatrix} = P, \quad (3.32)$$

where W , composed of the first row of each A_i , is termed the polarimetric measurement matrix, or more simply the “instrument matrix”, and P is the vector of measured

intensities. Note that the dimension of W is $N \times 4$, and the dimension of P is $N \times 1$. To reconstruct the unknown S from the known W and the measured P requires the transformation

$$S = W^{-1}P . \quad (3.33)$$

When W is not square a generalized inverse of W must be used. Of the infinite number of possible inverses, the pseudo-inverse of W , $W_p^{-1} = (W^T W)^{-1} W^T$ provides the best least square solution [131]. Thus the data reduction algorithm to determine S from the measured P becomes:

$$S = W_p^{-1}P = (W^T W)^{-1} W^T P . \quad (3.34)$$

In the case of a sample measuring polarimeter (Figure 3.3), the technique is similar.

Figure 3.3: Sample measuring polarimeter, where G_i and A_i are the composite Mueller matrices of the i th configuration of the PSG and PSA, M is the Mueller matrix of the sample, and P_i is the i th radiometer measurement.

The Mueller matrix of the system, M_{sys} , is now

$$M_{\text{sys}} = A_i M G_i, \quad (3.35)$$

where A_i is the Mueller matrix of the i th PSA state; G_i is the Stokes vector produced by the i th PSG state, and M is the Mueller matrix of the sample to be measured. As only intensity is measured, the equation for the i th configuration can be simplified as

$$a_i^T M g_i = P_i = \begin{pmatrix} a_{0,i} & a_{1,i} & a_{2,i} & a_{3,i} \end{pmatrix} \begin{pmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{pmatrix} \begin{pmatrix} g_{0,i} \\ g_{1,i} \\ g_{2,i} \\ g_{3,i} \end{pmatrix} = P_i, \quad (3.36)$$

where a_i is the first row of A_i , g_i is the first column of G_i , and P_i is the measured intensity.

This equation can be simplified by forming an appropriate 16-element product w_i for each configuration:

$$w_i = [a_{i,0}g_{i,0} \quad a_{i,0}g_{i,1} \quad a_{i,0}g_{i,2} \quad a_{i,0}g_{i,3} \quad a_{i,1}g_{i,0} \quad \cdots \quad a_{i,3}g_{i,3}]. \quad (3.37)$$

The instrument matrix W (dimension $N \times 16$) is then formed from the w_i as:

$$W = \begin{pmatrix} w_1 \\ w_2 \\ \vdots \\ w_N \end{pmatrix}. \quad (3.38)$$

The polarimeter equation then becomes

$$W^+ M_f = P, \quad (3.39)$$

where M_f is M flattened into a 16×1 vector. M_f is determined from the pseudo-inverse of W :

$$M_f = W_p^{-1} P = (W^T W)^{-1} W^T P. \quad (3.40)$$

For convenience in this dissertation, the symbol M is hereafter used to refer to both the 4x4 sample Mueller matrix and the 16x1 flattened sample vector, as they are informationally equivalent. The two equations 3.39 and 3.40 form the basis for polarimetry and polarimeter design using Mueller matrix techniques. It is clear from these equations that W must be well characterized, and the intensities P must be accurately measured, to provide an accurate reconstruction of M .

3.7: Physically realizable Mueller matrices

When using Equation 3.40 to reconstruct M from knowledge of W and measurement of P , there is error present due to non idealities in the system. When error is present, it is often the case that the reconstructed M is not physically realizable (“nonphysical”) – that is, it is not possible to generate this M using real components such as polarizers, retarders, and depolarizers. In this case degree of polarization is outside the range of 0–1, and/or the intensity is negative. The “nearest” physical matrix to M may be found, both to reduce the error when extracting polarization parameters, and to give a quantifiable metric for the error in the measurement of M .

The requirement for a Mueller matrix to be physical can be simply stated that a complex Hermitian matrix derived from its elements must have eigenvalues which are non-negative [132]. In particular, from the original Mueller matrix M with elements m_{ij} , i,j , in the range 0..3, the complex Hermitian matrix H is derived as

$$H = \frac{1}{2} \sum \sum m_{ij} (\sigma_i \otimes \sigma_j^*), \quad (3.41)$$

where the σ 's are the normalized Pauli matrices:

$$\sigma_0 = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \quad \sigma_1 = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} \quad \sigma_2 = \begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix} \quad \sigma_3 = \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix}, \quad (3.42)$$

and $\otimes$ indicates the outer product function flattened into a matrix, i.e.

$$\sigma_i \otimes \sigma_j^* = \begin{pmatrix} \sigma_{i,00} \sigma_{j,00}^* & \sigma_{i,00} \sigma_{j,01}^* & \sigma_{i,01} \sigma_{j,00}^* & \sigma_{i,01} \sigma_{j,01}^* \\ \vdots & \ddots & & \vdots \\ \vdots & & \ddots & \vdots \\ \sigma_{i,10} \sigma_{j,10}^* & \cdots & \cdots & \sigma_{i,11} \sigma_{j,11}^* \end{pmatrix}. \quad (3.43)$$

If any of the four eigenvalues of H is negative, then M is not physical. Often when a Mueller matrix is measured in the presence of noise, the Mueller matrix is close to physical. In this case three of the four eigenvalues are positive, and one is small and negative.

The Mueller matrix can be reconstructed from H as

$$m_{ij} = \text{Tr}\{H_p [\sigma_i \otimes \sigma_j^*]\}, \quad (3.44)$$

where Tr is the Trace function.

The method used in this work to construct a physical Mueller matrix M_p based on the measured nonphysical Mueller matrix M is to find that Hermitian matrix H_p which (a) has all positive eigenvalues (i.e. is positive semidefinite), and (b) has reconstructed M_p which is “closest” to the original M . Various metrics can be considered to define the meaning of “closest”, as discussed below.

The first step in finding the optimal H_p is to construct a generalized matrix H_{opt} which is by construction positive semidefinite. This can be done for example by forming H_{opt} from a Cholesky decomposition. The Cholesky decomposition of a complex matrix A is given by

$$A = C^\dagger C, \quad (3.45)$$

where $\dagger$ indicates conjugate transpose, and C is an upper triangular matrix (an equivalent decomposition exists which gives a lower triangular matrix). If the Cholesky decomposition of A exists, then A must be positive semidefinite. To preserve the magnitude of H_{opt} , its Cholesky decomposition is normalized by a constant,

$$H_{opt} = \frac{2C^\dagger C}{Tr[C^\dagger C]}, \quad (3.46)$$

where C is given by

$$C = \begin{pmatrix} h_1 & h_5 + ih_6 & h_{11} + ih_{12} & h_{15} + ih_{16} \\ 0 & h_2 & h_7 + ih_8 & h_{13} + ih_{14} \\ 0 & 0 & h_3 & h_9 + ih_{10} \\ 0 & 0 & 0 & h_4 \end{pmatrix}. \quad (3.47)$$

To perform the optimization, H_{opt} (having 16 variable parameters $h_1 - h_{16}$) is formed via Equations 3.46 and 3.47, M_p is formed via Equation 3.44, and the 16 parameters are varied until some defined difference metric between M_p and M is minimized. This type of optimization is usually performed using a mathematical software program.

It is necessary to provide some starting point for the 16 parameters. To do this a complex semidefinite Hermitian matrix H_{init} is formed from H :

$$H_{init} = \frac{2\sqrt{H}(\sqrt{H})^\dagger}{Tr[\sqrt{H}(\sqrt{H})^\dagger]}. \quad (3.48)$$

The Cholesky decomposition of H_{init} yields the upper triangular matrix C_{init} , and the 16 component values as defined in Equation 3.47 are used as the starting points.

Various metrics can be conceived to minimize the distance between M_p and M . Aiello et al. [132] suggest the use of a Maximum Likelihood metric which is analogous to that used in quantum physics. In this work it was found that minimizing the Frobenius distance F_d between M_p and M works well and is simpler to implement. Frobenius distance is defined as:

$$F_d = \frac{\|M - M_p\|}{\|M + M_p\|}, \quad (3.49)$$

where $\|\cdot\|$ is any appropriate matrix norm, such as the Euclidean norm, which for an $M \times N$ matrix A with elements a_{ij} is given by:

$$\|A\| = \sqrt{\sum_{i=1}^M \sum_{j=1}^N a_{ij}^2}. \quad (3.50)$$

The Cholesky decomposition method is computationally intensive. An alternate “quick and dirty” method for generating a physical M_p is to find the eigenvalues $(\lambda_1, \lambda_2, \lambda_3, \lambda_4)$ and eigenvectors (v_1, v_2, v_3, v_4) of H . Any negative eigenvalues are set to zero. The eigenvalues are formed into a diagonal matrix D :

$$D = \begin{pmatrix} \lambda'_1 & 0 & 0 & 0 \\ 0 & \lambda'_2 & 0 & 0 \\ 0 & 0 & \lambda'_3 & 0 \\ 0 & 0 & 0 & \lambda'_4 \end{pmatrix} \quad \text{where} \quad \lambda'_i = \begin{cases} \lambda_i & \text{if } \lambda_i \geq 0 \\ 0 & \text{if } \lambda_i < 0 \end{cases}, \quad (3.51)$$

and the eigenvectors are formed into a square unitary matrix P :

$$P = \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}. \quad (3.52)$$

A new physically realizable H_p is formed as the product

$$H_p = PDP^{-1}, \quad (3.53)$$

per the Eigen Decomposition Theorem [131]. Finally M_p is derived from H_p via Equation 3.44.

The latter method gives a dramatically faster algorithm, but unlike the original method the physical Mueller matrix calculated is not necessarily the closest one to the original unphysical M . So long as M is close to physical (i.e. one eigenvalue is at most only slightly negative) the two methods provide nearly identical results. In this work the

“quick and dirty” method is used, as the more precise method is prohibitively time consuming given the large amount of data.

3.8: Conclusions

In this chapter the basic theories of polarization and polarimetry have been presented. The Mueller calculus and Poincaré sphere were introduced as tools to describe polarization states and the polarization altering properties of samples, and methods were presented for extracting specific polarization parameters from Mueller matrices. The basic defining equations for light measuring and sample measuring polarimeters were developed, and shown to depend in a straightforward manner on the Mueller matrices of the generator and analyzer sections of the polarimeter as well as the vector of measured intensities. With these basic tools it is possible to design a working (though not optimized) polarimeter to measure the polarization properties of a sample. Finally, a method for converting a measured nonphysical Mueller matrix to the nearest physical Mueller matrix was presented.

CHAPTER 4: OPTIMIZATION OF MUELLER MATRIX POLARIMETERS

4.1: Introduction

Optimizing a Mueller matrix polarimeter requires careful selection of polarization components to ensure accurate reconstruction of a sample Mueller matrix M in the presence of error sources. In this chapter metrics to guide the design process for Mueller matrix polarimeters are presented. The goals are the following: specification of polarization elements, comparisons of polarimeter configurations, estimation of polarimeter errors, and compensation for known error sources. The use of these metrics is illustrated with analyses of two example polarimeters: a dual rotating retarder polarimeter, and a dual variable retarder polarimeter. The use of these metrics on the GDx-MM polarimeter is fully discussed in Chapter 9.

4.2: Background

The optimization of polarimeters has been widely discussed in the literature, and various forms of the condition number of the instrument matrix are most often presented as an appropriate optimization metric. Most articles have discussed light-measuring Stokes polarimeters in which the number of probing states is equal to the number of Stokes parameters to be determined. Azzam et al. [133] first discussed the choice of optimal Stokes vectors in the PSA, and demonstrated that these vectors inscribe a regular tetrahedron within the Poincaré sphere. Ambirajan and Look [134,135] obtained what they believed to be (but did not prove to be) an optimum rotating retarder Stokes polarimeter configuration by minimizing the condition number of the instrument matrix.

Sabatke et al. [136,137] derived the optimal configurations for a rotating retarder Stokes polarimeter using the condition number and related metrics in relation to the Singular Value Decomposition, and showed that there are two ways to inscribe the regular tetrahedron in the Poincaré sphere. Smith [138] optimized the dual rotating retarder Mueller matrix polarimeter using condition number, and treated optimization for the overspecified case where more than 16 measurements are made, demonstrating a substantial improvement in condition number with increasing number of measurements. Tyo [139-141] derived the optimal configuration for a number of Stokes polarimeters, including rotating retarder, variable retarder, multichannel linear, and hyperspectral dual variable retarder polarimeters. Savenkov [142] discussed the optimization of a general Mueller matrix polarimeter in terms of condition number for non-depolarizing samples. DeMartino et al. [143,144] and Garcia-Caurel et al. [145] applied the condition number minimization approach to spectroscopic polarimeters using photoelastic modulators and liquid crystal retarders. Many of these researchers have treated the effect of random detection noise on polarimeter performance. Sabatke et al. [137] discuss error propagation in terms of a noise covariance matrix. Tyo [146] introduced Stokes cones and tight frames to discuss the effect of systematic polarimeter errors. Zallat et al. [147] further developed the theory for random intensity measurement errors.

Sample measuring Mueller matrix polarimeters use variable polarization elements to generate and analyze a number of polarization states which have interacted with a sample of interest. The Mueller matrix of the sample is reconstructed from the vector of

measured fluxes. In practice there are systematic and random error sources which couple into the measurement and destabilize the reconstruction. Some errors are specific to the calibration and alignment of the polarization components, for example an inaccurate waveplate retardance or orientation. Error may also be present in the detection process, typically due to detector noise. To ensure accurate reconstruction it is necessary to consider error sources when designing a polarimeter. In this dissertation methods and metrics to guide the design process are presented and discussed, including the condition number, Poincaré sphere trajectories, and the Singular Value Decomposition. Error analysis techniques are developed which may be used to quantify overall error, evaluate relative contributions of the various error sources, and correct for error when the error sources are predictable and well characterized.

4.3: Rank, Range, and Nullspace

An examination of the rank, range, and nullspace of W is a useful method to determine whether a proposed polarimeter configuration is suitable to measure an M of interest.

The rank of W should be greater than or equal to the number of degrees of freedom of M (16 for a full Mueller matrix). The range and nullspace of a matrix A are defined as all vectors v such that

$$Av \neq 0 \quad (\text{range}), \quad Av = 0 \quad (\text{nullspace}), \quad (4.1)$$

and describe the set of vectors which can and cannot be reached via A . In the context of a polarimeter, any polarization state which lies in the nullspace of W cannot be measured by the polarimeter. A polarimeter is “complete” if the nullspace is empty.

M_R , the reconstruction of M as performed by the polarimeter, can be expressed as:

$$M_R = W_p^{-1} P = W W_p^{-1} M . \quad (4.2)$$

For an incomplete polarimeter, if M has components in the nullspace, then the data reduction will find a nearby $M_R \neq M$ in the range of W .

4.4: Stability in the Presence of Error

A consideration in polarimeter design is the loss of accuracy in the presence of error sources, which is related to the stability of W . Each row of W represents one polarimeter PSG/PSA state and forms one basis vector in the reconstruction. The measurement obtained with each state is the projection of M onto that basis vector. For the polarimeter to effectively reconstruct any M , there should be minimum correlation between basis vectors (i.e. they should be widely distributed so as to cover the space of all possible states), and well balanced in magnitude. In the case of an overspecified system with more than 16 measurements, the basis vectors provide redundant coverage of the polarization space, improving performance in the presence of noise. When a priori

knowledge of M exists, the basis states can be made to lie more densely in directions which provide the most information about M .

For a general purpose polarimeter W should be as far from singular as possible, i.e. it should be “well conditioned”. Various related metrics from linear algebra theory quantify this distance. The most widely used is κ_p , the condition number based on the L_p norm of the matrix A , which is defined as [131]

$$\kappa_p(A) = \|A\|_p \|A^{-1}\|_p, \quad (4.3)$$

where the bars signify the p -norm

$$\|A\|_p = \sup_{x \in D(A)} \frac{\|Ax\|_p}{\|x\|_p} \quad (\text{matrix p-norm}), \quad \left(\|x\|_p\right)^p = \sum_i x_i^p \quad (\text{vector p-norm}), \quad (4.4)$$

and where x is a vector, $D(A)$ is the domain of A , and sup is the supremum, defined as the limiting maximum value. Where A is not square a generalized inverse (usually the pseudo-inverse) is used.

Each of the four condition number definitions in general use has smallest value for a unitary matrix, and largest value for a singular matrix. An important tool in polarimeter

design is minimization of the condition number to balance the reconstruction basis set over the range of W and minimize the mapping of errors into the reconstruction.

The L_1 condition number ($p = 1$) is based on the maximum absolute column sum:

$$\|A\|_1 = \max_{1 \leq j \leq n} \sum_{i=1}^n |a_{ij}| . \quad (4.5)$$

The L_∞ condition number ($p = \infty$) is based on the maximum absolute row sum:

$$\|A\|_\infty = \max_{1 \leq i \leq n} \sum_{j=1}^n |a_{ij}| . \quad (4.6)$$

The L_2 condition number ($p = 2$) or spectral norm is related to the Euclidean length of the rows of A . It ranges from 1 for a unitary matrix, to infinity for a singular matrix:

$$\|A\|_2 = \sup_x \frac{\|Ax\|_2}{\|x\|_2} . \quad (4.7)$$

The Frobenius norm, not a p -norm, has no row or column dependence. For A square and invertible the Frobenius norm is inversely related to the determinant of A :

$$\|A\|_F = \left(\sum_{i,j} |a_{ij}|^2 \right)^{1/2} . \quad (4.8)$$

Each norm provides a different measure of system conditioning. Though the magnitudes of the various condition numbers are different for a given matrix, they are similarly bounded, related by [131]:

$$\begin{aligned}
 \frac{1}{n} \kappa_2(W) &\leq \kappa_1(W) \leq n \kappa_2(W) , \\
 \frac{1}{n} \kappa_\infty(W) &\leq \kappa_2(W) \leq n \kappa_\infty(W) , \\
 \frac{1}{n^2} \kappa_1(W) &\leq \kappa_\infty(W) \leq n^2 \kappa_1(W) .
 \end{aligned} \tag{4.9}$$

In this work the L_2 condition number is used. It has an intuitive range of 1 (perfect conditioning) to infinity (singular), and has a clear relationship to the Singular Value Decomposition as discussed in the following section.

Section 4.5: Singular Value Decomposition

Insight is provided into the conditioning of W via its Singular Value Decomposition (SVD), which has been discussed by Tyo [146] and Sabatke et al. [137] in relation to polarimeter design. The SVD allows any $N \times K$ matrix A to be factored into the form:

$$A = UDV^T = U \begin{pmatrix} \mu_0 & 0 & 0 & 0 \\ 0 & \mu_1 & 0 & 0 \\ & & \cdots & \\ 0 & 0 & 0 & \mu_{16} \\ & & \vdots & \end{pmatrix} V^T , \tag{4.10}$$

where U and V are $N \times N$ and $K \times K$ unitary matrices, and D is an $N \times K$ diagonal matrix. The number of nonzero diagonal elements $\mu_k, k:1..K$, termed singular values (SVs), is equal to the rank of W , and so for a full-rank W there are 16 singular values. Those columns of U associated with nonzero singular values form an orthonormal basis for the range of W , and those columns of V (rows of V^T) associated with zero-valued singular values form an orthonormal basis for the nullspace of W . An important interpretation of the SVD is that the columns of V associated with nonzero singular values form an orthonormal basis which spans the full vector space of W and thus can be used to reconstruct M ; each singular value gives the relative strength of the corresponding vector in this basis set; and the columns of U form a mapping from the V matrix basis set back to the original basis set of W . Further, since

$$P = WM = U \begin{pmatrix} \mu_1 & 0 & 0 & 0 \\ 0 & \mu_2 & 0 & 0 \\ & & \dots & \\ 0 & 0 & 0 & \mu_{16} \\ & & \vdots & \end{pmatrix} V^T M, \quad (4.11)$$

the rows of U corresponding to zero-valued singular values describe basis vectors which are not generated by M , so that their presence in a polarimetric measurement can only be due to noise. Based on this interpretation any basis vector in V which is associated with a relatively small singular value is near to the nullspace, and having little information content simply amplifies error into the reconstruction of M . Error sources which produce

projections (intensity vectors) which are similar to the intensity vectors generated by the basis vectors in V (particularly those which correspond to large singular values) will couple strongly into the reconstruction of M . Our experience indicates that the SVD of an “ideal” rank 16 instrument matrix W would have 15 equal nonzero singular values and one with larger value. The L_2 condition number is equivalent to the ratio of the largest to smallest singular value [131], and thus minimizing the condition number is equivalent to equalizing (to the extent possible) the range of singular values so that the basis vectors have similar weight.

In the case where relatively small singular values do exist, it is often preferable to set them to zero in D , remove the corresponding columns of U and V , and reconstruct a new lower-rank instrument matrix W from the modified SVD. Another option is to weight the singular values and the associated columns of U and V on the basis of the signal to noise ratio, for example giving lowest weight to those basis vector patterns which are most strongly associated with system noise. These techniques make the reconstruction less sensitive to error, but will introduce error if the sample to be measured contains components in the direction of the zeroed or re-weighted basis vectors.

W_p^{-1} can be expressed in terms of D^* , the transpose of D with the singular values replaced by their reciprocals:

$$W_p^{-1} = VD^*U^T = V \begin{pmatrix} 1/\mu_0 & 0 & 0 & 0 \\ 0 & 1/\mu_1 & 0 & 0 \\ 0 & 0 & \ddots & 0 \\ 0 & 0 & 0 & 1/\mu_{16} \end{pmatrix} U^T. \quad (4.12)$$

Multiplication by W_p^{-1} involves division by the singular values, and emphasizes that small singular values in W have large multiplicative effect in the inverse matrix.

4.6: Geometrical Interpretation

A geometrical interpretation relating the stability of W to the trajectory on the Poincaré sphere was first proposed by Azzam et al. [133] and later extended by Ambirajan and Look [134,135], Sabatke et al. [136], and Tyo [139]. If the four rows of W (which form the basis set for the Stokes polarimeter) are interpreted as Stokes vectors and plotted on the Poincaré sphere, they form the vertices of a (generally) irregular tetrahedron. The volume of the tetrahedron is proportional to the determinant of W , and is maximized when the vertices form a regular tetrahedron (Figure 4.1). In this case the maximum distance from any point on the sphere to one of the vertices is minimized, and the condition number is also minimum. The selection of PSA polarization elements may thus be limited by considering only those combinations providing appropriate degrees of freedom such that a regular tetrahedron may be inscribed in the Poincaré sphere trajectory. For a rotating retarder in front of a polarizer (one degree of freedom), Sabatke et al. demonstrated a “figure eight” trajectory with varying aspect ratio dependent on retardance [136]. An optimal trajectory enclosing a regular tetrahedron was identified for

exactly two retardances: 132° and 228° . Tyo showed that for two degrees of freedom (such as a dual variable retarder Stokes polarimeter) the trajectory forms a two-dimensional surface on the Poincaré sphere, giving a continuum of optimum configurations in which the tetrahedron may be inscribed [138].

Figure 4.1: The trajectory of an optimized rotating retarder Stokes polarimeter with retarder retardance of 132° (green line) plotted on the Poincaré sphere, demonstrating that it is possible to inscribe a regular tetrahedron. The vertices of the tetrahedron determine the optimal retarder orientations (red circles).

The geometrical interpretation does not address the effect of increasing the number of measurements N to overspecify the polarimeter. It was demonstrated by Sabatke et al. [136] that additional measurements “fill out” the trajectory, which in the presence of error does result in improved accuracy, but (at least in the case of independent identically distributed “random” measurement noise) a similar improvement would be obtained by taking additional measurements at the original four orientations and averaging.

To develop a geometrical interpretation for a Mueller matrix polarimeter, consider that W is formed as the outer product of the generator and analyzer portions of the polarimeter:

$$W = G^T \otimes A . \quad (4.13)$$

The condition number of the polarimeter is thus related to the condition numbers of the generator portion $\kappa(G)$ and analyzer portion $\kappa(A)$ as

$$\kappa(W) = \kappa(G^T \otimes A) = \kappa(G^T)\kappa(A) = \kappa(G)\kappa(A) . \quad (4.14)$$

Therefore the PSG and PSA should be individually optimized in terms of Poincaré sphere trajectory.

4.7: Error Analysis

The metrics reviewed in the previous section guide a polarimeter design toward higher stability. However, errors in the polarimeter and/or detection process drive W away from optimal conditioning, and thus the condition number and other metrics will not necessarily give the best solution when errors are present. The goals of an error analysis are: to quantify the accuracy in reconstructing M ; to identify those error sources with highest contribution; to compensate W for known error; and to optimize the polarimeter design with consideration for error.

At any use, W is not known exactly and may have changed since calibration. For example, a rotating retarder may have slightly different orientation, rays for a given sample may take different paths through the polarimeter, and the spectral distribution of return light may vary with the sample. Variable measurement error is also present, due for example to detector noise and source fluctuation. The basic instrument equation can be modified to include these effects:

$$(W + \delta W)M + \delta P = P_M , \quad (4.15)$$

where δW is a $N \times 16$ matrix representing the difference between the W matrix in use and the true W matrix, δP is a $N \times 1$ vector representing intensity measurement error, and P_M is the $N \times 1$ vector of intensities measured in the presence of instrument error.

M_R , the reconstruction of M , is given by

$$\begin{aligned} M_R &= W_p^{-1} P_M \\ &= W_p^{-1} [(W + \delta W)M + \delta P] \\ &= M + \delta M = M + W_p^{-1} [\delta W M + \delta P] \end{aligned} \quad (4.16)$$

where δM is the difference between M_R and M . From this equation it is clear that there are two error terms, one dependent on δW and M , and the other on δP .

Assuming the errors are small, they may be represented by a first order Taylor expansion. The error for the j th component of the i th polarimeter state, having R variables x_r (such as a retardance magnitude), each with nominal value ϕ_r , and error magnitude δ_r , is then

$$\delta W_{ij} \approx \sum_{r=1}^R \delta_{r,ij} \left. \frac{\partial W_{ij}}{\partial x_r} \right|_{x_r = \phi_r} . \quad (4.17)$$

Clearly to improve accuracy the derivative dW/dx must be minimized. The error in P is assumed independent of the polarimeter elements, and is given by

$$\delta P_i = \varepsilon_i , \quad (4.18)$$

where ε_i is the i th intensity measurement error.

The error in reconstructing each of the $k = 1..16$ elements of M in terms of the errors in the instrument and detection process is then

$$\delta M_k = \sum_{i=1}^N W_{ki}^{-1} \left[\sum_{j=1}^{16} \sum_{r=1}^R \delta_{r,ij} \left. \frac{\partial W_{ij}}{\partial x_r} \right|_{x_r = \phi_r} M_j \right] + \sum_{i=1}^N W_{ki}^{-1} \varepsilon_i \quad k: 1..16 . \quad (4.19)$$

To obtain an estimate of the typical error, the statistics of the error sources should be considered. Consider the Expectation E (mean) and standard deviation SD , which for a random variable x are given by:

$$E(x) \equiv \langle x \rangle = \sum_i p_i x_i, \quad SD(x) \equiv \sigma^2(x) = \langle x^2 \rangle - \langle x \rangle^2, \quad (4.20)$$

where p_i is the probability of each element x_i of x . The mean and standard deviation of δM due to errors in δW and δP are

$$\langle \delta M \rangle = \langle W^{-1} \delta W M \rangle + \langle W^{-1} \delta P \rangle, \quad SD[\delta M] = SD[W^{-1} \delta W M] + SD[W^{-1} \delta P]. \quad (4.21)$$

In this equation the variables are the polarimeter and measurement error sources δW and δP ; W and M are assumed known and fixed. The error mean and SD can be quantitatively estimated where the polarimeter state variables and the statistics of the error sources are approximately known. It is necessary to assume one or more values for M , either from knowledge of the sample, or from appropriate combinations of ideal diattenuators, retarders, and depolarizers.

Where there is a priori knowledge of a systematic error source, it is common to remove the mean error from the calculation of M_R by calculating a new instrument matrix W_q given by $W + \langle \delta W \rangle$. Then

$$M_R = W_q^{-1}[(W + \delta W)M + \delta P] = M + W_q^{-1}\delta P . \quad (4.22)$$

The effectiveness of the compensation depends on the accuracy of estimating $\langle \delta W \rangle$. For example when using a liquid crystal retarder with a known profile of retardance magnitude as a function of temperature, an error in retardance magnitude can be calculated at every use given the temperature at that time. Other examples include compensation for a rotating element which has an angular position which is known to be systematically different than at calibration, or a waveplate with wavelength-dependent retardance.

4.8: Forming a Minimizable Error Metric

A metric based on the error covariance matrix may be used to optimize a polarimeter in the presence of known error. This method has been applied to random measurement noise in Stokes polarimetry by Sabatke et al. [137], and also to random instrument noise in Stokes polarimetry by Tyo [146].

Given a vector Q consisting of N real-valued random variables (RVs) $\{q_1, q_2 \dots q_N\}$, the covariance matrix is defined as [148]

$$C_{ij} = \langle q_i q_j \rangle - \langle q_i \rangle \langle q_j \rangle \quad i, j : 1 - N . \quad (4.23)$$

The covariance matrix is a symmetric matrix which describes the correlation between RVs which have been centered about their means. In the polarimetry case the 16x16 covariance matrix of interest, C_M , is

$$C_{M,jk} = \langle \delta M_j \delta M_k \rangle - \langle \delta M_j \rangle \langle \delta M_k \rangle \quad j, k : 1-16 . \quad (4.24)$$

To derive C_M , consider the total error δE for the i^{th} polarimeter state:

$$\delta E_i = \sum_{j=1}^{16} \delta W_{ij} M_j + \delta P_i . \quad (4.25)$$

An error covariance matrix C_E based on δE is given by

$$C_{E,jk} = \langle \delta E_j \delta E_k \rangle - \langle \delta E_j \rangle \langle \delta E_k \rangle \quad j, k : 1-16 . \quad (4.26)$$

If all error sources are assumed to be uncorrelated and have the same statistical properties among all polarimeter states, then the error may be simplified to a sum of terms, each involving the polarimeter errors δ_r and detection error ε :

$$\delta E_i = \sum_{j=1}^{16} \sum_{r=1}^R a_{rji} \delta_r m_j + b_i \varepsilon , \quad (4.27)$$

where the a 's and b 's are constant coefficients derived from δW (typically many are zero) and the m 's are the 16 elements of M .

When the covariance matrix C_E is formed, the only nonzero terms are on the diagonals.

Each diagonal term has the form

$$C_{E,ii} = \sum_{k=1}^{16} \sum_{j=1}^{16} m_j m_k \left[\sum_{r=1}^R c_{rjki} \sigma_r^2 + d_i \sigma_\varepsilon^2 \right], \quad (4.28)$$

where the c 's and d 's are constant coefficients derived from δE (typically many are zero), the σ_r^2 's are the standard deviations of the polarimeter error sources, and σ_ε^2 is the standard deviation of the detection process error. It is straightforward to show that C_M may be expressed as

$$C_M = W^{-1} C_E (W^{-1})^T. \quad (4.29)$$

The on-diagonal elements of C_M represent gain factors in the propagation of error into M_R . One possible error metric (here termed EM) is given by the sum of the diagonal elements:

$$EM = \sum_i C_{M,ii}. \quad (4.30)$$

EM depends on the polarimeter configuration, the number of states, the polarization properties of the sample, and the statistical properties of the error sources. Minimization of EM with respect to a polarimeter variable gives the optimal value of that variable in the presence of error.

To effectively utilize EM it is useful to consider the statistical character of typical polarimeter error sources, including polarization element non idealities such as variations in retardance or diattenuation magnitude and/or orientation as elements are rotated or otherwise modified to produce different polarization states. Polarization leakage and/or impurity such as nonzero diattenuation and/or depolarization in an “ideal” retarder may also be present. Most often, errors due to the common non idealities can be represented by different distributions depending on whether the errors are random or systematic.

Examples of random errors include detector thermal noise and incorrect orientation of a rotating retarder due to inaccuracy in the driving motor. A random error has mean of zero, and SD of σ^2 . If these errors are assumed to be independent between measurements and have identical SD from measurement to measurement, then

$$\langle \delta_i \delta_j \rangle = \begin{cases} \sigma^2 & i = j \\ 0 & i \neq j \end{cases}, \quad \langle \delta_i \rangle = 0, \quad SD[\delta_i] = \sigma^2. \quad (4.31)$$

Examples of systematic errors include a fixed constant offset in the rotational position of a component, or a temperature-induced change in a polarization property. Systematic errors have mean equal to their value, and SD of zero. If each such error is assumed to be the same from measurement to measurement, then

$$\langle \delta_i \delta_j \rangle = \delta^2, \quad \langle \delta_i \rangle = \delta, \quad SD[\delta_i] = 0. \quad (4.32)$$

Error sources with other statistics can be readily handled by this theory. For example, avalanche photodiode performance is generally limited by shot noise with Poisson statistics, in which case the SD is equal to the mean.

4.9: Illustrative Examples

As an illustration of the techniques presented in this paper, two Mueller matrix polarimeter configurations are analyzed: dual rotating retarder (DRR) and dual variable retarder (DVR). These configurations utilize different types and combinations of polarization components (Table 4.1), and as such different polarimeter errors are present. For the error analyses which follow only the positive range of these errors is considered for simplicity (for example a positive temperature drift). The presented calculations of overall error range should be considered as due to a positive error, and an error of equal magnitude and opposite sign is implied for the case of a negative error. The types and magnitudes of the errors assumed to be present in these example polarimeters are summarized in Table 4.2.

