Abstract

Nonlinear Polarimetric Microscopy for Biomedical Imaging

Masood Samim
Doctor of Philosophy
Graduate Department of Physics
University of Toronto
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A framework for the nonlinear optical polarimetry and polarimetric microscopy is developed. Mathematical equations are derived in terms of linear and nonlinear Stokes Mueller formalism, which comprehensively characterize the polarization properties of the incoming and outgoing radiations, and provide structural information about the organization of the investigated materials. The algebraic formalism developed in this thesis simplifies many predictions for a nonlinear polarimetry study and provides an intuitive understanding of various polarization properties for radiations and the intervening medium.

For polarimetric microscopy experiments, a custom fast-scanning differential polarization microscope is developed, which is also capable of real-time three-dimensional imaging. The setup is equipped with a pair of high-speed resonant and galvanometric scanning mirrors, and supplemented by advanced adaptive optics and data acquisition modules. The scanning mirrors when combined with the adaptive optics deformable mirror enable fast 3D imaging. Deformable membrane mirrors and genetic algorithm optimization routines are employed to improve the imaging conditions including correcting the optical aberrations, maximizing signal intensities, and minimizing point-spread-functions of the focal volume. A field-programmable-gate array (FPGA) chip is exploited to rapidly acquire and process the multidimensional data.

Using the nonlinear optical polarimetry framework and the home-built polarization microscope, a few biologically important tissues are measured and analyzed to gain insight
as to their structure and dynamics. The structure and distribution of muscle sarcomere myosins, connective tissue collagen, carbohydrate-rich starch, and fruit fly eye retinal molecules are characterized with revealing polarization studies. In each case, using the theoretical framework, polarization sensitive data are analyzed to decipher the molecular orientations and nonlinear optical susceptibilities.

The developed nonlinear optical polarimetric microscopy is applicable to a wide variety of structural studies on ordered materials, and provides a non-invasive possibility to study the structural organization and dynamics within biological samples. For example, the technique is well suited for studies of a muscle contraction, histopathology of collagen structure for cancer tissue diagnostics, investigations of the polysacharide structural organization within a starch granule of a plant, or developmental study of the retina in an eye, among other applications.
to my family

for their limitless support
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“Creativity always comes as a surprise to us; therefore we can never count on it and we dare not believe in it until it has happened. In other words, we would not consciously engage upon tasks whose success clearly requires that creativity be forthcoming. Hence, the only way in which we can bring our creative resources fully into play is by misjudging the nature of the task, by presenting it to ourselves as more routine, simple, undemanding of genuine creativity than it will turn out to be.”

ALBERT O. HIRSCHMAN
The Principle of the Hiding Hand
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Chapter 1

Introduction

Light interacts with matter and the interaction is fundamental to numerous phenomena observed in everyday life. Properties of the material can be probed by light, and measurements of the optical response of a material and the polarization state of light are indispensable research methods in material science, biology and remote sensing. In this thesis, nonlinear interactions induced by high-intensity laser radiations are investigated to determine the structure of materials. In particular, polarization-dependent nonlinear interaction of light with matter and the generated nonlinear response are employed to reveal molecular organization of the material. The ultrastructural investigations, using noninvasive light-matter interactions, are particularly valuable when applied to biological structures. Nonlinear polarimetry can determine the molecular ultrastructure as well as structural changes occurring in living organisms during their physiological activities.

Polarimetry measures the polarization response of a material to the electromagnetic radiation.\(^1\) Although polarization is most often referred to the “sidedness”\(^2\) of electrical component of electromagnetic spectrum, other vector waves, including the magnetic component of the spectrum as well as elastic and spin waves in solids, can also possess polarization-like properties [1]. In this thesis, the polarimetry of electric field of the optical spectrum in conjunction with polarization-dependent response of the intervening medium is of primary concern, and therefore, the term polarization refers to these attributes of the light-matter interaction.

\(^1\)Ellipsometry is polarimetry of surfaces and thin films.
\(^2\)Sir Issac Newton referred to polarization intuitively by this term.
In polarimetry, an optical radiation is characterized by measuring light intensities at several polarization states, and the intervening material is determined by a number of parameters that indicate a change in aspects of the incoming radiation polarization. For example, an incident radiation’s electric field phase retardance, its intensity diattenuation, and its state depolarization are characterized by measuring the radiation polarization changes due to the medium. The basic operating principle of polarimetry has been as follows: \( \text{outgoing radiation} = \text{material} \times \text{incoming radiation} \). This principle is based on a linear relationship between an incoming and the outgoing electric fields. The mathematical details of the linear polarimetry will be discussed in Section 1.2.

The polarization response of a material is not always linear, however. Nonlinear optics is now a mature field in which the nonlinear response of a material to multiple interacting electric fields is described. In general, a light-matter interaction can be described by the induced polarization density. The generated electric field of the outgoing radiation is proportional to the nonlinear response function multiplied by multiple electric fields of incoming radiations. Thus, the induced polarization can be expressed as an \( n \)-order polynomial of electric fields multiplied by the corresponding nonlinear response functions; only one of the terms accounts for the linear relationship between the incoming and outgoing electric fields. Second-, third-, and higher-order terms account for increasingly higher nonlinear polarization responses. The mathematical details of the background theory of nonlinear optics is provided in Section 1.3.

The key challenge addressed in the thesis is how to construct and analyze more conveniently and in proper basis the nonlinear optical response of the intervening material to the electric fields of the incoming radiation. The solution presented in this thesis provides a theoretical framework describing the polarization state of outgoing and incoming light in a vector form and the intervening medium in a matrix form. The formalism describes each vector and matrix component by observable parameters, which can be conveniently related to the experiment. The theory extends the formal description of linear polarimetry by including nonlinear interactions that underlie important optical phenomena such as second-, third-, and higher-harmonic generation, sum- and difference frequency generation, and the coherent anti-Stokes Raman scattering. Chapter 2 will provide all details of the theory as well as the underlying mathematical formalisms.

\(^3\text{Note, the term polarization refers to a property of light and is different from polarization density which refers to a property of the material.}\)
Before delving into the mathematical details of linear polarimetry and nonlinear optics, the following historical perspective is provided to help appreciate the key breakthroughs and developments that are the foundation for this thesis. The overview will contextualize the work presented herein, and familiarize the reader with key terms and ideas referred to in subsequent chapters.

1.1 History and Overview

The basics of optical polarimetry rely on the wave theory of light, first developed and presented in 1678, by Christiaan Huygens who built the idea from the interference property of light and the observation of resulting intensity variations [2, 3]. In 1808 Étienne-Louis Malus discovered the polarization property of light and used the term “polarized light” [3]. David Brewster was the first to relate the polarization of light with the property of a medium [4]. In 1852 George Gabriel Stokes presented his discovery that the polarization properties of light can be obtained from measuring its intensities [5], and before the momentous culmination of the electromagnetic theory by James Maxwell who formalized the wave vector nature of electric field and polarization density in his famous equations in 1865 [6]. Finally, in 1892 Henri Poincaré’s sphere gave a geometrical means to depict the $4 \times 1$ Stokes vector and the polarization state of light [1, 7, 8]. An excellent account of early polarimetry and ellipsometry and their intertwined history is described by Azzam [3]. In this thesis, the details of Stokes vector notation and Poincaré sphere representation are provided in Section 1.2.

Continuing on the path of history, in 1941 in his seminal papers Clark Jones described in precise mathematical (vector and matrix) terms the theory of an electric field interaction with a medium [9, 10]. The $2 \times 2$ Jones matrices provided mathematical equivalence and intuitive understanding of physical systems when studying optical interactions. Although the $4 \times 4$ matrix transforming the incoming Stokes vector to the outgoing one is widely credited to Hans Mueller, it was in fact Francis Perrin who formalized the scattering behaviour of a medium with sixteen coefficients in the form of a matrix that related the incoming and outgoing radiations each defined by a Stokes vector. In his paper Perrin credits Paul Soleillet for generally formulating that a set of sixteen coefficients can characterize an optical phenomena when the beam radiating from an intervening medium
is due to an incoming radiation [11,12]. The idea that it was Perrin, rather than Mueller, who first used $4 \times 4$ matrix is indeed acknowledged by Nathan G. Parke, a student of Mueller, and whose doctoral dissertation is frequently referenced when referring to Mueller matrices [13].\(^4\) In his “Matrix Optics” thesis N. Parke described in algebraic terms the interaction of a radiation with matter in terms of products of matrices that describe the action of media on the incoming radiation. Parke’s work combined the Jones matrix formalism together with the Mueller matrix through the Wiener coherency matrix as well as the Stokes vector. By then, vector and matrix algebra was of course not new to physics, particularly to the particle and quantum physics. Not surprisingly in hindsight, Fano and others found relations between quantum mechanics, particle theory, and Stokes parameters [16–18].

Mueller’s remarkable success in popularizing the concept seems to be in his ability to understand that these $4 \times 4$ matrices are observables and describe phenomenologically optical interactions. Mueller also suggested that the Stokes vector should be connected to an alternative mode of describing light: termed as the “coherency matrix” by Norbert Wiener is a complex valued matrix notation for description of electric field components of light [19]. This realization was also paralleled with a phenomenological understanding of wave optics. Hence, the $4 \times 4$ Mueller matrix became the basis for an intuition into the transformation of polarization of a beam as had the Jones matrix for the electric field wave component of the light. The relevant mathematical descriptions are elaborated upon in Section 1.2.3.

Turning the attention to applications of polarimetry, and not surprisingly, one will find a broad range of applications in the areas of astronomy, chemistry and biology. For example, Stokes vectors were used for circular polarimetry of infrared light to discover the helical magnetic fields of a stellar nebular object [20]. Polarimetry has also been used to study the symmetry of chemical bonds and structure of proteins [21], and applied to measure sugar concentration on an industrial scale [22]. Furthermore, polarimetry has extensive applications in remote sensing as well [23], as non-contact and non-invasive methods for identifying and characterizing distant objects.

Since in this thesis important biological tissue are investigated exploiting the theory\(^4\)An obscure declassified World War II report is seldom cited as the publication authored by H. Mueller himself on Mueller algebra [14]. Another reference to Hans Mueller is the abstract of his talk at the Optical Society of America meeting in 1948 [15].
of nonlinear polarimetry, a background history of experimental techniques using Stokes Mueller polarimetry for biomedical application may be helpful and of interest to the reader.

The chiral nature investigations of molecules in biological samples, ranging from dermatology to cancer tissue studies and a variety of other biomedical applications, have benefited from techniques of linear polarimetry [24–28]. Various strategies have been developed to measure the Stokes vector of the radiations and to determine the Mueller matrix of samples. One of the earliest use of Stokes Mueller polarimetry as a biophysical tool was described by Bickel, et al [29, 30]. The technique was limited to obtaining scattering signal. Polarization modulation technique offered another way to measure all four Stokes parameters relatively faster and more reliably [31]. Although Hzerbroek and Holcer was first to use interferometry to characterize the Stokes vector [32], de Boer was able to extract all four Stokes parameters in an optical coherence tomography (OCT) study [33]. Meanwhile, the extraction of Mueller matrix elements was demonstrated by Yao and Lihong in OCT [34]. A Jones-matrix approach for four-channel OCT was demonstrated later by the same group as well [35]. Lu-Chipman decomposition of a Mueller matrix describing diattenuation, retardation and depolarization effect made it easy to interpret the physical meaning of the Mueller matrices [36]. Mueller matrix approach has been used to study the chirality and optical rotation in scattering media [37], as well as for optical activity and birefringence in multiply scattering media [26]. The propagation of polarized light in birefringent media was studied using a Monte Carlo method to quantify the expected corresponding Mueller matrix [38]. Optical rotation effects on linear and circular polarization by chiral and achiral media was studied by Hadely and Vitkin [39]. Sankaran et al. studied the degree of polarization due to scattering from various biological systems such as blood, fat, tendon, artery and heart muscle tissue [40]. Degree of polarization of laser-speckles from scattering media was studied by Li et al. [41]. Automated skin cancer detection using Mueller matrix was developed with an accuracy 97.96 % with just one set of Mueller matrix, and achieved a higher accuracy of 99.44 % with 36 images for Mueller matrix [42]. Imaging of skin with polarized light was performed to detect cancer by Steven Jacques [25]. Recently, Mueller matrix decomposition to characterize biological tissue, for example to determine birefringence properties of stems cell from rat heart cells, were shown by Alex Vitkin and colleagues from the University of Toronto [27]. Interested readers may find several ex-
cellent recent comprehensive reviews about various techniques and subtleties of linear polarimetry [23,28].

In addition to linear polarimetry, a brief history of nonlinear optics is also informative in this context. Early nonlinear effect discoveries date back to the late 19th century when Pockels and Kerr effects were related to the refractive index of crystals and liquids [43]. However, the optical harmonic generation and the rest of nonlinear optical effects owe their experimental confirmation to the laser, invented by Charles Townes and constructed by Theodore Maiman in 1960 [44–46]. Franken et al. in 1961 first observed second harmonic generation (SHG) in the ultraviolet spectral region at \( \lambda = 347.2 \) nm, at twice the frequency of a ruby laser radiation \( \lambda = 694.3 \) nm, from a quartz crystal [47]. This also marked for the first time that a coherent nonlinear effect was observed due to a coherent input radiation from a laser. Soon after in 1962 Terhune et al. observed third harmonic generation (THG) at thrice the frequency from calcite [48]. Armstrong, Bloembergen, Ducuing and Pershan formulated the general permutation symmetry for nonlinear susceptibilities in 1962 [49], which made it easier to predict the nonlinear response based on the symmetry of a system. Further details about the symmetry of structures in the context of thesis and how they can simplify some of calculations are provided in Section 1.3.2. The Stokes and Mueller polarimetry formalism combined with nonlinear optical polarimetry has been rare, however, and extend only to the second-harmonic ellipsometry. Quantum-mechanical description of Stokes vector formalism for SHG was detailed by Shi et al. [50], and SHG optical activity from chiral surfaces using Stokes notation is studied by Hecht et al [51]. The description of SHG radiation by means of linear Stokes vector has been experimentally studied by Mazumder et al. [52, 53]. In this thesis, the description of an \( n \)th-order light matter interaction in the context of a generalized Stokes Mueller polarimetry is formulated. Chapter 2 is entirely dedicated to the development of nonlinear optical polarimetry theory, and constitutes the key focus for this thesis.

Similar to linear polarimetry, nonlinear optical microscopy for biomedical applications has also come a long way. In early days of nonlinear microscopy, Hellwarth and Christenson used the nonlinear microscopy to examine the structure of ZnSe polycrystalline [54], and Sheppard et al. used SHG microscopy to study KDP, zinc oxide, and quartz [55]. Tsang showed that strong THG can be generated from interfaces when significant differences exist between refractive indices of two media [56]. However, these studies did
not concern any biological sample. In 1982 Duncan and colleagues used CARS to image onion-skin-cell [57], and later Sunny Xie and colleagues at Harvard applied CARS to the vibrational chemical bonds for three-dimensional imaging [58]. SHG microscopy was used for tendon collagen imaging by Freund in 1986 [59], and THG microscopy was developed later in 1997 [60], and was used for biological samples soon afterwards by Squier and colleagues [61]. The success of multi-photon fluorescence microscopy by Denk and Webb in 1990 brought a renewed attention to the nonlinear techniques in microscopy [62]. Due to lower scattering effects at longer wavelengths, the optical fields penetrate deeper into the samples. Additionally, nonlinear processes generate signal from a very thin layer of sample, and therefore, avoid issues associated with out-of-focus signal, bleaching, and blurring effects on images. Since each nonlinear technique provides a unique perspective, various modalities were combined to gain multifaceted insights from the specimen. Multi-modal SHG and MPF [63], THG and MPF [64], and the combined SHG,THG and MPF modalities [65] were demonstrated successfully by imaging various biological samples. The design of a unique and custom-built optical system for multi-modal polarimetric microscopy will be revealed in Chapter 3.

Essentially polarimetric microscopy is implemented in two modes: sequential or parallelized measurements. In sequential polarimetry mode, measurements are taken one at a time. This technique is simpler and requires fewer optomechanical components, and therefore, can be employed quicker compared to a parallelized system. However, the measurement time is long and only limited real-time studies can be performed with such systems. The solution for a fast imaging polarimetry is provided by the differential polarization microscopy technique.

A major focus of microscopy, including polarimetric microscopy, is on developing techniques to acquire information from a rapidly-changing system due to physiological activity of an organism. The polarimetry technique developed in this thesis is an effort in this direction. Recent developments in laser scanning microscopy have pushed the imaging speeds particularly for biological applications into new frontiers. Hardware and software used for various detection schemes have been upgraded to perform multi-foci, multicontrast nonlinear imaging. The first multi-focal scanning was performed with light passing through a rotating wheel also known as Nipkow disc [66]. Micro-lens and rotating Nipkow disc was used to perform parallel imaging in 3D [67]. Sensitive measurements of differential polarizations can be performed on a pixel-by-pixel basis by combining an optical
modulator of polarization and a lock-in amplifier in a laser-scanning microscope [68]. The differential polarization technique has been employed in this context for measuring fluorescence linear and circular dichroism, anisotropy of fluorescence, as well as the degree of polarization of fluorescence [68–70]. One of the first approaches to nonlinear multi-focal microscopy employed a micro-lens for time-multiplexing of the incoming radiation [71]. Beam multiplexing was also used as a solution to the well-known problem of budgeting the laser power and its distribution for proper sectioning and 3D imaging [72]. The term differential polarization was first used in 1988 in microscopy [68]. The microscope used to image linear and circular dichroism of individual chloroplasts, and revealed the chiral structure of the grana [73]. The approach involved one beam, and the polarization state of the beam was rapidly changed by a acousto-optic modulator. While birefringence and dichroism can be conveniently investigated with polarization microscopy, for thicker samples, where scattering becomes an issue, confocal laser scanning polarization microscopy presents an alternative. However, all these techniques are limited to distributing a single beam of the laser and are all concerned with multi-photon fluorescence. The breakthrough for harmonic generation microscopy came with the construction of a multi-beam laser. Fast electronics and clever techniques for multi-focal imaging [74], and multi-beam scanning technique allowed parallelization of incoming radiation [75]. Up to six beams from a laser cavity have been used to image simultaneously six different spots on a microscope imaging plane [75]. The field of differential microscopy is growing rapidly, and more interested readers are referred to the recent comprehensive reviews of multifocal nonlinear microscopy [76, 77]. The principles and design of the differential polarization technique for the custom-built polarimetric microscope will be revealed in Section 3.3.

Various other indispensable modules and capabilities are being added very quickly to the microscope setups as well. For example adaptive optics (AO) and on-chip computation allows for three-dimensional imaging and fast processing of the data. AO was added to a multi-photon confocal microscopy by Albert in 2000 to correct aberrations [78]. In this thesis, the multibeam polarimetric microscopy has taken advantage of deformable membrane mirrors for AO and multidepth focusing and beam overlapping [79,80]. All of these advancements are important for differential polarization microscopy as they allow precise 3D overlap of beams to perform polarimetry. Additionally, for nonlinear polarimetry experiments performed in this thesis a custom-programmed field-programmable gated-array (FPGA) chip was used to acquire the polarization data from samples in the multiplexed
microscope. The technique enabled, for example, the real-time 3D imaging of contracting myocytes [81,82]. A similar approach for multi-photon fluorescence shows great potential for calcium imaging in the brain [83]. Random-access scanning using acousto-optics, capable of continuous 3D scanning for two-photon calcium imaging [84], and sub-millisecond SHG holographic 3D imaging of a contracting muscle have been shown by others [85]. More recently, the 2014 Chemistry Nobel laureate Eric Betzig, who was credited with the invention of a super-resolution microscope, and his group built a new setup that acquire images ranging from molecules to embryo level with high spatio-temporal resolution [86]. All these technical advancements indicate a very fertile field and the need for increasing the potentials of microscopes. Therefore, the implementation of the nonlinear polarimetry is an inevitable development that may unleash a new wave of research in the biomedical field in order to study the ultra-structure of biological samples. The details of multiple depth-focusing with optimized resolution as well as intensity utilizing the AO technology is presented in Section 3.4. Furthermore, the description of logics for implementation of the FPGA programming for the differential polarization microscopy is provided in Section 3.3.3. Equipped with these modules in a novel way, the custom-built microscope has become a very versatile tool for various investigations of biological samples, particularly for nonlinear polarimetry. The system remains flexible and other useful upgrades are being considered for even further developments.

As mentioned earlier, biomedical applications of nonlinear polarimetry is a key focus of this thesis. More importantly, sub-micrometer structural properties of important biological tissue are studied with microscopic details. Recently, linear polarization-in/polarization-out (PIPO) technique has been successfully applied to determine some polarization-dependent attributes of biological samples [87–90]. For example, SHG microscopy has been used to study birefringent biological structures such as muscle cells [88]. Hierarchical structure of collagen in a variety of samples including in lung carcinoma tissue was recently investigated by SHG polarization microscopy as well [89,91]. The linear PIPO has also been applied to investigate starch granules [90]. In this thesis, detailed structure of muscle myosins were studied in the wild-type as well as in the mutant fruit fly. Nonlinear susceptibility ratiometric value changes were measured to probe and reveal organization of myosin molecules within the muscle cell sarcomere and published elsewhere [87]. In the context of this thesis, the nonlinear polarimetry theory from Chapter 2, and the polarization differential microscopy shown in Chapter 3, are employed to measure
and analyze various biological structures including myosin, collagen, starch and retinal molecules, and presented in Chapter 4.

In summary, the remaining of this chapter will provide the mathematical background for linear polarimetry and nonlinear optics in order to familiarize the reader to terms which will be used in the development of theoretical framework for nonlinear optical polarimetry in Chapter 2. The details of linear Stokes Mueller polarimetry is given in Section 1.2, and nonlinear optics is discussed in Section 1.3. Chapter 2 of the thesis shows the development of a theoretical framework for nonlinear optical polarimetry and will generalize the connection to the linear Stokes Mueller polarimetry. Chapter 3 will provide details of the methods used for polarimetric microscopy and reveal a parallelized data acquisition system for differential polarization microscopy. Chapter 4 will begin with the analyses of nonlinear polarimetric microscopy images and will follow up with experimental verifications of the nonlinear polarimetry theory. Detailed analyses of molecular susceptibilities and orientations will be presented for a class of biologically important molecules, including muscle myosin, collagen, starch, and retinal molecules. Chapter 5 will bring a brief discussion and conclusion to this thesis.