Table 4.1. Summary of polarimeter polarization components

Polarimeter	Fixed parameters in generator and analyzer	Variable parameters in generator and analyzer
DRR	Retarder retardance γ Polarizer orientation θ	Retarder increments $\Delta\phi_g(i)$, $\Delta\phi_a(i)$
DVR	Retarder 1 orientation ϕ_1 Retarder 2 orientation ϕ_2 Polarizer orientation θ	Retarder 1 retardance $\gamma_{g1}(i)$, $\gamma_{a1}(i)$ Retarder 2 retardance $\gamma_{g2}(i)$, $\gamma_{a2}(i)$

Table 4.2: Summary of polarimeter error sources

Polarimeter	Systematic error sources	Worst Case Mean Error	Random error sources	Worst Case SD Error
DRR	None	N/A	Retarder position Detector noise	$\sigma_{g,a}^2 = 0.5^\circ$ $\sigma_p^2 = 0.01$
DVR	Retardance drift	$\langle\delta_{1,2}\rangle = 2.5\%$	Detector noise	$\sigma_p^2 = 0.01$

The DRR polarimeter is shown in Figure 4.2. This common polarimeter consists of a PSG with fixed polarizer followed by a rotating retarder, and a PSA having the same elements but in reverse order. For this example it is assumed that both retarders have the same retardance, and the generator and analyzer retarders rotate in different uniform increments. The error sources are random positioning error in the retarders and random noise in the detector. It is desired to determine the optimal values of three variables: γ , $\Delta\phi_g$ and $\Delta\phi_a$ and predict the resulting polarimeter accuracy.

Figure 4.2: Schematic of Dual Rotating Retarder (DRR) polarimeter. Components include fixed polarizers at orientation θ and rotating retarders with retardance γ and orientations ϕ_g and ϕ_a which vary with state i : $1..N$.

The DVR polarimeter is shown in Figure 4.3. This polarimeter consists of a PSG with fixed polarizer followed by two variable retarders (often liquid crystal devices) at different fixed orientations, and a PSA having the same elements but in reverse order. The sequence of 16 pairs of generator and analyzer retardances are formed from permutations of retardance pairs $(\gamma_A \ \gamma_A)$, $(\gamma_A \ \gamma_B)$, $(\gamma_B \ \gamma_A)$, $(\gamma_B \ \gamma_B)$. The error sources are systematic retardance error due to temperature drift and random detector noise. It is desired to determine the optimal values of four variables: γ_A , γ_B , ϕ_1 , and ϕ_2 and predict the resulting polarimeter accuracy.

Figure 4.3: Schematic of Dual Variable Retarder (DVR) polarimeter. Components include fixed polarizers with orientation θ and variable retarder pairs. The outer retarders have fixed orientation ϕ_1 and retardance γ_{g1} and γ_{a1} which vary with state $i: 1..16$. The inner retarders have fixed orientation ϕ_2 and retardances γ_{g2} and γ_{a2} which vary with state $i: 1..16$.

Geometrical Interpretation

The DRR polarimeter having a PSG with one variable parameter (retarder orientation) traces a figure-eight type trajectory (Figure 4.4a). Through appropriate choice of retardance (i.e. via a condition number analysis) a regular tetrahedron may be inscribed.

The DVR polarimeter having a PSG with two variable parameters (retardances of the two variable retarders) traces a surface on the Poincaré sphere (Figure 4.4b) within which a regular tetrahedron may be inscribed.

Figure 4.4: Sample trajectories on the Poincaré sphere. (a): DRR polarimeter with one variable traces a figure-eight trajectory. (b): DVR polarimeter with two variables traces a surface.

Condition number

To evaluate the condition number, a W is assembled which is a function of the polarimeter variables. One or more variables (A) are fixed at reasonable “initial guess” values, and the remaining variables (B) are varied. The W having rank 16 and lowest condition number gives the optimal values for the B variables. The process is then repeated to find the optimal values for the A variables. A few iterations may be required if the initial guesses are not sufficiently close to the calculated optimum. Initial guesses based on evaluation of Poincaré sphere trajectories aid in limiting the variable ranges.

The DRR polarimeter has been analyzed in terms of condition number for $N = 16$ and $N = 30$ by Smith [138]. For the $N = 16$ case an optimal retardance of $\gamma = 127^\circ$ and optimal angular increments of $\Delta\theta_g = 34^\circ$ and $\Delta\theta_a = 26^\circ$ were found. Multiple solutions for angular increment were found for the $N = 30$ case. The minimum L2 condition number is

16.7 for $N = 16$, and 3.48 for $N = 30$. Figure 4.5 reproduces Smith's results for condition number as a function of γ . Figure 4.6 reproduces Smith's results for condition number as a function of $\Delta\theta_g$ and $\Delta\theta_a$. In these graphs, blue indicates a high (undesirable) condition number solution, and red indicates a low (desirable) condition number solution.

Figure 4.5: Log condition number as a function of waveplate retardance for the DRR polarimeter. The optimum solution is a retardance of 127° .

Figure 4.6: Condition number as a function of rotating retarder increments for the DRR polarimeter. Horizontal and vertical axes are the rotational increments of the two retarders ($\Delta\theta_g$ and $\Delta\theta_a$) in degrees. Blue indicates a high (undesirable) condition number, and red indicates a low condition number. Color scale is nonlinear to emphasize the low condition number solutions. (a): For $N = 16$, the best solutions are highly localized; one example is $\Delta\theta_g = 34^\circ$ and $\Delta\theta_a = 26^\circ$. (b): For $N = 30$ there are regions of good solutions.

The DVR polarimeter has been analyzed by De Martino et al. [144]. Optimal values of $\gamma_1 = 225^\circ$, $\gamma_2 = 45^\circ$, $\theta_B = 27.4^\circ$, and $\theta_C = 72.4^\circ$ were found. The minimum L2 condition number is 3, lower than for the DRR polarimeter, indicating better performance in the presence of error. Figure 4.7a shows the optimization with respect to angles when retardances are fixed; Figure 4.7b shows the optimization with respect to retardances when angles are fixed. As compared to the DRR polarimeter, the low condition number solutions are more closely clustered near fewer optimal values.

Figure 4.7: Condition number as a function of polarimeter variables for the DVR polarimeter, for $N = 16$. Blue indicates a high (undesirable) condition number, and red indicates a low condition number. Color scale is nonlinear to emphasize the low condition number solutions. (a): optimization of retarder angular orientations ϕ_1 and ϕ_2 assuming retardances of $\gamma_1 = 225^\circ$, $\gamma_2 = 45^\circ$. Horizontal and vertical axes are the angular orientations of the two retarders in degrees. (b): optimization of retarder retardances γ_1 and γ_2 for the optimum orientations of $\phi_1 = 27.4^\circ$, $\phi_2 = 72.4^\circ$. Horizontal and vertical axes are the retardances of the two retarders in degrees.

Singular Value Decomposition

The singular values (SVs) for each condition number optimized polarimeter are shown in Figure 4.8. For each polarimeter there are 16 nonzero SVs, indicating a rank 16 instrument matrix. The SV profiles for the $N = 30$ DRR and DVR polarimeters are closest to optimal, as they exhibit low slope for singular values 2-7 and 8-16, whereas the SV profile for the $N=16$ DRR polarimeter decreases monotonically.

Figure 4.8: Singular Values for the example polarimeter configurations.

Error Mean and Standard Deviation

The polarimetric measurement error is evaluated for two samples: a linear polarizer, rotated from 0° to 90° in steps of 15° ; and a quarter wave plate, rotated from 0° to 90° in steps of 15° . Figure 4.9 shows the worst case mean and standard deviation error for each polarimeter, assuming the error sources of Table 4.2. For the first sample, worst case mean and standard deviation of diattenuation magnitude and orientation are calculated as a function of polarizer orientation. For the second sample, worst case mean and standard deviation of retardance magnitude and orientation are calculated as a function of retarder orientation.

The DRR polarimeter has zero mean error, but the standard deviation is large due to error in retarder orientation. By comparison, the DVR polarimeter has measurable mean error due to the retardance magnitude error of the variable retarders, but the standard deviation is small since the only contribution is detector noise.

Figure 4.9: Calculated mean and SD error. (a),(c): sample is a linear polarizer rotated from 0° to 90° in 15° increments. (b),(d): sample is a quarter wave plate rotated from 0° to 90° in 15° increments. Solid red and blue lines: worst case mean error deviations; error bars: worst case random error deviations.

Optimization in the presence of known error

For the DRR polarimeter the error metric EM was minimized to determine the optimal retardance γ for various targets. Figure 4.10a shows that the ideal retardance varies from 117° for a linear polarizer target at 45° orientation, to 128° for a quarter wave plate target at 45° orientation. The average is 122.5° over all four sample targets, which is slightly less than the value of 127° found using the condition number metric, in which polarimeter error is not considered. A comparison of EM for $N = 16$ and $N = 30$ for a horizontal linear polarizer target is shown in Figure 4.10b. A $1/N^2$ improvement in EM is seen, as well as a slight shift in optimized retardance, from 122° to 125° . Thus taking additional measurements improves immunity to error sources and pulls the optimized retardance back towards the value found using the condition number metric (127°).

Figure 4.10: EM metric to optimize retardance for the DRR polarimeter when information is known about the error source and/or sample to be measured. (a): EM minimum occurs for different values of retardance depending on the sample to be measured. (b): EM minimum occurs at different values of retardance depending on the number of polarimeter states N . Here $N = 16$ and $N = 30$ are compared.

4.10: Conclusions

The techniques presented in this chapter can be used to optimize a polarimeter design, with the goal of providing good accuracy in sample Mueller matrix reconstruction. The most important design requirements are: (1) achieving a complete rank 16 polarimeter, so that no polarization properties fall into the nullspace and thus introduce error into the Mueller matrix reconstruction; and (2) minimizing the condition number of the instrument matrix to ensure stability in the presence of error sources. Poincaré sphere trajectories may be used as a visualization tool to limit the potentially large solution space. The Singular Value Decomposition gives insight into the stability of the instrument matrix via the balancing of the basis vectors. An error analysis can be performed to assess the individual and/or combined effects of the various error sources, and may be useful to estimate the magnitude of the error in the reconstruction, or to compensate for the error where error sources are predictable and well characterized. Two examples of polarimeter design optimization and error analysis have been presented which illustrate the concepts presented in this chapter.

CHAPTER 5: THE LASER DIAGNOSTICS GDx

5.1: Introduction

The Laser Diagnostics GDx retinal nerve fiber analyzer was the first commercially available instrument utilizing polarization methods for the diagnosis and monitoring of retinal eye disease, in this case glaucoma. This scanning laser ophthalmoscope is used in this work as a starting point for development of the GDx-MM, an instrument with more comprehensive polarization capability. In this chapter the Laser Diagnostics GDx is discussed in terms of its optical paths, polarization paths, and electronic functionality, with a view toward providing a foundation for understanding the GDx-MM instrument which is described in the following chapter. Particular emphasis is placed on a description of the functionality and limitations of the polarization section of the GDx.

5.2: Background and basic operation

The GDx (Figure 5.1) was manufactured by Laser Diagnostic Technologies Inc. (later acquired by Carl Zeiss Meditec Inc.). The objective is to map the retardance of the RNFL and derive its thickness. A first generation device, the Nerve Fiber Analyzer (NFA I), became commercially available in 1992. A later device, the NFA II, utilized two detectors rather than one as well as a fixed corneal compensator. The next generation device, the GDx Nerve Fiber Analyzer used in this research, retained the fixed corneal compensator and incorporated a normative database of 400 eyes classified by age and race. It was first patented in April 1994 (5,303,709) [149], received 510(k) clearance from the FDA in July 1994, and became commercially available in 1996. It was re-

patented with more specific information on optical path and mechanical design in August 1998 (patent 5,787,890) [150] and in August 2000 (patent 6,112,114) [151], and finally repatented with inclusion of an algorithm for cancelling system birefringence in March 2004 (patent 6,704,106) [152]. It has since been replaced by a newer instrument, the GDx-VCC, which incorporates variable corneal compensation. The Nerve Fiber Analyzer device in all of its forms has been used on thousands of patients, though due in part to high cost and uncertainty regarding its sensitivity and specificity, it is used in only a small percentage of ophthalmic clinics.

Figure 5.1: Laser Diagnostics GDx Retinal Nerve Fiber Analyzer, showing scan head and computer on portable cart.

The GDx is a scanning laser ophthalmoscope with polarization capability. The retina is scanned with a 780nm laser beam with variable linear polarization orientation. When the light interacts with the retina, the component of the light oscillating along the birefringent

nerve fibers travels at a different velocity from the component oscillating perpendicular to the fibers and is retarded. The backscattered light from the retina which passes through the pupil is collected by the GDx. Forty intensity images (twenty each at two detectors), with each pair collected at a different linear polarization orientation, are analyzed using an algorithm to determine retardance. The RNFL thickness, demonstrated to decrease with progression of glaucoma [89,153], is calculated from the measured retardance through a fixed birefringence factor. The retinal scan is non-contact, uses barely visible light, is performed on an undilated pupil, and requires only 0.7 seconds (though a scan time of up to a minute or longer may be required to align the device), making the procedure relatively comfortable for the patient.

Following each scan, the GDx device performs image processing tasks requiring at most one minute. In these procedures, a retardance image is calculated and converted to a thickness image for straightforward interpretation by an ophthalmologist. Comparisons are made to a normative database which takes into account the patient's age, sex, and race. Differentiation between normal and glaucomatous eyes is based on several parameters, including RNFL thickness, RNFL region area and volume, and symmetry of the RNFL thickness in the four quadrants (inferior, superior, nasal, and temporal). Various graphs are presented to the physician (Figure 5.2). Color-coded thickness maps are designed to emphasize those areas of the retina which are most likely glaucomatous. Bright colors (red, yellow) are associated with normal RNFL, and dark colors (blue) are associated with thinner RNFL. "Double hump" graphs show the RNFL thickness along a

circle centered around the optic nerve head, together with a normal range based on the normative database. The humps are due to the normal maxima of RNFL thickness superior and inferior to the optic nerve. Any individual parameter flagged as an outlier is identified to the physician, and a numeric value (“the number”) between 0 (normal) and 100 (glaucoma) is assigned based on the likelihood of glaucoma. The information provided by the GDx is intended to be used to locate areas of suspected glaucoma, and to monitor glaucoma progression.

Figure 5.2: Output data generated by the GDx. (a): fundus intensity image. (b): color-coded RNFL thickness map, showing areas of greater thickness in yellow and red. (c): double-hump graph showing thickness of RNFL along a circle centered on the ONH (dark green line) and normal range based on normative database (green shaded area). (inferior=I, superior=S, nasal=N, and temporal=T). Reprinted from [154].

The ability of the GDx to detect and monitor glaucoma has not been definitively determined. Thickness measurements have been demonstrated to be highly reproducible, with a typical standard deviation of 4 μm to 9 μm [155-157]. The sensitivity and specificity of the GDx in detecting glaucoma has been widely studied, and results have

varied significantly depending, for example, on the particulars of the neuropathies present in the study population, the choice of value for “the number” or other reported parameters to indicate disease, and the experience level of the examiner. Typical reported sensitivities and specificities are in the range of 70% to 90% [158-164], though some researchers have reported significantly lower numbers, particularly for specificity [109,165-167]. Lower than desired accuracy in detecting retinal pathology is largely due to several device limitations, discussed in the following section. The consensus on the GDx appears to be that it is probably helpful in locating potential areas of nerve fiber damage related to glaucoma, but should not be used as a stand-alone device to make a definite diagnosis [165,168]. One reason for this conclusion is the lower than desired accuracy, but another reason is that the progression of glaucoma takes many different forms, and while thinning of the RNFL is a strong indication of glaucoma, it is neither a definitive indication, nor is its absence a definitive contraindication.

5.3: Limitations of the GDx

The GDx has a number of limitations which contribute to error when performing polarimetry. The major limitations are:

1. Anterior segment birefringence if not perfectly compensated gives incorrect retinal retardance calculation.
2. RNFL thickness calculation incorrectly assumes RNFL birefringence is constant across the retina.

3. There is a wide range in RNFL thickness values among normal individuals, leading to an overlap of typical thickness values between normal and glaucomatous eyes.
4. The polarimetry is incomplete and so ocular diattenuation and depolarization lead to inaccurate retinal retardance calculation. This is of particular concern in older, diseased, or post-surgery eyes which are more likely to have higher depolarization.
5. Polarimeter design and reconstruction algorithm have high sensitivity to noise.

These limitations are readily apparent and have been discussed in the literature. This section provides an overview of these limitations; the latter two derive from polarimeter design issues which are discussed at length in Section 5.6.

One of the most significant problems with the GDx is that measured retardance includes the retardance of the anterior segment (in particular the cornea and lens), which is not of interest in retinal diagnosis, but which may be larger than the RNFL retardance and has been demonstrated to vary substantially in magnitude and orientation between subjects [78]. The GDx utilizes a fixed corneal compensator with fast axis oriented 15° nasally downward and retardation of 60 nm [49], designed to cancel an “average” magnitude and orientation of corneal retardance, where “average” was determined by direct measurement of a large number of subjects [23]. It is straightforward to demonstrate that when a subject’s corneal magnitude and axis deviate from these “average” values

significant error is introduced into the retardance measurement [25,31,169,170]. It has also been demonstrated that the fixed compensator allows for acceptable cancellation of anterior segment retardance in not more than 60-70% of patients [25], and thus it is reasonable to assume that anterior segment related errors have skewed the normative database [171,172]. A later version of the GDx, the GDx-VCC, utilizes a variable corneal compensator which provides custom compensation for each patient [78,173]. This custom compensation has been demonstrated to improve the discriminating power for glaucoma detection [169,172,174], but is reliant on the visualization of a clear Henle fiber bowtie pattern, which may not be possible in the presence of retinal disease [175,176]. Atypical retardance patterns are often noticed which manifest as variable areas of high retardation arranged in a spoke-like pattern, and/or splotchy areas of high retardation nasally and/or temporally [177,178]. In these cases the brightest areas of the thickness maps are not consistent with the histologically thickest portions of the RNFL [179], and in the presence of these patterns interpretation of results may be difficult or impossible [177,180]. To mitigate this problem a new computer algorithm referred to as “enhanced corneal compensation” (ECC) has been introduced [181]. With this method a bias retardance is added to the variable compensator to increase the total retardation to a region with less noise sensitivity; the bias is then removed mathematically from the measured results. This method has been demonstrated to improve glaucoma detection [179,182], but is still dependent on the presence of an intact Henle layer. An alternative “screen” method in which the anterior segment compensation is based on an average over the full retardance image is used where the Henle layer is distorted or absent [183].

However, this technique is sensitive to variations in the RNFL, which are significant outside the immediate foveal region. Methods for anterior segment compensation are discussed in further detail in Chapter 12.

Retardance is the product of birefringence and thickness; the GDx assumes a constant value of birefringence when calculating RNFL thickness from the retardance measurement. This value was derived from studies in monkey eyes [70,184]. However, it has been since demonstrated that birefringence of healthy RNFL varies significantly with position around the ONH. For example, in one study double-pass retardation per unit depth measured at 840 nm ranged between 0.1 and 0.35 $^{\circ}/\mu\text{m}$, with highest values occurring superior and inferior to the ONH [49].

The GDx is an incomplete polarimeter, and so does not measure retardance accurately in the presence of diattenuation and depolarization [31]. This issue is of particular concern in opacified eyes such as those with cataracts, in which significant differences between pre-surgery and post-surgery RNFL measurements have been demonstrated [185,186]. Spurious results have also been reported in the presence of various ocular pathologies including corneal edema, vitreous opacity, peripapillary atrophy, and anterior uveitis [187]. The polarimetry-based limitations of the GDx will be further discussed in Section 5.6.

5.4: Optical paths

The optical paths in the GDx are shown diagrammatically in Figure 5.3 (scanning path) and Figure 5.4 (imaging path). The outgoing optical path of the GDx consists of a linearly polarized 780 nm diode laser; a nonpolarizing beamsplitter (NPBS); a two-lens adjustable focus telescope; a two-dimensional scanner; two reflective filters which also function as turn mirrors; a rotating 180° waveplate; a 30 Diopter aspheric Volk lens; and a fixed waveplate for corneal compensation. The beam emerging from the laser is 3 mm in diameter, and diverges to a value of 5 mm at the NPBS. The NPBS reflects the laser beam without change in polarization state. The two-lens telescope (consisting of a collimating lens and focusing lens) focuses the beam to an image point located between the rotating waveplate and Volk lens. The telescope may be translated to obtain best focus on the retina using an adjustment knob. The scanner system consists of a resonant scanner with metal mirror operating at 4 kHz for fast axis motion in x , and a galvanometer scanner with dielectric coated mirror driven by a triangle wave at 26 Hz for the slow axis motion in y . With this combination, a single retinal scan of 300 scan lines is accomplished every 38 milliseconds. The reflective filters are presumably bandpass filters at 780 nm. The rotating waveplate is 6 mm thick, and is mounted on a belt-driven rotational mount, with rotational position controlled via a stepper motor; angular increment is 2.432° per step over 20 steps for a total of about 46° rotation. The Volk lens refocuses the beam through the corneal compensator and cornea onto the retina with low spherical aberration. The corneal compensator retarder has retardance of 60 nm (27.7°), and is mounted on a belt-driven rotational mount. The rotation is limited to two

positions: 15° nasally downwards with respect to left eye and 15° nasally downwards with respect to right eye, and is set automatically according to which eye (left or right) is being scanned. The cornea is positioned at the external pupil, about 30 mm from the instrument; the beam size at the cornea is about 2.5 mm. The scanned field of view on the retina is about 15° (corresponding to an extent of about 4.5 mm). The spot size on the retina is $15\text{ }\mu\text{m}$, and the $f/\#$ at the retina is about $f/24$.

The incoming path includes several common elements with the outgoing path, including: the corneal compensator, Volk lens, rotating waveplate, reflective filters, scanners, two-lens telescope, and NPBS. Additional components include a sealed detector assembly containing a pinhole, focusing lens, polarizing beamsplitter (PBS), and two detectors. After transmission through the NPBS, the beam passes through the $25\text{ }\mu\text{m}$ pinhole, which is confocal with the retina. The pinhole passes only light emanating from the focal spot on the retina, and rejects all other light. The focusing lens focuses the beam onto the detectors. The PBS directs the horizontally polarized portion of the light towards the “co-polarized” detector, and the vertically polarized portion of the light towards the “cross-polarized” detector. The detectors are silicon avalanche photodiode (APD) type, with approximately 1 mm^2 active area. Data acquisition occurs at a rate of 8 MHz, which provides 4x oversampling of 256×256 pixel images.

Figure 5.3: Diagram of GDx scanning path, showing the range of scan angles for the normal incidence path of the outgoing and return light. NPBS: nonpolarizing beamsplitter; PBS: polarizing beamsplitter; APD: avalanche photodiode.

Figure 5.4: Diagram of GDx imaging path for the outgoing and return light, for the scanners at their central position.

5.5: Radiometry

Simple radiometric calculations can be used to predict the expected optical power at each GDx detector, given the approximately 3 mW of optical power at 780 nm exiting the GDx, a few basic properties of the eye, and the approximate efficiencies of the optical components in the optical path.

The beam size at the eye pupil is about 2.5 mm, sufficiently small for the entire beam to enter the eye and proceed through the ocular media to the retina. The ocular media has

transmission T_{eye} , and the beam is focused to a spot size A_{spot} at the retina, giving an irradiance E of

$$E = \frac{T_{eye} P_{las}}{A_{spot}} \quad (\text{in Watt}/\mu\text{m}^2), \quad (5.1)$$

where P_{las} is the laser power and A_{spot} is the area of the spot at the retina. Assuming the retina is an approximately Lambertian reflector with reflectance ρ [5], the exiting radiance L is given by

$$L = \frac{\rho E}{\pi} \quad (\text{in Watt}/\mu\text{m}^2\text{-sr}). \quad (5.2)$$

The throughput of the eye U_{eye} is determined by the product of A_{spot} and the solid angle Ω_{eye} of the emission from the eye. Solid angle is determined by the eye pupil with area A_{pupil} and the distance from retina to pupil D_{eye} . Using a simple approximation to the solid angle formula,

$$U_{eye} = A_{spot} \Omega_{eye} = A_{spot} \frac{A_{pupil}}{D_{eye}^2} \quad (\text{in } \mu\text{m}^2\text{-sr}). \quad (5.3)$$

The throughput of the eye must be compared to the throughput of the GDx collection optics U_G , to determine which limits collection. The GDx throughput is determined by

the product of the Volk lens aperture A_G and the solid angle Ω_G . Solid angle is determined by the eye pupil with area A_{pupil} and the distance from pupil to instrument D_G .

$$U_G = A_G \Omega_G = A_G \frac{A_{pupil}}{D_G^2} . \quad (5.4)$$

The expected light power Φ that is returned from the eye and collected by the GDx is then

$$\Phi = LT_{eye} U \quad (\text{in Watt}) , \quad (5.5)$$

where U represents the smaller of U_{eye} and U_G (it is assumed that the GDx optics are properly designed to conserve throughput along the optical path). The GDx return optical path consists of 8 transmissive optical components, with each surface assumed to have high-efficiency anti-reflection coating with transmission T_{lens} ; 4 reflective optical components, with each surface assumed to have high-efficiency reflective coating with reflectance R_{mirr} ; and one beamsplitter, with each surface assumed to have transmission 0.5. The efficiency of this stack T_{opt} is thus

$$T_{opt} = 0.5 T_{lens}^{16} R_{mirr}^4 . \quad (5.6)$$

Thus the sum of the power reaching the two detectors P_{det} (and split in some way between them, depending on the polarization state) is

$$P_{det} = T_{opt} \Phi . \quad (5.7)$$

The parameters discussed in this section have been left as variables to emphasize their function. Table 5.1 shows appropriate values for each of the variables. Calculated radiometric quantities are shown in Table 5.2.

Table 5.1: Values of parameters for calculating GDx radiometry

Parameter	Description	Value
T_{eye}	Transmission of ocular media at 780 nm	0.80 [188]
P_{las}	Laser power exiting GDx	3 mW
A_{spot}	Spot area on retina (spot diameter 15 μm)	176 μm^2
ρ	Reflectance of ocular fundus	0.10 [189]
A_{pupil}	Area of ocular pupil (pupil diameter 7 mm)	38.5 mm ²
D_{eye}	Length of eye, retina to pupil	16 mm
A_G	GDx collection aperture (collection diameter 25 mm)	490 mm ²
D_G	Distance eye to GDx aperture	30 mm
T_{lens}	Transmission of each lens surface	0.99
R_{mirr}	Reflectivity of each mirror	0.99

Table 5.2: Calculated radiometric quantities

Radiometric Qty	Description	Calculated value
E	Irradiance incident on retina	$1.36 \times 10^{-5} \text{ W}/\mu\text{m}^2$
L	Radiance exiting from retina	$4.34 \times 10^{-7} \text{ W}/\mu\text{m}^2\text{-sr}$
U	Throughput of eye / GDx collection optics	$26.47 \mu\text{m}^2\text{-sr}$
Φ	Power collected by GDx	$9.19 \mu\text{W}$
T_{opt}	Throughput of GDx optics	0.41
P_{det}	Power incident on detectors	$3.76 \mu\text{W}$

P_{det} gives a reasonable estimate of the largest signal power to be expected, assuming ideal conditions (no vignetting, high fundus reflectivity, etc.). As the signal level is split between the two detectors, each detector will encounter a power level which lies between 0 and P_{det} . The range of incident powers measurable by the detector system is limited on the lower end by the minimum detectable power of the detector system P_{min} (calculated in Chapter 7), and on the upper end by saturation of the detector system with a ceiling determined by the power supply voltage V_p .

The detector circuit is designed with avalanche photodiodes having adjustable responsivity R_{apd} via a user-accessible knob, within a circuit having a fixed gain of $G_{circuit}$. The voltage output to the data acquisition circuit $V_{circuit}$ is

$$V_{circuit} = P_{det} R_{apd} G_{circuit} \quad (5.8)$$

Reasonable values for detector system parameters are listed in Table 5.3.

Table 5.3: Values of detector parameters.

Detector Qty	Description	Value
R_{apd}	Responsivity of APD detector	0-1 V/ μ W
$G_{circuit}$	Gain of electronic circuit	40
V_p	Power supply voltage	12 V
P_{min}	Minimum detectable power	0.2 nW

The detector system must be able generate a reasonable number of counts in the 11-bit analog-to-digital converter, in response to incident powers ranging from 0.2 nW to 3.76 μ W. Now for 0.2 nW incident power and R_{apd} adjusted to the maximum 1 V/ μ W, the voltage generated will be 8 mV giving 2 counts. For 3.76 μ W incident power, R_{apd} may be adjusted down to 0.08 V/ μ W to generate the maximum voltage of 12 V giving 2048 counts. Thus it is reasonably within the system capability to measure the expected range of incident flux.

5.6 Polarization path model and analysis

The GDx measures two retinal polarization parameters: linear retardance δ and retardance orientation θ . The Mueller matrix of a linear retarder $LR[\delta, \theta]$ is given by:

$$LR[\delta, \theta] = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos^2 2\theta + \cos \delta \sin^2 2\theta & (1 - \cos \delta) \cos 2\theta \sin 2\theta & -\sin \delta \sin 2\theta \\ 0 & (1 - \cos \delta) \cos 2\theta \sin 2\theta & \sin^2 2\theta + \cos \delta \cos^2 2\theta & \sin \delta \cos 2\theta \\ 0 & \sin \delta \sin 2\theta & -\sin \delta \cos 2\theta & \cos \delta \end{pmatrix}. \quad (5.9)$$

All other polarization parameters (i.e. circular retardance, diattenuation, and depolarization) are not measured by the GDx. The GDx polarization path is shown diagrammatically in Figure 5.5. The outgoing path forms the “generator” or PSG portion of the polarimeter. The polarization components in this path are a horizontally linearly polarized source; a half wave plate with rotational increment of $\Delta\phi$; and a fixed 27.7° retarder at $\alpha = +15^\circ$ or -15° orientation. The Mueller matrix of the i th state of the PSG (G_i) can be modeled as

$$G_i = LR[27.7^\circ, \alpha] LR[180^\circ, i\Delta\phi] LP[0^\circ], \quad (5.10)$$

where $LP[\theta]$ is a linear polarizer with orientation θ . The action of the first two elements of the PSG is to deliver linearly polarized light and to rotate its orientation via the rotating half wave plate. The action of the last element is to compensate the corneal retardance.

The incoming polarization path forms the “analyzer” or PSA portion of the polarimeter. The primary polarization components in this path are the fixed 27.7° retarder, rotating half wave plate, and polarizing beamsplitter. There are two analyzer paths, one for each

beam exiting the beamsplitter. These two paths are termed the “co-polarized” path $A_{co,i}$ (transmitted through the PBS) and “cross-polarized” beam path $A_{cross,i}$ (reflected by the PBS). The Mueller matrices of the two analyzer paths are modeled as

$$\begin{aligned} A_{co,i} &= LP[0^\circ]LR[180^\circ, -i\Delta\varphi]LR[27.7^\circ, -\alpha], \\ A_{cross,i} &= LP[90^\circ]LR[180^\circ, -i\Delta\varphi]LR[27.7^\circ, -\alpha]. \end{aligned} \quad (5.11)$$

The action of the co- and cross- polarized analyzers is to determine the retardance of the RNFL, as discussed in the following paragraphs.

Figure 5.5: GDx polarization path. The outgoing path consists of the source, horizontal polarizer, and rotating waveplate. The incoming path consists of the rotating waveplate, horizontal polarizer, and co detector (co-polarized path); or rotating waveplate, vertical polarizer, and cross detector (cross-polarized path).

The GDx retardance calculation is based primarily on the $N = 20$ images acquired by the cross-polarized detector (the co-polarized signal gives a reasonable measure of the total reflected intensity of the return beam and is used only for normalization). Each image is acquired at a different half wave plate position, which moves in 20 angular steps of $\Delta\phi = 2.432^\circ$, for a total span of 48.64° . The polarization function is most clearly illustrated by neglecting the corneal compensator retarder and the retardance of the cornea, in which case the generator and cross-polarized analyzer states are given by

$$\begin{aligned} G_i &= LR[180^\circ, i\Delta\phi] LP[0^\circ] , \\ A_{cross,i} &= LP[90^\circ] LR[180^\circ, -i\Delta\phi] . \end{aligned} \quad (5.12)$$

Two methods are presented to analyze the GDx polarization system. The first method, in which the instrument matrix W is assembled based on the known values of the system variables, illustrates the system effectiveness in measuring a linear retarder (i.e. the retina). The second method, in which the measured intensity is expressed as a function of the system variables and retarder variables, illustrates the measurement methodology employed by the system's designers.

As discussed in Chapter 3, the instrument matrix for the co-polarized analyzer W_{co} (cross-polarized analyzer W_{cross}) and can be assembled as a series of vectors $w_{co,i}$ ($w_{cross,i}$), each the outer product of G_i and $A_{co,i}$ ($A_{cross,i}$) at the known waveplate orientation of $i\Delta\phi$. The generalized vectors are given by

$$w_{co,i} = \begin{pmatrix} 1 \\ \cos 4i\Delta\varphi \\ \sin 4i\Delta\varphi \\ 0 \\ \cos 4i\Delta\varphi \\ \cos^2 4i\Delta\varphi \\ \frac{1}{2}\sin 8i\Delta\varphi \\ 0 \\ -\sin 4i\Delta\varphi \\ -\frac{1}{2}\sin 8i\Delta\varphi \\ -\sin^2 4i\Delta\varphi \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \quad w_{cross,i} = \begin{pmatrix} 1 \\ \cos 4i\Delta\varphi \\ \sin 4i\Delta\varphi \\ 0 \\ -\cos 4i\Delta\varphi \\ -\cos^2 4i\Delta\varphi \\ -\frac{1}{2}\sin 8i\Delta\varphi \\ 0 \\ \sin 4i\Delta\varphi \\ \frac{1}{2}\sin 8i\Delta\varphi \\ \sin^2 4i\Delta\varphi \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \quad (5.13)$$

showing that only eight Mueller matrix components are encoded, with encodings of cosine and sine of 4φ and 8φ . Some Mueller Matrix components share encodings with others, so that component scrambling will occur at reconstruction. Other Mueller matrix elements have no effect on the signal.

The instrument matrices for the full polarimeter, cross polarized section, and co polarized section (W , W_{cross} and W_{co}) can be assembled and evaluated. A weighted condition number, calculated as the ratio of the L2 condition number to the matrix rank, gives a metric for the polarimeter performance in the presence of noise when the instrument

matrix is not full rank (a smaller weighted condition number indicates better performance). W_{cross} , W_{co} , and W have the following properties:

W_{co} and W_{cross} :

Rank: 5 (incomplete polarimeter)

L2 Condition number: 17.6 (5 singular values ranging from 6.7 to 0.4)

Weighted condition number: 3.5

W :

Rank: 8 (incomplete polarimeter)

L2 Condition number: 18.7 (8 singular values ranging from 7.4 to 0.4)

Weighted condition number: 2.3

The singular values for W_{co} , W_{cross} , and W are plotted in Figure 5.6. The large negative slope clearly indicates that the basis vectors comprising this polarimeter are unequally weighted and thus do not provide a robust reconstruction. Given that retardance measurement requires two degrees of freedom, the rank of 5 shows that it may be possible to measure retardance, depending on whether the degrees of freedom to be measured correspond to those within the polarimeter's measurement space.

Figure 5.6: Singular values for GDx polarimeter. Many singular values are zero, indicating that the polarimeter is not full rank. The high slope for nonzero singular values indicates unequal weighting of the polarimeter basis vectors leading to high sensitivity to noise.

When W is used to measure a generalized sample Mueller matrix M , a vector of measured intensities P_m is recorded:

$$WM = W \begin{pmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{pmatrix} = P_m . \quad (5.14)$$

When P_m is then used with the inverse of W to reconstruct the sample matrix M_R , it is not possible to retrieve information on circular polarization properties (last row and column are all zeros), and the m_{12} and m_{21} elements are scrambled:

$$M_R = W^{-1}P_m = \begin{pmatrix} m_{00} & m_{01} & m_{02} & 0 \\ m_{10} & m_{11} & \frac{1}{2}(m_{12} - m_{21}) & 0 \\ m_{20} & \frac{1}{2}(m_{21} - m_{12}) & m_{22} & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}. \quad (5.15)$$

For example, when this system is used to measure the Mueller matrix of a pure linear retarder with retardance δ and orientation θ (such as the RNFL):

$$W LR[\delta, \theta] = P_{m,LR}, \quad W^{-1}P_{m,LR} = M_{R,LR}, \quad (5.16)$$

all elements are zero except for

$$\begin{aligned} m_{00} &= 1, \\ m_{11} &= \cos^2 2\theta + \frac{1}{2} \cos \delta (1 - \cos 4\theta), \\ m_{22} &= \frac{1}{2} (1 + \cos \delta + \cos \delta \cos 4\theta - \cos 4\theta). \end{aligned} \quad (5.17)$$

The desired parameters δ and θ can be found from these equations as

$$\delta = \cos^{-1} [m_{11} + m_{22} - 1], \quad \theta = \frac{1}{4} \frac{m_{11} - m_{22}}{1 - \cos \delta}. \quad (5.18)$$

However, if there is now a small depolarization component, which can be simulated by premultiplying $LR[\delta, \theta]$ by the Mueller matrix of a pure depolarizer $DEP[d]$ which imparts a DoP of $d \approx 1$ to all states:

$$DEP[d] = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & d & 0 & 0 \\ 0 & 0 & d & 0 \\ 0 & 0 & 0 & d \end{pmatrix}, \quad (5.19)$$

then the reconstructed Mueller matrix is given by

$$W DEP[d] LR[\delta, \theta] = P_{m, dep+LR}, \quad W^{-1} P_{m, dep+LR} = M_{R, dep+LR}, \quad (5.20)$$

and the measured components become

$$\begin{aligned} m_{00} &= 1, \\ m_{11} &= d \left[\cos^2 2\theta + \frac{1}{2} \cos \delta (1 - \cos 4\theta) \right], \\ m_{22} &= d \left[\frac{1}{2} (1 + \cos \delta + \cos \delta \cos 4\theta - \cos 4\theta) \right]. \end{aligned} \quad (5.21)$$

Now there is an error in the calculation of δ and θ dependent on the departure of d from 1. This error is substantial even for small amounts of depolarization. As calculation is based on the m_{11} and m_{22} components which have a cosine dependence on δ , the error is

enhanced for small values of δ , which is particularly undesirable when measuring retinal retardance (Figure 5.7). From this W matrix based analysis the following conclusions are drawn: the polarimeter is incomplete, but linear retardance can be measured reasonably accurately so long as retinal diattenuation, retinal depolarization, and any system noise sources are negligible. However, if these parameters are not negligible, error may be substantial.

Figure 5.7: Simulated error in measurement of linear retardance as a function of degree of polarization, for three different values of linear retardance within the typical range for retinal measurement.

The above analysis demonstrates that the GDx can measure linear retardance using the instrument matrix approach, but this is not the approach used by the instrument designers.

To understand the utilized algorithm, first consider the full system Mueller matrix M_{sys} consisting of the PSG, retina, and cross-polarized PSA:

$$M_{sys} = \left(LP[90^\circ] LR[180^\circ, -\varphi] \right) \left(LR[\delta, -\theta] LR[180^\circ, 0] LR[\delta, \theta] \right) \left(LR[180^\circ, \varphi] LP[0^\circ] \right), \quad (5.22)$$

A_{cross} retinal model with linear
retarder in front of mirror G

where the retina is modeled as a linear retarder with fixed retardance δ and fixed retardance orientation θ followed by a perfect reflector modeled as a horizontal linear retarder with 180° phase shift; light passes through the retinal retarder, reflects from the reflector, and passes back through the retinal retarder (Figure 5.8).

Figure 5.8: Simple model for GDx measurement of retinal retardance. Src: laser source; HLP: horizontal linear polarizer; HWP: half wave plate; NFL: nerve fiber layer modeled as a linear retarder; M: fundus modeled as an ideal mirror; VLP: vertical linear polarizer; Det: detector.

The intensity I_{sys} measured through this system is

$$I_{sys} = 2 \sin^2 \delta \sin^2[2(\theta - 2\varphi)] . \quad (5.23)$$

This result shows that the intensity measured by the GDx as a function of rotating waveplate position ϕ follows a $\sin^2$ curve, with the maximum intensity dependent on RNFL retardance δ , occurring when twice the waveplate orientation angle is 45° different from the RNFL orientation θ . This explains why the rotating waveplate rotates through approximately 45° rather than a more intuitive 90° (Figure 5.9a).

For each pixel in the image set, one of the 20 images has highest intensity, because the waveplate orientation is closest to being 45° from the nerve fiber layer at that rotating waveplate orientation. A map is assembled of the brightest intensity achieved for each pixel among the 20 images, and each pixel is then normalized to the co-polarized analyzer signal for that pixel, which gives a measure of the total reflected intensity of the return beam for that pixel. This brightness map represents the retardance of the RNFL, and is multiplied by a constant birefringence factor to obtain RNFL thickness. While this method works in theory, in practice it is highly sensitive to noise since only a single value of the 20 available is used to determine the maximum of the $\sin^2$ curve (Figure 5.9b). If the designers had chosen to perform a curve fit to the 20 measured intensity points at each pixel the noise level would have been $\sqrt{20}$ lower, though at the cost of longer processing time.

Figure 5.9: Theoretical intensity measurement of a linear retarder using the GDx polarimeter. (a): Ideal intensity measurement follows a $\sin^2$ curve. (b): Simulated intensity measurements with random noise added demonstrates that measurement error due to noise varies depending on the distribution of noise during the measurement.

To determine the effectiveness of the GDx and its polarization algorithm, one must consider possible non idealities. For example, there may be residual birefringence of unknown axis in the GDx optics, the corneal compensator may not completely cancel the corneal birefringence, or there may be partial depolarization by the system optics, anterior segment, or retina. Under these conditions there is significant error in the calculation of δ . Error may take the form of a bias on the measured retinal retardance due to the addition of linear retardance from other parts of the system, or it may take the form of leakage into the retardance measurement by other polarization elements, such as depolarization, which are not correctly measured by this incomplete polarimeter.

The GDx algorithm utilizes idealized polarization component models to determine the measurement algorithm. To address the most significant non idealities, the designers

have instituted a few methods such as use of a corneal compensator to approximately cancel corneal birefringence and averaging several measurements to approximately cancel system birefringence. As will be seen, this methodology is different from the measurement methodology developed for the GDx-MM, in which the polarization state of the generator and analyzer are measured directly and highly accurately, and used in the form of the W instrument matrix to calculate the polarization properties of the retina.

5.7: Electronics function

A microprocessor-driven electronics system consisting of several printed circuit boards controls the GDx electronics function. The GDx electronics are something of a black box; however, thorough knowledge was not necessary as much of the function was replaced by a new electronics system in the GDx-MM. The electronics functions which are relevant to this dissertation are as follows:

1. The x resonant scanner oscillates at 3.93 kHz.
2. The y galvonometric scanner is driven at 26 Hz, synchronous with the x scanner. The image acquisition time is 38 ms.
3. The laser is blanked for 6 ms at the beginning of each scan to provide a dark signal, which is used as the reference for the detector circuit.
4. The half wave plate stepper motor is driven in 2.432° steps between images
5. The two detectors are sampled into memory at 8 MHz and 8 bits, synchronous with the scanners.

The scanner frequency ratio gives 300 scan lines in an image, but as the laser is blanked for part of the scan there are only about 256 useful scan lines.

In addition, the electronics must monitor the buttons and knobs available to the user on the GDx instrument. Of these, the ones relevant to this dissertation are:

1. Start button, pressed to initiate acquisition of an image set
2. Gain knob, adjusted to increase or decrease gain of detectors
3. Alignment button, pressed to align the front and back scans

In addition to the electronic functions listed above, the GDx converts raw scanner data to various diagnostic images which are displayed to the operator within a few seconds. This function is completely replaced in the GDx-MM.

5.8: Reverse Engineering

A major challenge in the development of the GDx-MM was that it is based on an existing commercial instrument for which only general information is available. The basic mode of operation is known from various patents [149-152], company marketing materials [154], and numerous journal articles. However, the GDx optical and polarization paths, and the GDx electronic functionality, needed to be understood in greater detail. The information that was needed was obtained with a substantial reverse engineering effort, but as this effort is not central to this dissertation work, it will only be described briefly.

The optical path consists of a large number of individual elements. Unfortunately, it was not feasible to disassemble the optical train to characterize every element, as the instrument alignment and calibration would have been lost. Therefore a best effort was made to reconstruct the optical path by examining each element in terms of shape and appearance, accurately measuring the distances between elements, and tracing the beam size through the system using either an infrared sensor card or a knife edge as appropriate to estimate beam position and size. Image planes were located where the beam comes to a focus. The external pupil was located by noting where the scanner pattern collapses to a point, about an inch beyond the outer window. Scan angles were estimated by measuring the aspect ratio of the scan pattern and its growth with distance from the pupil. Given these observations, an approximate model was assembled in Code V (Optical Research Associates). This model will be presented in Chapter 6 together with the changes made for GDx-MM function. Some aspects of the optical system could not be determined, such as component coatings and the types of elements between the pinhole and detector, as these were enclosed in a housing which could not be opened. The polarization function was well established from the literature search. The orientation of the linear polarization emerging from the laser source was established using an infrared linear polarizer.

The electronic control function was significantly more difficult to reverse engineer. The general method was to infer the presence of a certain type of signal – for example a high frequency signal from the resonant scanner or a digital pulse when the Start button is

pressed – and probe the circuit boards with an oscilloscope until this signal was located. Particular signals of interest were scanner control and feedback; motor control for the rotating waveplate; and user interface control including buttons and knobs. Datasheets for all elements (such as motors and integrated circuits) were obtained and studied to further understand the function. The detector circuitry was “ohmed out” with an ohmmeter to give an exact understanding of its function. This electronic reverse engineering required several months of concentrated effort (and exceptional tolerance for ear-piercing 4 kHz scanner noise). Care was taken throughout to prevent electrostatic damage to the circuitry.

5.9: Conclusions

In this chapter the basic operational function of the GDx has been described, in terms of optical path, radiometry, polarization, and electronics. Limitations in the polarization measurement capability are clearly outlined, providing a baseline for the improved polarization function of the GDx-MM. Despite the lack of exact information about the system, a reverse engineering effort was able to determine enough information to be able to utilize the GDx as a platform for developing the GDx-MM.

CHAPTER 6: GDx-MM DESIGN AND DEVELOPMENT

6.1: Introduction

The goal of the modifications to the Laser Diagnostics GDx is to create a complete and well conditioned polarimeter, in order to measure all retinal polarization properties accurately in the presence of error sources. As demonstrated in the previous chapter, the original GDx is an incomplete polarimeter which has potentially high inaccuracy in the presence of error. The challenges to be overcome in creating the GDx-MM included polarimeter design within the limitations imposed by the GDx framework, and a substantial electrical system design. In this chapter GDx-MM design issues are detailed, including a discussion of the requirements and the processes used to establish the final polarimetric, optical, and electronic designs.

6.2: Polarization analysis

When this project was begun, the initial thought was that a few relatively simple changes could be made to the GDx over a period of a few months to produce a complete polarimeter. It soon became clear that the effort required was much more substantial, and in the end the development time was about three years. The challenge in retrofitting the GDx involved determining a system of polarization components to give a rank 16 well conditioned polarimeter, which could be mounted within the available space, and which would have minimal impact on the imaging properties of the device. In this section the methods used to design the new polarimeter are outlined step by step.

To measure circular and elliptical polarization properties it is necessary to generate and analyze elliptical states, requiring the insertion of retarders in the outgoing and incoming paths. Of the various possibilities, two variable retarders were inserted, one in the PSG and the other in the PSA. Variable retarders generally have fixed retardance with variable axis (i.e. motor driven waveplate), or variable retardance with fixed axis (i.e. liquid crystal retarder or photoelastic modulator). Liquid crystal retarders (LCRs) were the only feasible choice for this system as they are small enough and simple to control. The response time is marginally fast enough, and since they do not require a motor drive system they can fit in the existing frame. Following a product search it was determined that there was just enough space within the GDx outgoing and incoming optical paths to insert 1" diameter $\frac{1}{4}$ " thick LCRs, available from a few different vendors (the source used here was Bolder Vision Optik, Boulder, CO). No other viable technology was identified, given the tight space in the GDx frame. The many difficulties encountered in working with LCRs significantly prolonged the completion of this work, as will be discussed in Chapter 7.

With these LCRs inserted, there are three variable polarization elements in the system: the two variable retarders, and the rotating half wave plate. The new system is shown in Figure 6.1. The time to change the retardance of the variable retarders (on the order of 30-80 ms) is significantly longer than the time to move the waveplate (on the order of 5 ms). Thus it is most time efficient to generate a range of polarimeter states by setting a

LCR retardance, rotating to several waveplate orientations, changing the LCR retardance to a new value, rotating the waveplate to several orientations, etc.

Figure 6.1: GDx-MM polarization component layout, showing outgoing and incoming paths. RLR: rotating linear retarder; LR: fixed linear retarder; HLP: horizontal linear polarizer; VLP: vertical linear polarizer.

Given this philosophy, the Mueller matrices for the PSG and PSA become (neglecting the corneal compensator):

$$\begin{aligned}
 G_{ij} &= LR[180^\circ, i\Delta\varphi] LR[\beta_j, \sigma_G] LP[0^\circ], \\
 A_{cross,ij} &= LP[90^\circ] LR[\alpha_j, \sigma_A] LR[180^\circ, i\Delta\varphi], \\
 A_{co,ij} &= LP[0^\circ] LR[\alpha_j, \sigma_A] LR[180^\circ, i\Delta\varphi].
 \end{aligned} \tag{6.1}$$

where β_j and α_j are the variable retardances of the generator and analyzer LCRs, σ_G and σ_A are the fixed orientation angles of the LCRs, and $\Delta\phi = 2.432^\circ$ is the rotating half wave plate rotational increment. This increment is fixed due to the dimensions of the belt drive system which couples the stepper motor to the rotating waveplate in the GDx.

The original GDx system utilizes 20 states, requiring 0.7 seconds. The improved system could perform more accurate polarimetry by using more states, but the measurement duration is limited by saccadic eye movement and blinking. A maximum measurement time of 4 seconds was selected. Based on the required time to rotate the half wave plate and the time to electrically change the retardance of the LCRs, a measurement plan was initially proposed using 20 half wave plate positions, at each of four different LCR pair retardance (β_j and α_j) combinations, for a total of 80 states, requiring slightly under 4 seconds.

An optimization process was performed to determine β_j and α_j combinations and σ_G and σ_A fixed orientation angles to produce a well conditioned polarimeter. Since the solution space for each LCR is four dimensional, and thus quite large and difficult to visualize, insight into where the best solutions might lie was obtained by considering trajectories on the Poincaré sphere. Following the procedure outlined in Chapter 4, the accessible portion of the Poincaré sphere was determined for various combinations of the generator or analyzer variables. For $\sigma_G = 0^\circ$ it is only possible to access the equator of the sphere (Figure 6.2a). The accessible portion expands in a partial hemispherical band extending

from the equator towards the poles as σ_G increases, with the band encompassing an entire sphere for $\sigma_G = 45^\circ$ (Figures 6.2b and 6.2c). Thus with a variable retarder mounted at 45° it is possible to access any point on the surface of the Poincaré sphere.

Figure 6.2: Accessible portion of Poincaré sphere for different orientations of the variable retarders. (a): variable retarders at 0° orientation. (b): variable retarders at 15° orientation. (c): variable retarders at 45° orientation.

However, when the limitation is imposed that there are only four variable retardance settings which are fixed during rotation of the half wave plate, the accessible region collapses to four separate arcs with trajectories which follow latitude lines. For a 45° rotation of the half wave plate the latitude lines extend for a distance of halfway around the sphere (Figure 6.3a). When the waveplate rotation is doubled from 20 steps of 2.432° (48.6°) to 20 steps of 4.864° (97.2°), the full latitude line is accessible. The particular latitude section followed depends on the choice of variable retardance (Figure 6.3b).

Coverage can be further improved by reducing the retardance of the half wave plate. For an appropriate retardance choice, figure eight patterns are generated in which a regular tetrahedron can be inscribed (Figure 6.3c), forming an optimizable rank 16 polarimeter. From this Poincaré sphere analysis, it is clear that the GDx-MM polarimeter design with inserted LCRs operating in the sequence of 4 retardance combinations and 20 waveplate orientations will form a rank 16 polarimeter, so long as the waveplate full rotation is 97.2° and the half wave plate is replaced with a waveplate of lower retardance.

Figure 6.3: Trajectories on Poincaré sphere for LCR retardances ($3/8$, $4/8$, $5/8$, and $7/8$ wave shown) and total rotations of the rotating waveplate. (a): half wave plate rotating over 45° (Laser Diagnostics GDx uses two of these trajectories on the equator); (b): half wave plate rotating over 90° ; (c): 145° waveplate rotating over 90° .

Analysis using the Poincaré sphere has given insight into the best general structure for the polarimeter, but does not provide exact values for the waveplate and variable retarder retardances. To obtain optimal values, an instrument matrix W is assembled with the retardances as variables, and a condition number analysis is performed. To assemble W , the starting point is the Mueller matrices of the PSG and PSA:

$$\begin{aligned}
G_{ij} &= LR[\gamma, i4.864^\circ] LR[\beta_j, 45^\circ] LP[0^\circ], \\
A_{cross,ij} &= LP[90^\circ] LR[\alpha_j, 45^\circ] LR[\gamma, -i4.864^\circ], \\
A_{co,ij} &= LP[0^\circ] LR[\alpha_j, 45^\circ] LR[\gamma, -i4.864^\circ].
\end{aligned} \tag{6.2}$$

where γ is the rotating waveplate retardance, i ranges from 1 to 20 and j ranges from 1 to 4. These sets of equations still have nine variables: γ , β_1 - β_4 , and α_1 - α_4 . This set defines a large space which must be limited. This was done by restricting the values of β_1 - β_4 and α_1 - α_4 to members of the set: {3/8 wave, 5/8 wave, 7/8 wave}. These values were chosen as providing reasonable coverage on the Poincaré sphere, and by considering the range of accurately obtainable LCR retardances. As will be discussed in Chapter 9, restricting the LCR set to just a few members also makes the calibration process easier and less time consuming.

Given that there are two detectors, the question also arises as to whether data from both detectors can be combined into a single instrument matrix. The problem with combining data from both detectors is that the offset and/or gain are almost certainly different, leading to bias in the collected data which will couple as error into the polarization calculations. As the offset/gain differences may be large due to the lack of temperature compensation (discussed in Chapter 7), the decision was made to utilize only one of the two detectors. Since the eye has relatively small effect on the polarization properties of the incident beam, the co-detector will usually have the larger average signal strength; therefore the data from this detector was chosen for processing, even though data from

both detectors was collected. The cross detector signal certainly contains unique information however, which might be incorporated into a more sophisticated reconstruction algorithm in future work.

Given that only one detector is used, the instrument matrix with variables γ , β_1 - β_4 , and α_1 - α_4 was assembled from G and A_{co} . Then, several trial instrument matrices were assembled using a best initial guess value for γ of 135° and all possible combinations of β_j and α_j from the set {3/8 wave, 5/8 wave, 7/8 wave}. The rank and condition number were calculated for each combination. The results show that a large number of β_j and α_j combinations produce the same rank 16 instrument matrix, with minimum condition number of 20.6. Representative of the best-case combination found is:

$$\begin{aligned}\beta_j &= \{3/8 \text{ wave}, 3/8 \text{ wave}, 7/8 \text{ wave}, 5/8 \text{ wave}\} , \\ \alpha_j &= \{3/8 \text{ wave}, 5/8 \text{ wave}, 5/8 \text{ wave}, 3/8 \text{ wave}\} .\end{aligned}\tag{6.3}$$

Now, several trial instrument matrices were assembled using this best-case combination, but with γ ranging from 0° to 180° . The rank and condition number were calculated for each value of γ . Results show that $\gamma = 147^\circ$ is optimal, and that the minimum condition number of 14.5 grows rapidly away from this value (Figure 6.4).

Figure 6.4: Log condition number as a function of rotating waveplate retardance, assuming the LCR retardances are selected according to Equation 6.3. A retardance of 147° is optimal.

To compare possible configurations, the rank and condition number of the instrument matrix was calculated with different combinations of retardance and orientation of the rotating waveplate (Table 6.1). Reducing the waveplate retardance allows the system rank to increase from 14 to 16, and increasing rotation from 48.6° to 97.2° gives a dramatic improvement in condition number, from 349 to 14.

Table 6.1: Polarimeter rank and condition number vs. retardance and orientation of rotating waveplate assuming the LCR retardances of Equation 6.3.

Waveplate retardance	Waveplate rotation	Rank	Condition number
180°	48.6°	14	72
180°	97.2°	14	7
147°	48.6°	16	349
147°	97.2°	16	14.5

The last row of Table 6.1 shows the preferred polarimeter configuration with rank 16 and condition number of 14.5, described as:

$$\begin{aligned}
 G_{ij} &= LR[147^\circ, i4.864^\circ] LR[\beta_j, 45^\circ] LP[0^\circ], \\
 A_{co,ij} &= LP[0^\circ] LR[\alpha_j, 45^\circ] LR[147^\circ, -i4.864^\circ].
 \end{aligned} \tag{6.4}$$

where α_j and β_j are selected from a subset of combinations of the set {3/8 wave, 5/8 wave, 7/8 wave}. The final selection of α_j and β_j was done at calibration, as described in Chapter 9. In summary, the physical modifications to the GDx polarization system are:

1. Insert LCRs in generator and analyzer path at 45° orientation
2. Replace rotating half wave plate with 147° plate
3. Double step the rotating waveplate to double the total rotational range
4. Remove the corneal compensator waveplate (replaced with clear window)

To support these modifications, mechanical design was required. To install the LCRs in the system, mechanical mounts were designed which bolt to the GDx frame. Given the very tight space and the need to remove the LCRs if necessary, the mount design was difficult. The mount for the generator LCR holds the element such that the laser angle of incidence is 3° , to prevent direct back-reflections into the laser cavity. Replacing the rotating and corneal compensator waveplates required removing the old plates from their mounts and glueing in the new plate / window. Mechanical design support was provided by Charles Burkhardt at the University of Arizona machine shop.

6.3: Optical path

Replacing the rotating waveplate with one of different retardance but same optical thickness and appropriate coatings should have no effect on imaging properties. However, the insertion of the LCRs into the outgoing and incoming paths must be carefully considered.

Due to space constraints the generator and analyzer LCRs are both located in non-collimated space. As the bulk of the LCR is glass with index of refraction n of about 1.5 and thickness of $t = 5.2$ mm, it shifts the beam focus outward by a distance s :

$$s = \frac{n-1}{n} t = 1.7 \text{ mm} . \quad (6.5)$$

This shift results in the beam focal point falling well behind the retina (when the eye pupil is properly placed at the instrument external pupil). Since the LCRs are not normal to the beam path, they introduce a lateral translation of the beam D of

$$D \approx \frac{n-1}{n} t \theta , \quad (6.6)$$

where θ is the tilt angle of the plate. The lateral translation is calculated as 30 μm per degree of tilt. While a small translation of the beam is not of importance at the retina, it is critical in the return path, as only a very slight translation will cause the return beam to be misaligned with the 25 μm pinhole, resulting in little or no signal at the detectors.

The focal shift and lateral translation must be compensated to restore the outgoing and incoming focal points to their proper positions. To correct the focal shift, a weak negative lens was inserted between the telescope lenses and the scanner mirrors, and installed into the telescope assembly so that it translates with the telescope lenses as the system focus is adjusted. The weak lens slightly refocuses the beam, drawing its focus back in towards the original location. To optimize its focal length and determine the effect on imaging, a Code V model was assembled of the outgoing and incoming optical paths. As the optical component parameters are not known precisely, this model is approximate. The weak lens focal length optimized using Code V was found to be $f = -1980$ mm. This focal length was realized as well as possible using commercially available lenses, one plano convex (Lambda Research Optics PCX-12.7B-250,

$f_1 = 252.0$ mm) and one plano concave (Lambda Research Optics PCC-12.7B-150, $f_2 = -293.5$ mm) with the plano surfaces in contact, yielding a combined focal length at 780 nm of $f = -1782$ mm. The model suggests that the focal spot at the retina (nominally 15 μm) grows by about 35% and shifts toward the cornea by about 0.5 mm. The GDx telescope focus control is able to accommodate for the slight inward shift; the most important effect of the addition of the LCRs and weak lens is that the optical resolution decreases from 15 μm to 20 μm .

To address beam translation at the pinhole, a standard 1.5 mm thick glass slide mounted on a small tip/tilt stage was installed between the analyzer LCR and the pinhole. The rationale is that the slide can be tipped and tilted to introduce a counter-translation which realigns the beam to the pinhole. The orientation of the slide is manually adjusted during instrument calibration to give the maximum signal at the detectors. The slide is not an elegant solution, particularly because of slight focal shift and slightly increased spherical aberration, coma, and astigmatism, which increase the beam size at the pinhole plane. Code V analysis shows that the focal spot at the pinhole plane (nominally 15 μm) shifts inward about 0.3 mm, resulting in a doubling of the spot size at the pinhole. This implies that the signal strength for the GDx-MM is about one quarter the signal strength for the original GDx. In practice the signal strength was observed to decline to about one third. A better solution would be to move the pinhole so that it is centered on the beam and located at best focus, and then to align the polarizing beamsplitter and two detectors to the new beam center. Unfortunately, pinhole translation requires disassembly of the

sealed detector assembly with many associated risks, and would require fabrication of a custom alignment fixture. It was decided that the difficulty, cost, and extra time involved to move the pinhole were not justified, particularly as the glass slide solution provides reasonable results. The final GDx-MM optical and polarization component layout is shown in Figure 6.5.

Figure 6.5: Optical and polarization component imaging path in the GDx-MM for the scanners in the central position. Components notated in red indicate modifications to the Laser Diagnostics GDx. These changes include: replacing corneal compensator with window; replacing half wave plate with 145° plate; rotating plate through 90° rather than 45° ; inserting weak lens; inserting glass slide; and inserting LCRs in generator path (LCR-G) and analyzer path (LCR-A).

Code V simulations of the outgoing and incoming beam paths of the GDx-MM are shown in Figures 6.6, 6.7, and 6.8. The eye model is the “Arizona Eye” developed by Jim Schwiegerling [190].

Figure 6.6: Code V model of the GDx-MM outgoing imaging path, for scanners in the central position.

Figure 6.7: Code V model of the GDx-MM outgoing imaging path, for scanners giving maximum field of view on the retina.

Figure 6.8: Code V model for the GDx-MM incoming path, for scanners in the central position.

Section 6.4: Radiometry

There is a difference in radiometry between the original GDx and the GDx-MM which is due to transmissive loss of the additional elements in the optical path, and due to slight beam defocus, aberration, and translation at the pinhole plane. The radiometric calculations for the GDx-MM presented here make reference to the GDx calculations in Section 5.4. In the GDx-MM outgoing path, there is transmissive loss due to the addition of one LCR and the weak lens. The LCR has Fresnel loss due to reflection from the surface, as well as retardance-dependent loss through the liquid crystal medium. The new outgoing laser power is then

$$P'_{las} = T_{lens}^4 T_{LCR} P_{las} , \quad (6.7)$$

where P_{las} is the original output laser power, T_{LCR} is the LCR efficiency, and T_{lens} is the transmission of each optic face. New calculations can be made for the irradiance E' (Equation 5.1), radiance L' (Equation 5.2), and power Φ' (Equation 5.5), using the new value of P'_{las} .

In the incoming path, there is transmissive loss due to the addition of three transmissive optics: the weak lens, one LCR, and the glass slide; as well as loss at the pinhole plane. Referring to Equation 5.6, the new incoming path throughput becomes

$$T'_{opt} = T_{opt} T_{lens}^6 T_{LCR} T_{pinhole} , \quad (6.8)$$

where $T_{pinhole}$ indicates the change in the percentage of optical power passing through the pinhole. The new power at the detector is then

$$P'_{det} = T'_{opt} \Phi' . \quad (6.9)$$

Assuming the new components have the same high efficiency antireflection coating as the original components, the only new variables here are T_{LCR} and $T_{pinhole}$. T_{LCR} was measured directly using a Newport power meter to be in the range 82% - 93%, depending on retardance setting (3/8, 5/8, or 7/8 wave). $T_{pinhole}$ is not accurately known, but is

estimated from the Code V simulations to be 0.25. The values for those variables with modified radiometry are summarized in Table 6.2.

Table 6.2: Variables which modify the radiometry for GDx-MM

Radiometric Qty	Description	Calculated value
P'_{las}	Power exiting GDx	2.3 – 2.7 mW
E'	Irradiance incident on retina	$1.04 - 1.22 \times 10^{-5} \text{ W}/\mu\text{m}^2$
L'	Radiance exiting from retina	$3.32 - 3.90 \times 10^{-7} \text{ W}/\mu\text{m}^2\text{-sr}$
Φ'	Power collected by GDx	7.04 – 8.27 μW
T'_{opt}	Throughput of GDx optics	0.078 – 0.090
P'_{det}	Power incident on detectors	0.55 – 0.74 μW

Thus it is estimated that the power incident on the detectors is 15-20% of that seen in the original system (in actual use this figure was observed to be approximately 30%). The detector system is still capable of producing a full 11-bit range of counts with this smaller signal, provided the gain knob is adjusted higher.

6.5: System timing

Significant changes were made to the instrument electronics to support the polarization function. The new electronics were designed based on a timing sequence which was structured to support all needed instrument functions. Although the electronics

description makes for dry reading, it provides an understanding of instrument limitations, and contributes information to the error analysis.