1.2 Introduction to Linear Polarimetry

1.2.1 Electromagnetic Wave Equations

The phenomena governing electromagnetic fields, derived from first principles, are described by Maxwell’s equations:

\[
\vec{\nabla} \times \vec{E}(\vec{r}, t) + \frac{\partial \vec{B}(\vec{r}, t)}{\partial t} = 0, \quad \text{(Faraday’s law)}
\]

\[
\vec{\nabla} \times \vec{H}(\vec{r}, t) - \frac{\partial \vec{D}(\vec{r}, t)}{\partial t} = \vec{J}(\vec{r}, t), \quad \text{(Ampere’s law)}
\]

\[
\vec{\nabla} \cdot \vec{D}(\vec{r}, t) = \rho(\vec{r}, t), \quad \text{(Gauss’s law)}
\]

\[
\vec{\nabla} \cdot \vec{B}(\vec{r}, t) = 0, \quad \text{(No magnetic monopole)}
\]

\[\text{(1.1)}\]

\footnote{Some excerpts in above paragraphs have been previously published in articles elsewhere [79,80,87].}
where $\rho(\vec{r}, t)$ is the free charge density, and $\vec{J}(\vec{r}, t)$ is the corresponding current density. $\vec{E}$ and $\vec{H}$ are the electric and magnetic fields, $\vec{D}$ and $\vec{B}$ are the electric displacement and magnetic induction, and $\nabla \times$ and $\nabla \cdot$ are the mathematical operations for the curl and the divergence of the fields, receptively. In Maxwell’s equations the continuity equation for the charge and current density is implicitly given as: $\partial \rho / \partial t + \nabla \cdot \vec{J} = 0$.

In general, $\vec{D}$ and $\vec{H}$ are derived from $\vec{E}$ and $\vec{P}$ (polarization), and $\vec{B}$ and $\vec{M}$ (magnetization), respectively, and can have linear and nonlinear components, shown through the constitutive relations [92]:

$$\vec{D} = \epsilon_0 \vec{E} + \vec{P}$$
$$\vec{H} = \frac{1}{\mu_0} \vec{B} - \vec{M}$$

where $\epsilon_0 = 8.854 \times 10^{-12} \text{ F/m}$ is the permittivity of vacuum, $\mu_0 = 4\pi \times 10^{-7} \text{ Vs/(Am)}$ is the permeability of vacuum, and therefore, the speed of light in vacuum is $c = (\mu_0 \epsilon_0)^{-1/2}$ in SI units. These macroscopic fields are averaged over a volume larger than the dimensions of an atom or molecule, yet smaller than a wavelength-cube. Additional simplifications can be made: For some materials, including biological samples, free current density is negligible ($\vec{J} = 0$), and the sample is assumed to be nonmagnetic ($\vec{M} = 0$). Thus, by taking the curl of both side of the equation of Faraday’s law, as well as the time-derivative of the equation of Ampere’s Law, and using the simplification relations, the electric-field wave equation is:

$$\nabla \times \nabla \times \vec{E} + \frac{1}{c^2} \frac{\partial^2 \vec{E}}{\partial t^2} = -\mu_0 \frac{\partial^2 \vec{P}}{\partial t^2}$$

In this thesis, the primary concern is with the induced polarization on the right hand-side of Eq. 1.3. This induced polarization can be expanded in terms of linear and nonlinear contributions due to the applied electric field, which will be discussed further in Section 1.3.

---

6Further information regarding other contributions such as magnetization and quadrupole effects are detailed by Jackson [92], and local field effects are discussed by Jackson [92], Boyd [93] and Bloembergen [94].
1.2.2 Interaction of Light and Matter: Susceptibility and Response Function

As indicated earlier, the polarization density is a function of position and time as well as the electric displacement. The linear polarization density $P^{(1)}_\mu$ along a direction is generally given as [43,95,96]:

$$P^{(1)}_\mu(\vec{r}, t) = \epsilon_0 \int_{-\infty}^{\infty} dt_1 R^{(1)}_{\mu\alpha}(t; t_1) E_\alpha(\vec{r}, t_1)$$  \hspace{1cm} (1.4)

where $R^{(1)}_{\mu\alpha}$ is the element of the first order response function. Since the response function depends on the time difference, or $t - t_1 = t'_1$, then by defining the response function as $R^{(1)}(t; t'_1) \equiv R^{(1)}(t - t_1)$, the polarization density becomes:

$$\vec{P}^{(1)}(\vec{r}, t) = \epsilon_0 \int_{-\infty}^{\infty} dt'_1 R^{(1)}(t'_1) \vec{E}(\vec{r}, t - t'_1)$$  \hspace{1cm} (1.5)

Using the identity $\delta(\omega - \omega') = \frac{1}{2\pi} \int dt e^{i(\omega - \omega')t}$, the Fourier transform of electric field and polarization density, the linear susceptibility is defined according to:

$$\chi^{(1)}(-\omega_\sigma, \omega) = \int_{-\infty}^{\infty} dt'_1 R^{(1)}(t'_1) e^{i\omega_\sigma t'_1}$$  \hspace{1cm} (1.6)

where $\omega_\sigma = \omega$. In frequency domain the polarization density, where a monochromatic light\footnote{Monochromatic light is expressed as:

$$\vec{E}(\omega) = \frac{1}{2} \sum_{\omega' \geq 0} \left[ \vec{E}_{\omega'}(\vec{r}) \delta(\omega - \omega') + \vec{E}^*_\omega(\vec{r}) \delta(\omega + \omega') \right]$$} (i.e. only one frequency) is considered, becomes:

$$\vec{P}^{(1)}(\omega) = \epsilon_0 \chi^{(1)}(-\omega_\sigma; \omega) \cdot \vec{E}(\omega)$$  \hspace{1cm} (1.7)

Thus, the macroscopic linear susceptibility is obtained from the linear response function of the media. Similar analogous arguments for higher order polarization density can be made. Additionally, the rigorous derivation described above show how the interaction of an electromagnetic field can be characterized by combining the electric field with the matter response function. The resulting polarization density becomes the source for the signal, which is then measured. Note, the vector notation of electric field and the
resultant polarization density, which is proportional to the generated outgoing signal, in the linear case, is related by the susceptibility matrix $\chi^{(1)}$. This useful relationship is a good segue to the Jones formalism for transformation of electric fields.

1.2.3 Electric Field Notations: Jones and Coherency Matrices

**Jones Vector**

The electric field function for the electromagnetic wave, which satisfies the wave equation, is sinusoidal. In the most general form, the complex-valued quantity for the electric field components along the Cartesian coordinates can be written as:

$$E_i = \tilde{E}_i e^{i\phi_i}$$  \hspace{1cm} (1.8)

where $\tilde{E}_i(\zeta, t) = A_i e^{-i(k\zeta - \omega t)}$ contains the angular frequency $\omega$ and propagation vector $k$ information; $\zeta$ is the distance measured along the direction of propagation; and the two orthogonal components of the electric field have individual phases $\phi_i$. The real-valued electric field as a function of position and time can be expressed as [1]:

$$\vec{E}(\vec{r}, t) = A \cos (\omega t - \vec{k} \cdot \vec{r})$$  \hspace{1cm} (1.9)

where $\vec{k}$ is the direction of propagation and $|\vec{k}| = \frac{2\pi}{\lambda}$, and $\lambda$ is the wavelength. The phase velocity is $v \equiv \frac{d\zeta}{dt} = \frac{\omega}{2\pi} = f\lambda$. Note, all scaling constants can be included in the parameter $A$.

In a monochromatic field all points in space oscillate simultaneously with time, the temporal component can be suppressed (i.e let $t = 0$ in Eq. 1.9), and the spatial part can be factored out [1]:

$$\vec{E}(\zeta) = e^{-i2\pi\zeta/\lambda} \begin{bmatrix} A_1 e^{i\phi_1} \\ A_2 e^{i\phi_2} \end{bmatrix}$$  \hspace{1cm} (1.10)

where the exponential factor contains the frequency and the propagation information.

An electric field represented by the Jones vector can be transformed after interacting with a material, and its transformation can be handled by the corresponding material

\footnote{Tilde ($\sim$) over electric field $E$ and elsewhere indicates that the quantity is rapidly varying over time.}
Jones matrix $J$:

$$\vec{E}' = J\vec{E}$$  \hspace{1cm} (1.11)

where the symbol prime $'$ here denotes an outgoing, transformed, or an observable parameter. The transformation of the electric field vector using Jones calculus is a well-known and a developed technique in optics [9,10,97,98]. Eq. 1.11 is informative in that it shows how the incoming electric field component of light is related to the outgoing one due to the response of a medium. It is obvious that Eq. 1.8 is a complex-valued quantity, and therefore, in experiments the real-valued electric field is only indirectly measured through intensities.

**Coherency Notation**

A very useful notation for the state of electric field is the coherency matrix [13,19,97,99,100]. The coherency matrix is defined as following:

$$C = \langle \vec{E} \times \vec{E}^\dagger \rangle = \begin{bmatrix} \langle E_1 E_1^* \rangle & \langle E_1 E_2^* \rangle \\ \langle E_2 E_1^* \rangle & \langle E_2 E_2^* \rangle \end{bmatrix}$$  \hspace{1cm} (1.12)

In the above equation $\langle \cdot \rangle$ signifies a time average over an interval long enough to make the time-averaging independent of the interval and fluctuations. Henceforth, for simplicity the brackets may be omitted, but the time average is assumed for all coherency matrices. The coherency matrix for the outgoing field can be found using Eq. 1.11 and substituting it into Eq. 1.12. Consequently, the outgoing coherency matrix becomes:

$$C' = \langle \vec{E}' \times \vec{E}'^\dagger \rangle = J \langle \vec{E} \times \vec{E}^\dagger \rangle J^\dagger = JCJ^\dagger$$  \hspace{1cm} (1.13)

The intensity $I$ of the field measured along two mutually orthogonal directions is calculated according to [1]:

$$I = \langle \vec{E}^\dagger \vec{E} \rangle = \langle E_1^* E_1 + E_2^* E_2 \rangle = \langle |E_1|^2 + |E_2|^2 \rangle$$  \hspace{1cm} (1.14)

where $\dagger$ denotes here and elsewhere the complex-conjugate of a transposition: $\vec{E}^\dagger = (\vec{E}^*)^T = [E_1^* \ E_2^*]$. It is obvious from Eq. 1.14 that the intensity of an optical field is
a real-valued quantity and can be directly measured. This intensity is a preferred and
the main choice of determining the state of an optical field. However, a single intensity
measurement can rarely tell all about the polarization state of the optical field. Multiple
measurements are often made to determine the polarization state. A convenient and
comprehensive set of measurements to determine fully the polarization state of a radiation
is given by the Stokes vector which is discussed next.

1.2.4 Stokes Vector

In 1852 George Gabriel Stokes introduced a four-element vector that characterizes the
polarization state of light [5]. A key advantage of using Stokes vector over Jones is that
the Stokes vector is real and its elements are observables in experiments, while the Jones
vector deals with complex parameters. Albeit this comes at a cost of complicating the
phase information and is less explicit in the Stokes vector notation [8]. In general any
$2 \times 2$ square matrix $L$ can be expanded as a linear combination of its basis, which can
be formally stated as following [100,101]:

$$L = \sum_{\nu=0}^{3} \tau_{\nu} L_{\nu}$$

$$L_{\nu} = \frac{1}{2} \text{Tr}(\tau_{\nu} L)$$

(1.15)

where $\nu = 0, \cdots, 3$ represent the four $2 \times 2$ identity and Pauli matrices:

$$\tau_0 = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \quad \tau_1 = \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} \quad \tau_2 = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} \quad \tau_3 = \begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix}$$

(1.16)

The matrices in Eq. 1.16 form a complete orthonormal basis set with the property
$\text{Tr}(\tau_{\mu} \tau_{\nu}) = 2\delta_{\mu\nu}$. This formalism can be extended for nonlinear polarimetry purposes
to a $3 \times 3$ matrix (see Section 2.2.4) by using Gell-Mann matrices.\(^9\) In Section 2.1.7, a
general form of the matrices is provided such that any square matrix can be decomposed
in terms of its basis.

\(^9\)Linear Mueller matrices can be expanded in terms of $4 \times 4$ Dirac matrices [50,102,103].
More specifically, the $2 \times 2$ coherency matrix $C$ can be expanded as:

$$ C = \frac{1}{2} \sum_{\nu=0}^{3} \tau_\nu \text{Tr}(\tau_\nu C) = \frac{1}{2} \sum_{\nu=0}^{3} \tau_\nu s_\nu $$

$$ = \frac{1}{2} \left( \begin{array}{cc}
    s_0 + s_1 & s_2 + is_3 \\
    s_2 - is_3 & s_0 - s_1 \\
\end{array} \right) \quad (1.17) $$

The coefficients $s_\nu$ are defined to be the Stokes parameters and can now be formally stated in terms of $2 \times 2$ coherency, identity, and Pauli matrices via:

$$ s_\nu = \text{Tr}(\tau_\nu C) = \text{Tr}(C \tau_\nu) \quad (1.18) $$

Hence, the Stokes vector is real-valued, and this follows from the fact that both $\tau$ and $C$ are hermitian.\(^{10}\) This key feature of Stokes vector to represent light is advantageous as it suggests that the polarization state of light can be fully characterized by observables in an experiment. The Stokes parameters for a plane monochromatic wave are:

$$ s = \left( \begin{array}{c}
    s_0 \\
    s_1 \\
    s_2 \\
    s_3 \\
\end{array} \right) = \left( \begin{array}{c}
    \langle \tilde{E}_1^2 \rangle + \langle \tilde{E}_2^2 \rangle \\
    \langle \tilde{E}_1^2 \rangle - \langle \tilde{E}_2^2 \rangle \\
    \langle 2\tilde{E}_1\tilde{E}_2 \cos \phi \rangle \\
    \langle 2\tilde{E}_1\tilde{E}_2 \sin \phi \rangle \\
\end{array} \right) = \left( \begin{array}{c}
    I_0 + I_{90} \\
    I_0 - I_{90} \\
    I_{45} - I_{-45} \\
    I_{RCP} - I_{LCP} \\
\end{array} \right) \quad (1.19) $$

where $\phi = \phi_1 - \phi_2$. The first component $s_0$ is a measure of total intensity of the optical field. In the convenient basis in which the parameters in Eq. 1.19 are defined, the second and third components indicate the extent to which the optical field is linearly polarized, while the fourth component relay information about the circular polarization contribution to the vector. For a purely polarized light the following relation holds:

$$ s_0^2 = s_1^2 + s_2^2 + s_3^2 \quad (1.20) $$

On the other hand, for partially polarized or unpolarized light the inequality $s_0^2 \geq s_1^2 + s_2^2 + s_3^2$ is valid. An important and very useful parameter known as the degree of polarization is defined as:

$$ dop = \frac{\sqrt{s_1^2 + s_2^2 + s_3^2}}{s_0} \quad (1.21) $$

\(^{10}\)A matrix is hermitian when it is equal to its corresponding transposed complex conjugate.
This \( dop \) ranges from 0 to 1 for unpolarized to fully polarized light, respectively. Similarly, the degree of linear polarization \( dolp = \sqrt{s_1^2 + s_2^2}/s_0 \) and the degree of circularly polarization \( docp = s_3/s_0 \) provide information regarding the linear and circular polarizations contributions, respectively. Equation 1.21 together with 1.19 and the inequality points to an important relation between polarized and unpolarized light. The Stokes vector notation allows to split the two contributions according to the following relation [1,97,104]:

\[
\begin{align*}
\mathbf{s} = \mathbf{s}_p + \mathbf{s}_u &= \begin{pmatrix}
 s_0 \\
 s_1 \\
 s_2 \\
 s_3
\end{pmatrix} = s_0 \text{dop} \begin{pmatrix}
 1 \\
 s_1/(s_0 \text{dop}) \\
 s_2/(s_0 \text{dop}) \\
 s_3/(s_0 \text{dop})
\end{pmatrix} + (1 - \text{dop})s_0 \begin{pmatrix}
 1 \\
 0 \\
 0 \\
 0
\end{pmatrix} \\
\text{Eq. 1.22}
\end{align*}
\]

Therefore, for an optical field, once the Stokes vector is determined, the extent to which the light is polarized can be determined and separated out from the unpolarized part. The \( s_p \) vector components relay information about the specific polarization state that the polarized light has, and in the normalized form, each of the last three components go from \(-1\) to \(+1\). For example, any nonzero value in the fourth component indicate an elliptically polarized contribution, and the limit values of \(-1\) or \(+1\) point to the left circularly-polarized (LCP) or right circularly-polarized (RCP) light, respectively. The relation in Eq. 1.22 is used in Section 4.1 to analyze the data from the nonlinear polarimetric microscopy.

### 1.2.5 Mueller Matrix

As mentioned earlier, the desired method to describe the light-matter interaction is using parameters that are real and observables in an experiments. It was shown in the previous section (1.2.4) that the Stokes vector fully describes the polarization state of light using intensities. Mueller matrix is the complementary notation for the optical properties of a material that transforms the incident radiation Stokes vector to the outgoing radiation Stokes vector. The general form of the Stokes Mueller formalism is presented as:

\[
\mathbf{s}' = \mathbf{M}
\]

\[
\text{Eq. 1.23}
\]

where \( s \) is the Stokes vector defined according to Eq. 1.18 and \( M \) is a matrix representing the intervening material. In the following, the derivation of the Mueller matrix \( M \) is
presented using the aforementioned discussion of transformation of an electric field vector, the coherency matrix and Stokes vector.

By multiplying both sides of Eq. 1.13 for the transformation of coherency matrix with \( \tau_{\mu} \) from the left side, followed by taking the trace, and finally substituting the result into Eq. 1.17, the Stokes component for the outgoing beam can be written as:

\[
s'_\mu = \text{Tr}(\tau_{\mu}JCJ^\dagger) = \text{Tr}(\tau_{\mu}J(\frac{1}{2} \sum_\nu \tau_\nu s_\nu)J^\dagger) = \sum_\nu \frac{1}{2} \text{Tr}(\tau_{\mu}J\tau_\nu J^\dagger) s_\nu \equiv \sum_\nu M_{\mu\nu} s_\nu
\]

where the trace relation \( \text{Tr}(A+B) = \text{Tr}(A) + \text{Tr}(B) \) is used in going from the second line to the third. Thus, given the \( 2 \times 2 \) transformation matrix for Jones formalism, the transformation of the Stokes vector can be achieved by means of a \( 4 \times 4 \) Mueller matrix, and its element can be determined as following:

\[
M_{\mu\nu} = \frac{1}{2} \text{Tr}(\tau_{\mu}J\tau_\nu J^\dagger)
\]

It can be shown that the conjugate of a Mueller matrix element is also \( M_{\mu\nu}^* = \frac{1}{2} \text{Tr}(\tau_{\mu}J\tau_\nu J^\dagger) \), which means Mueller matrix is real [105]. An alternative derivation showing the relationship between Jones and Mueller, where the coherency vector rather than the coherency matrix is used, can be described for the linear polarimetry [1,99]. The analogous nonlinear formalism is developed in the Section 2.1.3.

If Eq. 1.25 is expanded for the most general form of the Jones matrix \( J \), the general form of a Mueller matrix \( M \) that transforms a Stokes vector can be obtained for the Jones matrices. The general form a Mueller matrix in terms of Jones matrix elements is [1,106]:

\[
M = \left( \begin{array}{cccc}
\frac{1}{2}(x_1 + x_2 + x_3 + x_4) & \frac{1}{2}(x_1 - x_2 - x_3 + x_4) & R_{13} + R_{42} & -S_{13} - S_{42} \\
\frac{1}{2}(x_1 - x_2 + x_3 - x_4) & \frac{1}{2}(x_1 + x_2 - x_3 - x_4) & R_{13} - R_{42} & -S_{13} + S_{42} \\
R_{14} + R_{32} & R_{14} - R_{32} & R_{12} + R_{34} & -S_{12} + S_{34} \\
S_{14} + S_{32} & S_{14} - S_{32} & S_{12} + S_{34} & R_{12} - R_{34}
\end{array} \right)
\]

(1.26)
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where \( x_i = J_i J_i^* = |J_i|^2 \), \( \Re_{ij} = \Re_{ji} = \text{Re}(J_i J_j^*) = \text{Re}(J_j J_i^*) \) and \( \Im_{ij} = -\Im_{ji} = \text{Im}(J_i J_j^*) = -\text{Im}(J_j J_i^*) \) for \( i, j = 1 \ldots 4 \); \( J_1, J_2, J_3, \) and \( J_4 \) represent \( J_{11}, J_{22}, J_{12}, \) and \( J_{21} \), respectively [1]. The general form of this matrix is explained elsewhere [101].\(^{11}\) Suffice to say that the Mueller matrix as defined in Eq. 1.26 is responsible for transforming an optical field, represented by a \( 4 \times 1 \) Stokes vector into another \( 4 \times 1 \) outgoing Stokes vector. Each component in each row in the Mueller matrix determines the extent of contribution of the incoming Stokes vector to the respective outgoing Stokes vector element. For example, if all components in the fourth row are zero, then there will not be any elliptical polarization contribution in the outgoing light. Similar relation applies to other rows as well.

Interestingly, a similar approach can also be taken to derive the matrix for the nonlinear processes. An alternative derivation showing the relationship between Jones and Mueller, where a coherency vector rather than the coherency matrix, can be described for linear polarimetry [1,99]. The analogous nonlinear formalism is developed and shown in Section 2.1.6. In the context of this thesis, there is an important relation between the components of Mueller matrix and the components of the Jones matrix for the linear case as described below. This is elaborated in more details in Section 2 for the nonlinear case and will reveal important implications.

An important realization about the linear Mueller matrix in Eq. 1.26 is that all elements with \( x_i \) are real regardless of whether \( J_{ij} \) is real or complex. Meanwhile, if \( J_{ij} \) is real, then all elements with \( \Im_{ij} \) vanish, while those with \( \Re_{ij} \) are maximum. Furthermore, it is worth repeating that the derivation above is for a Mueller matrix \( M \) in terms of the most general case of Jones matrix \( J \); however, the transformation from \( M \) to \( J \) is generally impossible as there are more independent elements in \( M \) than in \( J \). This is why a Mueller matrix \( M \) can describe the depolarizing effects of a system, while the Jones matrix \( J \) can only describe the non-depolarizing interactions of light with matter; that is, the description is for the purely polarized states of light [101].