”Timing” refers to the sequence in time of various control signals and the data acquisition clock, with respect to the scanner motion. Reliable timing is critical to ensure that the composition of the data stream is well understood and can be “unwrapped” into discrete images. The basic timing elements include:

1. Start of data acquisition
2. Rate of data acquisition
3. Moving the motor at the correct times
4. Changing the LCR drive voltage at the correct times

The frequency of the resonant fast scan can vary by up to several percent depending on ambient temperature, and so it is critical to base the GDx-MM timing in a closed loop manner on the scanners themselves. Therefore the data acquisition rate is tied to the fast scan frequency, and the start of data acquisition, motor motion, and LCR drive are tied to the slow scan frequency. The original GDx acquired detector data at a sampling rate of 8 MHz, giving 1024 measured points per line for 300 lines (4 μm sampling resolution over the approximately 4.5 mm wide field). It appears that the 1000 points were then averaged in groups of 4 to give 16 μm resolution in a 256 pixel wide scan line. The 44 scan lines which occur during laser blanking (i.e. when the laser is off during the first

6 ms of each scan to provide a dark reference for the detector) are discarded to produce a 256 x 256 pixel final image. Due to limitations in the capabilities of readily available data acquisition cards (both in terms of data rate and on-board memory), it was necessary to reduce the GDx-MM sampling rate to 4 MHz, giving 512 measured points per line (averaged in groups of 2) over 300 lines to give 16 μm resolution in a 256 x 256 pixel final image. While this reduction in sampling frequency is undesirable there is no simple workaround; resolution is unaffected though with fewer pixels averaged the signal-to-noise ratio is lower.

The timing structure is based on the polarization algorithm as presented in Section 6.2. There are four identical sequences, in which the LCR retardances are held fixed, and the waveplate spans approximately 90° via 18 double pulses of the stepper motor (spanning 18 slow scan cycles). Between each sequence, the retardance of the LCRs must be electrically changed, then the LCRs require 4 slow scan cycles (about 150 ms) to settle. This collection of $18 \times 4 = 72$ double pulses, plus 1 single pulse between each sequence, moves the waveplate exactly 360° , back to the start position. This sequence is slightly different from the 80-image sequence that was proposed in the polarization theory, but has negligible effect on the condition number of the instrument matrix. Data acquisition occurs continuously from the detectors direct into on-board memory throughout the entire timing structure. The start of the timing structure is synchronized to the first slow scan cycle occurring after the Start button is pressed. The full timing sequence is shown

diagrammatically in Figure 6.9. Unwrapping image data from the datastream is described in detail in Chapter 10.

Figure 6.9: Diagram of the GDx-MM timing structure. When the Start button is pressed the LCR retardance and motor drive signals (consisting of four sequences) are initiated. Each sequence contains multiple motor drive double pulses synchronized with the slow scanner.

6.6: Electronics

The GDx-MM electronics implement the timing structure in support of the image acquisition and polarization measurement. The most significant new requirements are:

1. Fast acquisition and storage of a larger number of images (100 vs. 20)
2. Custom movement of the rotating waveplate stepper motor
3. Electronic control of the LCRs

In addition, several other functions are required:

1. Synchronization of data acquisition with the scanners and the Start button
2. Homing of the motor to an accurate and reproducible starting position
3. Direct readout of the detectors for calibration purposes
4. Manual control of the laser for calibration purposes

Finally, several functions were implemented that are not mandatory but desirable:

1. Monitoring the front panel Gain knob, which sets the detector gain
2. Monitoring the laser output power
3. Monitoring the ambient temperature

The need for greatly expanded image storage as well as analog and digital control pointed toward the development of a custom data acquisition and control system housed in a separate computer. The need for a very high speed acquisition board with the ability to acquire directly to a large on-board memory (bypassing operating system interrupts) mandated the use of particular National Instruments boards for control and acquisition. Labview software was used to program the National Instruments boards.

The electronic effort can be separated into three sections: reverse engineering of the GDx instrument; selection and programming of the National Instruments boards; and custom circuit design for additional functionality. The final electronics system was fully

connectorized and packaged to be viable for use in a clinical setting. The final system consists of:

1. Original GDx instrument, consisting of scan head, computer, monitor (GDx)
2. Data acquisition computer (DAC)
3. Electronics box (EB)

The original GDx was received without documentation, such as schematics or wiring diagrams. A thorough visual examination of the instrument followed by many hours of probing with an oscilloscope were necessary to understand the subset of its electronic functionality that was necessary to the modifications. Once this function was understood, certain signals from the GDx were routed to the DAC. The following is a brief summary of the interface points that were established between the GDx instrument and the DAC.

Motor control

1. The rotating waveplate motor step drive is disconnected from GDx control and instead controlled by the DAC.
2. A signal indicating that the motor is in the home position is sent to the DAC.
3. A signal indicating that the Start button has been pressed is sent to the DAC.

Detector

1. The control signal which strobes the detector synchronously with the scanner is placed on a switch. Depending on switch position, the detector is controlled synchronously (as before) by the GDx or enabled on command from the DAC.
2. The detector output is put on a T connection, so that it is split between its original connection to the GDx computer, and a new connection to the DAC.

Laser

1. The power to the laser, which is enabled synchronously with the scanner, is placed on a switch. Depending on the switch position, the laser is blanked synchronously with the laser (as before) by the GDx, powered continuously, or turned off.
2. A small optical detector is placed behind the nonpolarizing beamsplitter to monitor the laser power.

Scan head controls

1. A signal indicating that the Start button has been pressed is sent to the DAC.
2. The setting of the front panel Gain knob is monitored.
3. A small temperature sensor is placed in the scan head.

The DAC functionality can be separated into several areas: analog input, analog output, digital pulse train generation, digital input/output, and timebase generation. To satisfy all

requirements, three National Instruments boards were selected: the 6115 multifunction data acquisition board to provide analog input/output and digital input/output; the 6602 counter board to provide digital pulse train generation; and the 5401 function generator board to provide timebase generation. The analog input is 12 bits with input voltage range of $\pm 5\text{V}$; since the detector output is always positive the effective depth is 11 bits or 2.4 mV per bit. This is an improvement over the original GDx system with 8 bits and 19.5 mV per bit. Four seconds of 4 MHz samples are transferred directly to on-board memory. The analog output generates a high resolution, variable magnitude 2 kHz square wave required for driving the LCRs. The counter board provides single pulses and pulse trains which are internally or externally triggered to implement assorted timing functions. The function generator board provides the timebase for data acquisition, and is adjusted in 1 kHz increments to match the timebase to the slightly variable scanner frequency. These boards are housed in a single Windows based desktop computer, and are programmed using Labview. The system architecture is shown in Figure 6.10. Figures 6.11 and 6.12 show the GDx-MM scan head and full system as packaged and connectorized. In these photos the cover has been removed to emphasize internal scan head wiring.

Figure 6.10: Electronics system architecture for the GDx-MM. Items in gray are original GDx components; items in red are newly added components.

Figure 6.11: The modified scan head with cover removed, viewed from both sides. New wiring has been connectorized to interface cleanly with the bottom bracket of the scan head assembly.

Figure 6.12: The GDx-MM system. A: scan head (here with cover off); B: electronics box; C: data acquisition computer; D: original GDx computer; E: monitor, keyboard, and mouse.

Several functions required additional custom circuitry, including: Start button, Motor homing, Motor double pulsing, and Detector manual control. Each of these functions is described briefly below.

Start Button External Circuit

This circuit is needed because the scanners are asynchronous with respect to when the Start button is pressed. However, it is necessary to start the data acquisition synchronously with the scanners so that the acquired data can be correlated with scanner position. The trigger signal generated by this circuit is synchronous with the leading

Motor Double Pulsing Circuit

The polarimetry algorithm requires double stepping the waveplate stepper motor. One simple way to do this is to send two pulses to the motor between images rather than one pulse. However, the National Instruments board which generates motor pulses does not have the ability to generate double pulses. For this reason a circuit was designed which allows for either single pulsing of the motor (for motor homing) or double pulsing (for polarization measurement sequence). The double pulser portion utilizes three monostable multivibrators. The schematic for this circuit is shown in Figure 6.14.

Figure 6.14: Motor double pulsing circuit

All custom circuitry and the breakout boxes for the National Instruments boards were housed in a single connectorized circuit box (Figure 6.16).

Figure 6.16: Custom circuit box, which houses breakout boxes for the National Instruments boards as well as custom circuit boards.

6.7: Conclusions

The modifications to the GDx to implement the GDx-MM polarimeter were extensive and included changes to the polarization, optical, mechanical, and electronic systems. In this chapter the methodology for developing the new polarization algorithm was described, and was shown to be based on choosing appropriate components for the available space; Poincaré sphere analysis for insight into the choice of some parameters; and condition number analysis for selecting final component values. Optical modifications were required to support the polarization function, with the challenge of

obtaining imaging performance near to that of the original instrument while using commercially available optics. Electronic modifications were extensive, requiring the design, assembly, and programming of a new data acquisition system containing National Instruments boards as well as custom circuitry.

CHAPTER 7: COMPONENT ANALYSIS

7.1: Introduction

The performance of the GDx-MM is dependent on the properties of its optical and polarization components. This chapter describes the characterization and analysis of relevant components, to understand their effect on polarimetry measurement and accuracy. Characterized components common to the original GDx and GDx-MM include the laser, scanners and scan mirrors, reflective filters, beamsplitters, lenses, and detectors. Characterized components new to the GDx-MM include the LCRs and rotating waveplate. The characterization process (by manufacturer data, theoretical analysis, laboratory measurement, and/or educated guess) is described; limitations and non idealities are identified; and the effect on polarimetry and imaging is discussed. Information on component non idealities is utilized in Chapter 9, where a detailed error analysis of the GDx-MM is performed.

7.2: Liquid Crystal Retarders

The GDx-MM polarimeter requires variable retardance elements in optical path, one in the generator and one in the analyzer. These elements should ideally have the following characteristics: retardance varying from at least $3/8$ to $7/8$ wave at 780 nm; high spatial uniformity over a clear aperture of at least $3/4$ "; high optical throughput; and good optical quality (i.e. surface figure and flatness). As the GDx was not designed to accommodate these components, the space in which they may be mounted is limited. Therefore the retarders must be small (under 1" diameter and $1/4$ " thickness). As the incident beams are

converging or diverging, the retardance should vary as little as possible with incident angle, over a 10° angle of incidence range. Finally, the elements should have fast response time when retardance is changed by computer control, ideally settling to the desired retardance in under 100 ms.

A few options are available for implementing a computer controlled variable retarder: liquid crystal retarders, Pockels cells, Soleil-Babinet compensators, and photoelastic modulators. Pockels cells, consisting of a photorefractive electro-optic crystal which exhibits birefringence proportional to an applied voltage, were rejected due to the large size, need for a kiloVolt power supply, and high cost. Soleil-Babinet compensators, consisting of crystalline wedges which are translated with respect to each other to produce true zero-order retardance of up to one wave, were rejected due to the large size, high cost, and requirement for a large housing to accommodate a computer controlled micrometer interface. Photoelastic modulators, consisting of bars of optical material such as glass which exhibit stress birefringence when they are driven to resonance, are a more likely possibility. However, working with these devices is tricky as the birefringence modulates sinusoidally, peak retardance is fixed by the material and dimensions of the bar, and retardance varies along the length of the bar. The need for sophisticated electronic control and vibration mounts makes this option quite expensive.

Pockels cells, Soleil-Babinet compensators, and photoelastic modulators have fast response time, high throughput, high uniformity over a large aperture, and wide

acceptance angle, but they are large and costly. Liquid crystal retarders (LCRs) were chosen for this project because they are small, relatively inexpensive, and relatively simple to control. These advantages come at the expense of slower response time, lower throughput, and more variability in polarization properties. LCRs can be thought of as tunable waveplates, with retardance in the range of zero to several waves determined by an applied square wave voltage, usually in the range 0-10 V. These devices utilize the “Fredericks” cell configuration, which is different from the twisted nematic configuration used in liquid crystal displays. There are four components (Figure 7.1): two glass plates which form a cavity, Indium Tin Oxide coatings on the plates which form transparent electrodes, a polyimide layer on each plate which acts to align the liquid crystal molecules parallel to the plates, and a high birefringence liquid crystal material sandwiched between the plates. When no voltage is applied the liquid crystal molecule’s directions are aligned parallel to the plates and the retardance is maximum. When a voltage differential is applied between the plates an electric field is induced which supplies a torque to the liquid crystal molecules, increasing the angle of the molecules with respect to the plates resulting in a decreased retardance. When the majority of the molecules are nearly perpendicular to the plates, retardance is minimum.

Figure 7.1: LCR composition and liquid crystal orientation as a function of applied voltage

Those molecules which are near to the surface of the plates are unable to fully rotate, and so it is not possible to obtain a zero phase shift. If a DC electric field is applied continuously then impurity ions in the liquid crystal material will tend to migrate towards the plates. These migrating ions may damage the helical liquid crystal structure, and once arrived at the plates will set up a permanent electric field which reduces the dynamic range of the device. To avoid this problem an alternating square wave voltage at approximately 1 kHz is applied. Retardance is a nonlinear function of applied voltage, and the relationship is highly variable from device to device, so that individual calibration of each device is required. Although optical quality and surface figure are generally very good, spatial uniformity is often poor due to the difficulty of obtaining an even distribution of the liquid crystal material between the plates. As the cells are fairly thick (about $\frac{1}{4}$ "), they introduce potentially undesirable focal shifts and tilts when used in uncollimated space. Transmission is fairly high (typically 80-90%), but can vary by up to several percent with applied voltage. Temperature dependence of the retardance is specified by the manufacturer to be typically 0.5% per $^{\circ}\text{C}$, a significant issue. The liquid crystal material scatters, leading to some depolarization of the polarized beam.

Of particular importance is the switching time, i.e. the time for the retardance to settle to within a small percentage of its final value after a new voltage is applied. Switching time should be as fast as possible to minimize measurement time and thus minimize eye movement and blinking. Switching time in LCRs is highly variable, depending on cell thickness, liquid crystal viscosity, temperature, and applied voltage. Switching time is also asymmetric: when voltage and thus electric field is increased (retardance is decreased) the torque applied to the molecules determines the response time, but when voltage is decreased (retardance is increased) a slower interactive restoring force between molecules determines response time. The restoring force has fastest response for those molecules near the plate surfaces, and slowest response for those near the center. LCR switching times are typically on the order of 1-100 ms.

LCR devices, available from a few suppliers, are not widely used and are generally “hand made” and thus somewhat variable in their properties. For this work seven LCRs were obtained from Bolder Vision Optik in Boulder, Colorado (the two with highest uniformity and degree of polarization were chosen for use in the instrument). Several measurements were done to quantify performance, including: retardance vs. voltage; depolarization vs. voltage, retardance vs. angle of incidence; switching time; and uniformity over clear area. In addition a study was done to determine whether changing retardance causes measurable warping of the device, which might induce aberration in the imaged beam.

Retardance vs. voltage, depolarization vs. voltage, and uniformity were tested by placing each LCR in a calibrated imaging dual rotating retarder Mueller matrix polarimeter in transmission configuration. The field of view of the polarimeter was set to 10 mm, equal to the clear aperture specification for the devices. The devices were mounted at normal incidence to the probing beam, with fast axis vertical. A Meadowlark Optics D3040 controller was used to adjust the magnitude of the applied 2 kHz square wave voltage waveform in the range 0V to 10V. A typical measured retardance vs. voltage curve is shown in Figure 7.2. The most effective portion of the curve is for applied voltages of 1V - 5V, yielding retardances of 1.5 to 0.25 waves. The slope of the curve in the 1V to 3V region is about 0.5 waves per volt, and in the 3V to 5V region is about 0.125 wave per volt. In other regions of the curve the slope is low and less useful. All devices displayed a similar retardance vs. voltage response to within $\pm 5\%$. The measured response curve for each cell was fitted to a high order polynomial, which was later used to determine the applied voltage to obtain a desired retardance.

Figure 7.2: Typical retardance vs. voltage curve for Bolder Vision Optik liquid crystal variable retarders at 780 nm.

All LCRs exhibited significant nonuniformity in polarization parameters across the clear aperture; a few examples are shown in Figure 7.3. Some devices were only slightly nonuniform, while others showed significant nonuniformity. The maximum variation in retardance magnitude across the clear aperture is about 5%. The depolarization index (DPI) also varied significantly from device to device. Three devices had DPI of 1%, three had DPI of 5%, and one had DPI of 10%. The large variation in uniformity and DPI is a clear indication of the “handmade” quality of the cells, and is likely associated with the glass spacer spheres and capacitive force between the plates. A further study of DPI in cells 2 and 4 as a function of applied voltage shows that cell 4 had nearly constant DPI of 1% over a voltage range of 2-6V, but cell 4 DPI increased from 2% at 2V to 8% at 6V. Depolarization images for these cells clearly indicate that depolarization is nonuniform.

Figure 7.3: Examples of nonuniformity in retardance magnitude across clear aperture of two representative Bolder Vision Optik liquid crystal variable retarders at 780 nm. Applied voltage is 2V.

Retardance as a function of angle of incidence was tested for one device using an imaging dual rotating retarder polarimeter in transmission configuration. Microscope objectives with 50 mm focal length were used to focus polarized light from the generator with an $f/3$ cone through the LCR, and to recollimate the transmitted light for passage through the analyzer and into a camera. The collected images give polarization properties as a function of angle of incidence for a single point on the LCR. Radial distance from the center of the image is proportional to angle of incidence and radial position represents orientation of the plane of incidence. Data was collected for three different applied voltages: 1.6V, 2.8V, and 5.0V, corresponding roughly to 1.25 waves, 0.5 waves, and 0.25 waves. A plot of the change in retardance over a $\pm 10^\circ$ angle of incidence for each applied voltage is shown in Figure 7.4. Retardance varies substantially, both as a function of angle of incidence (with high linear slope of 2° to 3.5° change in retardance per degree of incidence angle about normal incidence), and of applied voltage. In practice this is a large problem. Since the LCRs are not used in

collimated space there will be a range of retardances seen by different parts of the beam, causing depolarization.

Figure 7.4: Change in retardance as a function of angle of incidence over a 10° cone for a Bolder Vision Optik liquid crystal variable retarder at 780 nm.

The switching time of the LCRs was tested using a polariscope. A monochromator at 780 nm and optical fiber with collimating lens formed a 1" diameter collimated source beam. This beam passed through a first horizontal polarizer, the LCR with fast axis oriented at 45° , and a second horizontal polarizer. The intensity through this system, dependent on LCR retardance, was measured by a fast Newport photodetector and viewed on an oscilloscope. The LCR voltage was varied using a Meadowlark D3040 liquid crystal controller. Typical switching profiles are shown in Figure 7.5. Over a wide range of applied voltages the settling time is in the range 40-50 ms for voltage increase, and in the range 30-80 ms for voltage decrease.

(a)

(b)

Figure 7.5: Typical switching profiles for Bolder Vision Optik liquid crystal variable retarders at 780 nm. Time scale is 20 ms per grid. Top trace: 2 kHz applied square wave control signal. Bottom trace: response of LCR to change in control signal magnitude. (a): typical response for drive voltage increase; (b): typical response for drive voltage decrease.

There was a concern that the torque generated within the LCR in response to a change in voltage might distort its shape, thus introducing wavefront aberration into the optical system. A knife edge test explored this issue. The generator path was simulated using the following components: a red HeNe laser; a 20X, 0.4 NA microscope objective; a LCR; and a 50 mm focal length lens to focus the beam onto a CCD camera. A knife edge was placed at the focal point of this system, i.e. at that location where insertion into the beam caused uniform darkening of the beam as viewed by the camera. The LCR voltage was increased in 1V increments from 0 - 5V, and the knife edge was translated laterally in and out of the beam at each increment. No measurable change in knife edge position was needed to maintain its location at the focal point. The knife edge was then translated to a

fixed position such that it blocked exactly half the beam (uniform half intensity image at camera), and the LCR voltage was varied as before. No measurable change in intensity was observed. The conclusion is that no measurable beam distorting effects occur as the LCR voltage is changed.

7.3: Laser source

From patents and marketing literature it is known that the GDx laser operates at 780 nm. It is clearly a diode type laser given the wavelength and form factor. The output power stability of this laser source is of particular interest. As the polarization processing relies on relative intensity changes from image to image, any drift in laser output over the 4 second measurement time will couple as error into the reconstruction of the Mueller matrix image. A drift in laser output over the few hours required to calibrate the system (discussed further in Chapter 8) would introduce a systematic error into the calibration data, which would then affect every measurement made with the instrument.

Laser power output as a function of time over five minutes (after system off for several hours) was measured with the scanner disabled using an Oriel optical power meter. The output rose steadily from 2.9 mW to 3.0 mW, a small rise which will not contribute significant error. After being on overnight, the laser output was observed to remain constant to within the resolution of the power meter over a period of several hours; this demonstrates that a warmed-up laser can be used for system calibration without concern for intensity drift.

A laser-related issue is subtraction of a dark reference level from each acquired image, so that, for example, measurements are not influenced by room lighting conditions. While the confocal nature of the instrument minimizes the need for dark level subtraction, it is still advisable. By viewing the laser output using a photodetector and oscilloscope during GDx normal operation, it was observed that the laser is blanked by the GDx control system for approximately 6 ms at the beginning of each scan, roughly corresponding to the time when the waveplate motor is moving. This blanking function allows the detector “zero” to be reset on a per scan basis. This function was maintained as is in the GDx-MM design.

The polarization state of the laser was confirmed to be linearly polarized (as assumed in the analysis of Chapter 5) by disabling the scanners, and rotating a piece of Polarcor polarizer material in the output beam. The intensity through the polarizer, as measured using the Oriel power meter, varied from a maximum of 3 mW with the polarizer horizontally oriented to a minimum of approximately 0 mW with the polarizer vertically oriented, demonstrating that the laser is horizontal linearly polarized with high degree of polarization.

7.4: Scanners and scan mirrors

The slow and fast scanners were identified as galvonometer and resonant (respectively) based on their form factor and manufacturer datasheets. The scan frequencies were measured as 26.4 Hz and 3.94 kHz (respectively) by monitoring the drive signal using an

oscilloscope. Because the frequency of the resonant scanner can drift, and because the frequency of the data acquisition system is not locked to the scanner frequency, there is an irregularity in the sampled data which must be compensated. To estimate the magnitude of the drift, the fast scan frequency was monitored over various time intervals. It was noted that, over any given hour, the frequency drifts downward by about 1 Hz, and from day to day, the frequency differs by at most ± 5 Hz. This magnitude is small enough to be partly compensated by sampling the scanner frequency before each measurement and setting the data acquisition frequency to the sampled value. Further compensation is performed as a post-processing step, described in Chapter 10.

The resonant scanner has a metallized mirror, and the galvanometer scanner has a multilayer dielectric coated mirror. The composition of these coatings is unknown, but they are presumed to have high reflectivity for 780 nm. Because the incident light is converging and at an oblique angle, the coatings may contribute diattenuation and retardance which is dependent on angle of incidence. Different parts of the beam would then be polarized differently, giving undesirable depolarization. As the mirrors could not be removed from the system to be characterized, polarization ray trace simulations using Code V were performed instead. Typical coatings were assumed: silver for the metallized mirror, and an 8-layer stack of high-low refractive index material for the dielectric mirror. Simulations show that for a horizontally polarized beam with angle of incidence in the range 35° - 55° , the rotation and increase in ellipticity are minimal,

implying that depolarization and variation of polarization across the field should be minimal. Polarization ray trace simulations were performed by Karlton Crabtree.

Finally, as the scanner motion is nonlinear (i.e. the scan speed is faster at the edges of the scan pattern than at the center), a simple scan reconstruction which assumes a linear scan will produce a distorted image. This distortion has no effect on polarization content but may confuse image interpretation. Distortion was quantified by scanning a 1 mm grid graph paper (Figure 7.6). The increase in square size from center to far edge was about 9% in both horizontal and vertical directions. A small curve in the graph lines was also observed. This level of distortion is minor and considered acceptable. It would be possible to compensate for distortion as a post-processing step (not done in this work).

Figure 7.6: Scan of graph paper with 1 mm squares showing slight scan distortion over the field of view.

7.5: Avalanche Photodiode Detector

The avalanche photodiode detector (APD) used in this system, while very sensitive, has several issues which can influence accuracy. In this chapter the signal detection range is calculated and demonstrated to be appropriate given the expected signal strength. Non idealities are then discussed. The manufacturer datasheet was used to analyze the particular APD detector used in the GDx-MM instrument.

Several parameters define the basic performance of a detector, including responsivity, dynamic range, bandwidth, and signal-to-noise. The 3 dB bandwidth of this detector is 50 MHz, amply larger than the system's 4 MHz sample rate, and so fine detail in the image should not be blurred by bandwidth limitations. The responsivity R is dependent on an applied bias voltage (0 - 400V), and is in the range of 0 - 1 V/ μ W. Given a linear output voltage swing of up to 0.7V, this implies a maximum input signal of 0.7 μ W incident optical power. However, as the detector is followed by an amplifier with a gain of 40 powered by ± 8 V, the maximum signal from the detector is actually 0.2V, corresponding to 200 nW incident optical power. Beyond 200 nW the amplifier will saturate, beyond 2 μ W the detectors will saturate, and beyond 5 μ W the detectors will be damaged. These levels are consistent with the radiometric analysis of Chapter 4.

Noise performance of APDs is an important consideration [191], as it limits the minimum optical intensity which can be accurately detected. Sensitivity at low incident power is limited by detector (thermal) noise, and at higher power levels by shot noise. Since the

APD is designed to be operated under reverse bias, the noise performance at low light levels will be limited by shot noise due to dark current fluctuations and leakage current. The noise level at higher light levels will be limited by photon shot noise on the current generated by the optical signal. In general, APDs have higher noise than PIN photodiodes because the bulk leakage current is multiplied by the gain M of the APD, which is typically on the order of 100. In addition, the avalanche process generates current fluctuations which lead to an excess noise factor F , typically on the order of 5, which further increases the bulk leakage current. However, in cases where the amplifier noise is high (typically in high bandwidth situations such as the GDx-MM), the APD gain can be increased to the point where detector and amplifier noise are similar, giving an overall improved signal to noise ratio as compared to a PIN diode. There is an ideal gain which provides the best signal to noise ratio; for this detector that value is 120, corresponding to an applied voltage of about 370V.

The minimum detectable power P_{min} is defined as the light level giving a signal to noise ratio (SNR) of 1. It is related to the Noise Equivalent Power (NEP), defined as the minimum detectable power for a 1 Hz signal bandwidth. For this detector with $NEP = 0.03 \text{ pW}/\sqrt{\text{Hz}}$ and bandwidth $B = 50 \text{ MHz}$,

$$P_{min} = NEP\sqrt{B} = 2.1 \times 10^{-10} \text{ W}, \quad (7.1)$$

which quantifies the dynamic range as 0.2 nW to 200 nW incident optical power. The SNR is calculated as the ratio of electrical signal power to the sum of thermal and shot noise terms, at the optimal wavelength of 780 nm, temperature of 273 K, gain of $M = 120$, and bandwidth $B = 50$ MHz. The electrical signal power is given by

$$S = \frac{(RP_s)^2}{R_L} = 2.5 \times 10^7 P_s^2, \quad (7.2)$$

where R is the responsivity in V/W (data sheet: 5×10^5 V/W), P_s is the incident optical power, and R_L is the load impedance (data sheet: 10 Kohm). The thermal noise power can be estimated as

$$N_{th} = 4kTB = 8.09 \times 10^{-13} \text{ W}. \quad (7.3)$$

The shot noise power can be estimated as

$$N_{shot} = 2qBF(RMP_s + I_d M^2 R_L), \quad (7.4)$$

where q is the electronic charge and I_d is the dark current. Since I_d and F are not specified in the datasheet, typical values of 0.2 nA and 5 are assumed. In this case

$$N_{shot} = 4.8 \times 10^{-3} P_s + 2.3 \times 10^{-12}. \quad (7.5)$$

Then SNR is given by

$$SNR = \frac{S}{N_{therm} + N_{shot}} = 5.2 \times 10^9 \frac{P_s^2}{P_s + 4.8 \times 10^{-10}} \quad (7.6)$$

For incident optical powers of greater than about 1 nW, SNR is linear in P_s and shot noise limited, increasing to a value of over 1000:1 at the maximum signal level of 200 nW.

Detector linearity is important, as a nonlinear responsivity will result in measured intensity distortion, giving systematic error in the polarimetry. To investigate the linearity of the detector a monochromator with adjustable slit was used to generate a variable intensity beam which was injected into the GDx-MM. The intensity range was similar to a typical retinal signal (0-500 counts from the analog to digital converter). Intensity was increased linearly in steps, and at each step the GDx-MM captured an image. The average intensity (in counts) of each image was calculated. A plot of measured counts vs. incident intensity (Figure 7.7) demonstrates that the detector is highly linear, with the departure from linearity on the order of 3%.

Figure 7.7: Measured linearity of the GDx-MM detectors, showing an approximately 3% departure from linearity.

A detector-related issue is the possible presence of system frequencies exceeding the Nyquist rate (i.e. exceeding half the sampling rate of 4 MHz). Any such frequencies which leak into the detector circuit would alias into the frequency region of interest (i.e. below 2 MHz) and couple into the measurement. To investigate this issue, the grid of Figure 7.6 was scanned at the highest scanning frequency available from the National Instruments board (9 MHz), and a fast Fourier transform was calculated (Figure 7.8). The only significant frequency component above the Nyquist rate, at about 3.73 MHz, is a factor of 6 smaller (about 8 dB down) from the main frequency components. Its contribution is small enough to be neglected.

Figure 7.8: Fast Fourier transform of the GDx-MM scan of a graph paper target. Most frequency components are below 500 kHz. There is one significant component above the Nyquist rate of 2 MHz, but it is small enough to be neglected.

A major problem with APD detectors is the strong dependence of gain on temperature.

With a fixed operating voltage the gain decreases at higher temperatures and increases at lower temperatures. This can lead to measured intensity distortions which cause polarimetry error. This issue is of particular concern during the half hour long calibration procedure for the analyzer. As the GDx detector circuitry was designed without temperature stabilization, gain drift is a significant problem in the GDx-MM. Given the lack of stabilization, the change in gain for a 1°C change in temperature would be expected to be about 2.5% to 5.0% depending on operating voltage. Gain drifts of this order were directly observed by disabling the scanners, placing a Spectralon target at the retina plane to diffusely scatter light back into the instrument, and monitoring the detector output with an oscilloscope. Options for addressing this issue include the use of thermoelectric coolers to stabilize temperature and the use of custom analog circuitry to adjust the bias voltage with temperature. The decision was made to keep the original GDx detector circuit as is despite lack of temperature stabilization due to the complexity

of redesigning the circuit, a lack of space for additional circuitry, and the difficulty in realigning the detector. As such, detector gain drift is one of the primary error sources in the GDx-MM polarimeter. The effect of this error on accuracy is discussed more fully in Chapter 9.

7.6: Rotating Wave plate

A new waveplate with retardance of 147° (314 nm at 780 nm) was made to custom specification and characterized using a calibrated imaging polarimeter. Since this component is central to the polarimetry, it is important that it have accurate retardance over a wide clear aperture and range of angle of incidence and temperature. A zero-order retarder made from a birefringent polymer material was selected because this type of waveplate has significantly lower variation in retardance with angle of incidence than crystalline materials (about 6% change from 0° to 30° angle of incidence). The temperature sensitivity is about 0.04% per $^\circ\text{C}$, which is negligible. The waveplate was specified to have less than 1/100 wave retardance variation over the clear aperture. Imaging polarimeter test results show that the waveplate has retardance magnitude of $147.2^\circ \pm 0.4^\circ$, diattenuation magnitude of 0.02 ± 0.01 , and depolarization index of 0.012 ± 0.004 . These values are well within the specified limits.

The repeatability and accuracy of the waveplate motor drive are important, since variations will contribute to error. Repeatability treats the ability of the motor system to start from the same “zero” position each time, and is dependent on the reliability of the

homing sensor. Repeatability was estimated by comparing the home sensor response time to the motor drive pulse time during the homing procedure. This comparison indicates that the sensor may lag enough to let an additional pulse through after the motor is home, thus advancing it one position too far. Thus the repeatability is 1 step, or 2.432° . Accuracy treats the variability in rotary distance travelled when one pulse is sent, and is dependent on the motor configuration. Accuracy was estimated from the motor datasheet to be $\pm 5\%$ per step, or about ± 0.002 rad. While accuracy and repeatability are not as good as desired, it was impractical to replace the motor control system. Homing error is one of the primary error sources in the GDx-MM polarimeter, as discussed in Chapter 9.

7.7: Conclusions

In this chapter GDx-MM component issues and their limiting effect on polarimeter performance were discussed. The liquid crystal variable retarders present particularly serious problems due to significant depolarization as well as variations in retardance with aperture, angle of incidence, and temperature. The APD detectors are predicted to have acceptable SNR and high linearity over a wide range of optical signals, but gain drift with temperature is a concern. The waveplate motor control system is not as accurate and repeatable as desired and may be a major source of error. The laser source and scan mirrors are not expected to have substantial effect on polarimetry accuracy. The component errors identified in this chapter are used to predict GDx-MM accuracy in an analysis described in Chapter 9.

CHAPTER 8: CALIBRATION

8.1: Introduction

Calibration must be performed to generate the instrument matrix which converts each raw dataset to a Mueller matrix image. Various techniques are commonly used to calibrate a polarimeter; the relative merits of several are discussed. The calibration method used for the GDx-MM, direct measurement of states by an external polarimeter, is described in detail with an emphasis on instrumentation and measures taken to reduce error in the calibration data. Metrics for assessing the accuracy of the calibration data are also presented, and the calibration data is analyzed in terms of these metrics. Finally, data processing methods for assembling the instrument matrix from the calibration data set are described.

8.2: Overview of calibration methods

To reconstruct sample Mueller matrices from polarimeter measurements, the polarization states of the generator and analyzer must be accurately determined and assembled into an instrument matrix. For some polarimeters the polarization components which generate the states are nearly ideal, and it is reasonable to construct the instrument matrix from mathematical models derived from ideal Mueller matrices of the components. Small corrections to the model can be made by measuring the Mueller matrices of the polarimetric components using an external polarimeter, then constructing the instrument matrix from these matrices. A more sophisticated approach requiring somewhat more effort involves using the polarimeter to measure a specific set of high quality (nearly

ideal) targets with known Mueller matrices (e.g. Polarcor polarizer and quartz waveplate at various orientations) [192,193]. The data measured by the polarimeter can be reduced via a numerical solution to a set of linear equations to yield the instrument matrix. This approach is reasonably accurate so long as the targets may be accurately modeled by Mueller matrices. An even more involved calibration method is to measure each state individually using an external calibrated polarimeter. This method may be difficult to set up due to the need for precise alignment between the polarimeters, and is more time consuming as each state must be measured individually. However, this method provides highest accuracy so long as the external polarimeter is accurate and the polarization states do not drift significantly during the course of the measurements.

The GDx-MM is not well suited for the modeling approach as the optical path has many components mounted at complex angles and having coatings exhibiting potentially complicated polarization behavior. As these components cannot be removed without losing alignment it is not possible to perform polarimetric testing, which would be necessary to construct an accurate model. Measurement of a set of ideal targets is a better possibility. However, as best possible accuracy is desired, the GDx-MM was calibrated via measurement of all possible states using an external polarimeter. An appropriate combination of states giving highest stability was then selected for final use using a procedure described below. Given an accurate measurement of each of the highest stability states, the GDx-MM calibration is very accurate.

The generator and analyzer states must be separately calibrated. The generator states are described by the Stokes vectors of the light emitted for each of the 72 states. In this case, an external calibrated Stokes polarimeter measures the Stokes vector (“generator vector”) of each state. The analyzer states, however, must first be described by the composite Mueller matrix of the analyzer path components for each of the 72 states. The first row of each Mueller matrix gives the vector for use in the calibration. In this case, an external calibrated Stokes vector generator injects several states into the GDx-MM, and its detectors measure a corresponding intensity vector. The analyzer Stokes vectors (“analyzer vectors”) are calculated from the intensity vector and the Stokes vectors of the injected states. The following sections describe in detail the specific methods for calibrating the generator and analyzer sections.

8.3: Generator calibration

The calibration of the generator assumes the use of an external Stokes polarimeter that has been calibrated for use at 780 nm. The GDx-MM scan head was mounted on an optical bench with its optic axis coincident with the optic axis of the external polarimeter. The external polarimeter for this work consisted of a rotating quartz waveplate followed by a Glan-Thompson fixed polarizer. An optical power meter (Newport 1830-C) with a 780 nm bandpass filter measured the intensity through the external polarimeter. The GDx-MM laser sensor was connected to an external power meter to monitor power fluctuations. The generator calibration setup is shown in Figure 8.1.

Figure 8.1: Generator calibration setup, showing the scan head and external Stokes polarimeter mounted on an optical bench.

After waiting several hours for the laser to reach steady state, the GDx-MM was set to the first state by moving the rotating waveplate to the starting position, and by setting the generator LCR retardance using a Meadowlark Optics D3040 Liquid Crystal Controller. The Stokes polarimeter measured the Stokes vector of this state, and the laser power was measured by the external power meter. This procedure was repeated for every possible generator state, including 72 different motor positions and 3 different generator retarder settings ($3/8$ wave, $5/8$ wave, and $7/8$ wave), for a total of 216 measured Stokes vectors. Although only 72 of these Stokes vectors are eventually used, all 216 were recorded so that the ones yielding highest stability could later be selected as described in Section 8.5. Following measurement the Stokes vectors were converted to a more convenient form.

First, the product of the S_0 component of each vector and the corresponding laser power measurement was calculated, giving a “strength factor” for each vector which reflects laser intensity fluctuation and variation in system throughput. All vectors were then normalized to the S_0 component. Strength factors and normalized vectors were saved into data files.

A validation process was performed to verify that the recorded Stokes vectors are reasonable. Both the vector direction (ellipticity and orientation) and vector magnitude (degree of polarization) were examined. In the first step, the Degree of Polarization (DoP) of each measured Stokes vector was calculated per Equation 3.4. A plot of DoP (Figure 8.2) shows variation in the range 89% to 98%, correlated with rotating waveplate position. In the second step, the Stokes vectors were compared to an “ideal” model on the Poincaré sphere. The model was constructed from the ideal Mueller matrices of the generator path polarization components. As the model does not perfectly represent the generator path, the model and measured data agree well by not exactly, as shown in Figure 8.3.

Figure 8.2: Degree of Polarization of measured generator states.

Figure 8.3: A comparison of measured generator states (blue) and ideal model (red) plotted on the Poincaré sphere. Three different orientations of the sphere are shown.

8.4: Analyzer calibration

The calibration of the analyzer assumes the use of an external Stokes vector generator which has been calibrated at 780 nm. The scan head was mounted on an optical bench so that its optic axis was coincident with the optic axis of the external generator. The external generator for this work consisted of a 780 nm source, a fixed linear polarizer, and a rotating retarder. The source assembly was constructed from a 780 nm LED [Roithner Lasertechnik, Vienna, Austria], a ground glass diffusor [Edmund Scientific], and a 25 mm focal length collimating lens. The collimated source beam had 5 mW power and 15 mm diameter. A fraction of the beam power was diverted via a beamsplitter to a photodiode to monitor power. Because of the small pinhole in the scan head, accurate co-alignment of the optic axes of the GDx-MM and external Stokes vector generator was critical. Acceptable co-alignment was achieved by methodical adjustment of the relative height, tip, tilt, and yaw of the two instruments. Following co-alignment a telecentric lens system (described in Section 11.2) was mounted at the GDx-MM external pupil. This lens system allowed uniform scanning by the GDx-MM across the entire source beam, producing images with uniform intensity. The calibration setup for the analyzer is shown in Figure 8.4.

Figure 8.4: Analyzer calibration setup, showing the scan head and external Stokes polarization state generator mounted on an optical bench.

After waiting several hours for the LED to reach steady state, the external Stokes vector generator was set to the first state (waveplate horizontal). A dataset was recorded with the rotating waveplate rotating to all 72 positions, and the analyzer LCR retardance held fixed. The external Stokes generator was set in turn to 37 different states (waveplate rotated in 5° increments from 0° to 180°), and a dataset was collected for each state. This procedure was repeated for LCR retardances of $3/8$ wave, $5/8$ wave, and $7/8$ wave. The collected data consisted of 111 datasets, each giving 72 images, and each image having different uniform intensity. The center 75% of each image was averaged, giving 111 72-element intensity vectors. Over the 111 scans there were a total of 7992 intensity values, corresponding to combinations of the 37 external generator vectors, the 72 rotating

waveplate positions, and the three LCR retardances. These 7992 intensities were appropriately rearranged into 216 37-element intensity vectors, one for each analyzer state.

To extract the analyzer vectors for calibration from these intensity lists, consider that the calibration system can be represented as

$$A_i S_j = I_{ij} , \quad (8.1)$$

where A_i is the analyzer vector each of the $i = 216$ GDx states, S_j is the 4-element Stokes vector for each of the $j = 37$ input Stokes vectors, and I_{ij} is the measured intensity.

In more convenient matrix form,

$$A_i S = I_i . \quad (8.2)$$

A_i is then calculated as:

$$A_i = S^{-1} I_i . \quad (8.3)$$

However, as S is not square, the pseudoinverse is used:

$$A_i = (S^T S)^{-1} S^T I_i. \quad (8.4)$$

Although only 72 of these analyzer Stokes vectors are eventually used, all 216 were calculated so that the ones yielding highest stability could later be selected.

As with the generator vectors, the product of the S_0 component of each vector and the corresponding source power measurement is formed, giving a “strength factor” for each vector. All vectors are normalized to the S_0 component. Strength factors and normalized vectors are saved into data files.

Validation of the analyzer calibration was performed in the same way as for the generator calibration. A plot of DoP (Figure 8.5) shows small cyclic variation in the range 93% to 100%. For comparison on the Poincaré sphere the analyzer model was constructed from ideal Mueller matrices of the analyzer path polarization components. As the model does not perfectly represent the analyzer path, the model and measured data agree well by not exactly, as shown in Figure 8.6. Correspondence is lower than for the generator calibration due to drift during the long calibration process, as well as the presence of noise and high dynamic range in the collected data images.

Figure 8.5: Degree of Polarization of measured analyzer states.

Figure 8.6: A comparison of measured analyzer states (blue) and ideal model (red) plotted on the Poincaré sphere. Three different orientations of the sphere are shown.

8.5: Creation of Calibration Dataset

The generator and analyzer calibrations produce Stokes vectors, which must be combined in pairs to describe polarimeter states. The number of generator and analyzer states calibrated (216 of each) is larger than the number of combined states needed (72). To choose states for the final calibration set, the 216^2 possible Stokes vector pairs are assembled into all possible 72-member collections. The instrument matrix for each collection is calculated, and that matrix having rank 16 and lowest condition number is selected. Stokes vector pairs are restricted by the established timing sequence, consisting of $i = 4$ sequences, each having fixed LCR retardance and $j = 18$ waveplate rotations. A given sequence of generator LCR retardance g_i and analyzer LCR retardance a_i may be described generally as: $((g_1, a_1), (g_2, a_2), (g_3, a_3), (g_4, a_4))$. g_i and a_i are chosen from the values $(3\lambda/8, 5\lambda/8, 7\lambda/8)$. A general expression for W is then

$$W = \begin{pmatrix} w_{1,1} \\ \vdots \\ w_{1,18} \\ w_{2,1} \\ \vdots \\ w_{2,18} \\ w_{3,1} \\ \vdots \\ w_{3,18} \\ w_{4,1} \\ \vdots \\ w_{4,18} \end{pmatrix} = \begin{pmatrix} w_{1,1} \\ \vdots \\ w_{i,j} \end{pmatrix}. \quad (8.5)$$

Each row of W is given by

$$w_{i,j} = g_{i,j} \otimes a_{i,j}^T . \quad (8.6)$$

Given the number of measured polarization states and the sequence constraints it is possible to assemble $(3*3)^4 = 6561$ different W 's. If the calibration data were ideal (zero error), some W 's would have rank lower than 16, and groups of W 's would have identical condition number. However, since noise is present, every W has rank 16 (though some are very nearly lower rank), and there is a continuum of condition numbers. All 6561 matrices were assembled, and the one with minimum condition number (of 15.1) was identified. The corresponding retardance sequence is $((7\lambda/8, 5\lambda/8), (7\lambda/8, 3\lambda/8), (5\lambda/8, 3\lambda/8), (3\lambda/8, 7\lambda/8))$. The final calibration data consists of this best case set of 72 generator and analyzer Stokes vector pairs, as well as the strength data for these vectors. This data is saved into a text file for later use by a separate application which converts image stacks to a Mueller matrix image, as described in Chapter 10.

8.6: Conclusions

The GDx-MM calibration process involves separate calibration of the generator and analyzer sections using an external polarimeter, together with appropriate data processing to produce the system instrument matrix. The high degree of polarization and good model fit suggest that the calibration process is accurate and effective. Calibration was a time-consuming task which required a large amount of external equipment and data

processing. The advantage is high calibration accuracy due to the individual measurement of each state, and high stability due to the selection of those states giving lowest condition number of the instrument matrix. A disadvantage is the drift in polarization state which occurs during the long calibration process, leading to systematic error in polarization measurements; this issue is discussed in Chapter 9.

CHAPTER 9: ERROR ANALYSIS

9.1: Introduction

The performance of the GDx-MM in the presence of error sources can be predicted based on the theory presented in Chapter 4. It is advantageous to do so, to gain a knowledge of expected performance and verify that the level is acceptable, and to identify the primary sources of error and minimize them to the extent possible. Systematic and random error sources are inherent in the polarimeter and also arise due to the calibration process. In this chapter the main error sources based on information presented in Chapters 7 and 8 are summarized, and a comprehensive error analysis is performed to estimate the mean and standard deviation error predicted to be present in GDx-MM measurements and reconstructions of sample polarization properties.

9.2: Polarimeter-based error sources

The GDx-MM polarimeter error sources can be divided into two groups: those that contribute a systematic error (mean error), and those that contribute a zero-mean error distribution (SD error). Tables 9.1 summarizes the three main systematic error sources: LCR retardance drift, incorrect waveplate start position, and detector noise. The bound for LCR retardance drift with temperature is based on the manufacturer specification of 2.5% drift over $\pm 5^{\circ}\text{C}$. The rotating waveplate start position uncertainty is due to the use of an inconsistent motor “home” position sensor; this error is 1 motor step, or about 0.04 rad. As the principal noise component of the APD detector is Poisson distributed, it has equal systematic and zero mean error components. For a typical signal to noise ratio

of 20:1, the bound on error is 0.05. For the error analysis which follows only the positive range of these errors is considered for simplicity. The calculations of error range presented here should be considered as due to a positive error; a negative error would give an error range with opposite sign.

Table 9.1: Systematic error sources

Error	Bound on mean error
LCR retardance drift with temperature	± 0.1 rad
Rotating waveplate motor start position	± 0.04 rad
APD detector noise as a percentage of signal	± 0.05

Table 9.2 summarizes the two main zero-mean error sources: waveplate orientation uncertainty and detector noise. The bound for uncertainty in rotating waveplate rotational movement is based on the manufacturer specification of 5% per step, or 0.002 rad in the GDx-MM configuration. The detector noise is the same as for systematic error.

Table 9.2: Zero mean random error sources

Error	Bound on SD error
Rotating waveplate incremental position	± 0.002 rad
APD detector noise as a percentage of signal	± 0.05

To calculate the expected error, the GDx-MM configuration selected in the calibration process was modeled using ideal polarization components, and an instrument matrix was assembled. This matrix has four variables subject to error: generator LCR retardance; analyzer LCR retardance; rotating waveplate starting orientation; and rotating waveplate angular increment. Given these variables the δW matrix was assembled per Equation 4.17, and given the detector noise the δP vector was assembled per Equation 4.18. Finally, the sample error Mueller matrix δM was calculated per Equation 4.19. The mean and SD matrices M_m and M_{SD} , which represent the sample matrices which would be reconstructed by the GDx-MM in the presence of (respectively) systematic and zero mean error sources, were calculated as

$$M_m = M + \langle \delta M \rangle , \quad M_{SD} = M + SD[\delta M] . \quad (9.1)$$

To calculate numeric values for M_m and M_{SD} , the error bounds given in Tables 9.1 and 9.2 were used in the error terms, and a few different sample matrices M (as described below) were selected to give an idea of performance in various situations. Finally, polarization parameters were extracted from M_m and M_{SD} and compared to the ideal values which would be extracted from M in the case of zero error. This gives the predicted worst case error in calculating polarization parameters of interest.

It is important to appropriately interpret the results of the error analysis. In the case of systematic error sources, the bounds presented in Table 9.1 represent the worst case error

which might be expected for each source, and so the error analysis calculates the worst case error in Mueller matrix reconstruction. The actual error in reconstruction will lie somewhere between zero and this worst case error, and thus on average will be significantly smaller than the calculated worst case error. In the case of zero mean error sources, the bounds presented in Table 9.2 represent standard deviations which might be expected for each source, and so the error analysis calculates a corresponding standard deviation in the Mueller matrix reconstruction. The actual error in reconstruction will be distributed according to this calculated standard deviation.

9.3: Simulation results

Six polarization targets were selected for the error analysis:

1. An ideal polarizer rotating from 0° - 90° in 15° steps
2. An ideal quarter wave plate rotating from 0° - 90° in 15° steps
3. An ideal half wave plate rotating from 0° - 90° in 15° steps
4. An ideal variable retarder oriented at 60° and retardance varying from $1/32$ to $12/32$ waves in $1/32$ wave steps
5. An ideal depolarizer
6. Air

These targets were selected to illustrate the reconstruction error for a wide range of polarization parameters, and because they are commonly available for use in validation testing as described in Chapter 11. Figure 9.1 shows the error analysis in the presence of all error sources. In these graphs, solid lines are mean values of polarization parameters

calculated from the reconstructed Mueller matrix (i.e. the worst case error due to systematic error sources), and error bars are SD values (i.e. standard deviation due to zero mean error sources). Light red and blue lines show parameter values in the presence of error, and dark red and blue lines show the parameter values in the absence of error. In a typical usage the error will lie between the dark and light lines.

Error analysis results for mean error are summarized in Table 9.3. The largest mean error is encountered in measurement of diattenuation magnitude of a linear polarizer and retardance magnitude of a half wave plate. These errors vary nonlinearly with component orientation. Somewhat smaller mean errors are encountered in measurement of diattenuation orientation and retardance orientation; these errors are independent of orientation. Little mean error is seen in retardance magnitude over $1/32$ wave to $12/32$ wave, the range encountered in ocular measurement.

Table 9.3: Worst case systematic error in polarization parameters

Parameter	Mean error range
Diattenuation orientation (over the range $0^\circ - 90^\circ$)	-4.5° to $+0.8^\circ$
Diattenuation magnitude (nominally 1)	-0.19 to -0.02
Retardance orientation (over the range $0^\circ - 90^\circ$)	-3.6° to -5.5°
Retardance magnitude (over the range $1/32 - 12/32$ wave)	-0.08 to $+0.04$ rad
Depolarization index (nominally 0)	0 to $+0.14$

Error analysis results for zero mean error are summarized in Table 9.4. The largest standard deviation errors are seen for the half wave plate, both in retardance magnitude and orientation. Diattenuation magnitude and retardance magnitude also have measurable SD, but the SD for diattenuation orientation and retardance orientation is negligible.

Table 9.4: Standard deviation error in polarization parameters

Parameter	Bound on SD error
Diattenuation orientation (over the range $0^\circ - 90^\circ$)	$\pm 1^\circ$
Diattenuation magnitude (nominally 1)	± 0.1
Retardance orientation (over the range $0^\circ - 90^\circ$)	$\pm 0.6^\circ$
Retardance magnitude (over the range $1/32 - 12/32$ wave)	± 0.08 rad
Depolarization index (nominally 0)	± 0.1

In the worst case (all error sources at their maximum limits), the GDx-MM is predicted to have lowest accuracy in the measurement of diattenuation magnitude (about -0.27 worst case) and depolarization index (about 0.22 worst case); and somewhat better accuracy in the measurement of diattenuation orientation (about -5.7° worst case). Polarimeter accuracy is poor when retardance approaches a half wave, but the accuracy over the expected ocular retardance range of $1/32 - 12/32$ wave is acceptable for retardance orientation (about -4.8° worst case) and retardance magnitude (about 0.16 rad worst case).

Figure 9.1: Effect of polarimeter error sources on reconstruction of the sample Mueller matrix and extracted polarization parameters. (a): target #1, rotating linear polarizer; (b): target #2, rotating quarter wave plate; (c): target #3, rotating half wave plate; (d): target #4, variable retardance wave plate oriented at 60°

Figure 9.2 shows error analysis results for each systematic error source individually, for the rotating linear polarizer and rotating quarter wave plate targets. Constant offsets in diattenuation orientation, retardance magnitude, and retardance orientation are seen as a result of error in waveplate start position. Error in diattenuation magnitude is due to LCR retardance drift. A mean offset in measured intensity due to detector noise gives a fixed offset in many of the polarization parameters. Error in waveplate start position leads to higher error in a larger number of polarization parameters, as compared to LCR retardance error or detector noise. Examination of this data shows that greatest improvement in polarimeter performance could be obtained by use of a more accurate motor homing mechanism and by compensation for LCR retardance drift with temperature.

Figure 9.2: Individual error source contributions to the error in reconstruction, for target #1 rotating linear polarizer and target #3 rotating quarter wave plate. (a) and (b): error due to motor start position; (c) and (d): error due to temperature dependence of LCR retardance; (e) and (f): error due to detector noise

9.4: Calibration Error

During the calibration process, polarimeter-based systematic and zero-mean errors as well as calibration-based errors are present. Calibration-based errors arise due to a drift in instrument polarization state across the many datasets recorded over the long calibration time of several hours. These errors give differences between the instrument matrix based on the calibration dataset and the true configuration at each use, and can be particularly damaging as they couple directly into every measurement. Though great care was taken to minimize error during calibration, it is important to understand the types of error that were present and their potential effects. In this section calibration error sources are classified, their magnitude is bounded, and their effect on subsequent measurements is discussed.

Calibration errors may be separated into three classes. The first is “systematic”, in which properties of the polarimeter polarization components during calibration are different than in use. An example would be use of the polarimeter at a temperature different from that at calibration, giving a systematic change in LCR retardance. The second class is “random”, in which error is due to zero mean error sources in the system such as uncertainty in waveplate rotational increment. The third class is “drift”, in which polarimeter properties drift during the course of the calibration process.

Systematic and random calibration error sources are the same as listed in Tables 9.1 and 9.2, and are evaluated as in Section 9.2. These error sources are discussed again here

because it is important to view them in terms of a calibration framework. For example, the error introduced due to LCR retardance drift may be significant if the temperature at calibration is different from the typical temperature during human subject use. In addition, the motor start position and rotational increment may vary over the many datasets collected during the calibration process. If for example the start position is incorrect for one or more calibration datasets, the mean error for all uses of the instrument will be consistently high. Random variations due to the zero mean noise sources have a similar effect, i.e. their presence couples into the fixed calibration set and presents an offset to the measured data, leading to higher overall error.

Drift type error sources have a more insidious effect on the calibration, as they are added into the calibration dataset in different amounts depending on the point in time when a particular calibration measurement is done. The main sources of drift are listed in Table 9.5, where a maximum temperature variation during calibration of $\pm 0.5^{\circ}\text{C}$ is assumed.

Table 9.5: Drift type error sources during the duration of calibration

Drift type error	Bound
LCR retardance drift (temperature)	± 0.01 radians
Detector gain drift (temperature)	$\pm 2\%$
Laser power drift (temperature)	$\pm 0.5\%$

Unlike the systematic and random noise sources associated with calibration, it is possible to minimize the magnitude of drift type sources by taking extra care to maintain stable conditions throughout the calibration process. To stabilize temperature and associated effects, the instrument and calibration equipment were warmed up overnight with the room temperature maintained as constant as possible. Calibration was performed in a large open room to reduce localized buildup of heat from the equipment. Monitoring during calibration indicated that the temperature varied between 77.2°F and 78.3°F, giving a small 0.25% change in LCR retardance. The temperature at calibration is most likely higher than the temperature encountered in a physician's office, but is the best which could be achieved given the limited ability to control temperature in the room. The laser power varied by less than 0.5% during calibration. The drift in detector gain is substantial even for very small temperature change, and so the analyzer calibration set was recorded as quickly as possible to minimize error due to this effect. Analyzer data is recorded in a different sequence than in typical GDx-MM operation to allow a large amount of data to be acquired in the minimum amount of time. Data is collected for many rotating waveplate positions at each polarimeter setting (during which time the detector has nearly constant gain), but when constructing the instrument matrix the data is reassembled in terms of multiple polarimeter settings for each waveplate position (during which time the detector has drifted considerably). As there is no external method of monitoring the detector gain, it is not possible extract this error from the data.

As drift type errors are spread unevenly throughout the calibration dataset, it is difficult to estimate their effect on later measurement of a sample. The effect of LCR retardance drift should have a form similar to Figure 9.2, but lesser magnitude since the temperature variation during calibration is a factor of 10 smaller. The effect of detector gain drift is more difficult to predict. Graphs of analyzer calibration intensity profile comparing model calculation to measured data (Figure 9.3), show that there are discontinuities in the measured data (on the order of a few percent) which are due principally to detector gain drift. The error in reconstruction of M due to detector drift is probably also a few percent.

Figure 9.3: Evidence of detector gain drift in the calibration dataset. (a): theoretical model prediction of measured intensity profiles across the first 36 polarimeter states for different settings of the external polarization state generator. (b): actual measured intensity showing discontinuities due mostly to temperature-induced drift in the detector gain.

To assess polarimeter state drift over time, the GDx-MM was set to a fixed state and the output Stokes vector was measured several times over a period of one hour by an external Stokes polarimeter. The trajectory on the Poincaré sphere is shown in Figure 9.4, and demonstrates that the polarization state of the system drifts measurably, on the order of a few percent.

Figure 9.4: Drift in output Stokes vector over one hour, plotted on the Poincaré sphere. Progression is from red at $t = 0$ to blue at $t = 1$ hour.

Finally, it must be mentioned that the Stokes polarimeter and Stokes vector generator utilized to perform the calibration are imperfect, and so introduce further inaccuracy into the calibration. As these systems have high quality components and see frequent use in a polarimetry laboratory, it is reasonable to assume that the error introduced is of the same order as or less than that of the GDx-MM polarimeter itself. No attempt has been made to analyze the error contributed by these instruments.

Assuming conditions (temperature, motor positioning) are similar between calibration and use, the largest contribution to error from the calibration process is drift of system properties, which may introduce error of up several percent. If conditions are not the same between calibration and use, additional error is introduced with bounds as given in Tables 9.3 and 9.4. It would be possible to reduce the error due to temperature variation by calibrating at several temperatures, or by measuring the LCR temperature profiles and calculating an adjustment to the instrument matrix as described in Chapter 4. As the accuracy of the GDx-MM was judged to be acceptable for proof of concept, these more complicated techniques were not pursued.

9.5: Conclusions

The major error sources in the GDx-MM polarimeter are: uncertainty in rotating waveplate motor position; LCR retardance drift with temperature; and detector noise. Given these errors, the worst case polarimetry measurement error is predicted to be on the order of 10%. When calibration errors are present the typical error seen will be closer to the high end of this error bound. While the worst case error is larger than desired, typical performance is expected to be better. In any case this level of error is judged to be acceptable for this proof of concept instrument. The main sources of error, in particular the waveplate motor drive and LCRs, could be mitigated or eliminated by improved design in a future version of the instrument.

CHAPTER 10: USE MODEL AND DATA PROCESSING

10.1: Introduction

There are many steps between collection of a raw dataset and presentation of polarization parameter images. Although these steps would ideally be automated, for this proof of concept instrument manual post-processing was done using a variety of specialized software applications. In this chapter the entire process is described, including data collection with a human subject, assembling the raw data into an image set, collapsing the image set into a Mueller matrix through use of calibration data, and extraction and presentation of polarization parameters images.

10.2: Use with human subjects

To understand the data reduction process it is helpful to understand how raw retinal polarization data is obtained. The GDx-MM consists of the original GDx (a wheeled cart holding scan head with chin and forehead rest, a computer “GDx computer”, monitor, keyboard, and mouse), plus an additional wheeled cart holding an electronics box and another computer “DAQ computer”. Use of the instrument is by a trained operator in a manner similar to that used for the original GDx. The operator runs the original GDx control application on “GDx computer” as well as the GDx-MM control application on “DAQ computer”. A KVM box is used for convenience to switch the monitor, keyboard, and mouse between the two computers. The human subject places his or her chin on the chin rest. The operator turns on the scan head and positions it approximately in line with the subject’s eye. A ring light is centered and focused on the subject’s cornea to establish

coarse placement of the scan head. The subject is then requested to look at the red scan pattern, adjusting head position until it seems as bright as possible (Figure 10.1). There should now be a vascular pattern visible in the front LCD panel. The operator adjusts the focus knob to bring this pattern to good focus. Better focus is obtained with assistance from the subject in cases where he or she is able to distinctly resolve the the scan pattern and provide feedback to the operator. The LCD image is fine tuned by moving the scan head slightly to optimize alignment with the subject's pupil, thus minimizing vignetting by the pupil. The subject may be asked to shift his or her center of vision to various portions of the scan pattern to image different regions of the retina. Once alignment is considered optimal, the operator presses the Start button, and the GDx-MM acquires data over the next four seconds. During this time the subject must keep their visual focus as constant as possible and attempt to refrain from blinking and head movements.

Figure 10.1: Use of the GDx-MM by an operator, and correct positioning of the subject on the chin rest.

Immediately following the capture of data an overlay of the first 20 recorded images is available for viewing on the “GDx computer” display. This overlay indicates data quality, in terms of brightness, focus, centering, and eye movement, assisting the operator to determine whether the dataset is acceptable or should be repeated. A Labview application may also be run to display average dataset intensity as a quality metric. An average intensity in counts of 300-400 (on a scale of 1-1024) is optimal (neither dark nor saturated), although 200-300 and 400-500 are also acceptable.

The Labview software application which runs the GDx-MM is shown in Figure 10.2. The front panel allows for modification of many system parameters, but these parameters are initialized to their preferred values at startup and are typically not modified by the operator. The application performs system initialization including measuring the resonant scanner frequency, setting the timebase to a multiple of this frequency, moving the rotating waveplate to its home position, and driving the liquid crystal retarders to their first retardance values. The operator is then prompted for a filename for data storage. The software then waits for a press of the Start button to initiate the timing and data collection sequence. When the sequence finishes, the position of the gain knob, the laser power, and the ambient temperature are recorded. The data file contains a header with system information and 64 Mbytes of recorded data (32 Mbyte from each of the two detectors).

Figure 10.2: Main control panel for the GDx-MM.

10.3: From raw data to image stack

The 32 Mbyte data stream from the co-polarized detector is reassembled into a stack of 72 images, one for each polarization state. Due to the structure of the timing sequence, lack of exact lock between scanner and acquisition frequencies, and the need to interleave forward and backward scans, this is not a trivial task. In this section the recipe for transforming a single data stream of 16 million 2-byte numbers into a set of 72 two dimensional images is presented. To begin, the following parameters are known from timing structure: size of each image in pixels, approximate length of data stream before

first image starts, approximate size of dark region between images in pixels, and which of the 107 collected images are discarded to form the final set of 72 (images collected while the LCRs are settling are discarded). Because the scanner frequency is not locked to the data acquisition frequency, there are slight variations in parameters between image sets, and so parameters are manually determined on an image set by image set basis.

Image stack generation is performed by a custom Labview application (Figure 10.3). The process may be divided into six steps. In Step 1 (discard initial region), 200 points are deleted from the beginning of the stream. In Step 2 (coarse alignment), the data stream is reshaped into 107 individual blocks, each of which contains the data for one image. In each block the front scan is separated out from the back scan, and a fixed number of points is removed between images to provide coarse registration. In Step 3 (fine alignment), the back scan is flipped over and skewed by an adjustable angle which is fine tuned to give best registration. In Step 4 (remove images), those images recorded during dead time (i.e. motor turning and liquid crystals settling) are removed, leaving 72 images. In Step 5 (background subtraction) the mean of the dark band (laser blank time) of each image is calculated and subtracted from each pixel. In Step 6 (region selection) the central region of interest (typically 200 x 200 pixels) is selected, and the 72-image stack is saved to a file.

Figure 10.3: Labview application for converting raw data stream of 32 Mbytes to an image stack of 72 images.

The images in the stack have mutual registration to within $\pm 1/2$ pixel over the field of view of the instrument. However, in practical use it is common for eye motion and blinking to give misregistration or very low intensity in some images. A custom Labview application addresses these issues to improve the quality of the image stack (Figure 10.4). Manual transformations may be applied to any image, including: translation right/left in one pixel increments, translation up/down in one pixel increments, and rotation by a variable angle about a variable rotation center. Resulting misregistration error is ± 1

pixel. Images which are dark due to blinking or overly skewed due to eye movement are flagged and do not contribute to the polarization analysis. Better registration could be obtained with a more sophisticated automated algorithm, not developed for this work. The effect of misregistration on polarization accuracy is discussed in Chapter 12.

Figure 10.4: Labview application for registration of the 72 images in the dataset.

10.4: From image stack to polarization images

The instrument matrix derived from the calibration set is used to produce a Mueller matrix image from the 72-image stack. This processing is performed in Mathematica [Wolfram Research]. First, any images which have been flagged as unacceptable (for example due to patient blinking, excessive eye movement, etc.) are removed from the dataset, and the corresponding rows of W are removed. Then for each pixel the data vector is assembled and combined with W to give a Mueller matrix M . The result is a Mueller matrix at each of the 40,000 pixels in a 200 x 200 pixel image. Each M is converted to a physical matrix M' using the “fast physical” process described in Section 3.7. The Frobenius error between each M and M' is calculated, and all pixels having error larger than 0.25 are excluded from further processing. A Mueller matrix image may now be assembled; this image consists of 16 images, each of which shows the per-pixel value of one of the 16 elements of the Mueller matrix. An example is shown in Figure 10.5. Insight into polarization properties can be gained by examination of the Mueller matrix image as discussed in Section 3.3.