\(^{11}\)For useful descriptions of necessary and sufficient conditions to describe a Mueller matrix in term of a Jones matrix see refs. [105,107]. For useful descriptions of deterministic and nondeterministic Mueller matrix in term of a Jones matrix see refs. [23,108,109]. The physical interpretation of Mueller is given in ref. [36,110]; and the geometrical interpretation on the Poincaré sphere is provided in ref. [111]. For a review of measurements of Mueller polarimetry see refs. [23,104,109,112].
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Chain and Transformation of Mueller Matrices

The Mueller matrix is a convenient tool to express the effect of polarization of light when Stokes vector notation is used. For the linear interaction of light with matter the Mueller matrix is a $4 \times 4$ matrix where the real-valued numbers are dependent on the propagation vector $\vec{k}$ and wavelength $\lambda$ [104]. Mueller matrices just like Jones matrices can be cascaded to denote a train of elements, including the sample under study, where the combined action is simply the right-to-left product of individual matrices:

$$M = M_t M_{t-1} \cdots M_2 M_1 = \prod_{i=1}^{t} M_i$$  \hspace{1cm} (1.27)

Mueller matrices generally do not commute, and therefore, the order of matrices in a cascaded system should be strictly followed. In the case where an element is rotated with respect to a pre-defined orientation, the corresponding Mueller matrix is simply rotated about the beam by the same angle [104]:

$$M(\theta) = R_M(\theta) M R_M(-\theta) =$$

$$\begin{bmatrix}
1 & 0 & 0 & 0 \\
0 & \cos(2\theta) & -\sin(2\theta) & 0 \\
0 & \sin(2\theta) & \cos(2\theta) & 0 \\
0 & 0 & 0 & 1
\end{bmatrix} \begin{bmatrix}
m_{00} & m_{01} & m_{02} & m_{03} \\
m_{10} & m_{11} & m_{12} & m_{13} \\
m_{20} & m_{21} & m_{22} & m_{23} \\
m_{30} & m_{31} & m_{32} & m_{33}
\end{bmatrix} \begin{bmatrix}
1 & 0 & 0 & 0 \\
0 & \cos(2\theta) & \sin(2\theta) & 0 \\
0 & -\sin(2\theta) & \cos(2\theta) & 0 \\
0 & 0 & 0 & 1
\end{bmatrix}$$  \hspace{1cm} (1.28)

where $\theta > 0$ is the angle by which the optical element represented by $M$ is rotated from the primary axis of the incident beam. $R_M(\theta)$ (where $R_M(-\theta) = (R_M(\theta))^T$) is the rotational matrices for changing the basis for Stokes vectors and Mueller matrices [104].

1.2.6 Poincaré Sphere Representation

Poincaré sphere representation is a complimentary and sometimes more useful way to geometrically describe the polarization of light when Stokes vector notation is employed. Figure 1.1 shows the Poincaré sphere representation of the Stokes parameters. On a Poincaré sphere the intersection of any two of the three perpendicular great circles provide the six polarization states as the basis for determining the Stokes vector. Conventionally, these six states are chosen to be $0^\circ$, $90^\circ$, $45^\circ$, $-45^\circ$, RCP and LCP (see Eq. 1.19).
Any point on the equator represents a linearly polarized light. North and South poles represent the right- and left-circularly polarized light, respectively. Any point in the northern hemisphere denotes a right elliptically-polarized, while those in the south represent left elliptically-polarized light. The representation also facilitates the polarization state transformations by geometrical means as well. For example, any transformation of polarization is the same as drawing an arc on the sphere between the points representing the states before and after transformation. In fact, in early days, wood or plastic spheres were used in the laboratories to qualitatively obtain the transformation of the polarization by a known rotator or retarder [8].

![Figure 1.1: Poincaré sphere for Stokes vector. The black arrowed lines indicate the linearly polarized and the circles indicate the circularly polarized light. These six states have been used as a standard set of intensities to determine the $4 \times 1$ Stokes vector in an experiment. The green markings in addition to the six standard states indicate the two-photon states (in Eq. 2.92), and the purple markings in addition to the green and the six standard states show the three-photon states (in Eq. 2.134) that form the orthogonal basis for the corresponding polarimetry. The two circles above and below the equator mark the $\pm 45$ degree latitude.](image)

A more rigorous analysis of polarization state transformation would require precise calculations. On a Poincaré sphere the Cartesian coordinates are represented by Stokes parameters $s_1, s_2, s_3$, and the radius of the sphere is represented by $s_0$ for purely polarized, or $dop \cdot s_0$ for partially polarized light.
The following recipe can be followed to obtain an arbitrary polarization state on the Poincaré sphere using a linearly polarized light, a half- and a quarter-waveplate. The linearly polarized light with the magnitude $E_0$ at the angle $\theta_0$ from the horizontal axis is given by:

$$\vec{E}(\theta_0) = E_0 \begin{pmatrix} \cos \theta_0 \\ \sin \theta_0 \end{pmatrix}$$  \hfill (1.29)

By passing this linearly polarized light first through a quarter waveplate and then through a half-waveplate, the emerging electric field in terms of Poincaré coordinates and the Jones notation becomes [1,113]:

$$\vec{E}(\Psi, \Omega) = H\left(\frac{1}{2}(\Psi - \Omega + \theta_0)\right) Q\left((-\Omega + \theta_0)\right) \vec{E}(\theta_0),$$

$$\vec{E}(\Psi, \Omega) = \frac{E_0}{\sqrt{2}} \begin{pmatrix} \cos (\Psi + \Omega) + i \cos (\Psi - \Omega) \\ \sin (\Psi + \Omega) + i \sin (\Psi - \Omega) \end{pmatrix}$$  \hfill (1.30)

where $\Psi$ is the azimuthal and $\Omega$ is the altitude angle on the Poincaré sphere. The formula for the half-waveplate and quarter waveplate are as [113]:

$$H(\theta_h) = i \begin{pmatrix} \cos (2\theta_h) & \sin (2\theta_h) \\ \sin (2\theta_h) & -\cos (2\theta_h) \end{pmatrix},$$

$$Q(\theta_q) = \sqrt{\frac{1}{2}} \begin{pmatrix} 1 + i \cos (2\theta_q) & i \sin (2\theta_q) \\ i \sin (2\theta_q) & 1 - i \cos (2\theta_q) \end{pmatrix}$$  \hfill (1.31)

Substituting Eq. 1.30 for electric fields into Eq. 1.19 results in the Stokes vector in terms of Poincaré (spherical) coordinates:

$$s = \langle E_0^2 \rangle \begin{pmatrix} 1 \\ \cos(2\Psi) \cos(2\Omega) \\ \sin(2\Psi) \cos(2\Omega) \\ \sin(2\Omega) \end{pmatrix}$$  \hfill (1.32)

Therefore, when the state of polarizations on the Poincaré sphere is chosen, the desired polarization state is obtained by orienting the quarter and half waveplates at the calculated $\theta_h$ and $\theta_q$ angle values, respectively. This exercise also provides a method to obtain the nonlinear Stokes vectors for incoming radiations in a nonlinear polarimetric experiment, which will be discussed in Section 2.2.
1.3 Introduction to Nonlinear Optics

In this section the theoretical background for the nonlinear optics, particularly the parts that are directly relevant to this thesis, will be presented. The emphasis has been put on key derivations and notations, which will be subsequently used in the other chapters, particularly in Chapter 2.

1.3.1 Nonlinear Wave Equation

The induced polarization density \( \vec{P} \) described in Eq. 1.2 can be expanded in terms of linear and nonlinear contributions due to the applied electric field:

\[
\vec{P} = \vec{P}^{(1)} + \vec{P}^{(2)} + \vec{P}^{(3)} + \ldots = \vec{P}^{(1)} + \vec{P}^{(NL)}
\]

Substituting Eq. 1.33 into Eq. 1.3, the nonlinear wave equation can be obtained:

\[
\hat{\nabla} \times \hat{\nabla} \times \vec{E} + \frac{\epsilon^{(1)}}{c^2} \frac{\partial^2 \vec{E}}{\partial t^2} = -\mu_0 \frac{\partial^2 \vec{P}^{(NL)}}{\partial t^2}
\]

where \( \epsilon^{(1)} = \delta_{\mu\alpha} + \chi^{(1)}_{\mu\alpha}(-\omega_s; \omega) \) is the relative electrical permittivity [96]. In the case of an isotropic non-dissipative (no absorption) media the (linear) refractive index can be defined as \( n^2 = \epsilon^{(1)} \), while in the case of absorptive media a complex refractive index can be defined as \( n' = n + i\alpha \). Then, the real part is responsible for the intensity-dependent refractive index, while the imaginary part, to which the imaginary part of a complex susceptibility contributes, determines the absorption coefficient \( \alpha \) [43].

Note, in Eq. 1.34 the left-hand-side is a wave equation for an electric field that is driven by a nonlinear response of the medium as the source. This is the origin of the nonlinear optical phenomena that can be described in terms of different orders of susceptibilities by expanding on the constitutive relation for \( \vec{D} \) (in Eq. 1.2). Similar to the derivation of the first-order (see Section 1.2.2), the second-order polarization density, in term of the response function and its corresponding second-order susceptibility tensor \( \chi^{(2)} \), is [43]:

\[
\vec{P}^{(2)}(\omega) = \varepsilon_0 \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} d\omega_1 d\omega_2 \chi^{(2)}(-\omega_s; \omega_1, \omega_2) : \vec{E}(\omega_1) \vec{E}(\omega_2) \delta(\omega - \omega_s)
\]

(1.35)
where $\omega_s = \omega_1 + \omega_2$. Similarly, the third-order polarization density is:

$$\vec{P}^{(3)}(\omega) = \varepsilon_0 \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} d\omega_1 d\omega_2 d\omega_3 \chi^{(3)}(-\omega_\sigma;\omega_1, \omega_2, \omega_3) \vec{E}(\omega_1) \vec{E}(\omega_2) \vec{E}(\omega_3) \delta(\omega - \omega_s)$$  \hspace{1cm} (1.36)

More generally the polarization density for the $n^{\text{th}}$-order interaction is given as [95]:

$$\vec{P}^{(n)}(\omega) = \varepsilon_0 \int_{-\infty}^{\infty} d\omega_1 \cdots \int_{-\infty}^{\infty} d\omega_n \chi^{(n)}(-\omega_\sigma;\omega_1, \ldots, \omega_n) \vec{E}(\omega_1) \cdots \vec{E}(\omega_n) \delta(\omega - \omega_s)$$  \hspace{1cm} (1.37)

where the nonlinear susceptibilities are defined from the response function of the media [95]:

$$\chi^{(n)}(-\omega_\sigma;\omega_1, \ldots, \omega_n) = \int_{-\infty}^{\infty} dt_1' \cdots \int_{-\infty}^{\infty} dt_n' R^{(n)}(t_1', \ldots, t_n') e^{i\omega j t_j}$$  \hspace{1cm} (1.38)

where the summation is assumed over the index $j$ in the exponential, and $\omega_\sigma = \omega_1 + \omega_2 + \cdots + \omega_n$. Note, $\chi^{(n)}$ and $R^{(n)}$ are tensors for $n > 1$; therefore, any $\chi^{(n)}$ with $n > 1$ that has more than two subscript indices is assumed to be a tensor from here on.

The total polarization density can be written as:

$$\vec{P} = \varepsilon_0 \chi^{(1)} \cdot \vec{E} + \varepsilon_0 \chi^{(2)} : \vec{E} \vec{E} + \varepsilon_0 \chi^{(3)} : \vec{E} \vec{E} \vec{E} + \ldots$$  \hspace{1cm} (1.39)

Equation 1.39 is informative and very useful for the derivation of nonlinear polarimetry equations. As was shown in Section 1.2.2 the first term leads to the equation for linear polarimetry; second, third, and higher terms can be used to derive two, three, and other nonlinear polarimetry equations, as will be shown in Chapter 2.

### 1.3.2 Susceptibility Symmetry Considerations

In this thesis, the following five types of symmetries may be implicitly or clearly referred to and are therefore useful to describe [95, 96]:

1. Intrinsic permutation symmetry: $\chi^{(n)}_{\mu_1 \cdots \mu_n}(-\omega_\sigma;\omega_1, \cdots, \omega_n)$ yields invariance under $n!$ possible permutations of the pairs $(\alpha_1, \omega_1), (\alpha_1, \omega_1), \cdots, (\alpha_n, \omega_n)$. This symmetry applies universally to resonant as well as nonresonant media.
2. Overall permutation symmetry: If all frequencies, including the outgoing ones, are far from the transition frequencies (i.e. in nonresonant media), an additional permutation to the intrinsic permutation symmetry is allowed; hence, this permutation symmetry is over all frequencies. In other words, \( \chi_{\nu_1 \cdots \nu_n}^{(n)}(-\omega_\sigma; \omega_1, \cdots, \omega_n) \) is invariant under the \((n+1)!\) possible permutations of \((\mu, -\omega_\sigma), (\alpha_1, \omega_1), (\alpha_1, \omega_1), \cdots, (\alpha_n, \omega_n)\). This permutation applies when all optical frequencies are much smaller than any of the transition frequencies of the sample, for example, when both the fundamental and the radiated frequencies are below the absorption bands.

3. Kleinman symmetry: This is a form of overall permutation symmetry where all frequencies are below any electronic transition frequencies (nonresonant media). It yields invariance under the \((n+1)!\) possible permutations of the pair \((\mu, -\omega_\sigma), (\alpha_1, \omega_1), (\alpha_1, \omega_1), \cdots, (\alpha_n, \omega_n)\).

4. Inversion symmetries for all even-ordered symmetry yield zero: \( \chi_{\nu_1 \cdots \nu_n}^{(n)} = 0 \). This is a general property for all even-ordered processes; however, it is mostly encountered in and a commonly known feature of second-harmonics. This is why the SHG process is only possible in non-centrosymmetric media.

5. Spatial symmetries, which are determined by the point and crystal symmetry class of media are described in the Box: Spatial Symmetries Frequently Used In This Thesis.

For more detailed description of various symmetries and different classes of materials the reader can consult Chapter 2 in Boyd [93], and Chapter 5 in Butcher & Cotter [95].

**Tensor Rotation**

Often the sample is not aligned along a desired set of coordinates, or that the axes of symmetries are not known. Therefore, the properties of the tensor in the new coordinate system becomes of interest. The general way to track the rotation of the axes are thus useful. The following tensor rotation formula can be used to find the susceptibilities tensor elements in the new \((IJK)\) coordinates when the rule for rotation from the index
i to I is governed by the matrix $R_{ii}$:

$$
\chi^{(2)'}_{IJK} = R_{Ii}R_{Jj}R_{Kk}\chi^{(2)}_{ijk}
$$

(1.40)

A commonly used set of rotation matrices are the Euler angles for the Cartesian coordinates. Euler angles provide the rotation matrices for the susceptibility tensor transformations into an alternative coordinate system [114]:

$$
R(\varphi, \theta, \psi) = A(\psi)B(\theta)C(\varphi),
$$

where

$$
A(\psi) = \begin{pmatrix}
\cos \psi & -\sin \psi & 0 \\
\sin \psi & \cos \psi & 0 \\
0 & 0 & 1
\end{pmatrix},
B(\theta) = \begin{pmatrix}
\cos \theta & 0 & \sin \theta \\
0 & 1 & 0 \\
-\sin \theta & 0 & \cos \theta
\end{pmatrix},
C(\varphi) = \begin{pmatrix}
\cos \varphi & -\sin \varphi & 0 \\
\sin \varphi & \cos \varphi & 0 \\
0 & 0 & 1
\end{pmatrix}.
$$

(1.41)

Spatial Symmetries Frequently Used in This Thesis

Second-order Susceptibility: Hexagonal

$C_6$: $zzz$, $xxx = zyy$, $zzx = yzy$, $xxz = xyz$, $xzx = -yzy$, $xzy = -yzx$

$C_{6v}$: $zzz$, $xxx = zyy$, $xxz = yzy$, $xxz = yyz$

Third-order Susceptibility:

Isotropic:

$xxxx = yyyy = zzzz = xxyy + xyyx + yxxy$, 
$yyzz = zyyz = zzzz = xzxy = xzyy = yxyy$, 
$yzyz = yzyz = zzzz = xzyx = yxyx$, 
$yzzy = yzyy = zzzz = xyyx = yxxy$, 

$C_6$: $zzzz$, $xxxx = yyyy = xxxy + xyyx + yxxy = yxxy + yxyx + yyyx$ 
$yyzz = xxzz$, $zyyz = xzxy = zzzz$, $zyzy = xxzz$, $zyzz = xxxz$, $zyyz = zzzz$ 
$xzzz = -yyzz$, $-zyzz = -zxxz$, $zxxz = -zyzz$, $xxzz = -zyzz$ 
$zzxy = -yxzz$, $-zzxz = -yyyx$, $zzxz = -yxzz$, $yyxx = -zzxx$ 
$xzzz = -yyxx = xxzz = yyyy = xxxy = yyxx = yxxy = yyyx - xyyx - yxyx - xxxz$

$C_{6v}$: $zzzz$, $xxxx = yyyy = xxxy + xyyx + yxxy = yxxy + yxyx + yyyx$ 
$yyzz = xxzz$, $zyyz = xzxy = zzzz$, $zyzy = xxzz$, $zyzz = xxxz$, $zyyz = zzzz$, $zyyz = zzzz$, $zyyz = zzzz$
Transfer of Symmetry

A susceptibility tensor that possesses overall, intrinsic or Kleinman symmetry also possesses them when it is transformed in the new coordinate system. This simple conclusion simplifies the application of symmetry, provided that the permutation and symmetries of the material is known, but the material has been rotated and its symmetry axes are not aligned along the laboratory coordinates. Below is an example of permutation for the second-order susceptibility element $\chi^{(2)}_{ijk}$ that exchanges the first index with the second.

\[
\chi^{(2)}_{IJK} = R_{Ii} R_{Jj} R_{Kk} \chi^{(2)}_{ijk} = R_{Ik} R_{Jj} R_{Kk} \chi^{(2)}_{jik} = R_{Jj} R_{Ik} R_{Kk} \chi^{(2)}_{jik} = \chi^{(2)'}_{JIK}
\]

(1.42)

In the example above the transformed tensor is written in the elemental form, and the sum over repeated indices are assumed. The relation shown in Eq. 1.42 is the justification for assuming, for example, the Kleinman symmetry for the rotated coordinate system $IJK$ when the original system $ijk$ allows for such symmetry. This relation is used when considering the derivation in Section 2.2.4, and when discussing the results in Chapter 4.

Neumann’s Principle

A foundational postulate known as the Neumann’s principle requires that any type of symmetry possessed by the point symmetry group of the medium is also possessed by every physical property including the susceptibility tensors of the medium. Therefore, if a tensor transformation is similar to point symmetry of the medium, then the susceptibility elements remain unchanged:

\[
\chi^{(n)'}_{\mu\alpha_1\ldots\alpha_n} (-\omega;\omega_1,\ldots,\omega_n) = R_{\mu u} R_{\alpha_1 a_1} \cdots R_{\alpha_n a_n} \chi^{(n)}_{u a_1\ldots a_n} (-\omega;\omega_1,\ldots,\omega_n) = \chi^{(n)}_{\mu a_1\ldots a_n} (-\omega;\omega_1,\ldots,\omega_n)
\]

(1.43)

where the $3 \times 3$ matrix $R$ must operate exactly the same as the point symmetry of the system. Almost all the symmetries described for the susceptibilities of the class material shown in the Box: Spatial Symmetries Frequently Used in This Thesis, derives from this principle. This principle allows, for example, to use the same susceptibilities for cylindrically symmetric material, despite the fact that the sample may have been rotated around its cylindrical z-axis.
Chapter 1. Introduction

1.3.3 SHG

Second-harmonic generation is an important nonlinear optical phenomena and used for measurements in Chapter 4 of this thesis. The SHG characterizes uniquely a number of material, particularly biologically important tissue, such as muscle, collagen, and starch. Using Eq. 1.33 the polarization density for SHG simplifies to [43]:

$$\vec{P}^{(2)}(2\omega) = \frac{\varepsilon_0}{2} \chi^{(2)}(-2\omega;\omega,\omega) : \vec{E}(\omega)\vec{E}(\omega)$$ (1.44)

As described in Section 1.3.2, under symmetry considerations, the second-harmonic polarization density, and the ensuant SHG signal from a centrosymmetric material is zero.

Contracted Notation for SHG

For the SHG process, as well as other two-photon processes, including in sum- and difference-frequency generations, where Kleinman symmetry is valid, a contracted notation for the second-order susceptibility is used. In the contracted notation the tensor elements are written in the form of a matrix [93].

$$d_{il} = \frac{1}{2} \chi_{ijk}, \quad \text{where} \quad ij : 11 22 33 23, 32 31, 13 12, 21 \quad l : 1 2 3 4 5 6$$ (1.45)

For SHG the polarization density components can be expressed as:

$$\begin{bmatrix} P_x(2\omega) \\ P_y(2\omega) \\ P_z(2\omega) \end{bmatrix} = 2\varepsilon_0 \begin{bmatrix} d_{11} & d_{12} & d_{13} & d_{14} & d_{15} & d_{16} \\ d_{21} & d_{22} & d_{23} & d_{24} & d_{25} & d_{26} \\ d_{31} & d_{32} & d_{33} & d_{34} & d_{35} & d_{36} \end{bmatrix} \begin{bmatrix} E_x(\omega)^2 \\ E_y(\omega)^2 \\ E_z(\omega)^2 \end{bmatrix}$$

(1.46)

The electric fields on the right side make up a vector that multiply to the matrix $d$, and produce the polarization response $P$. The form of this vector-matrix notation is useful to notice, because a similar approach is taken to introduce the state of the incoming radiation when deriving the formalism for the nonlinear polarimetry (in Chapter 2).
1.3.4 THG

Third harmonic generation (THG) is another very important process where, for example, the signal is most prominent at the interface of two different medium, such as an air-glass interface or the lipid membrane of a biological cell. The effect is mainly due to Gouy phase shift of a focusing beam, first described in 1890 [115, 116]. Similar to second-harmonic, the third-order nonlinear polarization density for THG can be expressed as [43]:

\[
\vec{P}^{(3)}(3\omega) = \frac{\varepsilon_0}{4} \chi^{(3)}(-3\omega;\omega,\omega,\omega) : \vec{E}_\omega \vec{E}_\omega \vec{E}_\omega
\]  

(1.47)

where \( \chi^{(3)} \) is a third-order tensor. In the context of nonlinear polarimetry formalism, a contracted notation similar to the second-harmonic will be defined for THG as well.