Figure 10.5: Example Mueller matrix image, showing each element of the Mueller matrix on a per-pixel basis. Element m_{00} shows intensity, and the other elements are normalized to m_{00} and have relative intensity given by the scale at right.

To extract individual polarization parameters each Mueller matrix is decomposed into diattenuation, retardance, and depolarization component matrices per the Lu-Chipman algorithm. Polarization properties are then extracted from the appropriate component matrix as discussed in Section 3.4. The end result is a set of polarization parameter images which show retinal polarization properties on a per-pixel basis. An example image (for linear retardance) is shown in Figure 10.6. The full process of converting raw data into polarization images is summarized in Figure 10.7.

Figure 10.6: Example polarization parameter image, showing a parameter (here linear retardance) on a pixel-by-pixel basis. Bright red points are regions of excessively high error which are masked from data processing.

Figure 10.7: Summary of the process of converting the 72-image stack into a polarization parameter image: generating a Mueller matrix at each pixel, finding the closest physical Mueller matrix, decomposing the physical Mueller matrix, then extracting the polarization property of interest.

10.5: Conclusions

In this chapter the methods for processing raw data into polarization parameter images have been reviewed. This process is straightforward and able to consistently generate good quality polarization parameter images. However, as there are many steps requiring manual intervention and a significant amount of computer processing, the entire process from collection of the raw data to creation of polarization parameter images is estimated to require about 30 minutes per dataset. With automated software algorithms (in particular for image assembly and co-registration) this time could be reduced significantly.

CHAPTER 11: INSTRUMENT VALIDATION

11.1: Introduction

As a wide range of errors in the polarimeter and the calibration process affect GDx-MM performance, it is important to obtain a measure of accuracy in polarization parameter measurement. A validation process was performed to assess typical accuracy and repeatability. In this process several high quality polarization targets with well known properties were measured. An analysis of the collected data quantified the accuracy and repeatability of the instrument in measuring various polarization properties.

11.2: Validation Hardware

Because the GDx-MM is a scanning instrument which measures back-reflected light, there are special considerations in validation design. Because targets must be measured in reflection, a reflective element (mirror) is placed at the retinal plane and transmissive test targets are placed between the instrument and the mirror. In this configuration targets are measured double pass. A lens system (Figure 11.1) provides a telecentric scan, allowing specular reflections from the mirror to return along the same path and into the collection aperture of the instrument. Ideally this lens system would cause only minimal change to focal point location and spot size at focus. As the targets to be measured are uniform over the extent of the scan pattern these are not strong requirements. A compact lens system using off-the-shelf components was designed, consisting of a plano concave lens with -30 mm focal length (Thorlabs LC2679) and an achromatic doublet with +30 mm focal length (Thorlabs AC254-30). With the system

placed at the GDx-MM external pupil to prevent translation of the focal point, the telecentric scan extent is about 12 mm. A Code V simulation indicates that this system performs well except for high field curvature. Spot size at the retinal plane is about $40\text{ }\mu\text{m}$ at center scan and $500\text{ }\mu\text{m}$ at edge of scan, considered adequate for measuring uniform targets.

Figure 11.1: Code V model of the GDx-MM outgoing path including two-lens telecentric system, for scanner position giving maximum field of view on the retina.

To mount the mirror, targets, and lens system coaxial with the scan head optic axis, a validation rail was designed as shown in Figure 11.2. This rail consists of a commercially available dovetail optical rail with several rail carriers and holders for optical elements. A custom bracket mounts the rail directly to the scan head. The validation targets are as follows:

1. A Polarcor polarizer (Corning Inc. Corning, NY) in a rotation mount for measurement of diattenuation parameters as a function of orientation
2. A high precision birefringent polymer quarter wave retarder (Meadowlark Optics, Frederick, CO) in a rotation mount for measurement of retardance parameters as a function of orientation
3. A liquid crystal variable retarder (Bolder Vision Optik, Boulder, CO) in a fixed mount for measurement of retardance parameters as a function of magnitude
4. A 99% Spectralon reflectance standard (Labsphere, North Sutton, NH) in a fixed mount for measurement of depolarization

Figure 11.2: The validation rail mounted on the scan head. A: rail mount; B: telecentric lens system located at system external pupil; C: polarization target; D: mirror on tip/tilt stage.

The alignment of the validation rail is as follows. Lens 1 of the telecentric system is positioned at the system external pupil. Lens 2 is positioned to make the scan as close as possible to telecentric. With the scanners disabled, the laser beam is centered and focused on the mirror. A 0.5 neutral density filter is mounted near the lens system to bring the return signal within the dynamic range of the detector. The scanners are enabled, and the mirror is tipped/tilted until normal to the the optic axis as indicated by a bright central spot visible on the LCD viewer.

11.3: Validation Measurements

Each validation target was mounted in turn on the validation rail, and datasets were collected using the procedure described in Chapter 10. The front gain knob was adjusted to give an appropriate gain setting (images neither dark nor saturated), and the optical components on the rail were tilted slightly as necessary to avoid back-reflections. The following validation measurement sequences were performed:

1. Polarizer: orientations of 0° to 90° in steps of 15°
2. Quarter wave plate: orientations of 0° to 90° in steps of 15°
3. Liquid crystal retarder: fixed orientation of 60° and variable retardance of $3/16$ to $12/16$ wave in steps of $1/16$ wave
4. Air (all targets and mirror removed): one dataset
5. Spectralon (all targets and mirror removed): one dataset
6. Polarizer: 10 identical datasets recorded at orientation of 45°

Each of the 72 images in the processed image stack for a given dataset is approximately uniform with average intensity dictated by the polarization properties of the target. A 20x20 pixel uniform region of each image was selected and averaged to reduce the effect of noise. This process gives a 72-value intensity vector P_{meas} for each dataset. The measured sample Mueller matrix M_m was then calculated using P_{meas} and the calibrated instrument matrix, and converted to a physical matrix M' . Polarization parameters were then extracted from M' . An ideal intensity vector P_{ideal} was calculated using the ideal Mueller matrix M and the calibrated instrument matrix. Metrics for evaluating polarimetry accuracy for each target include: difference between P_{meas} and P_{ideal} ; Frobenius difference between M and M_m and between M_m and M' ; and difference between measured and ideal polarization parameters. The results for each target are discussed in the following paragraphs.

Set 1: Polarizer with variable orientation

An overview of P_{meas} and P_{ideal} for all polarizer orientations is shown in Figure 11.3; the 15° orientation plot is shown individually in Figure 11.4. The measured and ideal plots are similar, but there are small phase offsets and differences in magnitude. The Frobenius distance between M and M_m was 13% to 17%, and between M_m and M' was 3% to 7%. Diattenuation magnitude (ideally 1), diattenuation orientation (ideally linear from 0° to 90°), and depolarization index (ideally 0) are shown as a function of polarizer orientation in Figure 11.5. These figures show -0.05 ± 0.03 error in measurement of diattenuation magnitude, a systematic $-9.9^\circ \pm 0.9^\circ$ error in measurement of diattenuation

orientation, and 0.05 ± 0.03 error in measurement of depolarization index. Compared to the worst case error prediction, diattenuation magnitude and depolarization index error are smaller. Diattenuation orientation error is about twice as large as the maximum predicted error. Given that the error is twice as large as expected, the motor start position error was probably two steps rather than one step as assumed in the error analysis.

Figure 11.3: Intensity profiles over the 72 polarimeter states for a rotating linear polarizer target. (a): measured intensity; (b): ideal intensity

Figure 11.4: Comparison of ideal and measured intensity profiles for polarizer target at 15° orientation. Error is suspected to be due to incorrect rotating retarder home position.

(a)

(b)

(c)

Figure 11.5: Error in polarization parameters for a rotating linear polarizer target, as a function of polarizer orientation. (a): diattenuation magnitude; (b): diattenuation orientation; (c): depolarization index

Set 2: Quarter wave plate with variable orientation

An overview of P_{meas} and P_{ideal} for all waveplate orientations is shown in Figure 11.6; the 15° orientation plot is shown individually in Figure 11.7. The measured and ideal plots are similar, but there are small phase offsets and differences in magnitude. The Frobenius distance between M and M_m was 12% to 20% and between M_m and M' was 8% to 16%. Retardance magnitude (ideally 180°), retardance orientation (ideally linear from 0° to 90°), and depolarization index (ideally 0) are shown as a function of orientation in Figure 11.8. As measurements were double pass, the measured retardance is one half wave rather than one quarter wave. These figures show -0.07 ± 0.05 rad error in measurement of retardance magnitude, a systematic $-3^\circ \pm 2^\circ$ error in measurement of retardance orientation, and a small 0.01 ± 0.01 error in measurement of depolarization index. These results are better than predicted for a half wave plate, and more similar to predictions for a quarter wave plate. The systematic error in orientation is consistent with a motor start position error.

Figure 11.6: Intensity profiles over the 72 polarimeter states for a rotating quarter wave plate target. (a): measured intensity; (b): ideal intensity

Figure 11.7: Comparison of ideal and measured intensity profiles for quarter wave plate target at 15° orientation

(a)

(b)

(c)

Figure 11.8: Error in polarization parameters for quarter wave plate target as a function of wave plate orientation. (a): retardance magnitude; (b): retardance orientation; (c): depolarization index. Retardance magnitude is one half wave rather than one quarter wave due to the measurement being double pass.

Set 3: Variable liquid crystal retarder

The results for this target are qualitatively similar to the quarter wave plate results. The Frobenius distance between M and M_m was 17%, and between M_m and M' was 8% to 16%. Double pass retardance magnitude (ideally linear from $3/8$ to $12/8$ waves in $1/8$ wave steps), retardance orientation (ideally constant at approximately 60°), and

depolarization index (ideally 0) are shown as a function of retardance in Figure 11.9.

These figures show a 0.09 ± 0.1 rad error in measurement of retardance magnitude, a $-1^\circ \pm 3^\circ$ error in measurement of retardance orientation, and a small 0.02 ± 0.01 error in measurement of depolarization index. The measured error is lower than predicted by the worst case error analysis.

Figure 11.9: Error in polarization parameters for variable wave plate target, as a function of waveplate retardance. (a): retardance magnitude; (b): retardance orientation; (c): depolarization index. Measured retardance is twice the set waveplate retardance due to the double pass measurement.

Set 4: Air

The measured intensity for open air should ideally be zero in all 72 images. However, as shown in Figure 11.10, there was a small measured intensity of 11-28 counts (where a typical intensity is 300-500 counts), which varies with polarimeter state. This effect is due to coherent normal incidence back-reflections from the optics in the GDx-MM.

Linearly polarized laser light becomes elliptical on passing through the generator LCR, with ellipticity dependent on LCR retardance. Back-reflections (particularly from the telescope lenses) preserve the polarization state and are separated by the polarizing beamsplitter to the two detectors according to the ellipticity. Thus there is a different fixed offset in the detectors for each of the liquid crystal settings. The effect of this error is predicted to be small, about 0.05 in depolarization index and 0.03 in diattenuation.

Figure 11.10: Air profile, showing a small variation in intensity due to normal incidence back-reflections from the GDx optics (on the order of 10-30 counts, where a typical intensity measurement is 300-500 counts).

Set 5: Spectralon

As Spectralon is a highly reflecting and depolarizing target [194] the measured intensity should have a constant high value in all 72 images (as Spectralon is not completely depolarizing a small intensity variation would be expected). However, as shown in Figure 11.11, there is a significant intensity variation of up to 100 counts (about 20% of the signal) which depends on both liquid crystal retardance and waveplate orientation. This effect is due to coherent back-reflections from the GDx-MM optical components. The effect is more pronounced than with the air measurement as the return light from the target generates additional back-reflections. The error in measurement of depolarization index due to this intensity variation is about 0.2, very significant. No practical method was identified to reduce this error, but given its significance methods of reducing or eliminating back-reflections should be implemented in a future redesign.

Figure 11.11: Spectralon profile, showing an up to 20% variation in intensity with polarization state. Variation is due to back-reflections from the GDx optics as well as incomplete depolarization by the spectralon.

Set 6: Ten identical measurements of polarizer

For this study, ten identical datasets of a linear polarizer oriented at 45° were collected.

Prior to each measurement, the scanners were off for a period of three minutes. A further measurement was recorded after the scanners had been operating continuously for 10 minutes. As shown in Figure 11.12, the magnitude of the intensity vector changes by 0.4% to 2.2% from measurement to measurement, but the shape changes little. This suggests that the detector gain drifts significantly between measurements (3 minutes), but not significantly during the course of one measurement (4 seconds). The standard deviation over the eleven datasets was: for diattenuation magnitude, 0.02; for diattenuation orientation, 0.9° ; and for depolarization index, 0.01; indicating that repeatability is on the order of 1-2%.

Figure 11.12: Measured intensity profiles over the 72 polarization states for a linear polarizer oriented at 45° . Variability in magnitude is significant, but variation in normalized shape is small, giving good repeatability of 1-2%.

11.4: Conclusions

The validation results quantify the accuracy of the GDx-MM in the measurement of common polarization parameters, including diattenuation magnitude and orientation, retardance magnitude and orientation, and depolarization index. Based on comparison with error analysis predictions, it appears that the most significant errors are a difference in motor start position between calibration and use, and to a lesser extent LCR retardance variation due to a temperature difference between calibration and use. The maximum depolarization index error of 0.05 is consistent with the error analysis prediction, implying that polarization properties must be reasonably consistent across the aperture and not affected significantly by (for example) liquid crystal retarder variations and polarization effects in the mirror coatings. The measured accuracy based on validation testing is summarized in Table 11.1.

Table 11.1: Validation measurement results

Parameter	Error analysis worst case error prediction	Validation error measurement
Diattenuation magnitude	-0.25	-0.05 ± 0.03
Diattenuation orientation	-6°	$-9.9^\circ \pm 0.9^\circ$
Retardance magnitude	0.15 rad	$\pm 0.09 \pm 0.1$ rad
Retardance orientation	-5°	$-3^\circ \pm 2^\circ$
Depolarization index	0.2	0.05 ± 0.03

CHAPTER 12: HUMAN SUBJECT USE AND DATA ANALYSIS

12.1: Introduction

To demonstrate the functionality of the GDx-MM, retinal polarization data was collected on several normal human subjects. First, data was collected at the University of Arizona in December 2006 over a wide field of view on the retina on both eyes of two subjects. After delivery to Indiana University School of Optometry, researchers there under the direction of Dr. Ann Elsner collected data in the spring of 2007 on the ONH and macula regions of the right eyes of 15 subjects. The two subjects from the University of Arizona are principal researchers on this project and do not require approval to participate. At Indiana University this project has full board approval from the Indiana University Bloomington Institutional Review Board, and all subjects received training and provided informed consent. In this chapter the raw data as well as retardance, diattenuation, and depolarization images are presented and discussed. Retardance images require post-processing to compensate for anterior segment birefringence; several processing methods and their relative merits are analyzed. Error due to noise and misregistration is quantified, and its effect on accuracy is discussed. Linear retardance images are compared to images of the same eyes obtained at Indiana University using a Laser Diagnostics GDx to illustrate the differences in results. The characteristics of each polarization parameter image are discussed in terms of the relationship to retinal structure and the potential value of each parameter image in retinal disease diagnosis. Of particular interest are the retardance images, which may be used to indicate the health of

the nerve fiber layer and Henle fiber layer, and the depolarization images, which may be used to identify regions of cellular disruption.

12.2: Human subject interface issues

To properly align and focus the GDx-MM to a subject's retina the assistance of the subject is conducive to obtaining good results. The best images were obtained when the subject adjusted his or her eye position to make the scan pattern appear brightest, and adjusted the focus knob to make the scan lines appear sharpest. The subject's pupil would on occasion vignette part of the beam (often from high sensitivity to 780 nm), leading to uneven illumination. Polarization imaging calculations are not affected if the vignetting remains constant throughout the measurement, but the signal to noise ratio is lower. The most persistent problem is saccadic eye movement and blinking during the four second measurement. Such motions affect the polarization computation, but may be compensated when not extreme by selective image removal. Some eye movement was seen in every dataset, showing that it is quite difficult to keep one's eye still for four seconds. A future redesign of the instrument should implement a shorter data collection time (under 2 seconds), and provide a more effective method for alignment to the eye. In a few eyes the processed datasets exhibited oval-shaped artifacts with rapidly varying intensity as a function of polarization state. It is conjectured that these artifacts are caused by a tear film. As the underlying retinal polarization was obscured, these datasets were not usable. At this stage of GDx-MM development it is necessary to record several datasets of the same subject to obtain one or two that are bright and well focused.

The data collected at the University of Arizona shows polarization properties over a large retinal region, obtained by superimposing into mosaics several different measurements recorded with the subject centering his or her visual field on different regions of the scan pattern. For example, with the scan pattern in the center of the subject's field of view the macula is imaged, and with the scan pattern in the upper left corner of the subject's field of view the optic nerve head is imaged. It can be somewhat difficult for the subject to fixate anywhere but in the center of the pattern, so a small target was often placed on the wall behind the operator to help focus the subject's visual field. In the end many very good datasets were obtained, and many lower quality sets were discarded. The success rate for good quality measurement with a well trained operator and a cooperative subject was about 50-75%.

Section 12.3: From image stacks to Mueller matrix images

The raw data analyzed in this chapter consists of right eye and left eye data from two normal University of Arizona subjects, identified as Subjects A1 and A2, and right eye data from 15 Indiana University subjects, identified as Subjects 1-15. Subject 4 has an epiretinal membrane, and subject 14 is hyper-myopic, but otherwise all subjects are normal and free of retinal disease. The subjects are of Caucasian or Asian descent, with age in the approximate range 20 to 55 years. A typical GDx-MM raw data sequence of 72 images is shown in Figure 12.1 for ONH, and Figure 12.2 for macula. The presence of polarization information is immediately observed from the progression of region-specific intensity change. From the large discontinuities in intensity from image to image the

location of the liquid crystal retarder voltage changes are observed to be per the timing sequence in four groups of 18 images each. Within groups of 18 there is a continuum of smooth intensity change from image to image. Groups 1 and 2 (images 1-18 and 19-36) are the best balanced in terms of larger variations in intensity and overall high brightness, and so these groups are probably most effective in the reconstruction. Group 3 has overall lower intensity, and group 4 has overall lower intensity variation. This is potentially useful information for a future redesign of the system. A rotating bowtie-shaped intensity pattern can be seen most clearly in Group 1; this feature will be discussed in Section 12.4. Composite intensity images (i.e. the average of all 72 images) for ONH and macula as shown in Figure 12.3 allow visualization of specific features such as blood vessels.

Figure 12.1: Raw data sequence for ONH. Data for Subject 3.

Figure 12.2: Raw data sequence for macula. Data for Subject 3.

Figure 12.3: Mean intensity images (a): ONH and (b): macula. Data from Subject 3.

Representative Mueller matrix images (“MM images”) for ONH and macula are shown in Figure 12.4. In these images bright red corresponds to +1, bright blue corresponds to -1, and dark colors are near zero. The first row and column are mostly dark, demonstrating that diattenuation and polarizance (respectively) are low but nonzero. The diagonal elements are all near +1, showing that depolarization is low. A bowtie pattern for both ONH and macula is clearly visible in the off-diagonal elements in the second through fourth rows and columns, demonstrating that there is a radial retardance variation (as expected due to fiber orientations) in these areas of the retina. The bowtie pattern and its usage in compensating for anterior segment retardance will be discussed in the next section.

Figure 12.4: Example Mueller matrix images for Subject 12, showing each element of the Mueller matrix on a per-pixel basis. Element m_{00} shows gray-scale intensity, and the other elements are normalized to m_{00} and have relative intensity given by the scale at right. (a): ONH; (b): macula.

A useful metric for polarization data validity is the Frobenius error when converting from measured to physical MM. Typical Frobenius error images are shown in Figure 12.5, where a cutoff value of 0.25 (i.e. 25% difference between measured and physical) has been arbitrarily selected to indicate excessive error. A typical mean error is on the order of 0.1. High error in blood vessel regions occurs for two reasons: first, the signal to noise ratio is low because much of the light is reflected specularly at an angle which is outside the collection solid angle; and second, imperfect image registration gives higher error at regions of high intensity gradient such as vessel boundaries. The higher error in the central circular regions is due to laser back-reflections inside the instrument from optical interfaces near normal incidence. Higher error along the edges of some images is due to image translations in the co-registration process, resulting in some regions which lack data from one or more of the 72 images. In all polarization component images presented in this chapter regions of excessive error are bright red, and the values at the corresponding pixels are excluded from statistical calculations.

Figure 12.5: Typical Frobenius error images, showing high error (greater than 25%) in bright yellow (typically regions of blood vessels and the central back-reflection spot), and low error in bright blue. Data from Subject 3. (a): ONH; (b): macula.

Section 12.4: Anterior segment compensation

As discussed in Chapter 5 the anterior segment (AS), in particular the cornea, may have considerably higher retardance than the retina, and so it is necessary to compensate for AS retardance (ASR) in each dataset to obtain accurate retinal retardance images.

Methods for AS compensation used in the past have included external polarization elements to “neutralize” the ASR so that it does not appear in the dataset (GDx [169] and GDx-VCC [195]), and mathematical methods to extract the ASR from the dataset after it has been collected (GDx-ECC [181]). Given that ASR is by design incorporated into GDx-MM data, and given that a direct measurement of ASR (for example by viewing the fourth Purkinje image) is not possible here, it is necessary to estimate the ASR and then extract it as a post-processing step. There are several complicating factors which make this process difficult. The ASR profile depends on two variables, retardance magnitude and orientation, which must both be accurately known as errors of only a few percent can measurably affect the compensation. The ASR profile may vary significantly across the region of the cornea through which the probing light passes, depending on the size of this region and its location on the cornea. Regions off the central cornea have higher spatial retardance variation [65], and there are additional distorting effects due to the inclination and declination of the corneal surface with respect to the probing beam [26]. The retinal tissue also exhibits structural-based retardance variations which complicate ASR estimation. Two methods have been developed in previous work to estimate ASR, though with variable results: the “bowtie” method, and the “screen” method. These methods are discussed in this section and extended for use with GDx-MM data.

The experimental basis for the bowtie method was established by Klein Brink and van Blokland [26]. They used a Mueller matrix ellipsometer to irradiate retinal fields in an annular area around the fovea, taking care that the entry and exit positions at the cornea remained unchanged. A Fourier analysis of the data showed a large invariant corneal term, a smaller corneal term varying with direction through the cornea, and a small retinal term on the second harmonic. The retinal term was ascribed to the radial Henle's fiber layer. This type of analysis to separate the corneal and retinal components of the retardance profile was later adapted by Zhou and Weinreb for use with the GDx-VCC [78]. Retardance images of a normal macula exhibit a distinct bowtie-shaped pattern (i.e. the second harmonic pattern observed by Klein Brink and van Blokland), which can be modeled as the overlay of the AS as a uniform linear retarder with fixed retardance magnitude and fixed axis, and the retinal Henle layer as a uniform linear retarder with fixed retardance magnitude and radial slow axis centered on the fovea. Where the axes of the two retarders are aligned, the overall retardance is a maximum equal to the sum of the two retardances, and where the axes are orthogonal the overall retardance is a minimum equal to the difference between the two retardances. It is straightforward to model the two retarders using Mueller matrices; the calculated retardance magnitude through the cascade then exhibits the bowtie pattern. A sample simulation is shown in Figure 12.6. Comparison to a GDx-MM uncompensated retardance image demonstrates that the model prediction and measured data are similar.

Figure 12.6: Example of a bowtie model simulation, based on measured parameters from Subject 3. A: The anterior segment has uniform double-pass retardance of 23° , and uniform fast axis orientation of -38° (52° ND). B: The Henle fiber layer has uniform double-pass retardance of 12° , and radially symmetric fast axis orientation centered on the fovea. C: The combined retardance profile shows a bowtie pattern in both magnitude and orientation. D: The measured data for Subject 3, showing good agreement with the model, particularly for the magnitude plot

Using the bowtie method, the magnitude and orientation of the bowtie lobes are determined from the retardance magnitude image. These values are then used to compute ASR magnitude, ASR orientation, and Henle layer retardance magnitude. The procedure used with the GDx-MM data is similar to that described by Zhou and Weinreb [78], and is illustrated in Figure 12.7.

Figure 12.7: General bowtie method, using macula linear retardance image from Subject 3. (a): Step 1: select a processing region centered on the fovea; the two circles drawn delimit this region. (b): Step 2: plot the retardance profile along several concentric circles within the processing region. (c): Step 3: fit higher order polynomials to the profiles, or perform a Fourier analysis.

To analyze the linear retardance image the center of the bowtie pattern is first located by visual examination. Several concentric circles are then drawn around the center point, at a continuum of radii in increments of 1 pixel (Figure 12.7a). The retardance magnitude pattern around each circle is plotted, showing the expected minima and maxima (Figure 12.7b). The intensity plots are fit to high order polynomials (Figure 12.7c). These polynomial curves are averaged together to give one composite curve, which has a sine-like form with two maxima $I_{\max}$, two minima $I_{\min}$, and a distinct phase $\theta_{\max}$. Using derivatives to find the maxima and minima, the ASR magnitude δ_{AS} , orientation θ_{AS} , and Henle layer retardance magnitude δ_H are calculated as

$$\delta_{AS} = \frac{I_{\max} + I_{\min}}{2}, \quad \theta_{AS} = \theta_{\max}, \quad \delta_H = \frac{I_{\max} - I_{\min}}{2}. \quad (12.1)$$

Ambiguities clearly occur if the bowtie lobes are not regularly spaced at 90° , which is a common occurrence. A less noisy method for evaluating the composite curve is with a Fourier analysis, in which case δ_{AS} , θ_{AS} , and δ_H are given by

$$\delta_{AS} = F_0 + \frac{F_2}{2}, \quad \theta_{AS} = \theta_2, \quad \delta_H = F_0 - \frac{F_2}{2}, \quad (12.2)$$

where F_0 is the magnitude of the DC component, and F_2 and θ_2 are the magnitude and phase of the second harmonic.

The bowtie method gives the desired ASR parameters, but can give variable results depending on the quality of the image and the character of the retinal tissue in the macula region. Elsner has observed that the Henle layer is most uniform at a visual angle of about 1° to 1.2° radius from the fovea [196]. Beyond this radius there is a spatially varying retardance contribution from the ganglion cells of the nerve fiber layer which causes asymmetries in the bowtie pattern. Thus it is advisable with the GDx-MM to restrict circle diameter to about 13-16 pixels when performing the bowtie ASR calculation. However, given that this range is narrow the result is sensitive to image noise, and may not be usable due to obscuration from the back-reflection spot. In any case the bowtie method is not usable if the bowtie is weak, distorted, or indeterminate such as in the presence of retinal neuropathies.

The screen method is an alternative method to estimate ASR which does not require the presence of a bowtie pattern. This method was first suggested by Knighton and Huang [183] for use with the GDx, and is currently implemented in the GDx-VCC instrument as an alternative to the bowtie method when the bowtie pattern is weak or indeterminate.

The basis of the method is that the retinal retardance magnitude in the macula region is sufficiently lower than that of the AS, so that δ_{AS} and θ_{AS} may be approximately given by simple averages over some portion of the macula-region retardance magnitude and orientation images. Knighton and Huang averaged over a large portion of the macula image (i.e. nearly 15°), masking only the central back-reflection spot, a narrow slice at the edges, and any saturated pixels. Since the averaging area is large the effect of noise is considerably reduced as compared to the bowtie method. However, this large averaging area contains significant retardance variability due to the nerve fiber layer. Higher accuracy might be obtained by averaging over a region within a 1.2° radius of the fovea; however this method would have higher sensitivity to noise. In this work, estimations of δ_{AS} and θ_{AS} were obtained for each macula dataset using four methods:

1. Bowtie: bowtie method with circle radii chosen to encompass the most distinct region of the bowtie
2. Fovea Bowtie: bowtie method with circle radii in the range 1° to 1.2°
3. Full Screen: screen method using the full image excluding high Frobenius error points
4. Fovea Screen: screen method using a square $1^\circ \times 1^\circ$ square region centered on the fovea

A comparison of the results obtained with these methods for six of the 15 datasets is shown in Table 12.1.

Table 12.1: Comparison of results for AS compensation methods

Method	Subject 1 δ_{AS} θ_{AS}	Subject 3 δ_{AS} θ_{AS}	Subject 4 δ_{AS} θ_{AS}	Subject 7 δ_{AS} θ_{AS}	Subject 8 δ_{AS} θ_{AS}	Subject12 δ_{AS} θ_{AS}
Bowtie	30.1 81.9	23.3 -38.3	60.3 -63.0	43.4 -58.8	33.1 -60.1	25.9 -19.3
Fovea Bowtie	27.4 79.6	21.3 -38.2	63.0 -44.0	36.4 -42.1	32.6 -61.4	23.1 -21.6
Full Screen	25.2 73.0	21.0 -43.5	58.6 -61.4	47.3 -61.4	36.3 -57.9	18.8 -24.8
Fovea Screen	18.1 73.0	20.4 -40.0	64.0 -66.9	28.1 -25.9	35.0 -70.9	18.8 -19.5

The results for the different methods are usually similar, typically within approximately 5° . However, when the image is noisy and/or the bowtie is somewhat indeterminate near the fovea, the “Fovea bowtie” and “Fovea screen” methods produce substantially different values. Each of the four ASR estimates obtained for each dataset was used to generate an ASR compensated dataset, and the appearance of the retardance images from these datasets dictated which estimate was finally selected. These processes are described in the following paragraphs.

To extract δ_{AS} and θ_{AS} from the dataset, each Mueller matrix in the composite MM image is first decomposed into diattenuation, retardance, and depolarization matrices using the Lu-Chipman algorithm [129]:

$$M = M_{dep} M_{ret} M_{diatt} . \quad (12.3)$$

New retardance matrices M'_{ret} are then constructed from the original retardance matrices

M_{ret} as

$$M'_{ret} = \left(LR \left[\frac{\delta_{AS}}{2}, -\theta_{AS} \right] \right)^{-1} M_{ret} \left(LR \left[\frac{\delta_{AS}}{2}, \theta_{AS} \right] \right)^{-1} , \quad (12.4)$$

where $LR[\delta, \theta]$ indicates a linear retarder with retardance δ and orientation θ . Finally each Mueller matrix is reassembled from the new retardance matrix and the original diattenuation and depolarization matrices as

$$M' = M_{dep} M'_{ret} M_{diatt} . \quad (12.5)$$

The composite MM image assembled from the M' matrices is the full retinal polarization image with ASR extracted.

The criterion for choice of ASR estimate (among the four available) is that the compensated linear retardance image should have a residual pattern within a 1.5° radius of the fovea that is as circularly symmetric (i.e. “donut-like”) as possible. A comparison of compensated retinal retardance images using the various estimates shows that even a

few percent difference in δ_{AS} and θ_{AS} affects the compensation measurably (Figure 12.8). For most datasets the most symmetric pattern was obtained using the Fovea Screen estimate, though where the fovea region was noisy or obscured by the central back-reflection spot the Full Screen estimate provided better results. For some datasets no methods provided an acceptable result; in this case the Full Screen estimate was used as a starting point and δ_{AS} and θ_{AS} were varied within $\pm 8^\circ$ to identify the most symmetric pattern (Figure 12.9). The final values of δ_{AS} and θ_{AS} for the 15 datasets from Indiana University were obtained as follows: Fovea Screen: 9 datasets; Full Screen: 2 datasets; Bowtie: 1 dataset; further variation with Full Screen starting point: 3 datasets. The compensated images showed clear donut patterns in 3 datasets, were at least reasonably circularly symmetric in 9 datasets, and were not very symmetric in 3 datasets. δ_{AS} ranged from 12° to 64° , and θ_{AS} ranged from -17° NU to 88° ND. The wide variety of AS compensation results among these 15 normal patients clearly illustrates the difficulty in obtaining a reliable, accurate method for AS compensation.

(a)

(b)

Figure 12.8: Various methods for anterior segment compensation, demonstrating significant differences in compensation results. Images from left to right are: no compensation, Bowtie method, Fovea Bowtie method, Full Screen method, and Fovea Screen method. (a): Subject 12. (b): Subject 8.

Figure 12.9: Varying δ_{AS} and θ_{AS} within $\pm 8^\circ$ of the values found using the screen method demonstrates the wide range of compensation obtained. Data for Subject 4.

12.5: Image Gradients and Noise Analysis

As the intensity vector for each pixel is determined across many images, random noise between adjacent pixels and/or misregistration error from image to image will decrease the accuracy. To determine the level of random noise in the datasets, an analysis process was performed on Spectralon and macula datasets as shown in Figure 12.10. First, reasonably uniform 30 x 30 pixel subregions of the image were selected. Histograms of the intensity distribution in these regions were then calculated. Each subregion was then translated to the right or upwards and subtracted from the original image,. Histograms of the intensity ratio of subtracted images to original images were then calculated.

Figure 12.10: Random noise analysis method for Spectralon (top row) and macula (bottom row). (a), (d) : original image showing subregion for analysis in red box; (b), (e): subregion of image; (c), (f): histogram of percent change in pixel intensity with vertical shift.

As seen in the intensity histogram, the measured intensity in counts is roughly equivalent for Spectralon and macula. The noise ratio, defined as the ratio of the image mean to the standard deviation, is 13 for Spectralon and 6 for macula. The retinal image is clearly noisier than the Spectralon image. This is seen most clearly in the shifted image histogram, where for Spectralon most pixels are less than 10% different from their neighbors, but for the macula a substantial number of pixels are more than 10% different and many are 10% to 40% different. Noisy variation is probably due to low signal level and the presence of laser speckle reflected from the target. For the macula, noise is also due to variation in the underlying tissue.

To estimate the effect of the observed noise on accuracy, the error analysis techniques described in Chapter 9 were employed. First a Mueller matrix eye model was assembled based on typical measured polarization parameters from GDx-MM data. The model consists of a cascade of an ideal depolarizer with depolarization index of 0.25; an ideal linear retarder with retardance magnitude of 30° and orientation of 15° ; and an ideal diattenuator with diattenuation magnitude of 0.08. An error analysis was performed assuming errors from Tables 9.1 and 9.2, but with detector noise error increased to 0.3.

The following errors are predicted:

1. Diattenuation magnitude: 0.02 ± 0.1
2. Diattenuation orientation: $5^\circ \pm 19^\circ$
3. Linear retardance: $1.2^\circ \pm 1.7^\circ$
4. Retardance orientation: $6.3^\circ \pm 1.7^\circ$
5. Depolarization index: 0.17 ± 0.08

To evaluate the effect of noise on polarization parameters directly, relatively uniform regions of macula region polarization parameter images were evaluated. Within these subregions diattenuation magnitude had standard deviation of 0.04; linear retardance had standard deviation of 7° ; and depolarization index had standard deviation of 0.07 (no uniform subregions could be identified in the orientation plots). This suggests that the level of noise present in GDx-MM images measurably limits the accuracy.

Misregistration error for the datasets using the manual registration process is ± 1 pixel. To gauge the effect of this error on polarimetry accuracy, a sub-pixel registration process was performed on one ONH dataset and one macula dataset by Steve Burns at Indiana University. In this process the co- and cross- polarized images are averaged together to decrease the dynamic range. Six 10x10 pixel subregions of the image are manually selected (in particular regions with distinct features such as bifurcated vessels), and an automated cross-correlation algorithm calculates optimal translation for each image. Accuracy is estimated at about $\frac{1}{4}$ pixel. Figure 12.11 shows a comparison of Frobenius error and linear retardance plots for the two registration methods. To quantify polarization parameter variability, the ratio of mean to standard deviation was calculated for both methods as: for linear retardance, 7.2 manual (7.4 automated); for diattenuation magnitude, 2.5 manual (2.6 automated); and for depolarization index, 4.4 manual (5.5 automated). Subpixel registration provided slightly better results overall, with particularly noticeable improvement at vessel walls where the intensity gradient is highest.

Figure 12.11: Comparison of images for different registration methods. (a): Frobenius error, manual method; (b): Frobenius error, automated method; (c): Linear retardance, manual method; (d): Linear retardance, automated method. The automated method shows particular improvement at vessel walls.

12.6: Results for Indiana University subjects

A representative subset of the collected data is presented in the following figures:

1. Linear retardance plot for six ONHs and maculas (Figure 12.12)
2. Retardance orientation plot for six ONHs and maculas (Figure 12.13)
3. Diattenuation magnitude plot for six ONHs and maculas (Figure 12.14)
4. Diattenuation orientation plot for six ONHs and maculas (Figure 12.15)
5. Depolarization index plot for six ONHs and maculas (Figure 12.16)

Figure 12.12: Linear Retardance plots for subjects 1, 3, 7, 8, 11, and 12. Bright red points indicate high error. (a): macula region. (b): optic nerve head.

Figure 12.13: Retardance Orientation plots for subjects 1, 3, 7, 8, 11, and 12. (a): macula region. (b): optic nerve head.

Figure 12.14: Diattenuation Magnitude plots for subjects 1, 3, 7, 8, 11, and 12. Bright red points indicate high error. (a): macula region. (b): optic nerve head.

Figure 12.15: Diattenuation Orientation plots for subjects 1, 3, 7, 8, 11, and 12. (a): macula region. (b): optic nerve head.

Figure 12.16: Depolarization Index plots for subjects 1, 3, 7, 8, 11, and 12. Bright red points indicate high error. (a): macula region. (b): optic nerve head.

Table 12.2 summarizes statistical calculations of linear retardance, retardance orientation, diattenuation magnitude, and depolarization index across the 15 subjects. To construct this table the mean and standard deviation of each polarization parameter was calculated for each subject over the entire image (excluding high Frobenius error points), giving 15 "image mean" and "image standard deviation" values for each parameter. The minimum value of the image mean, the maximum value of the image mean, the mean value of the image mean, and the mean value of the image standard deviation are presented.

Table 12.2: Statistical polarization parameter values for Indiana University subjects.

Parameter	Minimum	Maximum	Mean, SD
ONH mean linear retardance	23.3°	34.9°	28.4° +/- 15.5°
Macula mean linear retardance	9.2°	21.1°	17.3° +/- 9.4°
ONH mean diattenuation magnitude	0.07	0.11	0.09 +/- 0.05
Macula mean diattenuation magnitude	0.07	0.10	0.08 +/- 0.05
ONH mean depolarization index	0.21	0.29	0.25 +/- 0.07
Macula mean depolarization index	0.21	0.32	0.25 +/- 0.07

12.7: Discussion of results

Macula region linear retardance plots demonstrate the range of residual patterns observed after AS compensation. Some plots exhibit clear donut shapes surrounding the fovea, while others are less symmetric. Asymmetries beyond an approximately 10-20 pixel

radius of the fovea are to be expected due to nerve fiber layer variations. The retardance magnitude of approximately 17° is comparable to the results of other researchers [26]. ONH linear retardance plots show significant asymmetric variation. Retardance magnitude highest in superior and inferior locations and lowest in nasal and temporal regions, as expected due to thickness variation of the retinal nerve fiber layer. As AS compensation is not perfect these images represent a blend of AS and retinal retardance; however, as AS retardance is approximately constant across the field of view virtually all variation is due to the retina. The mean linear retardance averaged over the 15 subjects was $30^\circ \pm 5^\circ$, and the variation around the ONH was in the approximate range 10° to 60° , consistent with previously published results (mean retardance of $33^\circ \pm 3^\circ$, variation from 10° to 50°) [37].

In the retardance orientation images the fast axis orientation is horizontal at 12 o'clock and 6 o'clock, and vertical at 3 o'clock and 9 o'clock, with smooth variation in between. This indicates that the fast axis exhibits circular symmetry about a center point (the fovea or the ONH). The slow axis is therefore radially symmetric about this center point, as would be expected given the radial orientation of the retinal fibers in these areas. The "pinwheel" patterns in the macula region are consistently well defined and symmetric over the 15 datasets, but those about the ONH are less symmetric, probably due to irregular fiber orientation.

The depolarization index images show weak features with mean value of approximately 25% (degree of polarization of 75%). Higher depolarization of up to 40% is seen particularly in annular regions around the ONH and in localized regions near the macula. Depolarization along major blood vessels is higher than in surrounding regions; this may be due to higher scattering from vessels, but may also be a result of lower accuracy due to the lower signal to noise ratio. These results are in agreement with measurements by van Blokland and Norren (80%-90%) [74] and Bueno (67% - 92%) [31]. Localized higher depolarization in the macula has also been observed by Elsner [197].

The diattenuation magnitude images show weak features with mean value of approximately 8%, in reasonable agreement with measurements by Park et al (6%) [72], Kemp et al (2%) [19], and Bueno and Artal (10%) [73]. Diattenuation is highest superior and inferior to the ONH, suggesting that the diattenuation profile may be primarily due to the nerve fiber layer. The diattenuation orientation images (though noisy due to low diattenuation magnitude) are similar to the retardance orientation images, strengthening this theory. Diattenuation magnitude images for the macula region show variation about the fovea, which may be due to nerve fiber layer variations. The orientation images are variable, showing either no preferred orientation, localized regions of preferred orientation, or a single preferred orientation. The diattenuation profiles may, as with the retardance profiles, be influenced by the interaction with the cornea. Corneal diattenuation has been measured by Bueno and Jaronksi as 0.06 double pass on bovine and porcine corneas [53], of a similar magnitude to the measurements made in this study.

12.8: Comparison to GDx

A comparison of polarization images obtained with the GDx-MM and the GDx provides qualitative validity for the GDx-MM results, and illustrates the expected measurement differences between the two instruments. Macula and ONH data for three GDx-MM subjects was acquired at Indiana University using a Laser Diagnostics GDx with the corneal compensator removed. With the compensator removed the linear retardance data from both instruments is directly comparable, containing both anterior segment and retinal components (presuming that the region of the cornea traversed by the probe beam is similar). The GDx raw datasets, consisting of 40 images each, were processed using the algorithm published by Knighton and Huang [183]. Direct comparisons of macula and ONH data are shown in Figures 12.17 and 12.18.

Figure 12.17: Comparison between GDx-MM (top row) and GDx (bottom row) uncompensated linear retardance data for the maculas of three subjects.

Figure 12.18: Comparison between GDx-MM (top row) and GDx (bottom row) uncompensated linear retardance data for the ONHs of three subjects.

From these figures it is clear that the GDx-MM and GDx polarization images are similar. Differences in retardance measurement between the two instruments are expected as the GDx is inaccurate in the presence of diattenuation and depolarization. The GDx-MM data is noisier than the GDx-MM data, most likely due to the lower signal at the detector.

12.9: Utility of polarization parameter plots

Although no diseased patients were evaluated for the initial GDx-MM study, it is important to consider how each polarization parameter plot might provide useful information in disease diagnosis and monitoring. Conjectures can be made based on prior use of similar instruments such as the Laser Diagnostics GDx together with general knowledge of polarization effects in the eye as discussed in Chapter 2. Retardance plots

would be expected to primarily provide information about the Henle layer in the macula region and the retinal nerve fiber layer in other regions. The ASR compensated linear retardance plot gives an indication of the thickness of the nerve fiber layer, which is useful in glaucoma diagnosis and monitoring. The uncompensated linear retardance plot and the retardance orientation plot for the macula region may be used to detect disruptions of the Henle layer, which would be evidenced as disruptions in or absence of the bowtie (linear retardance) or pinwheel (retardance orientation) patterns.

Diattenuation has not been explored as a disease diagnostic, and so it will be highly informative to discover whether certain disease features will present higher contrast in diattenuation magnitude and/or orientation images. It is reasonable to conjecture that disruption of the regular tissue structure due to disease may be evidenced as features of higher or lower diattenuation, and future studies of diseased eyes should investigate this hypothesis.

The depolarization plots are likely to be of particular interest as they emphasize deeper structures from which the return light has encountered more scattering events.

Researchers at Indiana University have observed that many disease-related features have measurably higher contrast in depolarized light images. These features include drusen, pools of fluid and leakage points, vessel abnormalities, edema, exudative lesions, peripapillary hyperpigmentation, and neovascular membranes [2,82,119]. It is thus reasonable to predict that depolarization images would facilitate detection and monitoring of disease processes which disrupt the regular structure of retinal tissue.

12.10: Other polarization parameters

In the previous sections a representative subset of polarization parameters was presented and discussed. However, several other parameters may be calculated from the raw data, including: retardance (magnitude, circular magnitude); diattenuation (linear magnitude, circular magnitude); and polarizance (magnitude, linear magnitude, circular magnitude, and orientation). Images of these parameters for the macula and ONH of Subject 12 are shown in Figures 12.19 and 12.20. Mean elliptical retardance magnitude is significant, on the order of -25° . Elliptical diattenuation is small, so that diattenuation magnitude and linear diattenuation are nearly the same. Circular polarizance is significant, on the order of 0.15. The polarizance orientation plot for the ONH shows a circularly symmetric fast axis similar to the retardance and diattenuation fast axis orientation. These other parameter plots may provide interesting information about tissue structure, and should be investigated in future uses of the GDx-MM.

Figure 12.19: Other polarization parameter images for macula region of Subject 12.

Figure 12.20: Other polarization parameter images for ONH of Subject 12.

12.11: Retina Mosaics

The data collected at the University of Arizona over a wide retinal field of view for Subjects A1 (left eye) and A2 (right and left eyes) was assembled into mosaics. The data for the right eye of Subject A1 had tear film artifacts which obscured the retinal polarization properties, and so was not evaluated. Mosaics were fit together from 5-10 datasets, covering roughly 45° of visual field (Figures 12.21 – 12.23). The polarization parameters often but do not always “match up” across image boundaries, indicating a variation in the accuracy of Mueller matrix reconstruction from measurement to measurement. It is clear from the intensity images that variations in reconstruction accuracy most often correspond to dim regions, while the “match up” across boundaries is better where both images have high brightness. The depolarization index and diattenuation orientation plots are particularly sensitive to low signal to noise. Linear retardance magnitude is greatest superior and inferior to the ONH, with regions of higher magnitude following the large blood vessels emanating from the ONH. Retardance orientation pinwheel patterns are localized at the ONH and macula. The fast axis tends to be vertical in nasal and temporal regions and horizontal in inferior and superior regions, and beyond the macula and ONH the orientation varies slowly across the retina. Localized regions with a particular orientation are often framed by large blood vessels. There is little variation in diattenuation magnitude. The high noise in the diattenuation orientation plots makes interpretation difficult, but there is clearly some organized variation across the retina. Depolarization index shows little variation, but is consistently lowest near the ONH and highest near the macula.

Figure 12.21: Subject A1 left eye mosaic. (a): Intensity; (b): Depolarization index; (c): Linear retardance; (d): Retardance orientation; (e): Diattenuation magnitude; (f): Diattenuation orientation.

Figure 12.22: Subject A2 right eye mosaic. (a): Intensity; (b): Depolarization index; (c): Linear retardance; (d): Retardance orientation; (e): Diattenuation magnitude; (f): Diattenuation orientation.

Figure 12.23: Subject A2 left eye mosaic. (a): Intensity; (b): Depolarization index; (c): Linear retardance; (d): Retardance orientation; (e): Diattenuation magnitude; (f): Diattenuation orientation.

12.12: Conclusions

This chapter presents a wide range of retinal polarization data to illustrate the capabilities of the GDx-MM retinal imaging polarimeter. The polarization parameter images for the normal eyes from this study demonstrate that the GDx-MM provides clear visualization and quantification of tissue properties, highly desirable for a diagnostic instrument. An important limitation to this technology is the difficulty in accurately compensating for anterior segment retardance. Image noise and misregistration error also contribute to inaccuracy. Better data processing methods and an improved instrument design should improve the ability to compensate the anterior segment, and to provide clearer images than were obtained with this first generation instrument. Ideally a larger, well designed study including both normal and diseased eyes will be performed in the future to more fully explore the potential of the various polarization parameters for revealing tissue properties and diagnosing disease. The important achievement illustrated by the data and analysis in this chapter is demonstration of proof of concept of complete retinal polarimetry in an instrument which is adaptable to clinic use.

CHAPTER 13: CONCLUSIONS AND FUTURE WORK

13.1: Introduction

This dissertation describes the development of the GDx-MM, a complete imaging polarimeter for measurement of the polarization properties of the retina. This work was performed as part of an NIH-funded study based at Indiana University School of Optometry to explore noninvasive methods to quantitatively map visual function and structure. The three major components of this dissertation are: 1. Development of techniques for Mueller matrix polarimeter optimization and error analysis; 2. Design, build, calibration, and validation of the polarimeter; and 3. Analysis of the data collected on normal human subjects. In this chapter the major challenges and accomplishments associated with each component are reviewed, and several opportunities for improvement in future designs are identified and discussed.

13.2: Review of accomplishments

Polarimeter design is straightforward in theory, but due to the large number of degrees of freedom and the effect of error sources it is necessary to employ appropriate techniques to properly optimize a polarimeter design. This dissertation presents a number of techniques for Mueller matrix sample measuring polarimeters, including matrix rank and range, condition number, singular value decomposition, and Poincaré sphere trajectories. For the design of the GDx-MM polarimeter, some parameters were determined from visualization of trajectories on the Poincaré sphere, and others were optimized using a

condition number analysis. A condition number analysis was also used in calibration to choose the set of parameters giving the most stable instrument matrix.

An error analysis method for sample measuring polarimeters was developed with consideration for both random and systematic error sources. Error sources in the polarimeter are estimated by the first term in a Taylor series expansion of the appropriate terms in the instrument matrix. The error analysis may be used to optimize the polarimeter in the presence of error, compensate for a known systematic error, predict the error in reconstruction of a sample Mueller matrix, and determine the relative contributions of each error source to the total error in reconstruction. An error analysis for the GDx-MM polarimeter revealed that the major sources of error are liquid crystal retarder drift and incorrect waveplate positioning, with predicted worst case accuracy on the order of 10%.

Developing the GDx-MM was a long and difficult process. Reverse engineering was first needed to understand the GDx platform and form a modification plan. Changes to the optical and polarization paths included the insertion of liquid crystal retarders, replacement of the rotating waveplate, and addition of a weak lens and tilted glass plate to compensate for focal shift and translation. A new timing sequence required significant electronic modifications, including capturing a large number of images, driving the liquid crystal retarders, and driving the rotating waveplate. A data acquisition system was designed and programmed to perform the electronics tasks. The GDx-MM was

calibrated using external polarimeters, requiring measurement of a large number of states. Those states giving an instrument matrix with lowest condition number were selected for use. A validation process established that the accuracy and repeatability of the polarimeter is on the order of $\pm 5\%$. Processing the raw data into polarization parameter images is a multi-step process requiring manual intervention, due to irregularities from scanner frequency drift and eye movement. Steps include conversion of the raw data stream to an image stack; image registration; application of calibration data to obtain a Mueller matrix image; conversion to physical Mueller matrices; and extracting polarization parameters to form polarization parameter images.

Data collection and analysis for two normal human subjects at the University of Arizona and 15 normal subjects at Indiana University is the culmination of this work. The polarization parameter images reveal useful information about the tissue microstructure. For example, linear retardance images show variation about the optic nerve head correlating to nerve fiber layer thickness; retardance and diattenuation orientation images exhibit circularly symmetric variation about the optic nerve head and macula correlating to fiber orientation; diattenuation magnitude images exhibit a nearly uniform diattenuation of approximately 10%; and depolarization index images show a moderate depolarization of approximately 25%, slightly higher in the macula region. Mosaics reveal polarization properties over a larger region of the retina. The summary of measured polarization parameters presented in Table 12.2 provides an integrated view of all retinal tissue polarization properties collected together over a large sample set.

13.3: Discussion and Future work

While the GDx-MM instrument is a step toward the use of polarization in the diagnosis of retinal disease, in its current form the accuracy may not be sufficiently high to be useful for large-scale clinical studies. Many of the instrument limitations derive from the fact that it is a retrofit of an existing instrument, with the particular problem that many optical and polarization components have unknown properties and cannot be removed or adjusted without losing alignment. An original ground-up design of a new polarimeter should easily overcome many of the limitations. Possible improvements to the instrument fall into the general categories of: polarimetry; optics; electronics; calibration; data processing; user interface; and data analysis. Each of these categories will be discussed in this section.

The GDx-MM was optimized polarimetrically with the constraint that polarization components in the generator and analyzer must fit within the GDx frame and be simple to control. This led to the use of liquid crystal retarders, which are problematic due to a number of issues including strong retardance variation with temperature and angle of incidence; retardance variation across the clear aperture; low throughput; slow response time; and measurable depolarization. It was also necessary to use the existing rotating waveplate to generate and analyze a sufficient number of states in a short time, leading to error from the inaccurate stepper motor drive. A polarimeter with lower condition number could be assembled (for example) from pairs of variable retarders (rather than from a single variable retarder and a single rotating waveplate as in the GDx-MM).

Better performance would also be obtained through the use of higher quality variable retarders such as photoelastic modulators which have fast reponse and are less sensitive to temperature. Redesign of the optic path should allow polarization components to be placed in collimated space, to eliminate angle of incidence variations. Polarization-dependent back-reflections should be reduced to the extent possible, for example by the use of improved coatings or tilting some components.

To improve the electronic function a custom control system would ideally be designed from scratch. The GDx-MM use of PC-based interface cards controlled by the Labview programming language was found to be problematic, as the cards were often “glitchy” at high data acquisition rates and the timing sequence was occasionally disrupted by computer-generated interrupts. The lack of temperature stabilization and gain/offset compensation in the detector circuit was a principal source of error and must be addressed in a future design. Image quality and data processing time would be improved by locking the data acquisition frequency directly to the varying resonant scanner frequency. Several computationally intensive data processing tasks, such as applying calibration data and making Mueller matrices physical, might be integrated into the instrument’s processors rather than being performed as post-processing steps by software applications.

Better alignment and calibration techniques could be implemented in a ground-up design. The GDx-MM was limited by the need to maintain the original optical alignment even

while installing new components in the optical path, as it was considered impractical to reproduce the original alignment without development of a complicated alignment fixture. As a result, “band aid” solutions such as the weak lens and tilted glass slide were used, reducing throughput and adversely affecting polarization and imaging performance. The calibration procedure used for the GDx-MM was onerous, requiring a large amount of equipment and a long time for alignment, measurement, and post-processing. To be able to calibrate easily and reliably a new calibration procedure and fixture should be implemented allowing for quick alignment and automatic data collection and processing. It would be advisable to calibrate at a few different temperatures and adjust the calibration set to the measured room temperature at each use.

The intended audience for GDx-MM data includes scientists and researchers, as well as health care professionals using the instrument with human subjects. It is therefore important that data collection be quick, comfortable, and reliable; and that the processed data be accurate and clearly presented. The GDx-MM does well in accomplishing these goals, but a future redesign could incorporate many improvements. Patient interface and data quality could be improved by limiting measurement time to two seconds and providing a camera-based alignment aid to ensure the probing beam is normal to the central cornea and centered on the pupil. Better data analysis could include for example more effective anterior segment compensation and sub-pixel image registration. Excessive noise in the polarization parameter images was a principal limitation to accurate interpretation; a new optical design with higher throughput as well as improved

detector circuitry could significantly improve the image quality. For effective presentation of data to health care professionals, algorithms may be created for such tasks as knitting together retinal mosaics; comparing data to a normative database; and using combinations of polarization parameter images for optimal visualization of retinal tissue structure and defects.

13.4: Overall conclusions

In conclusion, a Mueller matrix imaging polarimeter has been designed, optimized, built, calibrated, validated, and used on several human subjects to generate polarization parameter images. Though polarimetry has been used in the past to investigate retinal tissue, this research presents the first instrument which generates a full polarization signature of the retina in one measurement, using an instrument which obtains data rapidly and without need for uncomfortable procedures such as pupil dilation. As this instrument was developed through modification to an existing instrument, there were several design limitations leading to lower than desired accuracy. A number of improvements have been suggested for a ground-up design which would exhibit substantially improved accuracy. The polarization parameter images produced by the GDx-MM for several normal human subjects clearly demonstrate the potential of this technology to reveal the microstructure of retinal tissue, and represent a step forward in the use of retinal imaging polarimetry in the diagnosis and monitoring of retinal disease.

Figure 13.1: Karen is very happy that the GDx-MM is finally ready to pack up for the trip to Indiana University.

APPENDIX A: ACRONYMS

AMD	Age-related Macular Degeneration	Section 2.6
APD	Avalanche Photodiode Detector	Section 7.5
AS(R)	Anterior Segment (Retardance)	Section 12.4
DAC	Data Acquisition Computer	Section 6.6
DOF	Degrees of Freedom	Section 3.3
DoP	Degree of Polarization	Section 3.3
DR	Diabetic Retinopathy	Section 2.6
DRR	Dual Rotating Retarder polarimeter	Section 4.9
DVR	Dual Variable Retarder polarimeter	Section 4.9
LCR	Liquid Crystal Retarder	Section 7.2
ND	Nasally Downward	Figure 2.4
NPBS	Non Polarizing Beamsplitter	Section 5.4
NU	Nasally Upward	Figure 2.4
OCT	Optical Coherence Tomography	Section 2.4
ONH	Optic Nerve Head	Section 2.2
PBS	Polarizing Beamsplitter	Section 5.4
PSA	Polarization State Analyzer	Section 3.6
PSG	Polarization State Generator	Section 3.6
PS-OCT	Polarization Sensitive Optical Coherence Tomography	Section 2.4
RNFL	Retinal Nerve Fiber Layer	Section 2.2
RPE	Retinal Pigment Epithelium	Section 2.2
SD	Standard Deviation	Section 4.7
SLP	Scanning Laser Polarimeter	Section 2.4
SNR	Signal to Noise Ratio	Section 7.5
SV	Singular Value	Section 4.5
SVD	Singular Value Decomposition	Section 4.5

APPENDIX B: SYSTEM SPECIFICATIONS

Illumination source	780 nm laser diode (Class 1 laser)
Optical power delivered to eye	3 mW
Image size	256 x 256 pixels
Resolution at retina	20 μm
Measurement time	4 seconds
Beam size at cornea	2.5 mm
Retinal image field	15° x 15° (4.5 mm x 4.5 mm)
Accuracy, typical: Retardance magnitude Retardance orientation Diattenuation magnitude Diattenuation orientation Depolarization index	 0.09 $\pm$ 0.10 rad 3° $\pm$ 2° 0.05 $\pm$ 0.03 9.9° $\pm$ 0.9° 0.05 $\pm$ 0.03

REFERENCES

1. R. N. Weinreb, S. Shakiba, and L. Zangwill, "Scanning laser polarimetry to measure the nerve fiber layer of normal and glaucomatous eyes", *Am J Ophthalmol* **119**, 627–636 (1995).
2. S. A. Burns, A. E. Elsner, M. B. Mellem-Kairala, and R. B. Simmons, "Improved contrast of subretinal structures using polarization analysis", *Invest Ophthalmol Vis Sci* **44** (9), 4061-4068 (2003).
3. R. S. Snell and M. A. Lemp, *Clinical Anatomy of the Eye*, (Blackwell Publishing, Victoria, Australia 1997).
4. H. Kolb, E. Fernandez, and R. Nelson, "Webvision: The Organization of the Retina and Visual System", <http://webvision.med.utah.edu>.
5. D. A. Atchison and G. Smith, *Optics of the Human Eye*, (Butterworth Heinemann, Oxford, 2000).
6. D. Pipe and L. Rapley, *Ocular Anatomy and Histology*, (ABDO College of Education, Kent, England, 1984).
7. P. N. Bishop, "Structural macromolecules and supramolecule organisation of the vitreous gel", *Prog Retin Eye Res* **19** (3), 323-44 (2000).
8. R. Wehner, "Polarized-light navigation by insects," *Scientific American* **23** (1), 106-115, (1976).
9. M. F. Moody and J. R. Parriss, "Discrimination of polarized light by Octopus", *Nature* **186**, 839-840 (1960).
10. W.K. von Haidinger, "Ueber das directe Erkennen des polarisirten Lichts und der Lage der Polarisationssebene", *Annalen der Physik*, **139** (9), 29-39 (1844).
11. W. C. McCrone, "Light microscopy", in *Physical Methods of Chemistry*. B. W. Rossiter and J. F. Hamilton, eds. (John Wiley and Sons, New York, 1991), pp. 343–443.
12. Q. Zhou and R. W. Knighton, "Light scattering and form birefringence of parallel cylindrical arrays that represent cellular organelles of the retinal nerve fiber layer," *Appl Opt* **36**, 2273–2285 (1997).
13. O. Wiener, "Die Theorie des Mischkorpers fur das Feld der stationaren Stromung," *Abh Math-Phys Klasse Koniglich Sachsichen Des Wiss* **32**, 509-604 (1912).

REFERENCES, Continued

14. F. Horowitz, "Structure-Induced Optical Anisotropy in Thin Films", PhD Thesis, University of Arizona (1983).
15. R. P. Hemenger, "Dichroism of the macular pigment and Haidinger's brushes," *J Opt Soc Am* **72**, 734-737 (1982).
16. W. L. Bragg and A. B. Pippard, "The form birefringence of macromolecules", *Acta Cryst* **6**, 865-867 (1953).
17. R. Oldenbourg, E. D. Salmon, and P. T. Tran, "Birefringence of single and bundled microtubules", *Biophys J* **74**, 645-654 (1998).
18. X. R. Huang and R. W. Knighton, "Theoretical model of the polarization properties of the retinal nerve fiber layer in reflection", *Appl Opt* **42** (28), 5726-5736 (2003).
19. N. J. Kemp, H. N. Zaatari, J. Park, H. Grady Rylander III, and T. E. Milner, "Form-biattenuance in fibrous tissues measured with polarization-sensitive optical coherence tomography (PS-OCT)", *Optics Express* **13** (12), 4611-4628 (2005).
20. X. R. Huang and R. W. Knighton, "Diattenuation and polarization preservation of retinal nerve fiber layer reflectance", *Appl Opt* **42** (28), 5737-5743 (2003).
21. B. F. Hochheimer and H. A. Kues, "Retinal polarization effects," *Appl Opt* **21**, 3811-3815 (1982).
22. B. K. Pierscionek and R. A. Weale, "Investigation of the polarization optics of the living human cornea and lens with Purkinje images", *Appl Opt* **37**, 6845-6851 (1988).
23. R. W. Knighton and X. R. Huang, "Linear birefringence of the central human cornea", *Invest Ophthalmol Vis Sci* **43** (1), 82-86 (2002).
24. A. G. Bennett and J. L. Francis, "The Purkinje images" in *The Eye: Volume 4, Visual Optics and the Optical Space Sense*, H. Davson, ed. (New York, Academic Press, 1962), pp. 108-113.
25. D. S. Greenfield, R. W. Knighton, and X. R. Huang, "Effect of corneal polarization axis on assessment of retinal nerve fiber layer thickness by scanning laser polarimetry", *Am J Ophthalmol* **129**, 715-722 (2000).
26. H. B. Klein Brink and G. J. Van Blokland, "Birefringence of the human foveal area assessed in vivo with Muller-matrix ellipsometry", *J Opt Soc Am A* **5**, 49-57 (1988).

REFERENCES, Continued

27. G. J. van Blokland, "Ellipsometry of the human retina in vivo: preservation of polarization," *J Opt Soc Am A* **2**, 72-75 (1985).
28. G. J. van Blokland and S. C. Verhelst, "Corneal polarization in the living human eye explained with a biaxial model", *J Opt Soc Am A* **4**, 82-90 (1987).
29. H. B. Klein Brink, "Birefringence of the human crystalline lens in vivo," *J Opt Soc Am A* **8**, 1788- 1792 (1991).
30. S. Y. Lu and R. A. Chipman, "Interpretation of Mueller matrices based on polar decomposition", *J Opt Soc Am A – Opt Image Sci Vis* **13**, 1106-1113 (1996).
31. J. M. Bueno, "The influence of depolarization and corneal birefringence on ocular polarization", *J Opt A: Pure Appl Opt* **6**, S91-S99 (2004).
32. J. M. Bueno, E. Berrio, M. Ozolinsh, and P. Artal, "Degree of polarization as an objective method of estimating scattering", *J Opt Soc Am A* **21** (7), 1316-1321 (2004).
33. X. R. Huang and R. W. Knighton, "Linear birefringence of the retinal nerve fiber layer measured in vitro with a multispectral imaging micropolarimeter", *J Biomedical Optics* **7** (2), 199–204 (2002).
34. J. W. Jaronski and H. T. Kasprzak, "Linear birefringence measurements of the in vitro human cornea", *Ophthal Physiol Opt* **23**, 361–369 (2003).
35. J. M. Bueno, "Polarimetry in the human eye using an imaging linear polariscope", *J Opt A: Pure Appl Opt* **4**, 553–561 (2002).
36. R. N. Weinreb, C. Bowd, D. S. Greenfield, and L. M. Zangwill, "Measurement of the magnitude and axis of corneal polarization with scanning laser polarimetry", *Arch Ophthalmol* **120**, 901-906 (2002).
37. X. R. Huang, H. Bagga, D. S. Greenfield, and R. W. Knighton, "Variation of peripapillary retinal nerve fiber layer birefringence in normal human subjects", *Invest Ophthal Vis Sci* **45** (9), 3073-3080 (2004).
38. J. M. Bueno and B. Vohnsen, "Polarimetric high-resolution confocal scanning laser ophthalmoscope", *Vision Research* **45**, 3526–3534 (2005).
39. N. T. Choplin, D. C. Lundy, and A. W. Dreher, "Differentiating patients with glaucoma from glaucoma suspects and normal subjects by nerve fiber layer assessment with scanning laser polarimetry", *Ophthalmology* **105**, 2068–2076 (1998).