1.3.5 Intensity of Generated Harmonics with Focused Gaussian Beam

In this thesis the following considerations are made for the wave equation: A laser with the Gaussian transverse profile is used. Additionally, for the electric field, the identity \( \nabla \times \nabla \times \vec{E} = \nabla (\nabla \cdot \vec{E}) - \nabla^2 \vec{E} = -\nabla^2 \vec{E} \) applies. By letting \( \vec{E}_{\omega_n}(z,t) = A_{\omega_n}(z,t)e^{ik_nz}, \) and assuming a unidirectional propagation of the electric field in the positive z-direction,\(^{12}\) the term on the left hand side of the wave equation in Eq. 1.34 becomes:

\[
\nabla \times \nabla \times \vec{E}_{\omega_n}(z,t) = - \left( \frac{\partial^2 A_{\omega_n}}{\partial z^2} + 2ik_n \frac{\partial A_{\omega_n}}{\partial z} - k_n^2 A_{\omega_n} \right) e^{ik_nz}
\]  

(1.48)

The real-valued electric field takes the form:

\[
\vec{E}(\vec{r},t) = \sum_{\omega_n \geq 0} \text{Re}(\vec{E}_{\omega_n}(\vec{r},t)e^{(-i\omega_n t)})
\]

\[
= \sum_{\omega_n \geq 0} |A_{\omega_n}(z,t)| \cos(k_n z - \omega_n t + \phi(z))
\]  

(1.49)

where \( \phi \) is the phase of the slowly varying envelope function \( A_{\omega_n}(z,t) \) of the electric field [96]. Similarly, \( \vec{P}_n = \vec{P}_n e^{i(k'_n z - \omega_n t)} + c.c. \) for the polarization density [93]. Further-

\(^{12}\)Note, this choice for the propagation direction is a matter of historical convention, and in Chapter 2 the Y-axis of the laboratory coordinate system is adopted to simplify some of calculations.
more, by using the slowly varying envelope approximation: \( \left| \frac{\partial^2 A_{\omega n}}{\partial z^2} \right| \ll \left| k_n \frac{\partial A_{\omega n}}{\partial z} \right| \), the wave equation for the nonlinear field becomes [93]:

\[
2 i k_n \frac{\partial A_n}{\partial z} + \nabla_T^2 A_n = -\mu_0 \omega_n^2 p_n e^{i \Delta k z} \quad (1.50)
\]

where \( \nabla_T \) is the transverse laplacian (in \( x \) and \( y \)). In the constant pump-approximation the amplitude of the \( n^{th} \)-order electric field has the following Gaussian form [93]:

\[
A_n(r, z) = \frac{A_n(z)}{1 + i 2 z / \iota} e^{-nr^2/w_0^2(1+i2z/\iota)} \quad (1.51)
\]

where \( r^2 = x^2 + y^2 \), \( x \) and \( y \) being the polarization orientation of the electric field, and \( z \) is measured along the propagation direction. \( \iota = 2 \pi w_0^2 / \lambda \) is the confocal parameter for both fundamental \( A_1 \) beam as well as harmonic \( A_n \) beam [93]. \( w_0 \) is the radius of the beam waist at the focus, and \( \lambda \) and \( \omega \) is the wavelength and frequency of the fundamental beam, respectively. The following solves the wave equation [93]

\[
A_n(z) = \frac{i n \omega}{2 n c} \chi^{(n)}(n) A_1^n J_n(\Delta k, z_0, z) \quad (1.52)
\]

where\(^\text{13}\) \( n \) is the refractive index, \( \Delta k = nk - k_n \) is the phase mismatch, \( A_1 \) is the amplitude of the fundamental field and,

\[
J_n(\Delta k, z_0, z) = \int_{z_0}^{z} \frac{e^{i \Delta k z'}}{(1 + 2 i z'/\iota)^{n-1}} dz' \quad (1.53)
\]

where \( z_0 \) is the interface that the beam enters the nonlinear medium. Then, the integrated \( n \)-harmonic intensity for a focused Gaussian beam is:

\[
I_n = \int_{-\infty}^{\infty} |A_n(r, z)|^2 dr = |A_n(z)|^2 \int_{-\infty}^{\infty} e^{-2nr^2/w_0^2(1+2z/\iota)} dr \quad (1.54)
\]

\[
\propto |A_1^n J_n(\iota)|^2 \iota
\]

\(^{13}\)Note, the refractive index is denoted by \( n \), and the order of interaction is represented by \( n \). For a complete list of variables see Appendix A.
where the identity $\int_{-\infty}^{\infty} e^{-ar^2} dr = \pi/a$ is used. $A_1$ also depends on the confocal parameter $\imath$, and for a constant input power of fundamental beam: $\int_{-\infty}^{\infty} A_1^2 dr = C$, where $C$ is a constant. Thus, $A_1^2 \pi w_0^2 = C$ or $A_1^2 = 2C/\imath \lambda$. Then, the generated $n^{th}$-order intensity is:

$$I_n \propto \frac{1}{\imath^{n-1}}|J_n(\imath)|^2$$  \hspace{1cm} (1.55)

Numerical calculations and experimental results (see Section 3.4) show that for a given input power, the nonlinear signal decreases inversely with increasing confocal parameter $\imath$. This makes intuitive sense as well: it implies that for a constant fundamental power, if the beam focusing is not tight, the power of generated signal will go down due to a lower photon density, and the harmonic signal will go down with decreased fundamental intensity. Eq. 1.55 is used in Section 3.4 to fit the scatter plot of the THG intensity vs. the point-spread-function.

The background theory presented in this chapter will be used in Chapter 2 to develop the nonlinear polarimetry theory. In particular, the coherency matrix, the Stokes and Mueller notations will be expanded to include the nonlinear electric fields and susceptibilities. The symmetry relations and the contracted notation are exploited further to simplify the derived nonlinear formalism. The Stokes Mueller formalism will also be used in Chapter 4 to analyze the data from the biological samples.
Chapter 2

Theory of Nonlinear Optical Polarimetry

In this chapter the theory of nonlinear optical polarimetry will be developed.\(^1\) Polarimetry techniques entail the characterization of the polarization state of the optical response from a sample due to a known incoming radiation polarization state. Linear optical polarimetry is a well-established measurement technique that has found applications in various research fields including material science and biomedical imaging. For example in biomedical research, linear polarimetry has been used for structural characterization of biological tissue revealing important changes related to various diseases [26, 27, 29, 34, 117–119]. At the same time, nonlinear optical techniques, such as second-harmonic generation (SHG), third-harmonic generation (THG), coherent anti-Stokes Raman scattering (CARS), and others, have also provided indispensable possibilities to study materials including biological tissue [59, 63, 87, 120–124]. However, the amalgamation of the two techniques has been long overdue. Recently, attempts have been made to deal with the nonlinear polarization measurements in a linear fashion. For example SHG signal from samples have been characterized by a \(4 \times 1\) Stokes vector as well as for the incoming radiation [53, 125]. Consequently, characterizations of the samples have remained unresolved, mainly because of the nonlinear relationship between the

\(^1\)The following chapter was in part adapted from previously published work. Reprinted (adapted) with permission from Samim, M., S. Krouglov and V. Barzda (2015). “Double Stokes Mueller polarimetry of second-harmonic generation in ordered molecular structures.” *Journal of the Optical Society of America B* 32(3): 451–461.
incoming and outgoing radiations. In ellipsometry, for two-photon processes a nonlinear relationship have been attempted by using a quantum-mechanical framework and a Jones Stokes approach [50, 126]. Combined, these efforts demonstrate the need for a unifying and general framework for nonlinear optical Stokes-Mueller polarimetry. The union has the potential to broaden the scope to incorporate nonlinear light-matter interactions and their relevant polarimetry measurements. In addition, some methods developed in the field of linear polarimetry may be creatively applied for the nonlinear optical polarimetry.

In an optical setup the polarization-dependent interaction of light with matter can be described using the Stokes-Mueller, Poincaré, or Jones formalism [1,8,113]. Each formalism has unique advantages applicable for various circumstances. In the linear Stokes-Mueller formalism, the light is represented by a four-element Stokes vector, and its interaction with matter is represented by a $4 \times 4$ Mueller matrix. The Stokes vector can describe partially- or completely-polarized light, and operates with intensities, which are real numbers, and thus, observables in an experiment. On the other hand, Jones formalism is used to describe purely polarized states retaining the phase relations of the electric fields and requires working with complex variables.

The theoretical framework for nonlinear optical polarimetry is developed in this chapter. The nonlinear polarimetry framework, similar to the Stokes-Mueller formalism, has significant advantages. The polarization state of light as well as the response of a material are described with real-valued parameters. In the multi-photon polarimetry, the Jones and Stokes-Mueller formalism is analogous to the conventional linear polarimetry. The Jones formalism can be used to describe a nonlinear light-matter interaction using second-order susceptibilities and pure polarization states. However, the biological tissue is a highly heterogeneous scattering material; therefore, it advantageous to employ Stokes-Mueller formalism for polarization analyses of the nonlinear optical responses. Additionally, the linear polarimetry technique has extensive and comprehensive formulations for describing a measurement system, some of which are applicable to the nonlinear polarimetry experiments. For nonlinear polarimetry, describing the polarization density is the starting point; the incoming radiation interacts nonlinearly with the sample through susceptibilities and gives rise to the polarization density. Analogous to this paradigm, the derivations of vectors for polarization states of radiations, both incoming and outgoing, and the characterization of the matrix for the material, are the main focus of this chapter.
The contents of this Chapter are organized as follows: First, the formalism for an \(n^{\text{th}}\)-order process is developed. Expressions are derived from the basic relationship between the nonlinear electric field and interaction with nonlinear susceptibilities. The general derivations are then followed by two- and three-photon processes. In each section, detailed formula are provided for the incoming and outgoing radiations as well as for the intervening media. In particular, important processes such as SHG, SFG, DFG, THG and CARS are discussed. Additionally, in each section properties of the vector for nonlinear fields and the matrix for the material are described. The methods to measure the elements of the two- and three-photon matrices are also presented. A brief summary of the derived equations and key take-away messages will conclude this chapter.

### 2.1 Theory of Nonlinear Stokes-Mueller Polarimetry

The general nonlinear Stokes-Mueller equation, describing the relationship between the generated nonlinear signal radiation, the nonlinear properties of the media, and the incoming radiations can be written as:

\[
s'(\omega_\sigma) = M^{(n)}S(\omega_1, \omega_2, \cdots, \omega_n)
\]  

(2.1)

where \(s'\) is the Stokes vector describing the generated radiation at \(\omega_\sigma\) frequency and prime signifies the measured outgoing signal. \(M^{(n)}\) is the nonlinear Mueller matrix describing a material capable of an \(n^{\text{th}}\)-order light-matter interaction, while \(S\) is a vector representing the incoming electric fields that generate the light via nonlinear interactions. Henceforth, the \(s'\) and \(S\) are called the polarization state vectors for outgoing and incoming radiations, respectively.

The left hand side is the outgoing radiation, which once generated is simply represented by an ordinary \(4 \times 1\) Stokes vector, as was previously discussed in Section 1.2.4. The right hand side variables, at a more basic level, incorporate two physical quantities: the nonlinear susceptibility, which is directly related to the structure of the material, and the nonlinearly interacting electric field, which are from the incoming light radiation. At this level the two key variables are: \(\psi\), or the state function of fields that interact to produce a particular nonlinear phenomenon; and \(\chi\), the nonlinear susceptibility matrix that represents the material in the context of polarimetry. The definition of polarization
density that was derived earlier for nonlinear optical interactions can be stated as follows:

\[ P_i^{(n)} = \chi_{ijkm}^{(n)} E_j E_k \cdots E_m = \chi_{iA}^{(n)} \psi_A^{(n)} \]  \hspace{1cm} (2.2)

where (Einstein) summation is assumed over the repeated indices. The first index for \( \chi \) represents the orientation of the outgoing polarization, and the second index represents the contracted notation for the direction of polarization of incoming electric fields. The relation between the index \( A \) and \( j, k, \ldots, m \) is specific for a given particular nonlinear process. The dimensions of the variables and expressions of the state functions are provided explicitly for two- and three-photon processes in Sections 2.2 and 2.3, respectively. Essentially, for an \( n^{th} \)-order nonlinear optical phenomena \( A \) runs from 1 to \( n + 1 \), and \( i \) represents two orthogonal vectors expanding the plane of polarization perpendicular to the direction of light propagation.\(^2\)

The Stokes vector deals with the light intensity, and the nonlinear intensity depends on the susceptibility and the interacting electric fields according to the following equation:

\[ I \propto |P_i|^2 = \chi_{iA} \chi_{iB}^* \psi_A \psi_B \]  \hspace{1cm} (2.3)

Thus, it is obvious that the Stokes and Mueller notations are composed of products of electric fields vectors, and products of susceptibilities components, respectively.

At the level of individual electric fields, the outgoing field, which is denoted by the state vector \( \Phi' \), is related to the products of incoming nonlinear electric fields, denoted by the state vector \( \psi^{(n)} \), which interacts with the nonlinear susceptibility that is denoted by \( \chi^{(n)} \):

\[ \Phi'(\omega_\sigma) = \chi^{(n)} \psi^{(n)}(\omega_1, \omega_2, \cdots, \omega_n) \]  \hspace{1cm} (2.4)

In this framework, each component of vector of the generated electric field \( \Phi' \) is proportional to the polarization density described in Eq. 2.2. Moreover, \( \Phi' \) depends on the susceptibility tensor components and the polarization state of the laser radiation \( \psi^{(n)} \), which has \( n + 1 \) components composed of interacting incoming electric fields (see Eq. 2.2). In general, the state vector for the nonlinear combination of electric fields can be written

\(^2\)Thus, any matrix notation of \( \chi_{iA}^{(n)} \) (i.e. with two indices) represent the contracted notation of \( n^{th} \)-order nonlinear susceptibilities.
as:

\[
\psi^{(n)}(\omega_1, \omega_2, \cdots, \omega_n) = \begin{pmatrix}
\psi_1^{(n)} \\
\psi_2^{(n)} \\
\vdots \\
\psi_{n+1}^{(n)}
\end{pmatrix}
\] (2.5)

Each element of the state vector \(\psi_A^{(n)}\) \((A = 1, \cdots, n + 1)\) is an \(n\)th-order function of one or more electric fields oscillation at frequencies \((\omega_1, \omega_2, \cdots, \omega_n)\). The nonlinear electric fields have a \((n + 1) \times (n + 1)\) coherency matrix which is defined as:

\[
\rho^{(n)}(\omega_1, \omega_2, \cdots, \omega_n) = \left\langle \psi^{(n)} \cdot \psi^{(n)*} \right\rangle = \begin{pmatrix}
\left\langle \psi_1^{(n)} \psi_1^{(n)*} \right\rangle & \cdots & \left\langle \psi_1^{(n)} \psi_{n+1}^{(n)*} \right\rangle \\
\vdots & \ddots & \vdots \\
\left\langle \psi_{n+1}^{(n)} \psi_1^{(n)*} \right\rangle & \cdots & \left\langle \psi_{n+1}^{(n)} \psi_{n+1}^{(n)*} \right\rangle
\end{pmatrix}
\] (2.6)

The coherency matrix, averaged over time (see Eq. 1.12), is the unified basis for defining the incoming radiations similar to the linear Stokes vector shown in Section 1.2.

### 2.1.1 Outgoing Radiation Stokes Vector \(s'\)

The Stokes vector \(s'\) for the outgoing electric field \(E(\omega_\sigma)\) is characterized by a \(4 \times 1\) vector similar to the linear Stokes vector. In terms of its coherency matrix and Pauli matrices using Eqs. 1.12 and 1.16, the outgoing Stokes vector is obtained according to:

\[
s'_t = \text{Tr} \left( C' \tau_t \right) = C'_{ab} (\tau_t)_{ba} = \left\langle \Phi'(\omega_\sigma) \Phi'^*_b \right\rangle \left( \tau_t \right)_{ba} = \left\langle \Phi'^* \tau_t \Phi' \right\rangle
\] (2.7)

where \(a\) and \(b\) each run from 1 to 2, representing the orthogonal outgoing polarization orientations perpendicular to the propagation direction. \(C'(\omega_\sigma)\) is the coherency matrix; \(\Phi(\omega_\sigma)\) is the state (or simply the electric field) vector of the outgoing beam; and \(\tau_t\) \((t = 0...3)\) denote the \(2 \times 2\) Pauli matrices (see Eq. 1.16).

### 2.1.2 Real-valued Vector \(S\) forIncoming Radiation

For nonlinear interaction of electric fields the coefficients of expansion for the coherency matrix forms a real-valued vector similar to the Stokes vector. The nonlinear coherency
matrix can be expanded by the basis that have higher dimensions than the Pauli’s matrices. Leaving aside the details of the dimension for now, and simply denoting this set as $\eta$, the nonlinear vector can be written as:

$$S_N = \text{Tr} \left( \rho \eta_N \right) = \rho_{AB} \left( \eta_N \right)_{BA} = \langle \psi_A \psi^*_B \rangle \left( \eta_N \right)_{BA} = \langle \psi^\dagger \eta_N \psi \rangle$$  \hspace{1cm} (2.8)

where $N = 1, \cdots, (n + 1)^2$ for each element of the nonlinear vector representing the $n^{\text{th}}$-order electric fields. The $\eta$ matrices similar to Pauli’s expand higher dimension states.

A subset of properties of $\eta$ matrices that are essential in deriving an $n^{\text{th}}$-order process is:

1. They are square matrices with dimension $(n + 1) \times (n + 1)$.
2. They are hermitian: $\eta^\dagger = \eta$.
3. There are $(n + 1)^2$ of $\eta$ matrices which form the basis.
4. They obey the orthogonality relation $\text{Tr}(\eta_\mu \eta_\nu) = c_\eta \delta_\mu\nu$, where $c_\eta$ is a constant and a real number, and $\delta_\mu\nu$ is the Kronecker delta ($\delta_\mu\nu = 1$ when $\mu = \nu$, and 0 otherwise).

The recipe for finding these matrices is given in Section 2.1.7 under $\eta$ Matrices for Nonlinear Polarimetry.

Similar to the linear Stokes parameters the nonlinear vector obeys the following relation$^3$:

$$n S_1^2 \geq \sum_{N=2}^{(n+1)^2} S_N^2$$  \hspace{1cm} (2.9)

where the equality is valid for the purely polarized state. Therefore, it is helpful to use the degree of polarization (DOP) parameter to characterize the fundamental radiation using the nonlinear vector:

$$DOP(\omega_1, \omega_2, \cdots, \omega_n) = \sqrt{\sum_{N=2}^{(n+1)^2} \frac{S_N^2}{n S_1^2}}$$  \hspace{1cm} (2.10)

where $DOP$ ranges from 0 to 1 for unpolarized to fully polarized fundamental radiation, respectively.

$^3$For any vector $\psi$ of length $n + 1$, let $\rho = \langle \psi \otimes \psi^\dagger \rangle$: then $n \left[ \text{Tr}(\eta_1 \rho) \right]^2 = \sum_{N=2}^{(n+1)^2} \left[ \text{Tr}(\eta_N \rho) \right]^2$. The set of $\eta$ matrices are defined in Section 2.1.7
2.1.3 Real-valued Matrix $\mathcal{M}^{(n)}$ for Intervening Medium

By substituting linear and nonlinear Stokes vector expressions (Eq. 2.7 and 2.8, respectively) into the general nonlinear polarimetry Eq. 2.1, the following expression is obtained:

$$\langle \Phi^\dagger \tau \Phi' \rangle = \mathcal{M}^{(n)}_{tN} \langle \psi^\dagger \eta_N \psi \rangle$$

(2.11)

In this frame, each component vector $\Phi'$ of the generated electric field is proportional to the polarization, which depends on the susceptibility tensor components and the polarization state radiation for incoming nonlinear electric fields. By substituting explicit expressions of $\Phi'$ and $\Phi'^\dagger$ into Eq. 2.11 in the elemental form:

$$\langle \chi^{(n)*}_{aA} \psi^*_{A}(\tau_{t})_{ab} \chi^{(n)}_{bB} \psi_{B} \rangle = \mathcal{M}^{(n)}_{tN} \langle \psi^*_{A} (\eta_N)_{AB} \psi_{B} \rangle$$

(2.12)

where $A$ and $B = 1, \cdots, n + 1$. Since Eq. 2.12 is written in terms of individual elements, the state functions of the fundamental radiation can be dropped, and the nonlinear Mueller matrix elements $\mathcal{M}^{(n)}_{tN}$ can be written in terms of the $n$th-order susceptibilities as:

$$\chi^{(n)*}_{aA} (\tau_{t})_{ab} \chi^{(n)}_{bB} = \mathcal{M}^{(n)}_{tN} (\eta_N)_{AB}$$

(2.13)

Note, in Eq. 2.13 the signal is assumed to be from a single generator. In the next section (2.1.4) an ensemble of scatterers will be considered and shown to have a similar derivation. Multiplying both sides by $(\eta_N)_{BC}$ and after summation over index $B$, and letting $A = C$:

$$\frac{1}{c_n} \chi^{(n)*}_{aA} (\tau_{t})_{ab} \chi^{(n)}_{bB} (\eta_N)_{BA} = \mathcal{M}^{(n)}_{tN}$$

(2.14)

where (Einstein) summation is implied over repeated indices (i.e. $a$, $b$, $A$ and $B$). Finally, the expression of a real-valued matrix element in terms of the susceptibilities is:

$$\mathcal{M}^{(n)}_{tN} = \frac{1}{c_n} \text{Tr} \left( (\tau_{t}) \chi^{(n)} \eta_N \chi^{(n)*} \right)$$

(2.15)

This expression is the general (nonlinear) form of the construction shown for the linear Mueller matrix element (see Eq. 1.25). In contrast to the linear Mueller matrix element, the nonlinear $\mathcal{M}^{(n)}$ is composed of nonlinear susceptibilities and $\eta$ matrices, which have a higher dimension than Pauli’s. Note that for linear polarimetry, the transformation Jones matrix $J$ (in Eq. 1.25) can also be represented by the linear susceptibility $\chi^{(1)}$. 

in which case the only difference between linear and nonlinear Mueller matrix elements would be the replacement of one Pauli matrix with an $\eta$ matrix. Thus, this familiar form of Mueller matrix elements can be investigated similar to the linear case. The elements of nonlinear matrix are real, which leads to a very useful and a much desired expression for determining the nonlinear susceptibilities.

2.1.4 Ensemble Representation

In a highly scattering media such as in biological tissue, the system may not be completely coherent, and the source of the signal may be an ensemble of scatterers. Therefore, an ensemble average of individual elements with probability $p_e$ may be more appropriate to consider [127]. The outgoing nonlinear radiation resulting from an ensemble is:

$$
\sum_e p_e \langle \chi^{(n)*}_{aA} \psi^*_A (\tau_{t})_{ab} \chi^{(n)}_{bB} \psi_B \rangle = \mathcal{M}_{tN}^{(n)} \langle \psi^*_A (\eta_N)_{AB} \psi_B \rangle
$$

(2.16)

Since the above equation is in the elemental form, it can be rewritten as:

$$
(\tau_{t})_{ab} \sum_e p_e \chi^{(n)*}_{aA} \chi^{(n)}_{bB} \langle \psi^*_A \psi_B \rangle = \mathcal{M}_{tN}^{(n)} (\eta_N)_{AB} \langle \psi^*_A \psi_B \rangle
$$

(2.17)

Dropping the incoming radiation from both sides, and following the derivation shown in Eqs. 2.12 to 2.14, the ensemble representation of the matrix element becomes:

$$
\mathcal{M}_{tN}^{(n)} = \frac{1}{c_q} \sum_e p_e \left( \chi^{(n)*}_{aA} (\tau_{t})_{ab} \chi^{(n)}_{bB} (\eta_N)_{BA} \right)
$$

(2.18)

By taking the constants $(\tau_{t})_{ab}$ and $(\eta_N)_{BA}$ out of the summation, and substituting the relation $\sum_e p_e \left( \chi^{(n)*}_{aA} \chi^{(n)}_{bB} \right) = \langle \chi^{(n)*}_{aA} \chi^{(n)}_{bB} \rangle_e$ in Eq. 2.18, the nonlinear Mueller element for the ensemble becomes:

$$
\mathcal{M}_{tN}^{(n)} = \frac{1}{c_q} \langle \chi^{(n)*}_{aA} \chi^{(n)}_{bB} \rangle_e (\tau_{t})_{ab} (\eta_N)_{BA}
$$

(2.19)

where $\langle \rangle_e$ stands for the average over the $e$ ensemble. The right-hand side of Eq. 2.19 has a similar form to Eq. 2.14, except that here an ensemble of $\chi^{(n)}$ are considered (the order of variables is a non-issue because both equations are in the elemental form). The correlation matrix $X$ forming from $\langle \chi^{(n)*}_{aA} \chi^{(n)}_{bB} \rangle_e$ contains all the information about the
ensemble, and in the case of a perfectly homogeneous medium reduces to a single source. Note, since the generated light is no longer due to a single source, but rather from an ensemble of sources that may not be necessarily coherent, then the outgoing radiation may not be fully polarized. This result is a desired and better representation of experimental data from a heterogeneous medium. Therefore, for the rest of this thesis, the ensemble average is assumed, but for the sake of simplifying the equations \( \langle \rangle \) may be omitted.

2.1.5 Expression of Susceptibilities in Nonlinear Polarimetry

Stokes polarimetry measures the Mueller matrix components, while nonlinear properties of the material are often described by \( \chi^{(n)} \) tensor component values. Thus, the next step is to derive expressions for \( \chi^{(n)} \) products in terms of \( \mathcal{M}^{(n)} \) component values. To this end, an equivalent expression for nonlinear \( \mathcal{M}^{(n)} \) can be used. Using a property of the trace operation,\(^4\) the nonlinear \( \mathcal{M}^{(n)}_{tN} \) element in Eq. 2.15 can be written as:

\[
\mathcal{M}^{(n)}_{tN} = \left( \mathcal{M}^{(n)}_{tN} \right)^* = \left( \frac{1}{c_n} \text{Tr} \left( \tau_t \chi^{(n)} \eta_N \chi^{(n)\dagger} \eta_N^* \right) \right)^* = \frac{1}{c_n} \text{Tr} \left( \tau_t^T \chi^{(n)*} \eta_N^* \chi^{(n)T} \eta_N \right) = \frac{1}{c_n} \text{vec}(\tau_t)^T (\chi^{(n)} \otimes \chi^{(n)*}) \text{vec}(\eta_N^*)
\]

(2.20)

where in the first line the hermitian properties of \( \tau \) and \( \eta \) are exploited. Note in the case of an ensemble using Eq. 2.18, Eq. 2.20 still holds and \( X^{(n)} = \left\langle \chi^{(n)} \otimes \chi^{(n)*} \right\rangle_e \). If Pauli matrices \( \tau \) are rearranged as:

\[
T \equiv \begin{pmatrix}
\text{vec}(\tau_0)^T \\
\text{vec}(\tau_1)^T \\
\text{vec}(\tau_2)^T \\
\text{vec}(\tau_3)^T
\end{pmatrix} = \begin{pmatrix}
1 & 0 & 0 & 1 \\
1 & 0 & 0 & -1 \\
0 & 1 & 1 & 0 \\
0 & i & -i & 0
\end{pmatrix},
\]

(2.21)

where the matrix \( T \) is invertible and obeys \( T^{-1} = \frac{1}{2} T^\dagger \), and \( [\text{vec}(\eta_1^*), \cdots, \text{vec}(\eta_N^*)] = H^\dagger \), the nonlinear matrix \( \mathcal{M}^{(n)} \) can be arrived at:

\[
\mathcal{M}^{(n)} = TX^{(n)}H^{-1}
\]

(2.22)

\(^4\)For any two matrices \( A \) and \( B \), \( \text{Tr}(AB) = \text{vec}(A^T)^T \text{vec}(B) \), where \( \text{vec}(A) = [a_{1,1}, \ldots, a_{s,1}, a_{1,2}, \ldots, a_{s,2}, \ldots, a_{1,t}, \ldots, a_{s,t}]^T \) is the vectorization of a \( s \times t \)-matrix \( A \) (in other words columns of a matrix are stacked below one another): a corollary is \( \text{Tr}(A^T BCD^T) = \text{vec}(A)^T (D \otimes B) \text{vec}(C) \).
where $H$ should be invertible and obey $H^{-1} = \frac{1}{c_{\eta}} H^\dagger$. Consequently, the susceptibility products can be easily found as:

$$X^{(n)} = T^{-1} M^{(n)} H$$ (2.23)

The relationship between the nonlinear susceptibilities in terms of Mueller matrix derived in Eq. 2.23 is a very useful one, because it shows that when the Mueller matrix is obtained in a polarimetry measurement for a sample, the explicit values for the corresponding susceptibilities can be determined. These susceptibilities are then used to characterize the sample under the investigation. In addition, susceptibility components may have phase relations with respect to each other or the radiations, and incorporate unique information for a given nonlinear phenomena. These phase relations can also be found in terms of the measured $M^{(n)}$ matrix.

In the case of a single source the elemental form of the susceptibility product is:

$$\chi^{(n)}_{aA} \chi^{(n)*}_{bB} = X_{ij}^{(n)} = \frac{1}{2} T_{ij}^{\dagger} M^{(n)}_{iN} H_{Nj},$$

where $i = (a - 1)2 + b$ and $j = (A - 1)(n + 1) + B$ (and $a$ and $b = 1, 2$; $A$ and $B = 1, \ldots, n + 1$). Since, $\chi^{(n)}_{aA} \chi^{(n)*}_{bB} = \chi^{(n)}_{aA} |\chi^{(n)}_{bB}| e^{i(\delta_{aA} - \delta_{bB})}$, then the relative phase between any two susceptibility elements $\chi^{(n)}_{aA}$ and $\chi^{(n)}_{bB}$ can be found according to \footnote{Note the difference in notations between the index $i$ and the imaginary $i$.}:

$$\delta_{aA} - \delta_{bB} = \Delta_{aA,bB} = \tan^{-1} \left( -i \frac{\chi^{(n)}_{bB} \chi^{(n)*}_{aA} - \chi^{(n)}_{aA} \chi^{(n)*}_{bB}}{\chi^{(n)}_{aA} \chi^{(n)*}_{bB} + \chi^{(n)}_{bB} \chi^{(n)*}_{aA}} \right) = \tan^{-1} \left( \frac{X_{kl}^{(n)} - X_{ij}^{(n)}}{X_{kl}^{(n)} + X_{ij}^{(n)}} \right)$$ (2.24)

where $k = (b - 1)2 + a$ and $l = (B - 1)(n + 1) + A$, and summations over repeated indices are assumed. Equation 2.24 shows that by measuring the material nonlinear matrix the relative phase of the susceptibility elements can be obtained.

In previous nonlinear polarimetry studies, it has been customary to characterize the nonlinear optical properties of a material using susceptibility values. Therefore, Eqs. 2.23 and 2.21 provide a mechanism to check and compare nonlinear polarimetry investigations with other previous studies and conventional nonlinear optics notations. As an example, the ratio of susceptibilities for cylindrically symmetric material is calculated and compared with another technique for a number of biological structures in Chapter 4.
2.1.6 An Alternative Derivation of Susceptibilities for Nonlinear Polarimetry

An alternative approach to derive the nonlinear $\mathcal{M}^{(n)}$ can be taken that involve the vectorization of the coherency matrices. This derivation is illustrated with an example of a right-hand coordinate system and symmetry properties that will be used later in Chapter 4. Important biological tissue molecules such as muscle myosin, collagen triple helix and starch carbohydrates have nonlinear susceptibilities that have cylindrical symmetry. For this class of material the molecular cylindrical axis is sometimes aligned with $Z$-axis, and therefore, their molecular susceptibilities can directly be obtained from measured data without any rotation. A convenient way is to assume $Y$-axis as the light propagation direction, and the $XZ$ plane as the polarization plane perpendicular to the light propagation direction (see Fig. 2.2). Thus the index $i = X, Z$. In this case, then the outgoing radiation elements including the respective Pauli matrices are changed compared to ones in Eq. 1.16 to account for the change of coordinates:

$$
\begin{align*}
\hat{\tau}_0 &= \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}, \\
\hat{\tau}_1 &= \begin{pmatrix} -1 & 0 \\ 0 & 1 \end{pmatrix}, \\
\hat{\tau}_2 &= \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}, \\
\hat{\tau}_3 &= \begin{pmatrix} 0 & i \\ -i & 0 \end{pmatrix}, \\
\text{and } \mathbf{\tau} &\equiv \begin{pmatrix} \text{vec}(\hat{\tau}_0)^T \\ \text{vec}(\hat{\tau}_1)^T \\ \text{vec}(\hat{\tau}_2)^T \\ \text{vec}(\hat{\tau}_3)^T \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 & 1 \\ -1 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \\ 0 & -i & i & 0 \end{pmatrix}
\end{align*}
$$

(2.25)

Correspondingly, the relation between electric fields and Stokes vector components in the coherency matrix becomes:

$$
\hat{C}(\omega) = \begin{pmatrix} (E_1^*(\omega) E_1(\omega)) & (E_2^*(\omega) E_2(\omega)) \\ (E_1^*(\omega) E_2(\omega)) & (E_2^*(\omega) E_2(\omega)) \end{pmatrix} = \frac{1}{2} \begin{pmatrix} s_0(\omega) - s_1(\omega) & s_2(\omega) - s_3(\omega) \\ s_2(\omega) + s_3(\omega) & s_0(\omega) + s_1(\omega) \end{pmatrix}
$$

(2.26)

Note, the relation between electric fields and Stokes vector components in coherency matrix $C$ of Eq. 1.17 is different from those defined in $\hat{C}$ Eq. 2.26. That is because in the first case the polarization plane is in $XY$ and an angle is measured from $X$, while in the latter case it is in $XZ$, and the angle is measured from $Z$.

The matrix $\mathcal{M}^{(n)}$ can be calculated more directly by noticing that the average of product of each side of Eq. 2.4 is:

$$
\langle \Phi' \otimes \Phi'^* \rangle = \langle \chi^{(n)} \psi \otimes \chi^{(n)*} \psi^* \rangle = \langle \chi^{(n)} \otimes \chi^{(n)*} \rangle_{\text{ensemble}} \langle \psi \otimes \psi^* \rangle_{\text{time}}
$$

(2.27)
Maintaining the conventional optics notation\(^6\), the coherency matrices (Eq. 2.26) can be written in vector forms:

\[
\vec{C}' = \langle \Phi' \otimes \Phi'^* \rangle = \begin{bmatrix}
\hat{C}'_{11} \\
\hat{C}'_{12} \\
\hat{C}'_{21} \\
\hat{C}'_{22}
\end{bmatrix} = \begin{bmatrix}
\hat{C}'_{XX} \\
\hat{C}'_{XZ} \\
\hat{C}'_{ZX} \\
\hat{C}'_{ZZ}
\end{bmatrix}, \quad \text{and} \quad \vec{\rho} = \langle \psi \otimes \psi^* \rangle = \begin{bmatrix}
\rho_{11} \\
\rho_{12} \\
\vdots \\
\rho_{(n+1)(n+1)}
\end{bmatrix} (2.28)
\]

Substituting these vectors into Eq 2.27, the coherency vector for outgoing radiation in terms of the nonlinear coherency matrix is:

\[
\vec{C}' = X^{(n)} \vec{\rho} \quad (2.29)
\]

Since \(s' = \hat{T} \vec{C}'\) and \(S = H \vec{\rho}\), then Eq. 2.27 or 2.29 can be written as:

\[
\hat{T}^{-1} s' = X^{(n)} H^{-1} S, \quad \text{or} \quad s' = \left( \hat{T} X^{(n)} H^{-1} \right) S \quad (2.30)
\]

Finally the nonlinear \(M^{(n)}\) can be obtained using:

\[
M^{(n)} = \hat{T} X^{(n)} H^{-1} \quad (2.31)
\]

Therefore, \(X^{(n)} = \hat{T}^{-1} M^{(n)} H\), which is exactly the same as Eq. 2.23. The difference between this derivation and the one provided before is that here the coherency matrices are vectorized, and so are the Pauli and \(\eta\) matrices in the form of \(\hat{T}\) and \(H\), respectively. Therefore, no trace operation is necessary. Note, this derivation is given simply for convenience and does not change the overall formalism, except that for a given choice of propagation direction, the corresponding notation is adopted for the outgoing radiation.

For the remaining of the thesis this notation (i.e. \(Y\)-axis as the propagation direction) is respected, particularly for the derivation of SHG, and calculations in Chapter 4, mainly because for the majority of biological samples described there, cylindrical symmetry for susceptibilities can be assumed.

\(^6\text{In the derivation above, the conventional optics notation for the coherency vector notation was sought out [1]}\)
2.1.7 $\eta$ Matrices for Nonlinear Polarimetry

The polarization state of incoming radiation $S$ (Eq. 2.8) as well as the matrix representing the nonlinear medium $M^{(n)}$ (Eq. 2.15) require the $(n+1)$-by-$(n+1)$-$\eta$ matrices in order to be defined from the nonlinear coherency and susceptibility matrices, respectively. The recipe for generating $\eta$ matrices has two steps: In Step 1 the matrix $\eta''_{jk}$ is defined such that the element $jk$ of the matrix $\eta''_{jk}$ is 1, and 0 otherwise. This creates a two dimensional set of matrices. Note that the $\eta''$ are also independent basis and can expand the coherency matrix. However, they are not hermitian and therefore the resulting Stokes vector and Mueller matrix will be complex. To obtain the desired hermitian matrices for an $n^{th}$-order process the following relation can be used:

$$
\eta'_{jk} = \begin{cases} 
\eta''_{jk} + \eta''_{kj}, & \text{if } j < k \\
i(\eta''_{jk} - \eta''_{kj}), & \text{if } j > k \\
\frac{2}{j^2 + j} \left[ \sum_{m=1}^{j} \eta''_{mm} \right] - j\eta''_{j+1,j+1}, & \text{if } 1 \leq k = j < (n + 1) \\
\frac{2}{n + 1} I_{n+1}, & \text{if } j = k = (n + 1)
\end{cases}
$$

(2.32)

where $I_{n+1}$ is the $(n + 1)$-by-$(n + 1)$ identity matrix. The first case (when $j < k$) the new matrices $\eta'_{jk} = \eta''_{jk} + \eta''_{kj}$ are real valued; the second case (when $j > k$), the new matrices $\eta'_{jk} = i(\eta''_{jk} - \eta''_{kj})$ are complex valued and have similar nonzero elements as to their real-value counterparts in the first case. In the third case, (when $1 \leq j = k < n + 1$), the new matrices are diagonal and real valued. Finally, in the last case an identity matrix is used. In Step 2 the two-dimensional $\eta'$ set is converted to a one-dimensional set of matrices $\eta'_{jk} \rightarrow \eta_N$. An explicit example is shown in Section 2.3

These matrices satisfy all the requirements as desired for expanding the nonlinear coherency matrix for the nonlinear polarimetry. The new matrices defined in Eq. 2.32 ensure that $\eta$ obey: $\text{Tr}(\eta_{\mu}\eta_{\nu}) = 2\delta_{\mu\nu}$. For linear polarimetry $n = 2$ and $\eta$ corresponds to Pauli matrices. For second-order process $n = 3$ and therefore the generated matrices are those of Gell-Mann’s. For the case of three-photon polarimetry $n = 4$, and there are

---

7This is to simplify the indices and to conform to a Stokes-Mueller notation of vector = matrix x vector. The matrices $\eta'$ can also be used directly for polarimetry, in which case there will be an additional index for the entity representing the incoming radiation as well as for the entity representing the medium.
sixteen $4 \times 4$ matrices, which will be shown in Section 2.3; and so forth. A useful relations between these matrices and Stokes-Mueller formalism is the following: The real-valued $\eta$ generate the Stokes vector components that depend on linear polarization, while the complex valued ones are responsible for circular components. Also, the real-valued ones are in part responsible for nonzero Mueller matrix elements, while the Mueller matrix component constructed from a complex-valued $\eta$ matrix may be zero if the involved nonlinear susceptibilities are real.

2.1.8 Combining Nonlinear and Linear Optical Elements

For a setup, containing a nonlinear optical medium followed by a train of linear optical components, the Mueller Stokes formalism can be used to relate vector of incoming radiation to the outgoing vector of the nonlinear radiation:

$$s'(\omega) = M_t \cdots M_1 M^{(n)} S(\omega_1, \omega_2, \cdots, \omega_n)$$

where $M_1 \cdots M_t$ are the $4 \times 4$ linear Mueller matrices that characterize the linear interactions, and $M^{(n)}$ is the $4 \times 9$ for the second-order matrix, $4 \times 16$ for the third-order matrix, and $4 \times (n+1)^2$ for the $n$th-order nonlinear interaction. Therefore, linear Stokes and the nonlinear vector for polarimetry can be appropriately combined. In the following, an example of combined nonlinear and linear Stokes polarimetry is discussed.

**Derivation of Linear PIPO for Harmonics Generation**

In linear polarization-in polarization-out (PIPO) microscopy typically a linearly polarized incoming beam is incident upon the sample that is very thin (< 10 µm), and the outgoing signal is measured after passing through a linear analyzer [87–89]. Measuring the angle of polarization from Z-axis, the electric field for the fundamental wavelength is:

$$E(\omega) = \begin{pmatrix} E_1(\omega) \\ E_2(\omega) \end{pmatrix} = E_0 \begin{pmatrix} \sin(\theta) \\ \cos(\theta) \end{pmatrix}$$
The $n$-harmonic electric field in terms of incoming fields and nonlinear susceptibilities is:

$$E(n\omega) = \begin{pmatrix} E_1(n\omega) \\ E_2(n\omega) \end{pmatrix} \propto \chi^{(n)}(\psi) = \begin{pmatrix} \chi_{1A}^{(n)}(\psi) \\ \chi_{2A}^{(n)}(\psi) \end{pmatrix}$$

(2.35)

The $n$-harmonic Stokes vector using Eq. 2.7 is:

$$s(n\omega) = \begin{pmatrix} \chi_{2A}^{(n)}(\psi)^* \chi_{2B}^{(n)} \psi_B \\ \chi_{1A}^{(n)}(\psi)^* \chi_{1B}^{(n)} \psi_B \\ \chi_{1A}^{(n)}(\psi) \chi_{1B}^{(n)} \psi_B \\ 0 \\ 0 \end{pmatrix} + \begin{pmatrix} \chi_{1A}^{(n)}(\psi)^* \chi_{1B}^{(n)} \psi_B \\ \chi_{1A}^{(n)}(\psi) \chi_{1B}^{(n)} \psi_B \\ 0 \end{pmatrix}$$

(2.36)

Passing this nonlinear signal through a linear analyzer the measured Stokes vector is:

$$s' = M_{LA} s(n\omega)$$

(2.37)

where $M_{LA}$ is the Mueller matrix for the linear analyzer oriented at an angle $\varphi$:

$$M_{LA} = \frac{1}{2} \begin{pmatrix} 1 & \cos(2\varphi) & \sin(2\varphi) & 0 \\ \cos(2\varphi) & \cos^2(2\varphi) & \frac{1}{2} \sin(4\varphi) & 0 \\ \sin(2\varphi) & \frac{1}{2} \sin(4\varphi) & \sin^2(2\varphi) & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$$

(2.38)

and the first component of vector in Eq. 2.37 is:

$$s'_0(n\omega) = I(n\omega) \propto |\cos(\varphi) E_2(n\omega) + \sin(\varphi) E_1(n\omega)|^2 \propto |\cos(\varphi) \chi_{2A}^{(n)}(\psi) + \sin(\varphi) \chi_{1A}^{(n)}(\psi)|^2$$

(2.39)

Similarly, when the outgoing signal experiences birefringence, a $4 \times 4$ Mueller matrix corresponding to the retardance can be used to analyze the measured signal. Previous studies have used SHG and THG PIPO measurements to analyze biological samples [87–89, 128]. Sections on SHG and THG in this chapter and in Chapter 4 will show the extended derivation that involve a combined linear and nonlinear effects, and present analyses of relevant experiments.
2.2 Two Photon Polarimetry

Second order nonlinear optical signals, schematically shown in Fig 2.1, are highly sensitive to the molecular symmetry and organization of molecules in the examined material. Polarization-dependent second-harmonic generation (SHG) and sum-frequency generation can be used to probe the symmetry of molecules and investigate the molecular organization of ordered aggregates. For example, measurements of the polarization-dependent SHG and hyper-Rayleigh scattering in solutions and at interfaces provide information about molecular symmetry [50,129–131]. Similarly, polarization sensitive measurements reveal organization of protein structures within various biological tissue [87–90].

![Figure 2.1: Schematics of a two-photon process analyzed by polarimetry. Two incoming beams with frequencies $\omega_1$ and $\omega_2$ are incident onto the sample that is represented by a the second-oder susceptibility $\chi^{(2)}$. The resulting outgoing signal from the intervening material is then measured. In two-photo polarimetry the incoming radiations are represented by $S$, the medium with $M^{(2)}$, and the outgoing measured signal by $s'$.](image)

For two-photon processes, the polarization-state vector describing the state of the incoming laser light is comprised of 9 components, while the intervening matrix $M^{(2)}$ has $4 \times 9$ components and describes the nonlinear properties of the material. The second-order matrix can be expressed in terms of susceptibility values, which are used to characterize nonlinear properties of the material. By performing nonlinear Stokes polarimetry measurements it is possible to examine nonlinear properties of the material and determine uniquely the nonlinear matrix components values.