REFERENCES, Continued

40. S. T. Hoh, D. S. Greenfield, A. Mistlberger, J. M. Liebmann, H. Ishikawa, and R. Ritch, "Optical coherence tomography and scanning laser polarimetry in normal, ocular hypertensive, and glaucomatous eyes", *Am J Ophthalmol* **129**, 129–135 (2000).
41. M. R. Hee, D. Huang, E. A. Swanson, and J. G. Fujimoto, "Polarization-sensitive low-coherence reflectometer for birefringence characterization and ranging", *J Opt Soc Am B* **9** (6), 903-908 (1992).
42. G. Yao and L. V. Wang, "Two-dimensional depth-resolved Mueller matrix characterization of biological tissue by optical coherence tomography," *Opt Lett* **24**, 537-539 (1999).
43. S. Jiao, G. Yao, and L. V. Wang, "Depth-Resolved two-dimensional Stokes vectors of backscattered light and Mueller matrices of biological tissue measured with Optical Coherence Tomography," *Appl Opt* **39**, 6318-6324 (2000).
44. E. Götzinger, M. Pircher, M. Sticker, I. Dejaco-Ruhswurm, S. Kaminski, C. Skorpik, A. F. Fercher, and C. K. Hitzenberger, "Imaging of polarization properties of the human cornea with phase resolved polarization sensitive optical coherence tomography", *Proc SPIE* **4951**, 21-26 (2003).
45. R. G. Cucu, A. Gh. Podoleanu, R. B. Rosen, A. B. Boxer, and D. A. Jackson, "*En face* polarization sensitive optical coherence tomography", *Proc SPIE* **5140**, 113-119 (2003).
46. M. Todorovic, S. Jiao, and L. V. Wang, "Determination of local polarization properties of biological samples in the presence of diattenuation by use of Mueller optical coherence tomography", *Opt Lett* **29** (20), 2402-2404 (2004).
47. N. J. Kemp, J. Park, H. N. Zaatari, H. G. Rylander, and T. E. Milner, "High-sensitivity determination of birefringence in turbid media with enhanced polarization-sensitive optical coherence tomography", *J Opt Soc Am A* **22** (3), 552-560 (2005).
48. M. Pircher, E. Goetzinger, O. Findl, S. Michels, W. Geitzenauer, C. Leydolt, U. Schmidt-Erfurth, and C. K. Hitzenberger, "Human macula investigated in vivo with Polarization-Sensitive Optical Coherence Tomography", *Invest Ophthalmol Vis Sci* **47** (12), 5487-5494 (2006).

REFERENCES, Continued

49. B. Cense, T. C. Chen, B. H. Park, M. C. Pierce, and J. F. de Boer, "Thickness and birefringence of healthy retinal nerve fiber layer tissue measured with polarization-sensitive optical coherence tomography", *Invest Ophthalm Vis Sci* **45** (8), 2606-2612 (2004).
50. J. M. Bueno and P. Artal, "Double-pass imaging polarimetry in the human eye", *Opt Lett* **24** (1), 64-66 (1999).
51. J. M. Bueno, "Measurement of parameters of polarization in the living human eye using imaging polarimetry", *Vision Research* **40**, 3791-3799 (2000).
52. J. M. Bueno and P. Artal, "Polarization and retinal image quality estimates in the human eye", *J Opt Soc Am A* **18** (3), 489-496 (2001).
53. J. M. Bueno and J. Jaronski, "Spatially resolved polarization properties for in vitro corneas", *Ophthalm Physiol Opt.* **21** (5), 384-392 (2001).
54. J. M. Bueno and M. C. W. Campbell, "Polarization properties of the in vitro old human crystalline lens", *Ophthalm Physiol Opt* **23**, 109-118 (2003).
55. J. M. Bueno and M. C. W. Campbell, "Confocal scanning laser ophthalmoscopy improvement by use of Mueller-matrix polarimetry", *Opt Lett* **27** (10), 830-832 (2002).
56. M. C. W. Campbell, J. M. Bueno, C. J. Cookson, Q. Liang, M. L. Kisilak, and J. J. Hunter, "Enhanced confocal microscopy and ophthalmoscopy with polarization imaging", *Proc SPIE* **5969**, 59692H-1 - 59692H-6 (2005).
57. J. M. Bueno, J. J. Hunter, C. J. Cookson, M. L. Kisilak, and M. C. W. Campbell, "Improved scanning laser fundus imaging using polarimetry", *J Opt Soc Am A* **24** (5), 1337-1348 (2007).
58. D. Lara and C. Dainty, "Double-pass axially resolved confocal Mueller matrix imaging polarimetry", *Opt Lett* **30** (21), 2879-2881 (2005).
59. D. Lara and C. Dainty, "Axially resolved complete Mueller matrix confocal microscopy", *Appl Opt* **45** (9), 1917-1930 (2006).
60. D. M. Maurice, "The structure and transparency of the cornea," *J Physiol* **136**, 263-286 (1957).
61. M. A. Jakus, "Further observations on the fine structure of the cornea," *Invest Ophthalmol Vis Sci* **1**, 202-225 (1962).

REFERENCES, Continued

62. C. C. Teng, "Fine structure of the human cornea," *Am J Ophthal* **54**, 969–1002 (1962).
63. V. Louis-Dorr, K. Naoun, P. Alle, A. M. Benoit, and A. Raspiller, "Linear dichroism of the cornea", *Appl Opt* **43** (7), 1515-1521 (2004).
64. G. Bairaktaris, D. Lewis, N. J. Fullwood, I. A., Nieduszynski, B. Marcyniuk, A. J. Quantock, and A. E. Ridgway, "An ultrastructural investigation into proteoglycan distribution in human corneas," *Cornea* **17**, 396–402 (1998).
65. M. Pircher, E. Goetzinger, R. Leitgeb and C. K. Hitzenberger, "Transversal phase resolved polarization sensitive optical coherence tomography", *Phys Med Biol* **49**, 1257–1263 (2004).
66. F. A. Bettelheim, "On the optical anisotropy of lens fiber cells", *Exp Eye Res* **21**, 231–234 (1975).
67. R. A. Weale, "Sex, age and the birefringence of the human crystalline lens", *Exp Eye Res* **29**, 449–461 (1979).
68. R. J. McNichols and G. L. Cote, "Optical glucose sensing in biological fluids: an overview", *J Biomedical Optics* **5** (1), 5–16 (2000).
69. J. S. Baba, B. D. Cameron, S. Theru, and G. L. Cote, "Effect of temperature, pH, and corneal birefringence on polarimetric glucose monitoring in the eye", *J Biomedical Optics* **7** (3), 321–328 (2002).
70. R. N. Weinreb, A. W. Dreher, A. Coleman, H. Quigley, B. Shaw, and K. Reiter, "Histopathologic validation of Fourier-ellipsometry measurements of retinal nerve fiber layer thickness," *Arch. Ophthalmol* **108** (4), 557–560 (1990).
71. A. M. Benoit, K. Naoun, V. Louis-Dorr, L. Mala, and A. Raspiller, "Linear dichroism of the retinal nerve fiber layer expressed with Mueller matrices", *Appl Opt* **40** (4), 565–569 (2001).
72. B. H. Park, M. C. Pierce, B. Cense, and J. F. de Boer, "Vector-based analysis for polarization-sensitive optical coherence tomography", *Proc SPIE* **5316**, 397-403 (2004).
73. J. M. Bueno and P. Artal, "Diattenuation of the human eye at the fovea", *Invest Ophthalmol Vis Sci* **46**, E-Abstract 2557 (2005).

REFERENCES, Continued

74. G. J. van Blokland and D. V. Norren, "Intensity and polarization of light scattered at small angles from the human fovea," *Vision Res* **26**, 485–494 (1986).
75. J. M. Bueno, C. J. Cookson, M. L. Kisilak, J. J. Hunter and M. C. W. Campbell, "Degree of polarization of light reflected from optic nerve head", *Invest Ophthalmol Vis Sci* **47**, E-Abstract 4059 (2006).
76. M. Pircher, E. Götzinger, R. Leitgeb, H. Sattmann, O. Findl, and C. K. Hitzenberger, "Imaging of polarization properties of human retina in vivo with phase resolved transversal PS-OCT", *Optics Express* **12** (24), 5940-5951 (2004).
77. G. P. Misson, "A Mueller matrix model of Haidinger's brushes", *Ophthal Physiol Opt* **23**, 441–447 (2003).
78. Q. Zhou and R. N. Weinreb, "Individualized compensation of anterior segment birefringence during scanning laser polarimetry", *Invest Ophthalmol Vis Sci* **43** (7), 2221-2228 (2002).
79. F. I. Harosi, "Microspectrophotometry and optical phenomena: birefringence, dichroism, and anomalous dispersion," in *Vertebrate Photoreceptor Optics*, J. M. Enoch and F. L. Tobey, Jr., eds. (Springer-Verlag, Berlin, 1981).
80. S. A. Burns, S. Wu, F. Delori, and A. E. Elsner, "Direct measurement of human-cone photoreceptor alignment", *J Opt Soc Am A* **12** (10), 2329-2338 (1995).
81. O. Strauss, "The retinal pigment epithelium in visual function", *Physiol Rev* **85**, 845–881 (2005).
82. A. E. Elsner, A. Weber, M. C. Cheney, D. A. VanNasdale, and M. Miura, "Imaging polarimetry in patients with neovascular age-related macular degeneration", *J Opt Soc Am A* **24** (5), 1468-1480 (2007).
83. S. Resnikoff, D. Pascolini, D. Etyaale, I. Kocur, R. Pararajasegaram, G. P. Pokharel, and S. P. Mariotti, "Global data on visual impairment in the year 2002", *Bulletin of the World Health Organization* **82** (11), 844-851 (2004).
84. D. A. Lee and E. J. Higginbotham, "Glaucoma and its treatment: A review", *Am J Health-Syst Pharm* **62**, 691-699 (2005).
85. A. Ulrich, C. Ulrich, T. Barth, and W. D. Ulrich, "Detection of disturbed autoregulation of the peripapillary choroid in primary open angle glaucoma", *Ophthalmic Surg Lasers* **27** (9), 746-757 (1996).

REFERENCES, Continued

86. J. E. Grunwald, C. E. Riva, R. A. Stone, E. U. Keates, and B. L. Petrig, "Retinal autoregulation in open-angle glaucoma", *Ophthalmology* **91** (12), 1690-1694 (1984).
87. A. Sommer, N. R. Miller, I. Pollack, A. E. Maumenee, and T. George, "The nerve fiber layer in the diagnosis of glaucoma", *Arch Ophthalmol* **95**, 2149-2156 (1977).
88. A. Sommer, H. A. Quigley, A. L. Robin, N. R. Miller, J. Katz, and S. Arkell, "Evaluation of nerve fiber layer assessment", *Arch Ophthalmol* **102**, 1766-1771 (1984).
89. A. Sommer, J. Katz, and H. A. Quigley, "Clinically detectable nerve fiber layer atrophy precedes the onset of glaucomatous field loss", *Arch Ophthalmol* **109**, 77-83 (1991).
90. M. C. Leske, A. Heijl, L. Hyman, and B. Bengtsson, "Early Manifest Glaucoma Trial: design and baseline data", *Ophthalmology* **106**, 2144-2153 (1999).
91. A. Sommer, J. M. Tielsch, and J. Katz, "Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans: The Baltimore Eye Survey", *Arch Ophthalmol* **109**, 1090-1095 (1991).
92. M. A. Kass, D. K. Heuer, and E. J. Higginbotham, "The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma", *Arch Ophthalmol* **120**, 701-713 (2002).
93. P. R. Lichter, D. C. Musch, and B. W. Gillespie, "CIGTS Study Group. Interim clinical outcomes in the Collaborative Initial Glaucoma Treatment Study comparing initial treatment randomized to medications or surgery", *Ophthalmology* **108**, 1943-1953 (2001).
94. Collaborative Normal-Tension Glaucoma Study Group, "Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures", *Am J Ophthalmol* **126**, 487-497 (1998).
95. The AGIS Investigators, "The advanced glaucoma intervention study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration", *Am J Ophthalmol* **130**, 429-440 (2000).
96. J. Ambati, B. K. Ambati, S. H. Yoo, S. Ianchulev, and A. P. Adamis, "Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies", *Survey Ophthalmol* **48** (3), 257-293 (2003).

REFERENCES, Continued

97. H. Lewis, B. R. Straatsma, and R. Y. Foos, "The prevalence of macular drusen in postmortem eyes", *Am J Ophthalmol* **102**, 801–803 (1986).
98. W. R. Green and C. Enger, "Age-related macular degeneration histopathologic studies: The 1992 Lorenz E. Zimmerman Lecture", *Ophthalmology* **100**, 1519–1535 (1993).
99. T. A. Ciulla, A. G. Amador, and B. Zinman, "Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies", *Diabetes Care* **26** (9), 2653–2664 (2003).
100. D. E. Singer, D. M. Nathan, H. A. Fogel, and A. P. Schachar, "Screening for diabetic retinopathy", *Ann Intern Med* **116**, 660–671 (1992).
101. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, "Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy", *N Engl J Med* **342**, 381–389 (2000).
102. Diabetes Control and Complications Trial Research Group, "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus", *N Engl J Med* **329**, 977–986 (1993).
103. UK Prospective Diabetes Study Group, "Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes: UKPDS 33", *Lancet* **352**, 837–853 (1998).
104. Early Treatment Diabetic Retinopathy Study Research Group, "Photocoagulation for diabetic macular edema: Early Treatment Diabetic Retinopathy Study Report No. 1", *Arch Ophthalmol* **103**, 1796–1806 (1985).
105. F. L. Ferris III, "How effective are treatments for diabetic retinopathy?", *JAMA* **269**, 1290–1291 (1993).
106. K. R. Denninghoff, M. H. Smith, and L. Hillman, "Retinal imaging techniques in diabetes", *Diabetes Technology & Therapeutics* **2** (1), 111–113 (2000).
107. S. H. Hardarson, A. Harris, R. A. Karlsson, G. H. Halldorsson, L. Kagemann, E. Rechtman, G. M. Zoega, T. Eysteinnsson, J. A. Benediktsson, A. Thorsteinsson, P. K. Jensen, J. Beach, and E. Stefansson, "Automatic retinal oximetry", *Invest Ophthalmol Vis Sci* **47** (11), 5011–5016 (2006).

REFERENCES, Continued

108. C. C. Bailey, J. M. Sparrow, R. H. Grey, and H. Cheng, "The National Diabetic Retinopathy Laser Treatment Audit, III: clinical outcomes", *Eye* **13**, 151–159 (1999).
109. R. N. Weinreb, L. Zangwill, and C. C. Berry, "Detection of glaucoma with scanning laser polarimetry", *Arch Ophthalmol* **116**, 1583–1589 (1998).
110. N. T. Choplin, D. C. Lundy, and A. W. Dreher, "Differentiating patients with glaucoma from glaucoma suspects and normal subjects by nerve fiber layer assessment with scanning laser polarimetry", *Ophthalmology* **105**, 2068–2076 (1998).
111. V. W. Lee and K. H. Mok, "Retinal nerve fiber layer measurement by nerve fiber analyzer in normal subjects and patients with glaucoma", *Ophthalmology* **106**, 1006–1008 (1999).
112. H. Bagga and D. S. Greenfield, "Retinal nerve fiber layer assessment using scanning laser polarimetry", *International Ophthalmology Clinics* **44** (2), 29-42 (2004).
113. M. C. Cheney, A. E. Elsner, S. A. Burns, M. B. Kairala, C. L. Trempe, K. Lashkari, J. J. Weiter, M. Miura, M. Osako and M. Usui , "Polarization amplitude and phase changes in retinal exudation", *Invest Ophthalmol Vis Sci* **44**, E-Abstract 5001 (2003).
114. M. C. Cheney, A. E. Elsner, A. Weber and Q. Y. J. Smithwick, "Discrimination of retinal pathology using scanning laser polarimetry analysis", *Invest Ophthalmol Vis Sci* **46**, E-Abstract 4304 (2005).
115. M. C. Cheney, A. E. Elsner, A. Weber, M. Miura, Q. Y. J. Smithwick, M. B. Mellem-Kairala, J. J. Weiter and S. A. Burns , "Analysis of scattered light in age-related maculopathy", *Invest Ophthalmol Vis Sci* **45**, E-Abstract 3120 (2004).
116. A. E. Elsner, D. A. VanNasdale, A. Weber and M. Miura, "Imaging diabetic retinopathy with near infrared light and polarimetry", *Invest Ophthalmol Vis Sci* **47**, E-Abstract 4062 (2006).
117. M. B. Mellem Kairala, A. E. Elsner, S. A. Burns and R. B. Simmons, "Polarimetric analysis of the peripapillary hyperpigmentation in glaucoma patients", *Invest Ophthalmol Vis Sci* **43**, E-Abstract 245 (2002).
118. M. B. Mellem Kairala, A. E. Elsner, S. A. Burns, C. L. Trempe, K. Lashkari, J. J. Weiter, M. C. Cheney, M. Miura, M. Osako and M. Usui , "Imaging polarimetry in macular exudation and atrophy", *Invest Ophthalmol Vis Sci* **44**, E-Abstract 1818 (2003).

REFERENCES, Continued

119. M. B. Mellem-Kairala, A. E. Elsner, A. Weber, R. B. Simmons, and S. A. Burns, "Improved contrast of peripapillary hyperpigmentation using polarization analysis", *Invest Ophthalmol Vis Sci*, **46** (3), 1099-1106 (2005).
120. A. Weber, A. E. Elsner, M. C. Cheney, M. Miura, A. Remky, K. Lashkari, P. Salvetti, Q. Y. Smithwick and S. Kompa, "Exudative lesions in age-related macular degeneration: comparison of fluorescein angiograms and polarimetry images", *Invest Ophthalmol Vis Sci* **45**, E-Abstract 3069 (2004).
121. M. Miura, A. E. Elsner, A. Weber, M. C. Cheney, O. Masahiro, T. Iwasaki and M. Usui, "Imaging polarimetry in central serous chorioretinopathy", *Invest Ophthalmol Vis Sci* **46**, E-Abstract 3545 (2005).
122. A. Weber, A. E. Elsner, M. Miura, M. C. Cheney, J. P. Walker, G. L. Wing, P. A. Raskauskas, T. Ghuman, D. C. Fletcher and S. Kompa, "Influence of macular pathology associated with age-related macular degeneration in scanning laser polarimetry", *Invest Ophthalmol Vis Sci* **46**, E-Abstract 230 (2005).
123. A. Weber, M. C. Cheney, Q. Y. J. Smithwick, and A. E. Elsner, "Polarimetric imaging and blood vessel quantification," *Opt Express* **12**, 5178–5190 (2004).
124. A. De Martino, Y.-K. Kim, E. Garcia-Caurel, B. Laude, and B. Drevillon, "Optimized Mueller polarimeter with liquid crystals", *Opt Lett* **28**, 616-618 (2003).
125. M. Born and E. Wolf, *Principles of Optics*, (Cambridge University Press, Cambridge, 7th ed. 1999).
126. R. A. Chipman, "Polarimetry" in *Handbook of Optics 2nd ed.*, M. Bass, ed. (McGraw-Hill, New York, 1995), pp. 22.1 – 22.37.
127. W. A. Shurcliff, *Polarized Light*, (Harvard University Press, Cambridge, Mass., 1962).
128. D. Goldstein, *Polarized Light, 2nd ed.*, (Marcel Dekker Inc., New York, 2003).
129. S. Y. Lu, and R. A. Chipman, "Interpretation of Mueller matrices based on polar decomposition", *J Opt Soc Am A – Opt Image Scie Vis* **13**, 1106-1113 (1996).
130. R. A. Chipman, "Depolarization index and the average degree of polarization," *Appl Opt* **44**, 2490-2495 (2005).

REFERENCES, Continued

131. G. H. Golub and C. F. Van Loan, *Matrix Computations*, (Johns Hopkins University Press, Baltimore, Maryland, 1983).
132. A. Aiello, G. Puentes, D. Voigt, and J. P. Woerdman, "Maximum-likelihood estimation of Mueller matrices," *Opt Lett* **31**, 817-819 (2006).
133. R. M. A. Azzam, I. M. Elminyaw, and A. M. El-Saba, "General analysis and optimization of the four-detector photopolarimeter", *J Opt Soc Am A* **5**, 681-689 (1988).
134. A. Ambirajan and D. C. Look, "Optimum angles for a polarimeter: part I", *Opt Eng* **34**, 1651-1655 (1995).
135. A. Ambirajan and D. C. Look, "Optimum angles for a polarimeter: part II", *Opt Eng* **34**, 1656-1659 (1995).
136. D. S. Sabatke, M. R. Descour, E. Dereniak, W. C. Sweatt, S. A. Kemme, and G. S. Phipps, "Optimization of retardance for a complete Stokes polarimeter", *Opt Lett* **25**, 802-804 (2000).
137. D. S. Sabatke, A. M. Locke, M. R. Descour, W. C. Sweatt, J. P. Garcia, E. Dereniak, S. A. Kemme, and G. S. Phipps, "Figures of merit for complete Stokes polarimeters", *Proc SPIE* **4133**, 75-81 (2000).
138. M. H. Smith, "Optimization of a dual-rotating-retarder Mueller matrix polarimeter", *Appl Opt* **41** (13), 2488-2493 (2002).
139. J. S. Tyo, "Noise equalization in Stokes parameter images obtained by use of variable-retardance polarimeters", *Opt Lett* **25** (16), 1198-2000 (2000).
140. J. S. Tyo, "Considerations in polarimeter design", *Proc SPIE* **4133**, 65-74 (2000).
141. J. S. Tyo, "Optimum linear combination strategy for an N-channel polarization-sensitive vision or imaging system", *J Opt Soc Am A* **15**, 359-366 (1998).
142. S. N. Savenkov, "Optimization and structuring of the instrument matrix for polarimetric measurements", *Opt Eng* **41** (5), 965-972 (2002).
143. A. De Martino, E. Garcia-Caurel, B. Laude, and B. Drevillon, "Optimized Mueller polarimeter with liquid crystals", *Opt Lett* **28**, 616-618 (2003).

REFERENCES, Continued

144. A. De Martino, B. Garcia-Caurel, B. Laude, and B. Drevillon, "General methods for optimized design and calibration of Mueller polarimeters", *Thin Solid Films* **455-456**, 112-119 (2004).
145. E. Garcia-Caurel, A. De Martina, and B. Drevillon, "Spectroscopic Mueller polarimeter based on liquid crystal devices", *Thin Solid Films* **455-456**, 120-123 (2004).
146. J. S. Tyo, "Design of optimal polarimeters: maximization of signal-to-noise ratio and minimization of systematic error", *Appl Opt* **41** (4), 619-630 (2002).
147. J. Zallat, S. Ainouz, and M. P. Stoll, "Optimal configurations for imaging polarimeters: impact of image noise and systematic errors", *J Opt A: Pure Appl Opt* **8**, 807-814 (2006).
148. A. Papoulis, *Probability, Random Variables, and Stochastic Processes* (McGraw-Hill, Boston, 1991).
149. A. W. Dreher and K. Reiter, "Retinal eye disease diagnostic system", patent 5,303,709 (1994).
150. K. Reiter and A. W. Dreher, "Eye examination apparatus employing polarized light probe", patent 5,787,890 (1998).
151. A. W. Dreher, "Eye examination apparatus employing polarized light probe", patent 6,112,114 (2000).
152. M. Anderson, Q. Zhou, and W. Papworth, "Method and system for cancelling system retardance error in an ophthalmological polarimeter", patent 6,704,106 (2004).
153. H. A. Quigley, E. M. Addicks, and W. R. Green, "Optic nerve damage in human glaucoma. III. Quantitative correlation of nerve fiber loss and visual field defect in glaucoma, ischemic neuropathy, papilledema, and toxic neuropathy", *Arch Ophthalmol* **100**, 135-146 (1982).
154. Carl Zeiss Meditec, "GDx: Precise RNFL analysis for glaucoma case detection, diagnosis, and management", <http://www.meditec.zeiss.com/gdx>.
155. S. T. Hoh, H. Ishikawa, D. S. Greenfield, J. M. Liebmann, S. J. Chew, and R. Ritch, "Peripapillary nerve fiber layer thickness measurement reproducibility using scanning laser polarimetry", *J Glaucoma* **7**: 12-15 (1998).

REFERENCES, Continued

156. A. Junghardt, M. K. Schmid, I. Schipper, H. Wildberger and B. Seifert, "Reproducibility of the data determined by scanning laser polarimetry", *Graefes Arch Clin Exp Ophthalmol* **234**: 628–632 (1996).
157. L. Zangwill, C. A. Berry, V. S. Garden and R. N. Weinreb, "Reproducibility of retardation measurements with the nerve fiber analyzer II", *J Glaucoma* **6**: 384–389 (1997).
158. M. J. Sinai, E. A. Essock, R. D. Fechtner and N. Srinivasan, "Diffuse and localized nerve fiber layer loss measured with a scanning laser polarimeter: sensitivity and specificity of detecting glaucoma", *J Glaucoma* **9**, 154–162 (2000).
159. R. Lauande-Pimentel, R. A. Carvalho, H. C. Oliveira, D. C. Goncalves, L. M. Silva and V. P. Costa, "Discrimination between normal and glaucomatous eyes with visual field and scanning laser polarimetry measurements", *Br J Ophthalmol* **85**: 586–591 (2001).
160. D. Poinoosawmy, J. C. Tan, C. Bunce and R. A. Hitchings, "The ability of the GDx nerve fibre analyser neural network to diagnose glaucoma", *Graefes Arch Clin Exp Ophthalmol* **239**: 122–127 (2001).
161. M. J. Tjon-Fo-Sang and H. G. Lemij, "The sensitivity and specificity of nerve fiber layer measurements in glaucoma as determined with scanning laser polarimetry", *Am J Ophthalmol* **123**, 62–69 (1997).
162. J. R. Tribble, R. O. Schultz, J. C. Robinson and T. L. Rothe, "Accuracy of scanning laser polarimetry in the diagnosis of glaucoma", *Arch Ophthalmol* **117**, 1298–1304 (1999).
163. T. P. Colen and H. G. Lemij, "Sensitivity and specificity of the GDx: clinical judgment of standard printouts versus the number", *J Glaucoma* **12** (2), 129–133 (2003).
164. N. Choplin, "The sensitivity and specificity of scanning laser polarimetry in the detection of glaucoma in a clinical setting", *Ophthalmology* **108** (5), 899–904 (2001).
165. C. Sanchez-Galeana, C. Bowd, E. Z. Blumenthal, P. A. Gokhale, L. M. Zangwill and R. N. Weinreb, "Using optical imaging summary data to detect glaucoma", *Ophthalmology* **108**, 1812–1818 (2001).
166. K. Fabre, I. Michiels and T. Zeyen, "The sensitivity and specificity of TOP, FDP, and GDX in screening for early glaucoma", *Bull Soc Belge Ophtalmol* **275**, 17–23 (2000).

REFERENCES, Continued

167. S. Funaki, M. Shirakashi, K. Yaoeda, H. Abe, S. Kunitatsu, Y. Suzuki, G. Tomita, M. Araie, N. Yamada, H. Uchida, T. Yamamoto, and Y. Kitazawa, "Specificity and sensitivity of glaucoma detection in the Japanese population using scanning laser polarimetry", *Br J Ophthalmol* **86**, 70-74 (2002).
168. L. M. Zangwill, C. Bowd, and C. C. Berry, "Discriminating between normal and glaucomatous eyes using the Heidelberg Retina Tomograph, GDx Nerve Fiber Analyzer, and Optical Coherence Tomograph", *Arch Ophthalmol* **119**, 985-993 (2001).
169. D. S. Greenfield, R. W. Knighton, W. J. Feuer, J. C. Schiffman, L. Zangwill and R. N. Weinreb, "Correction for corneal polarization axis improves the discriminating power of scanning laser polarimetry", *Am J Ophthalmol* **134** 27-33 (2002).
170. D. F. Garway-Heath, M. J. Greaney and J. Caprioli, "Correction for the erroneous compensation of anterior segment birefringence with the scanning laser polarimeter for glaucoma diagnosis", *Invest Ophthalmol Vis Sci* **43** 1465-74 (2002).
171. S. Miglior, "GDx in glaucoma", *Acta Ophthalmologica Scandinavica* **80**, 36-40 (2002).
172. N. T. Choplin, Q. Zhou, and R. W. Knighton, "Effect of individualized compensation for anterior segment birefringence on retinal nerve fiber layer assessments as determined by scanning laser polarimetry", *Ophthalmology* **110**, 719-25 (2003).
173. Q. Zhou, "System and method for determining birefringence of anterior segment of a patient's eye", patent 6,356,036 (2002).
174. R. N. Weinreb, C. Bowd, and L. M. Zangwill, "Glaucoma detection using scanning laser polarimetry with variable corneal polarization compensation", *Arch Ophthalmol* **120**, 218-24 (2003).
175. A. Katsanos, P. Kothy, A. Papp and G. Hollo, "Influence of subfoveal choroidal neovascularisation on macular imaging with scanning laser polarimetry of the retinal nerve fibre layer", *Eye* **19**, 117-122 (2005).
176. H. Bagga, D. S. Greenfield, and R. W. Knighton, "Scanning Laser Polarimetry with Variable Corneal Compensation: Identification and Correction for Corneal Birefringence in Eyes with Macular Disease", *Invest Ophthalmol Vis Sci* **44**, 1969-1976 (2003).

REFERENCES, Continued

177. H. Bagga, D. S. Greenfield, and W. J. Feuer, "Quantitative assessment of atypical birefringence images using scanning laser polarimetry with variable corneal compensation", *Am J Ophthalmol* **139**, 437–46 (2005).
178. S. Da Pozzo, R. Marchesan, T. Canziani, O. Vattovani and G. Ravalico, "Atypical pattern of retardation on GDx- VCC and its effect on retinal nerve fibre layer evaluation in glaucomatous eyes", *Eye* **20**, 769–775 (2006).
179. M. Sehi, D. C. Guaqueta, and D. S. Greenfield, "An enhancement module to improve the atypical birefringence pattern using scanning laser polarimetry with variable corneal compensation", *Br J Ophthalmol* **90**, 749–753 (2006).
180. C. Bowd, F. A. Medeiros, R. N. Weinreb, and L. M. Zangwill, "The effect of atypical birefringence patterns on glaucoma detection using scanning laser polarimetry with variable corneal compensation", *Invest Ophthalmol Vis Sci* **48** (1), 223–227 (2007).
181. N. J. Reus, Q. Zhou, and H. G. Lemij, "Enhanced imaging algorithm for scanning laser polarimetry with variable corneal compensation", *Invest Ophthalmol Vis Sci* **47** (9), 3870–3877 (2006).
182. M. Toth and G. Hollo, "Enhanced corneal compensation for scanning laser polarimetry on eyes with atypical polarisation pattern", *Br J Ophthalmol* **89**, 1139–1142 (2005).
183. R. W. Knighton and X. R. Huang, "Analytical methods for scanning laser polarimetry", *Opt Express* **10** (21), 1179–1189 (2002).
184. J. E. Morgan, A. Waldock, and G. Jeffery, "Retinal nerve fibre layer polarimetry: histological and clinical comparison", *Br J Ophthalmol* **82**, 684–690 (1998).
185. S. Kremmer, D. F. Garway-Heath, S. de Cilla, K. P. Steuhl, and J. M. Selbach, "Influence of cataract surgery with implantation of different intraocular lenses on scanning laser tomography and polarimetry", *Am J Ophthalmol* **136** (6), 1016–1021 (2003).
186. G. Gazzard, P. J. Foster, J. G. Devereux, F. Oen, P. T. K. Chew, P. T. Khaw and S. K. L. Seah, "Effect of cataract extraction and intraocular lens implantation on nerve fibre layer thickness measurements by scanning laser polarimeter (GDx) in glaucoma patients", *Eye* **18** (2), 163–168 (2004).
187. S. T. Hoh, D. S. Greenfield, and H. Ishikawa, "Factors affecting image acquisition during scanning laser polarimetry", *Ophthalmic Surg Lasers* **29**, 545–551 (1998).

REFERENCES, Continued

188. E. A. Boettner and J. R. Wolter, "Transmission of the ocular media", *Invest Ophthalmol* **1**, 776-783 (1962).
189. F. C. Delori and K. P. Pflibsen, "Spectral reflectance of the human ocular fundus", *Appl Opt* **28**, 1061-1077 (1989).
190. J. Schwiegerling, "Visual Performance Prediction Using Schematic Eye Models", PhD Dissertation, University of Arizona (1996).
191. K. Iizuka, *Elements of Photonics Volume II: For Fiber and Integrated Optics*, (Wiley-Interscience, New York, 2002).
192. A. De Martino, E. Garcia-Caurel, B. Laude, and B. Drevillon, "General methods for optimized design and calibration of Mueller polarimeters", *Thin Solid Films* **455–456**, 112–119 (2004).
193. E. Compain, S. Poirier, and B. Drevillon, "General and self-consistent method for the calibration of polarization modulators, polarimeters, and Mueller-matrix ellipsometers", *Appl Opt* **38** (16), 3490-3502 (1999).
194. "A Guide to Reflectance Coatings and Materials", Labsphere Inc., North Sutton, NH.
195. Q. Zhou, J. Reed, R. Betts, P. Trost, P. W. Lo, C. Wallace, R. Bienias, G. Li, R. Winnick, W. Papworth, and M. Sinai, "Detection of glaucomatous retinal nerve fiber layer damage by scanning laser polarimetry with variable corneal compensation", *Proc SPIE* **4951**, 32-41 (2003).
196. A. E. Elsner, A. Weber, and M. C. Cheney, "Changes in retinal birefringence with eccentricity", *Invest Ophthalmol Vis Sci* **46**, E-Abstract 2555 (2005).
197. A. E. Elsner, D. VanNasdale, Y. Zhao, M. Miura, A. Weber, K. M. Twietmeyer, and R. A. Chipman, "Retinal birefringence changes associated with exudative eye disease", in 2007 OSA Frontiers in Optics conference (Optical Society of America 2007).