In this section, first the overall expression for second-order light-matter interaction is derived using the formalism described earlier. Next, the $9 \times 1$ polarization-state vectors for SHG, SFG and DFG are derived. Then, the $4 \times 9$ matrix is derived from the second-order susceptibilities. Properties and symmetries of the matrix are also discussed. SHG polarimetry is given as an example of how to determine the elements of the polarization state vectors and the matrix. The derived equations in this section will be used in Chapter 4. A discussion of main results will conclude this section.
2.2.1 Derivation of Polarization State of Incoming Radiation

The polarization density for two photon process can be written as:

\[ P_i = \chi^{(2)}_{ijk} E_j E_k = \chi^{(2)}_{iA} \psi^{(2)}_A \]  

(2.40)

The state vector for electric fields of incoming radiation can be expressed as:

\[ \psi^{(2)}(\omega_1, \omega_2) = \begin{pmatrix} \psi^{(2)}_1 \\ \psi^{(2)}_2 \\ \psi^{(2)}_3 \end{pmatrix} \]  

(2.41)

Consequently, the coherency matrix of incoming radiation for two photon processes is:

\[ \rho^{(2)}(\omega_1, \omega_2) = \langle \psi^{(2)} \cdot \psi^{(2)\dagger} \rangle = \begin{pmatrix} \langle \psi^{(2)}_1 \psi^{(2)*}_1 \rangle & \langle \psi^{(2)}_1 \psi^{(2)*}_2 \rangle & \langle \psi^{(2)}_1 \psi^{(2)*}_3 \rangle \\ \langle \psi^{(2)}_2 \psi^{(2)*}_1 \rangle & \langle \psi^{(2)}_2 \psi^{(2)*}_2 \rangle & \langle \psi^{(2)}_2 \psi^{(2)*}_3 \rangle \\ \langle \psi^{(2)}_3 \psi^{(2)*}_1 \rangle & \langle \psi^{(2)}_3 \psi^{(2)*}_2 \rangle & \langle \psi^{(2)}_3 \psi^{(2)*}_3 \rangle \end{pmatrix} \]  

(2.42)

where time average is assumed for each element of the coherency matrix (see Eq. 2.6). Therefore, the elements of the vector for incoming radiation for two photon processes becomes:

\[ S^{(2)}_N(\omega_1, \omega_2) = \text{Tr}(\lambda_N \rho^{(2)}) \]  

(2.43)

where \( \lambda_N \) are the 3 x 3 identity matrix, and the Gell-Mann matrices:

\[
\begin{align*}
\lambda_3 &= \begin{pmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 0 \end{pmatrix}, & \lambda_4 &= \begin{pmatrix} 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}, & \lambda_6 &= \begin{pmatrix} 0 & 0 & 1 \\ 0 & 0 & 0 \\ 1 & 0 & 0 \end{pmatrix}, \\
\lambda_7 &= \begin{pmatrix} 0 & -i & 0 \\ i & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}, & \lambda_2 &= \sqrt{\frac{1}{3}} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -2 \end{pmatrix}, & \lambda_5 &= \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix}, \\
\lambda_9 &= \begin{pmatrix} 0 & 0 & -i \\ 0 & 0 & 0 \\ i & 0 & 0 \end{pmatrix}, & \lambda_8 &= \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & -i \\ 0 & i & 0 \end{pmatrix}, & \lambda_1 &= \sqrt{\frac{1}{3}} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}.
\end{align*}
\]  

(2.44)

Similar to Pauli matrices, the orthogonality condition for the matrices dictates \( \text{Tr}(\lambda_M \lambda_N) = 2\delta_{MN} \). As in the case for the degree of polarization for the linear Stokes parameters, the
double Stokes vector obeys the following relation:

\[ 2S_1^2 \geq \sum_{N=2}^{9} S_N^2 \]  \hspace{1cm} (2.45)

where the equality is valid for the purely polarized state. Therefore, it is convenient to use the degree of polarization (DOP) parameter to characterize the fundamental radiation using double Stokes vector:

\[ DOP(\omega_1, \omega_2) = \sqrt{\sum_{N=2}^{9} S_N^2 / 2S_1^2} \]  \hspace{1cm} (2.46)

where \( DOP \) ranges from 0 to 1 for unpolarized to fully polarized fundamental radiation, respectively.

**Second Harmonic Generation**

The electric field of the second harmonic radiation is proportional to a polarization \( P_i \), induced by the electric field \( E_i = \tilde{E}_i e^{i\phi_i} \), of the fundamental radiation, where \( \tilde{E}_i = A_i e^{-i(kY - 2\omega t)} \):

\[ P_i^{(2)} = \chi_{ijk}^{(2)} E_j E_k = \chi_{iA}^{(2)} \psi_A^{(2)} \]  \hspace{1cm} (2.47)

The polarization is expressed in the modified form of contracted notation, where \( \chi_{ijk}^{(2)} = \chi_{iA} \) are the susceptibility tensor components; \( i \) corresponds to \( X \) and \( Z \), and \( A \) is the contracted index representing \( XX \), \( ZZ \), and \( XZ \) or \( ZX \), respectively. The state function of the fundamental radiation at frequency \( \omega \) is:

\[ \psi(\omega, \omega) = \begin{pmatrix} E_1^2 \\ E_2^2 \\ 2E_1E_2 \end{pmatrix} \]  \hspace{1cm} (2.48)

where its conjugate transpose becomes:

\[ \psi^\dagger(\omega, \omega) = \left( E_1^{*2}, E_2^{*2}, 2E_1^*E_2^* \right) \]  \hspace{1cm} (2.49)
Finally, the real-valued vector for incoming radiation in SHG can be written as:

\[ I \propto |P_1|^2 \propto \langle \chi_i A \chi_i^* B \psi_A \psi_B^* \rangle \quad (2.50) \]

where index \( B \) also represents \( XX, ZZ, \) and \( XZ \) or \( ZX \). Similar to the general nonlinear coherency matrix in Eq. 2.6, the coherency matrix for SHG can be written as a dyadic product of the state function:

\[ \rho(\omega, \omega) = \langle \psi(\omega, \omega) \cdot \psi^\dagger(\omega, \omega) \rangle = \begin{pmatrix}
\langle E_1^2 E_1^2 \rangle & \langle E_1^2 E_2^* \rangle & \langle 2E_1^2 E_1^* E_2^* \rangle \\
\langle E_2^2 E_1^2 \rangle & \langle E_2^2 E_2^* \rangle & \langle 2E_2^2 E_1^* E_2^* \rangle \\
\langle 2E_1 E_2 E_1^* \rangle & \langle 2E_1 E_2 E_2^* \rangle & \langle 4E_1 E_2 E_1^* E_2^* \rangle
\end{pmatrix} \quad (2.51) \]

In the coherency matrix above the average is assumed for each element (see Eq. 1.12). Finally, the real-valued vector for incoming radiation in SHG can be written as:

\[ S_N(\omega, \omega) = \text{Tr} \left( \rho(\omega, \omega) \lambda_N \right) \\
= \rho_{AB}(\omega, \omega) (\lambda_N)_{BA} = \langle \psi_A(\omega, \omega) \psi_B^*(\omega, \omega) \rangle (\lambda_N)_{BA} \quad (2.52) \]

where \( \lambda_N \) is the coherency matrix relation. The intensity of the second harmonic can be expressed as:

\[ I \propto |P_1|^2 \propto \langle \chi_i A \chi_i^* B \psi_A \psi_B^* \rangle \quad (2.50) \]

The double Stokes vector can be expressed using Eq. 2.43 in terms of the fundamental radiation electric fields (Eqs. 2.48 and 2.49), and subsequently, can also be related to the linear Stokes parameters of the fundamental radiation:

\[
\begin{pmatrix}
S_1 \\
S_2 \\
S_3 \\
S_4 \\
S_5 \\
S_6 \\
S_7 \\
S_8 \\
S_9
\end{pmatrix} = \begin{pmatrix}
\sqrt{\frac{7}{3}} \left( \langle E_1^2 E_1^2 \rangle + \langle E_2^2 E_2^2 \rangle + \langle 4E_1 E_2 E_1^* E_2^* \rangle \right) \\
\sqrt{\frac{1}{3}} \left( \langle E_1^2 E_1^2 \rangle + \langle E_2^2 E_2^2 \rangle - \langle 8E_1 E_2 E_1^* E_2^* \rangle \right) \\
\langle E_1^2 E_1^2 \rangle - \langle E_2^2 E_2^2 \rangle \\
\langle E_1^2 E_2^2 \rangle + \langle E_2^2 E_1^2 \rangle \\
2 \left( \langle E_2^2 E_2^* \rangle + \langle E_1 E_2 E_2^* \rangle \right) \\
2 \left( \langle E_1^2 E_1^* \rangle + \langle E_1 E_2 E_1^* \rangle \right) \\
\frac{1}{2} \left( \langle E_1^2 E_1^2 \rangle - \langle E_2^2 E_2^2 \rangle \right) i \\
\frac{1}{2} \left( \langle E_1^2 E_2^2 \rangle - \langle E_2^2 E_1^2 \rangle \right) i \\
\frac{1}{2} \left( \langle E_1^2 E_2^* \rangle - \langle E_2^2 E_1^* \rangle \right) i
\end{pmatrix} = \begin{pmatrix}
\sqrt{\frac{1}{6}} (3s_2^2 - s_0^2) \\
\sqrt{\frac{1}{12}} (5s_1^2 - 3s_0^2) \\
-s_0 s_1 \\
\frac{1}{2} (s_2^2 - s_0^2) \\
s_2 (s_1 + s_0) \\
-s_2 (s_1 - s_0) \\
-s_2 s_3 \\
s_3 (s_1 + s_0) \\
s_3 (s_1 - s_0)
\end{pmatrix} \quad (2.53)
\]

where time average is assumed for each term. The double Stokes vector expression in terms of the linear Stokes parameters of fundamental radiation \((\omega)\) is obtained by using the coherency matrix relation \( \hat{C'} \) using Eq. 2.26 (see Section 2.1.6).
Sum Frequency Generation

In many respects sum frequency generation (SFG) is very similar to SHG, however, a key difference is the utilization of two incident beams with frequencies \( \omega_1 \) and \( \omega_2 \), which are not the same [93]. When Kleinman symmetry is valid (i.e. all frequencies are lower than the lowest optical transition frequency of the material), the state of the incoming radiation electric fields for SFG can be written as:

\[
\psi(\omega_1, \omega_2) = \begin{pmatrix}
E_1(\omega_1) E_1(\omega_2) \\
E_2(\omega_1) E_2(\omega_2) \\
E_1(\omega_1) E_2(\omega_2) + E_1(\omega_2) E_2(\omega_1)
\end{pmatrix}
\]  
(2.54)

The corresponding coherency matrix for the incoming radiations for in SFG is:

\[
\rho(\omega_1, \omega_2) = \langle \psi(\omega_1, \omega_2) \cdot \psi^\dagger(\omega_1, \omega_2) \rangle = \\
\begin{pmatrix}
\langle E^*_1(\omega_1) E^*_1(\omega_2) E_1(\omega_1) E_1(\omega_2) \rangle & \langle E^*_2(\omega_1) E^*_2(\omega_2) E_1(\omega_1) E_1(\omega_2) \rangle & \langle E_1(\omega_1) E_1(\omega_2) \sigma^* \rangle \\
\langle E^*_1(\omega_1) E^*_1(\omega_2) E_2(\omega_1) E_2(\omega_2) \rangle & \langle E^*_2(\omega_1) E^*_2(\omega_2) E_2(\omega_1) E_2(\omega_2) \rangle & \langle E_2(\omega_1) E_2(\omega_2) \sigma^* \rangle \\
\langle E^*_1(\omega_1) E^*_1(\omega_2) \sigma \rangle & \langle E^*_2(\omega_1) E^*_2(\omega_2) \sigma \rangle & \langle \sigma \sigma^* \rangle
\end{pmatrix}
\]  
(2.55)

where \( \sigma = E_1(\omega_1) E_2(\omega_2) + E_1(\omega_2) E_2(\omega_1) \), and time average is assumed. The element of vector for the state of incoming radiations for SFG is \( S_N(\omega_1, \omega_2) = \text{Tr}(\rho(\omega_1, \omega_2) \lambda_N) \):

\[
S(\omega_1, \omega_2) = \frac{1}{2} \begin{pmatrix}
\frac{\sqrt{3}}{2} [2 s_0(\omega_1) s_0(\omega_2) + s_2(\omega_1) s_2(\omega_2) + s_3(\omega_1) s_3(\omega_2)] \\
-\frac{\sqrt{3}}{2} [s_0(\omega_1) s_0(\omega_2) - 3 s_1(\omega_1) s_1(\omega_2) + 2 s_2(\omega_1) s_2(\omega_2) + 2 s_3(\omega_1) s_3(\omega_2)] \\
\frac{\sqrt{3}}{2} [2 s_1(\omega_1) s_2(\omega_2) + s_2(\omega_1) s_2(\omega_2) - 3 s_1(\omega_1) s_1(\omega_2) + 2 s_2(\omega_1) s_2(\omega_2) + 2 s_3(\omega_1) s_3(\omega_2)] \\
\frac{\sqrt{3}}{2} [2 s_2(\omega_1) s_3(\omega_2) + s_3(\omega_1) s_3(\omega_2) - 3 s_1(\omega_1) s_1(\omega_2) + 2 s_2(\omega_1) s_2(\omega_2) + 2 s_3(\omega_1) s_3(\omega_2)] \\
\end{pmatrix}
\]  
(2.56)

where \( s(\omega_1) \) is the Stokes vector for beam 1, \( s(\omega_2) \) is the Stokes vector for beam 2, and the coherency relations (Eq. 1.12) for the two frequencies have been used.

\[
C(\omega_1) = \begin{pmatrix}
\langle E^*_1(\omega_1) E_1(\omega_1) \rangle & \langle E^*_2(\omega_1) E_1(\omega_1) \rangle \\
\langle E^*_1(\omega_1) E_2(\omega_1) \rangle & \langle E^*_2(\omega_1) E_2(\omega_1) \rangle
\end{pmatrix} = \frac{1}{2} \begin{pmatrix}
[2 s_0(\omega_1) + s_1(\omega_1) + s_2(\omega_1) + s_3(\omega_1) i] \\
[s_2(\omega_1) - s_3(\omega_1) i] [s_0(\omega_1) - s_1(\omega_1)]
\end{pmatrix}
\]  
(2.57)
and
\[ C(\omega_2) = \begin{pmatrix}
\langle E_1^* (\omega_2) E_1 (\omega_2) \rangle & \langle E_2^* (\omega_2) E_1 (\omega_2) \rangle \\
\langle E_1^* (\omega_2) E_2 (\omega_2) \rangle & \langle E_2^* (\omega_2) E_2 (\omega_2) \rangle
\end{pmatrix} = \frac{1}{2} \begin{pmatrix}
s_0(\omega_2) + s_1(\omega_2) s_2(\omega_2) + s_3(\omega_2) i \\
s_2(\omega_2) - s_3(\omega_2) i, s_0(\omega_2) - s_1(\omega_2)
\end{pmatrix} \] (2.58)

### Difference Frequency Generation

A very similar approach to SFG can be taken to arrive at the vector for incoming radiation for difference-frequency generation (DFG). When Kleinman symmetry is valid, the state of the incoming radiation electric fields for DFG can be written as:

\[ \psi(\omega_1, -\omega_2) = \begin{pmatrix}
E_1(\omega_1) E_1^*(\omega_2) \\
E_2(\omega_1) E_2^*(\omega_2) \\
E_2(\omega_1) E_1^*(\omega_2) + E_1(\omega_1) E_2^*(\omega_2)
\end{pmatrix} \] (2.59)

Therefore the real-valued vector elements corresponding to the polarization state of incoming radiation for DFG becomes \( S_N(\omega_1, -\omega_2) = \text{Tr}(\rho(\omega_1, -\omega_2)\lambda_N) \):

\[ S(\omega_1, -\omega_2) = \frac{1}{2} \begin{pmatrix}
\sqrt{\frac{N}{\pi}} [2 s_0(\omega_1) s_0(\omega_2) + s_2(\omega_1) s_2(\omega_2) - s_3(\omega_1) s_3(\omega_2)] \\
\sqrt{\frac{N}{\pi}} [3 s_1(\omega_1) s_1(\omega_2) - s_0(\omega_1) s_0(\omega_2) - 2 s_2(\omega_1) s_2(\omega_2) + 2 s_3(\omega_1) s_3(\omega_2)] \\
[ s_0(\omega_1) s_1(\omega_2) + s_1(\omega_1) s_0(\omega_2) \\
s_2(\omega_1) s_2(\omega_2) + s_3(\omega_1) s_3(\omega_2) \\
s_0(\omega_1) s_2(\omega_2) + s_2(\omega_1) s_0(\omega_2) - s_1(\omega_1) s_3(\omega_2) - s_2(\omega_1) s_1(\omega_2) \\
s_0(\omega_1) s_3(\omega_2) + s_1(\omega_1) s_2(\omega_2) + s_2(\omega_1) s_1(\omega_2) + s_2(\omega_1) s_3(\omega_2) \\
s_3(\omega_1) s_0(\omega_2) - s_0(\omega_1) s_3(\omega_2) + s_1(\omega_1) s_3(\omega_2) - s_3(\omega_1) s_1(\omega_2) \\
s_0(\omega_1) s_3(\omega_2) - s_3(\omega_1) s_0(\omega_2) + s_1(\omega_1) s_3(\omega_2) - s_3(\omega_1) s_1(\omega_2)
\end{pmatrix} \] (2.60)

The vector \( S(\omega_1, -\omega_2) \) for DFG is similar to SFG and differs only in the sign of \( s_3(\omega_2) \). That is because for DFG, \( \omega_2 \) is the negative frequency (i.e. the electric field corresponding to \( \omega_2 \) is complex conjugated with respect to the electric field at \( \omega_1 \)). Therefore, the polarization states of incoming radiations to produce DFG can be obtained by changing the beam with \( \omega_2 \) to have the opposite ellipticity with respect to the case which produces SFG; and vice versa.
2.2.2 Derivation of Real-valued Matrix $\mathcal{M}^{(2)}$ for Medium

The nonlinear Mueller matrix for the case of two photon processes including SHG, SFG and DFG is derived here. For polarimetry purposes, the susceptibility matrix from the second-order susceptibility tensor can be constructed using a contracted notation. The contracted notation for SHG is straightforward and can be obtained similar to the matrix defined in Section 1.3.3. The same matrix is applicable to SFG and DFG when Kleinman symmetry is valid, and therefore, the constructed $\mathcal{M}^{(2)}$ matrix can be used in conjunction with the vector $S(\omega_1, \omega_2)$ and $S(\omega_1, -\omega_2)$, for SFG and DFG, respectively.

When $Y$-axis is the propagation direction and the polarization is in $XZ$ plane, there are six nonzero susceptibility tensor components. Putting them in a matrix format, the susceptibility matrix $\chi^{(2)}$ for the two-photon process becomes:

$$\chi^{(2)} = \begin{pmatrix} \chi^{(2)}_{xxx} & \chi^{(2)}_{xzz} & \chi^{(2)}_{xxz} \\ \chi^{(2)}_{xzx} & \chi^{(2)}_{zzz} & \chi^{(2)}_{zxz} \end{pmatrix}$$ (2.61)

In the contracted notation the second-order susceptibility matrix can be written as $\chi^{(2)}_{iA} = \chi^{(2)}_{ijk}$:

$$\chi^{(2)} = \begin{pmatrix} \chi^{(2)}_{11} & \chi^{(2)}_{12} & \chi^{(2)}_{13} \\ \chi^{(2)}_{21} & \chi^{(2)}_{22} & \chi^{(2)}_{23} \end{pmatrix}$$ (2.62)

where

$$jk : xx \ zz \ xz, zx$$

$$A : 1 \ 2 \ 3$$ (2.63)

Therefore, using Eqs. 2.15, the general formula for second-order material matrix elements becomes:

$$\mathcal{M}_{ul}^{(2)} = \frac{1}{2} \text{Tr}(\tau_l \chi^{(2)} \lambda_l \chi^{(2)\dagger})$$ (2.64)

Here a single source is shown and the extension to an ensemble of generators is straightforward (see Section 2.1.4). Note, the matrix $\mathcal{M}^{(2)}$ is composed of $4 \times 9$ elements, which characterize the nonlinear properties of the material. Each element of the double Mueller matrix contain a linear combination of the susceptibility component products of $\chi^{*}_{aA} \chi_{bB}$. In the matrix format the second-order matrix is $\mathcal{M}^{(2)} = \hat{T}X^{(2)} \Lambda^{-1}$, where $\hat{T}$ was defined in Eq. 2.25, and $X$ is the second-order susceptibility product matrix defined as:
\[ X^{(2)} = \langle \chi^{(2)} \otimes \chi^{(2)*} \rangle = \begin{pmatrix} \chi_{11}^{*} \chi_{11} \chi_{12}^{*} \chi_{12} \chi_{13}^{*} \chi_{13} \chi_{14}^{*} \chi_{14} \chi_{21}^{*} \chi_{21} \chi_{22}^{*} \chi_{22} \chi_{23}^{*} \chi_{23} \chi_{24}^{*} \chi_{24} \chi_{31}^{*} \chi_{31} \chi_{32}^{*} \chi_{32} \chi_{33}^{*} \chi_{33} \chi_{34}^{*} \chi_{34} \chi_{41}^{*} \chi_{41} \chi_{42}^{*} \chi_{42} \chi_{43}^{*} \chi_{43} \end{pmatrix} \] (2.65)

where an ensemble average is assumed for each element. The matrix \( \Lambda \) is made from the vectorization of \( \lambda \) matrices:

\[
\Lambda \equiv \begin{pmatrix} \text{vec}(\lambda_1)^T \\ \text{vec}(\lambda_2)^T \\ \text{vec}(\lambda_3)^T \\ \text{vec}(\lambda_4)^T \\ \text{vec}(\lambda_5)^T \\ \text{vec}(\lambda_6)^T \\ \text{vec}(\lambda_7)^T \\ \text{vec}(\lambda_8)^T \\ \text{vec}(\lambda_9)^T \end{pmatrix} = \begin{pmatrix} \sqrt{\frac{2}{3}} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{2}{\sqrt{3}} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{1}{2} & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{1}{2} & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & i & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & i & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & i & 0 & -i \end{pmatrix} \] (2.66)

The matrix \( \Lambda \) is invertible and obeys \( \Lambda^{-1} = \frac{1}{2} \Lambda^\dagger \). Each complex susceptibility component can be written as \( \chi_{aA} = |\chi_{aA}| e^{i\delta_{aA}} \), and the products of susceptibility components have associated phase differences \( \Delta_{aA,bB} = \delta_{aA} - \delta_{bB} \). The linear combination of \( |\chi_{aA}| |\chi_{bB}| e^{\Delta_{aA,bB}} \) terms for each element \( \mathcal{M}_{t,N} \) is determined by Pauli and Gell-Mann matrices with indices \( t \) and \( N \), respectively. Therefore, by replacing \( H \) in Eq. 2.24 with \( \Lambda \), the phase relationship between the second-order susceptibility tensor elements can be obtained.

The color in susceptibility product matrix (Eq. 2.65) highlights the phase relation between susceptibilities. Phase independent terms are colorless (white); those terms dependent on the phase difference of incoming index are yellow; those terms dependent on the phase difference of outgoing index are green; and finally those terms that depend on a relative phase of both incoming and outgoing indices are purple. The same color coding arrangement is highlighted in the symbolic matrix Eq. 2.67 representing nonlinear real-valued matrix as well. The expressions for each of the components are presented in the
Chapter 2. Theory of Nonlinear Optical Polarimetry

Elements of the Material Matrix $M^{(2)}$:

\[
M_{01}^{(2)} = M_{10}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} - \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{02}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxx} \chi_{xxz} + \chi_{xxz} \chi_{xxx} + \chi_{zzz} \chi_{zzz} - \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{03}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{zxz} \chi_{zxz} - \chi_{zzz} \chi_{zzz} - \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{04}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxz} + \chi_{xxz} \chi_{xxx} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{05}^{(2)} = \frac{1}{3} \left( \chi_{xxz} \chi_{xxz} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{06}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{07}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{08}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{09}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{11}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxz} \chi_{xxz} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{12}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxz} \chi_{xxz} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{13}^{(2)} = \frac{1}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{14}^{(2)} = \frac{1}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{15}^{(2)} = \frac{1}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{16}^{(2)} = \frac{1}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{17}^{(2)} = \frac{1}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{18}^{(2)} = \frac{1}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{19}^{(2)} = \frac{1}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{21}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{22}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{23}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{24}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{25}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{26}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{27}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{28}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{29}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{31}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{32}^{(2)} = \frac{\sqrt{3}}{6} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{33}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{34}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{35}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{36}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{37}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{38}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]

\[
M_{39}^{(2)} = \frac{1}{3} \left( \chi_{xxx} \chi_{xxx} + \chi_{xxz} \chi_{xxz} + \chi_{zzz} \chi_{zzz} \right)
\]
The summary of phase relations for double Mueller matrix components is presented as:

\[
\begin{pmatrix}
NP & NP & NP & I_\diamond^c & I_\Delta^c & I_\nabla^c & I_\nabla^s & I_\Delta^s & I_\diamond^s \\
NP & NP & NP & I_\diamond^c & I_\Delta^c & I_\nabla^c & I_\nabla^s & I_\Delta^s & I_\diamond^s \\
O^c & O^c & O^c & OI_\diamond^c & OI_\Delta^c & OI_\nabla^c & OI_\nabla^s & OI_\Delta^s & OI_\diamond^s \\
O^s & O^s & O^s & OI_\diamond^s & OI_\Delta^s & OI_\nabla^s & OI_\nabla^c & OI_\Delta^c & OI_\diamond^c \\
\end{pmatrix}
\]

where \(NP, O, I\) and \(OI\) represent no phase, outgoing, incoming and combined outgoing-incoming phase dependency, respectively. The phase differences of the susceptibility products \(\Delta_{aA,bB}\) scale as \(\cos\) or \(\sin\) function in part due to products of Pauli and Gell-Mann matrix elements that have real or imaginary values (see Eq. 2.25 and 2.44), respectively, and are denoted in the symbolic matrix 2.67 by \(c\) or \(s\) superscript, respectively.

Additionally, the following symmetry of the double Mueller matrix can be seen. The fourth, fifth, and sixth column of the double Mueller matrix \((N = 4, 5, 6)\) have the same susceptibility products as the seventh, eighth and ninth column \((N + 3)\), respectively, where the retardedness of the components in the last three columns acquire additional \(\pi/2\) phase shift relative to the middle three columns. The corresponding columns are indicated as subscripts \(\diamond, \Delta\) and \(\nabla\) in the symbolic matrix 2.67.

For example, it can be noted that Pauli matrices with index values \(t = 0, 1\) (Eq. 2.25) and Gell-Mann matrices with index \(N = 1, 2, 3\) (Eq. 2.44) contain only diagonal nonzero elements (i.e. \(a = b\) and \(A = B\)), and therefore, the first two rows and first three columns of the double Mueller matrix become \(\chi_{aA}^*\chi_{aA} = |\chi_{aA}|^2\). They do not contain phase relations, and therefore, are denoted as \(NP\) in the symbolic Mueller matrix representation 2.67. Squared values of each of the six susceptibilities can be deduced from the \(NP\) components for the pure polarized states as follows:

\[
\begin{pmatrix}
|\chi_{zxx}|^2 \\
|\chi_{zzz}|^2 \\
|\chi_{zxy}|^2 \\
|\chi_{zzy}|^2 \\
|\chi_{xxz}|^2 \\
|\chi_{xzz}|^2 \\
\end{pmatrix} = \begin{pmatrix}
\frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & \frac{1}{2} & \frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & \frac{1}{2} \\
\frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & -\frac{1}{2} & \frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & -\frac{1}{2} \\
\frac{\sqrt{6}}{6} & -\frac{\sqrt{3}}{3} & 0 & \frac{\sqrt{6}}{6} & -\frac{\sqrt{3}}{3} & 0 \\
\frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & \frac{1}{2} & -\frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & -\frac{1}{2} \\
\frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & -\frac{1}{2} & -\frac{\sqrt{6}}{6} & \frac{\sqrt{3}}{6} & \frac{1}{2} \\
\frac{\sqrt{6}}{6} & -\frac{\sqrt{3}}{3} & 0 & -\frac{\sqrt{6}}{6} & -\frac{\sqrt{3}}{3} & 0 \\
\end{pmatrix}\begin{pmatrix}
M_{0,1} \\
M_{0,2} \\
M_{0,3} \\
M_{1,1} \\
M_{1,2} \\
M_{1,3} \\
\end{pmatrix}
\]

Similarly, for the last two rows and first three columns of double Mueller matrix (i.e. \(t = 2, 3\) and \(N = 1, 2, 3\)), Pauli matrices have only the off-diagonal elements that are nonzero, while the respective Gell-Mann matrices have only diagonal elements that are
components can be deduced using the following relations:

\[ |\chi_{zxx}|^2 |\chi_{xxx}|^2 = \left( \frac{\sqrt{2}}{6} \mathcal{M}_{2,1} + \frac{\sqrt{2}}{6} \mathcal{M}_{2,2} + \frac{1}{2} \mathcal{M}_{2,3} \right)^2 + \left( \frac{\sqrt{2}}{6} \mathcal{M}_{3,1} + \frac{\sqrt{2}}{6} \mathcal{M}_{3,2} + \frac{1}{2} \mathcal{M}_{3,3} \right)^2 \]

\[ |\chi_{zzz}|^2 |\chi_{xxz}|^2 = \left( \frac{\sqrt{2}}{6} \mathcal{M}_{2,1} + \frac{\sqrt{3}}{6} \mathcal{M}_{2,2} - \frac{1}{2} \mathcal{M}_{2,3} \right)^2 + \left( \frac{\sqrt{2}}{6} \mathcal{M}_{3,1} + \frac{\sqrt{3}}{6} \mathcal{M}_{3,2} - \frac{1}{2} \mathcal{M}_{3,3} \right)^2 \]

\[ |\chi_{zzx}|^2 |\chi_{xxz}|^2 = \left( \frac{\sqrt{2}}{6} \mathcal{M}_{2,1} - \frac{\sqrt{3}}{3} \mathcal{M}_{2,2} \right)^2 + \left( \frac{\sqrt{2}}{6} \mathcal{M}_{3,1} - \frac{\sqrt{3}}{3} \mathcal{M}_{3,2} \right)^2 \]  

(2.69)

Their corresponding phase differences are:

\[ \tan \Delta_{zxx,xxx} = \left( \frac{\sqrt{2}}{6} \mathcal{M}_{3,1} + \frac{\sqrt{2}}{6} \mathcal{M}_{3,2} + \frac{1}{2} \mathcal{M}_{3,3} \right) / \left( \frac{\sqrt{2}}{6} \mathcal{M}_{2,1} + \frac{\sqrt{2}}{6} \mathcal{M}_{2,2} + \frac{1}{2} \mathcal{M}_{2,3} \right) \]

\[ \tan \Delta_{zzz,xzz} = \left( \frac{\sqrt{2}}{6} \mathcal{M}_{3,1} + \frac{\sqrt{3}}{6} \mathcal{M}_{3,2} - \frac{1}{2} \mathcal{M}_{3,3} \right) / \left( \frac{\sqrt{2}}{6} \mathcal{M}_{2,1} + \frac{\sqrt{3}}{6} \mathcal{M}_{2,2} - \frac{1}{2} \mathcal{M}_{2,3} \right) \]

\[ \tan \Delta_{zzx,xxz} = \left( \frac{\sqrt{2}}{6} \mathcal{M}_{3,1} - \frac{\sqrt{2}}{3} \mathcal{M}_{3,2} \right) / \left( \frac{\sqrt{2}}{6} \mathcal{M}_{2,1} - \frac{\sqrt{2}}{3} \mathcal{M}_{2,2} \right) \]  

(2.70)

Likewise, for the first two rows and the last six columns of the double Mueller matrix \((t = 0, 1, \text{ and } N = 4, \ldots, 9)\), the corresponding Pauli matrices have nonzero diagonal components while the Gell-Mann matrices have only the off-diagonal elements as nonzero (i.e. \(A \neq B\)). This results in the double Mueller matrix components containing products of susceptibilities only in terms of the incoming indices, while the outgoing polarization indices are equal (i.e. \(a = b\)). Therefore, these components contain the phase differences only with respect to incoming polarization components and are represented by \(I\) in the symbolic matrix 2.67. These susceptibility products can be deduced:

\[ |\chi_{za}|^2 |\chi_{zb}|^2 = (\mathcal{M}_{0,m} + \mathcal{M}_{1,m})^2 + (\mathcal{M}_{0,m+3} + \mathcal{M}_{1,m+3})^2 \]  

\[ |\chi_{xa}|^2 |\chi_{xb}|^2 = (\mathcal{M}_{0,m} - \mathcal{M}_{1,m})^2 + (\mathcal{M}_{0,m+3} - \mathcal{M}_{1,m+3})^2 \]  

(2.71)

The phases between the \(I\) components can be obtained:

\[ \tan \Delta_{za,zb} = (\mathcal{M}_{0,m+3} + \mathcal{M}_{1,m+3}) / (\mathcal{M}_{0,m} + \mathcal{M}_{1,m}) \]

\[ \tan \Delta_{xa,xb} = (\mathcal{M}_{0,m+3} - \mathcal{M}_{1,m+3}) / (\mathcal{M}_{0,m} - \mathcal{M}_{1,m}) \]  

(2.72)

where \(m = 4, 5, 6\) and for \(A, B = XX, ZZ, ZZ, XZ; XX, XZ\). For the remaining \(\mathcal{M}_{tN}\) components \((t = 2, 3\) and \(N = 4..9)\) \(a \neq b\) and \(A \neq B\). Thus, the double Mueller matrix components contain the linear combination of susceptibilities only with the cross products in terms of both incoming as well as outgoing indices, and their phase differences, and therefore are represented as \(OI\) in Eq. 2.67. The products of these susceptibility components can be deduced using the following relations:

\[ |\chi_{aA}|^2 |\chi_{bB}|^2 = (\mathcal{M}_{2,m} + \mathcal{M}_{3,m+3})^2 + (\mathcal{M}_{3,m} - \mathcal{M}_{2,m+3})^2 \]

\[ |\chi_{aA}|^2 |\chi_{bB}|^2 = (\mathcal{M}_{2,m} - \mathcal{M}_{3,m+3})^2 + (\mathcal{M}_{3,m} + \mathcal{M}_{2,m+3})^2 \]  

(2.73)
The phases between the OI components can be obtained:

\[
\tan \Delta_{aA,bB} = \left( M_{3,m} - M_{2,m+3} \right) / \left( M_{2,m} + M_{3,m+3} \right) \\
\tan \Delta_{aA,bB} = \left( M_{3,m} + M_{2,m+3} \right) / \left( M_{2,m} - M_{3,m+3} \right)
\]  

(2.74)

The ratios of absolute values for the susceptibility components can be deduced by taking the susceptibility product ratios. The relations may be useful when absolute values of the susceptibilities and the phase differences between the components are of interest. When all susceptibility tensor components are in phase, then it is clear that the components, denoted by the superscript \( c \) have maximum values. At the same time, the last three elements of the first three rows and the first six elements of the last row of the double Mueller matrix, which are denoted by superscript \( s \), vanish. This pattern of \( M^{(2)} \) arises for the real nonlinear susceptibilities.

It is also conceivable to rearrange the \( 4 \times 9 \) elements of Eq. 2.15 for the double Mueller matrix into a \( 36 \) element column vector, which together with the \( 36 \) element susceptibility products and a \( (36 \times 36) \) transformation matrix form a system of equations. The susceptibility products then can be found by inverting this system of linear equations (derivation shown in Appendix B). However, it is beneficial to keep the double Mueller matrix in the \( 4 \times 9 \) matrix form due to the correspondence with the conventional \( 4 \times 1 \) Stokes vector and polarimetry measurements.

### 2.2.3 Properties and Symmetries of Medium Matrix

The elements of \( M^{(2)} \) are composed of the susceptibility components products. Often for measurements a ratiometric susceptibility component relations is desired, and therefore, convenient expressions for double Mueller matrix elements in terms of the nonlinear susceptibility tensor components ratios are provided here. Additionally, properties of \( M^{(2)} \) are discussed, and matrix expressions for cylindrically symmetric materials, which are frequently encountered in the biological samples, are presented.
Double Mueller Matrix for Real Susceptibilities

When Kleinman symmetry is assumed, the six susceptibility components for SHG reduce to four, and can be further reduced to three susceptibility component ratios. In addition, when real-valued susceptibilities are assumed, the double Mueller components that have phase relations scaling as \( \sin \Delta_{aA,bB} \) vanish. The explicit form of the double Mueller matrix \( \mathcal{M}^{(2)} \) in terms of susceptibility ratio components for SHG is:

\[
\mathcal{M}_{\text{Kleinman}}^{(2)} = \chi_2^{ZXX} \\
\begin{pmatrix}
\frac{1}{\sqrt{6}} (a^2 + b^2 + 2c^2 + 2) & \frac{1}{\sqrt{12}} (a^2 + b^2 - c^2 - 1) & \frac{1}{2} (a^2 - b^2 + c^2 + 1) & b + ac & c + a & 0 & 0 & 0 \\
-\frac{1}{\sqrt{6}} (a^2 - b^2) & \frac{1}{\sqrt{12}} (a^2 - b^2 + 3c^2 - 3) & -\frac{1}{2} (a^2 + b^2 - c^2 - 1) & b - ac & c - a & 0 & 0 & 0 \\
\frac{\sqrt{2}}{\sqrt{3}} (a + c + bc) & \frac{1}{\sqrt{3}} (a - 2c + bc) & a - bc & c + ab & b + c^2 & 1 + ac & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & c - ab & b - c^2 & 1 - ac
\end{pmatrix}
\]  

(2.75)

where \( a = \chi_{XXX}/\chi_{ZXX}, b = \chi_{ZZZ}/\chi_{ZXX}, \) and \( c = \chi_{XZZ}/\chi_{ZXX} \). The characteristic pattern of Mueller matrix components with 0 values show that if the fundamental radiation is linearly polarized (where the last three components of double Stokes vector are zero), then there will be no circularly polarized component in SHG radiation. This predication can be used to verify whether the nonlinear susceptibilities are real-valued. On the other hand, the if the fundamental radiation is elliptically polarized (where \( S_7, S_8, \) and \( S_9 \) are nonzero), then there will be a nonzero circularly polarized SHG (i.e. a nonzero \( s'_3 \) component) proportional to \( \mathcal{M}_{37}^{(2)}, \mathcal{M}_{38}^{(2)} \) and \( \mathcal{M}_{39}^{(2)} \) elements. In any case, the susceptibility component ratio values depend on the ultrastructure of the material. In the following the polarimetry for the cylindrically symmetric materials, which often occur in biological structures, is discussed.

Two Photon Mueller Matrix for Cylindrically Symmetric Media

When a material has cylindrical symmetry (C\(_{6v}\)), for example in the case of oriented molecules around z axis, only \( \chi_{zzz} \) and \( \chi_{xxx} \) tensor components survive in the molecular frame of reference (denoted with lowercase letters xyz in Fig. 2.2). Therefore, the material is characterized by only one nonlinear susceptibility tensor component ratio \( R = \chi_{zzz}/\chi_{xxx} \). If the cylindrical material z axis is oriented along the Z axis of the laboratory frame of reference (denoted with uppercase letters XYZ Fig. 2.2) only \( b = \chi_{ZZZ}/\chi_{ZXX} \) survives and is equal to the ratio \( R \). The resulting double Mueller
matrix acquires the following form:

\[
M^{(2)}_{C_{6v}} = \chi_{ZXX}^{2} \begin{pmatrix}
\sqrt{\frac{7}{6}}(b^2 + 2) & \sqrt{\frac{1}{12}}(b^2 - 1) & \frac{1}{2}(1 - b^2) & b & 0 & 0 & 0 & 0 \\
\sqrt{\frac{7}{6}}b^2 & \sqrt{\frac{1}{12}}(b^2 + 3) & \frac{1}{2}(1 - b^2) & b & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & b & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & b & 1 & 0
\end{pmatrix}
\] (2.76)

By comparing the general double Mueller matrix expression Eq. 2.75 and its reduced form for cylindrical structures Eq. 2.76, it is clear that the nonzero matrix elements in Eq. 2.76 originate due to the real part of the dipolar molecular susceptibility as pointed out by Shi et al. [50].

The rotation of the cylindrical material in the laboratory frame of reference (XYZ frame) by angles \(\alpha\) and \(\delta\) (see the angle definition in the Fig. 2.2) result in three nonlinear susceptibility tensor component ratios \(a\), \(b\) and \(c\). The ratios can be expressed in terms of the molecular \(R\)-ratio by using the general tensor rotation theory (see Section 1.3.2), where \(\chi_{ijkl}^{(2)} = R_{II}R_{JJ}R_{KK}\chi_{ijk}^{(2)} [89]:

\[
a = \frac{\chi_{XXX}}{\chi_{ZXX}} = \frac{\sin \delta [(R - 3)\cos^2 \alpha \sin^2 \delta + 3]}{\cos \delta [(R - 3)\cos^2 \alpha \sin^2 \delta + 1]} \quad (2.77)
\]

\[
b = \frac{\chi_{ZZZ}}{\chi_{ZXX}} = \frac{(R - 3)\cos^2 \alpha \cos^2 \delta + 3}{(R - 3)\cos^2 \alpha \sin^2 \delta + 1} \quad (2.78)
\]

\[
c = \frac{\chi_{XZZ}}{\chi_{ZXX}} = \frac{\sin \delta [(R - 3)\cos^2 \alpha \cos^2 \delta + 1]}{\cos \delta [(R - 3)\cos^2 \alpha \sin^2 \delta + 1]} \quad (2.79)
\]

By taking the deduced values of the \(a\), \(b\) and \(c\) ratios, the rotation angle \(\delta\) and the molecular nonlinear susceptibility ratio \(R\) can be expressed as:

\[
\delta = \arctan \left( \frac{a + c}{b + 1} \right) \quad \text{and} \quad R = \frac{\chi_{ZZZ}^{(2)}}{\chi_{ZXX}^{(2)}} = \frac{2a + ab + 2bc + 3c}{ab - c} \quad (2.80)
\]

In Eq. 2.80, the equality for \(R\) is only valid when \(\alpha = 0\). The angle \(\delta\) in Eq. 2.80 is the same as “the asymmetry parameter” introduced in a previous study [89], and was phenomenologically understood as the asymmetrical distribution of fibers in a focal volume. \(R\) and \(\delta\) are important parameters of interest when performing a nonlinear polarization experiment. They provide information about the organization and orientation of...
molecules in cylindrically symmetric materials. The solution for $\delta$ is obtained via:

$$\frac{\cos \delta}{\sin \delta} (a + c) = \frac{(R - 3)cos^2 \alpha + 4}{(R - 3)cos^2 \alpha \sin^2 \delta + 1} = b + 1$$  \hspace{1cm} (2.81)

and the solution for $R$ is obtained by acknowledging:

$$\frac{a}{c} = \frac{(R - 3) + 2b + 6}{(R - 3)b + 2b - 2}, \quad \text{and} \quad \sin^2 \delta = \frac{(R - 3) - b + 3}{(R - 3)(b + 1)}$$  \hspace{1cm} (2.82)

### 2.2.4 Second Harmonic Generation Polarimetry

Investigations of biological structures with polarization-dependent SHG microscopy provide information about the molecular organization in the tissue [80, 87, 89, 121, 132–134]. These molecular organizations play a major role in determining mechanical and functional properties of the biomaterials. For example, SHG polarization microscopy revealed that the collagen organization in lung tissues is affected by cancer [135]. Thus, development of polarization-dependent nonlinear optical techniques for determining the molecular organization may find broad applications in the field of biology, material sciences and nanotechnology.

In the experimental setup, the electric fields of fundamental radiation are considered to be coplanar, and the propagating incoming beam at frequency $\omega$, as well as the outgoing beam at frequency of $2\omega$, have the same direction along the Y axis. Meanwhile, the radiation electric fields are oriented in the XZ plane of the laboratory frame of reference (Fig. 2.2). The laboratory frame of reference is denoted with capital letters XYZ for the Cartesian coordinate system, and the lowercase xyz denotes the coordinate system associated with the molecular frame of reference (Fig. 2.2). The Z-axis is considered as a primary axis of the right-hand coordinate system. The general nonlinear Stokes-Mueller equation describing the relation between the polarization of fundamental laser radiation, the generated nonlinear signal radiation and the nonlinear properties of the media can be written as follows:

$$s'(2\omega) = \mathcal{M}^{(2)} S(\omega)$$  \hspace{1cm} (2.83)

where $\mathcal{M}^{(2)}$ is the two-photon Mueller matrix, $S$ is the Stokes vector for incoming laser beams that generate SHG, and hereafter, called the double Stokes vector, while $s'$ is the Stokes vector describing the generated nonlinear radiation.
In the following a convenient expression for the double Stokes vector for SHG is derived. Subsequently, the method for obtaining double Mueller matrix component values in the double Stokes-Mueller polarimetry experiment will be derived. Expressions for double Stokes polarimetry, using arbitrarily oriented linear polarization-in polarization-out (PIPO) measurement configuration, will be provided. In the final section, the derivation of SHG Stokes-Mueller polarimetry equations for the birefringent material is discussed.

Poincaré Coordinates for Polarization State of Incoming Radiation for SHG

For SHG polarimetry the fundamental beam polarization state needs to be defined. In Stokes-Mueller polarimetry representation, the expression of double Stokes vector in terms of Poincaré coordinates is derived. Let us start with the linearly polarized light oriented at an angle \( \theta_0 = \pi/4 \) with respect to the primary axis. Its Stokes vector is:

\[
s_{\pi/4} = \left\langle E_0^2 \right\rangle \begin{pmatrix} 1 & 0 & 1 & 0 \end{pmatrix}^T
\]

This linearly polarized light is passed through a quarter-waveplate and a half-waveplate, resulting in a new Stokes vector for the fundamental beam:
\[
\mathbf{H}(\theta_h) = \begin{pmatrix}
1 & 0 & 0 & 0 \\
0 & \cos (4\theta_h) & \sin (4\theta_h) & 0 \\
0 & \sin (4\theta_h) & -\cos (4\theta_h) & 0 \\
0 & 0 & 0 & -1
\end{pmatrix}, \quad \mathbf{Q}(\theta_q) = \begin{pmatrix}
1 & 0 & 0 & 0 \\
0 & \cos^2 (2\theta_q) & \frac{1}{2} \sin (4\theta_q) & -\sin (2\theta_q) \\
0 & \frac{1}{2} \sin (4\theta_q) & \sin^2 (2\theta_q) & \cos (2\theta_q) \\
0 & \sin (2\theta_q) & -\cos (2\theta_q) & 0
\end{pmatrix}
\]

(2.85)

The \( \mathbf{H}(\theta_h) \) and \( \mathbf{Q}(\theta_q) \) matrices for the half- and quarter-waveplate can be derived separately by using the general Mueller expression for a retarder with its fast axis orientated at \( \theta_h \) and \( \theta_q \) angles from the primary axis of laboratory coordinate system, respectively [104]:

\[
s = \mathbf{H}(\theta_h) \mathbf{Q}(\theta_q) s_{\pi/4}
\]

Therefore, the linear Stokes vector for the fundamental beam takes the familiar form:

\[
s = \left\langle E_0^2 \right\rangle \begin{pmatrix}
1 \\
\cos 2(2\theta_h - \theta_q) \cos 2(\pi/4 - \theta_q) \\
\sin 2(2\theta_h - \theta_q) \cos 2(\pi/4 - \theta_q) \\
\sin 2(\pi/4 - \theta_q)
\end{pmatrix}
\]

(2.87)

\( \theta_h \) and \( \theta_q \) angles can be related to the Fig. 1.1 Poincaré sphere coordinates \((2\Psi, 2\Omega)\), where \( \theta_h = \frac{1}{2}(\Psi - \Omega + \pi/4) \) and \( \theta_q = (-\Psi + \pi/4) \) (compare Eqs. 1.32 and 2.87). The obtained linear form of Stokes vector (or the electric field in Eq. 1.32) is substituted into Eq. 2.53 to find the incoming radiation polarization state in terms of Poincaré coordinates:

\[
S(\Psi, \Omega) = \left\langle E_0^4 \right\rangle
\begin{pmatrix}
\frac{\sqrt{\pi}}{6} \left( 3 - (\cos (2\Psi) \cos (2\Omega))^2 \right) \\
\frac{\sqrt{3}}{6} \left( 5(\cos (2\Psi) \cos (2\Omega))^2 - 3 \right) \\
-\cos (2\Psi) \cos (2\Omega) \\
\frac{1}{2} \cos (4\Omega) - (\cos (2\Psi) \cos (2\Omega))^2 \\
\cos (2\Omega) \sin (2\Psi) \left( 1 + \cos (2\Psi) \cos (2\Omega) \right) \\
\cos (2\Omega) \sin (2\Psi) \left( 1 - \cos (2\Psi) \cos (2\Omega) \right) \\
-\frac{1}{2} \sin (2\Psi) \sin (4\Omega) \\
\sin (2\Omega) \left( \cos (2\Psi) \cos (2\Omega) + 1 \right) \\
\sin (2\Omega) \left( \cos (2\Psi) \cos (2\Omega) - 1 \right)
\end{pmatrix}
\]

(2.88)

This double Stokes vector is used to find the polarization state of the fundamental beam for double Stokes-Mueller polarimetry and SHG polarimetry analyses.
Complete Double Stokes SHG Polarimetry

The components of double Mueller matrix can be obtained by measuring nonlinear intensities of SHG radiations, which can be generated at various fundamental polarization states. As shown in Fig. 2.2, the fundamental polarization state is prepared by the polarization state generator (PSG), while the nonlinear signal is analyzed by passing it through the polarization state analyzer (PSA). The PSG and PSA can be constructed for example by using a linear polarizer, a quarter waveplate and a half waveplate oriented at desired angles. An example of constructing the PSG method for double Stokes vector was more discussed in previous section using a QWP and a HWP. A complete $4 \times 9$ double Mueller matrix can be deduced by using a minimum of 9 different fundamental radiation polarization states ($Q = 1...9$), each described by the double Stokes vector (and each vector with 9 components ($N = 1...9$)). At each polarization state of the fundamental radiation, all 4 Stokes vector elements of SHG radiation, $s'_t (t = 0, \ldots, 3)$, are measured, leading to 36 independent measurements:

$$s'_{\gamma,Q} = \mathcal{M}_{\gamma,N} S_{N,Q}$$

(2.89)

where summation is assumed over repeated index $N$. For example, the 9 different polarization states, shown in Fig. 1.1, for the fundamental radiation can be chosen with distinct Poincaré coordinates (where the azimuth and elevation coordinates are denoted by $2\Psi$ and $2\Omega$, respectively, and are given in Eq. 2.88). These polarization states are: linear polarizations along $Z$, along $X$, along $\pm 45^\circ$ with respect to $Z$, right and left circular polarization, linear polarization midway between $Z$ and $-45^\circ$, and two elliptical polarizations midway between $X$ and right circular polarizations, and between $45^\circ$ and left circular polarizations. In terms of Poincaré coordinates the set is:

$$(\Psi,\Omega)_Q = (0,0), ([\pi/2,0], [\pi/4,0], [-\pi/4,0], [0,\pi/4], [0,-\pi/4], [-\pi,0], [\pi,0], [\pi/2,\pi/8], [-\pi/4,-\pi/8])$$

(2.90)

The linear Stokes vector values for these chosen polarization states are:

$$s_{\gamma,Q} = \left(\begin{array}{c}
s_{\gamma,1} \\
s_{\gamma,2} \\
s_{\gamma,3} \\
s_{\gamma,4} \\
s_{\gamma,5} \\
s_{\gamma,6} \\
s_{\gamma,7} \\
s_{\gamma,8} \\
s_{\gamma,9}
\end{array}\right)$$

$$\propto \langle E_0^2 \rangle$$

(2.91)
Using Eq. 2.53 for the double Stokes vector and the values in Eq. 2.91 for the linear Stokes vector, the double Stokes vectors values for the chosen polarization states are:

\[
S_{N,Q} = \left( \begin{array}{c}
S_{N,1} \\
S_{N,2} \\
S_{N,3} \\
S_{N,4} \\
S_{N,5} \\
S_{N,6} \\
S_{N,7} \\
S_{N,8} \\
S_{N,9}
\end{array} \right) = \left( \begin{array}{cccccccc}
\sqrt{\frac{3}{2}} & \sqrt{\frac{3}{2}} & \sqrt{\frac{3}{2}} & \sqrt{\frac{3}{2}} & \frac{5\sqrt{6}}{12} & \frac{5\sqrt{6}}{12} & \frac{\sqrt{6}}{2} \\
\frac{\sqrt{3}}{2} & -\frac{\sqrt{3}}{2} & -\frac{\sqrt{3}}{2} & -\frac{\sqrt{3}}{2} & -\frac{\sqrt{3}}{12} & -\frac{\sqrt{3}}{12} & -\frac{\sqrt{3}}{12} \\
-1 & 1 & 0 & 0 & 0 & \frac{-\sqrt{2}}{2} & \frac{\sqrt{2}}{2} & 0 \\
0 & 0 & \frac{1}{2} & \frac{1}{2} & -\frac{1}{2} & -\frac{1}{2} & \frac{1}{4} & -\frac{1}{4} & 0 \\
0 & 0 & 1 & -1 & 0 & 0 & \frac{-1-\sqrt{2}}{2} & 0 & \frac{\sqrt{2}}{2} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{2} \\
0 & 0 & 0 & 0 & 1 & -1 & 0 & \frac{\sqrt{2}-1}{2} & -\frac{\sqrt{2}}{2} \\
0 & 0 & 0 & 0 & -1 & 1 & 0 & \frac{-1-\sqrt{2}}{2} & \frac{\sqrt{2}}{2}
\end{array} \right)
\]

\[
(2.92)
\]

The \( S_{NQ}^{(\Psi,\Omega)} \) matrix elements in Eq. 2.92 can also be obtained for the given Poincaré coordinates using Eq. 2.88. The 9 × 9 matrix in Eq. 2.92 has an inverse and satisfies the condition for Eq. 2.89. This matrix, together with the 4 × 9 matrix for the measured SHG polarization states \( s'_{t,Q} \), determines the experimental values of the 4 × 9 \( M^{(2)} \) uniquely, using the relation \( M_{tN} = s'_{t,Q} S_{Q,N}^{-1} \) (summation is assumed over index \( Q \)).

**Reduced Double Stokes SHG Polarimetry for Real Susceptibility**

Nonlinear interactions can be described with real nonlinear susceptibilities when fundamental and generated second harmonic waves are in phase and the frequencies are away from absorption bands. The real nonlinear susceptibility ratios \( a, b \) and \( c \) can be obtained from combinations of the double Mueller matrix elements (see Eq. 2.75). A reduced double Stokes polarimetry measurements can be performed since not all nonlinear Mueller matrix components have to be used for the calculation of the ratios. One set of solutions for the \( a, b \) and \( c \) ratios in terms of the measured SHG linear polarization states \( s'_{t,Q} \) is:

\[
a = \frac{s'_{t,2}}{2\mathcal{L}}, \quad c = \frac{s'_{t,1}}{2b\mathcal{L}},
\]

\[
b = \frac{s'_{t,2}^2 \left( 2s'_{t,3} + 2s'_{t,4} - s'_{t,2} - s'_{t,1} \right)^2 + 100 \left( s'_{t,1} s'_{t,2} \mathcal{L} + 5s'_{t,1} \mathcal{L}^2 \right) - 5s'_{t,2}^2 s'_{t,1}^2}{\left( 2s'_{t,3} + 2s'_{t,4} - s'_{t,2} - s'_{t,1} \right) \left( 100\mathcal{L}^2 + s'_{t,2}^2 \right)},
\]

\[
(2.93)
\]
where \( L = \frac{1}{2} (s'_{1,2} + \sqrt{s_{1,2}^2 + s_{2,2}^2}) \). Equation 2.93 shows that only six elements, namely \( s'_{1,1}, s'_{2,1}, s'_{1,2}, s'_{2,2}, s'_{2,3}, \) and \( s'_{2,4} \) are required to determine \( a, b \) and \( c \) ratios. These six SHG polarization measurements use the second and third Stokes vector components (i.e. \( s'_{1} \) and \( s'_{2} \)), which are the intensity differences between the linear analyzer orientation at 0 and \( \pi/2 \), and between \( \pi/4 \) and \( -\pi/4 \), respectively. Additionally, Eq. 2.93 shows that only four linear polarization states of fundamental radiation (\( S_Q \), where \( Q = 1, \ldots, 4 \) in Eq. 2.92) at angles \( \theta = 0, \pi/2, \pi/4, \) and \( -\pi/4 \) from the \( Z \) axis are required to measure the susceptibility component ratios. Combined, 12 intensity measurements at different polarization configurations for the fundamental and SHG are required to determine the nonlinear susceptibility ratios \( a, b \) and \( c \). For the case of cylindrical symmetry, those ratios uniquely determine the molecular susceptibility ratio \( R \) and the orientation angle of the cylindrical axis \( \delta \) (Eq 2.80). The described method for obtaining \( a, b \) and \( c \) ratios is not unique, and alternative sets of polarization measurements can be designed.

SHG Polarimetry for Linear PIPO Configuration

In the following the linear polarization-in polarization-out (PIPO) experiments using Stokes-Mueller formalism are discussed. Recently, PIPO measurements have been shown to provide a robust determination of the susceptibility ratios \( a, b \) and \( c \) as well as determination of the molecular susceptibility ratio and orientation angle for the cylindrically symmetric materials [87,89]. The PIPO setup is comprised of a PSG, containing a linear polarizer for the fundamental radiation, the medium with nonlinear optical properties, and a PSA containing a linear polarizer (analyzer) for the SHG as shown in Fig. 2.2. The SHG intensity is measured at different polarizer and linear analyzer orientations, and the surface plot of intensities as a function of incoming polarization and analyzer angles is constructed. The best fit of the intensity equation to the surface determines \( a, b \) and \( c \) ratio values [89].

The dependence of double Stokes vector on the orientations of the quarter- and the half-wave plate was derived in Section 2.2.4. The double Stokes vector expression for linear polarization at an angle \( \theta \) with respect to \( Z \) axis can be expressed as follows:
\[
S_{\phi_0=0} = \langle E_0^4 \rangle = \left( \begin{array}{c}
\frac{\sqrt{6}}{12} (5 - \cos(4\theta)) \\
\frac{\sqrt{3}}{12} (5 \cos(4\theta) - 1) \\
- \cos(2\theta) \\
\frac{1}{2} \sin^2(2\theta) \\
2 \cos^2(\theta) \sin(2\theta) \\
2 \sin^2(\theta) \sin(2\theta) \\
0 \\
0 \\
0 
\end{array} \right)
\]

By multiplying Eq. 2.94 above to Eq. 2.75 for double Mueller matrix with real susceptibilities, the Stokes vector for SHG is obtained:

\[
\left( \begin{array}{c}
s'_0 (2\omega) \\
s'_1 (2\omega) \\
s'_2 (2\omega) \\
s'_3 (2\omega)
\end{array} \right) \propto \left( \begin{array}{c}
\sigma_1^2 + \sigma_2^2 \\
\sigma_1^2 - \sigma_2^2 \\
2\sigma_1\sigma_2 \\
0
\end{array} \right)
\]

where

\[
\sigma_1 = a\sin^2\theta + \sin 2\theta + c\cos^2\theta \\
\sigma_2 = \sin^2\theta + c\sin 2\theta + b\cos^2\theta
\]

The double Stokes-Mueller equation for the combined nonlinear medium and linear polarizer can be obtained as:

\[
s' (2\omega) = M_{LA} M^{(2)} S(\omega)_{\phi_0=0} = \mathcal{L} \left( \begin{array}{c}
(\sigma_1 \sin \varphi + \sigma_2 \cos \varphi)^2 \\
\cos(2\varphi)(\sigma_1 \sin \varphi + \sigma_2 \cos \varphi)^2 \\
\sin(2\varphi)(\sigma_1 \sin \varphi + \sigma_2 \cos \varphi)^2 \\
0
\end{array} \right)
\]

where \( M_{LA} \) denotes the (4 x 4) Mueller matrix for the linear analyzer. Note that the \( s'_0 \) component is identical to the so-called “PIPO equation” in a previous study [89]. It is used in nonlinear microscopy for fitting the PIPO imaging data, and is more explicitly stated as follows:

\[
s'_0 (2\omega) = I_{2\omega}(\theta, \varphi) = \mathcal{L} |\sigma_1 \sin \varphi + \sigma_2 \cos \varphi|^2
\]

From Eq. 2.97 it follows that for real susceptibilities and linear incoming polarization
s'_3 = 0. Therefore, it is very informative to measure the s'_3 component; if the measured value is negligible the assumption that the material possess real-valued susceptibilities is valid. Additionally, it is assumed that the fundamental and SHG radiations do not experience any birefringence. Such an assumption applies often when measuring thin samples at the wavelength away from the fundamental and SHG absorption bands.

**SHG Polarimetry for Birefringent Media**

The linear laser polarization when passing through a birefringent material will acquire a phase shift between the X and Z components (where \( \phi_Z - \phi_X = \phi_\omega \); \( E_X = E_0 \sin \theta \), and \( E_Z = E_0 e^{i\phi_\omega} \cos \theta \); \( \theta \) is the angle of linearly polarized light before entering the material). Using these expressions for \( E_X \) and \( E_Z \) and Eq. 2.53, the double Stokes vector is:

\[
S = \left( E_0^4 \right) \begin{pmatrix}
\frac{\sqrt{6}}{12} (5 - \cos(4\theta)) \\
\frac{\sqrt{3}}{12} (5 \cos(4\theta) - 1) \\
-\cos(2\theta) \\
\frac{1}{2} \sin^2(2\theta) \cos(2\phi_\omega) \\
2 \cos^2(\theta) \sin(2\theta) \cos(\phi_\omega) \\
2 \sin^2(\theta) \sin(2\theta) \cos(\phi_\omega) \\
-2 \cos^2(\theta) \sin(2\theta) \sin(\phi_\omega) \\
2 \sin^2(\theta) \sin(2\theta) \sin(\phi_\omega) \\
\frac{1}{2} \sin^2(2\theta) \sin(2\phi_\omega)
\end{pmatrix}
\] (2.99)

Multiplying Eq. 2.99 to Eq. 2.75 for double Mueller matrix, the SHG Stokes vector is:

\[
\begin{pmatrix}
s'_0 (2\omega) \\
s'_1 (2\omega) \\
s'_2 (2\omega) \\
s'_3 (2\omega)
\end{pmatrix} \propto \begin{pmatrix}
|\zeta_1|^2 + |\zeta_2|^2 \\
|\zeta_1|^2 - |\zeta_2|^2 \\
\zeta_1 \zeta_2^* + \zeta_2 \zeta_1^* \\
(\zeta_1 \zeta_2^* - \zeta_2 \zeta_1^*) i
\end{pmatrix}
\] (2.100)

where

\[
\zeta_1 = \sin^2 \theta + ce^{i\phi_\omega} \sin 2 \theta + be^{2i\phi_\omega} \cos^2 \theta
\]

\[
\zeta_2 = a \sin^2 \theta + e^{i\phi_\omega} \sin 2 \theta + ce^{2i\phi_\omega} \cos^2 \theta
\] (2.101)

Thus, the birefringence in incoming radiation influences all 4 SHG Stokes components.
The effect of birefringence on generated SHG can be described using a combined linear and nonlinear Stokes-Mueller polarimetry similar to the shown in Eq. 2.97. When the incoming laser beam is linearly polarized, and the laboratory $Z$ axis orientation is matched both to the extraordinary axis of birefringent material and to the cylindrical axis of nonlinear medium ($\delta = 0$), an equation can be derived that accounts for the phase delay between the two perpendicular components of the SHG radiation. The Mueller matrix, describing the birefringence effect with a retardance of $\phi_{2\omega}$ between the components, where the orientation of extraordinary axis is matched to the laboratory $Z$ axis, is given as [104]:

$$
M_{\text{biref}} = \begin{pmatrix}
1 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 \\
0 & 0 & \cos(\delta_{2\omega}) & \sin(\delta_{2\omega}) \\
0 & 0 & -\sin(\delta_{2\omega}) & \cos(\delta_{2\omega})
\end{pmatrix}
$$

(2.102)

Multiplying the double Stokes expression for the linear polarization at orientation $\theta$ (Eq. 2.33), the nonlinear Mueller matrix (Eq. 2.75) and the birefringence Mueller matrix (Eq. 2.102), the SHG Stokes vector becomes:

$$
s'(2\omega) = M_{\text{biref}}M(2)S(\omega) = L \begin{pmatrix}
\sigma_1^2 + \sigma_2^2 \\
\sigma_1^2 - \sigma_2^2 \\
2\sigma_1\sigma_2 \cos \delta_{2\omega} \\
2\sigma_1\sigma_2 \sin \delta_{2\omega}
\end{pmatrix}
$$

(2.103)

Therefore, the phase delay can be calculated easily by obtaining Stokes parameters $s'_2$ and $s'_3$ of the SHG radiation and using $\tan \delta_{2\omega} = s'_{3,\theta}/s'_{2,\theta}$.

### 2.2.5 Discussion

The double Stokes-Mueller formalism can be applied to describe nonlinear interactions of the polarized light with a material. The incoming beam polarization state is described by a nine component double Stokes vector expressed in terms of the products of Stokes vector components for the fundamental radiation (Eq. 2.53). The outgoing SHG polarization state is represented by the conventional $4 \times 1$-component Stokes vector. The corresponding nonlinear optical properties of the material are described by the $4 \times 9$ components of the double Mueller matrix $M^{(2)}$. The double Mueller matrix components are related to
the second order susceptibility tensor components according to Eq. 2.64. The nonlinear optical properties of materials are often described using susceptibility tensor, which have complex component values. While it is customary to use nonlinear susceptibility tensor representation, experimentally measured values are real, and therefore, can be described using the double Stokes-Mueller formalism. The properties of double Mueller matrix are very informative for designing experiments of second-harmonic Stokes polarimetry.

Matrix 2.67 illustrates the relative phase dependency between the nonlinear susceptibility components. The NP components are independent of the phase relations and represent the linear combination of absolute values of the susceptibilities. The I components describe individually outgoing X and Z linear polarization components while including phase relations between different incoming polarization components XX, ZZ, and XZ. The O components describe the phase relations between outgoing polarizations for the same incoming polarizations. The OI components describe the remaining phase relations between outgoing polarizations for different incoming polarization components. This double Mueller matrix pattern provides information about various properties of the nonlinear material, and some will be discussed below.

The double-Mueller matrix components scale as cos or sin function for the phases of complex nonlinear susceptibility components. When the nonlinear susceptibility values are real, (Eq. 2.75), the first six elements in the fourth row and the last three elements in the first three rows of the Mueller matrix are zero. Thus, for a sample with real-valued susceptibilities and with Kleinman symmetry, any measured optical activity is reflected in the $s'_3$, which results from the last three elements of the fourth row in the double Mueller matrix. These elements can be evoked only when elliptical polarization is present in the fundamental radiation. The double Mueller matrix for the material with cylindrical symmetry Eq. 2.76 provides an informative framework for designing experiments to obtain the ratio $b = \chi_{ZZZ}/\chi_{XXX}$. For example, in a case where the cylindrically symmetric crystal axes are aligned with the laboratory axes, then the susceptibility component ratio can be measured by using Eq. 2.76 and recording $s'_{0.1}$ and $s'_{0.2}$, and then deducing $b = (s'_{0.1}/s'_{0.2})^{1/2}$. Alternatively, the ratio can be measured using Eqs. 2.76 and 2.92, and recording $s'_{2,Q}$ when $Q = 3, 4, 7, 9$, and deducing $b = (s'_{2,Q}/L) - S_{6,Q}/S_{5,Q}$; or similarly the ratio can be measured by recoding $s'_{3,Q}$ when $Q = 5, 6, 8, 9$, and deducing $b = ((s'_{3,Q}/L) - S_{9,Q})/S_{8,Q}$, where $S_{5,Q}$, $S_{6,Q}$, $S_{8,Q}$, and $S_{9,Q}$ are double Stokes vector elements for the chosen polarization states (see Eq. 2.92).
Cylindrically symmetric structures such as collagen, muscle, starch, and others are ubiquitous in biological samples. If the cylindrical axes of the material is not aligned to the laboratory coordinates, then the tensor is expressed in terms of rotated tensor elements along the laboratory coordinates. Equations 2.80 provides the solution to molecular parameters in terms of the measured ratio $a$, $b$ and $c$ for the cylindrical structure. In total 12 measurements of incoming and outgoing polarization combinations are required to determine the molecular susceptibility ratio and cylindrical axis orientation using Eq. 2.93. Namely, four linear incoming polarizations at angles $\theta$, $\pi/2$, $\pi/4$, and $-\pi/4$ and four linear outgoing polarization states at $0$, $\pi/2$, $\pi/4$, and $-\pi/4$ angle from the Z axis are measured. In Eq. 2.98 it was shown that $s_0^2 (2\omega)$ is connected to the PIPO analyses, which rely on the fitting of SHG intensity generated from the sample as a function of incident polarization angle $\theta$ and the outgoing analyzer angle $\varphi$ [89]. Since the reduced double Stokes-Mueller analysis depends on direct calculations to recover the molecular susceptibility ratio and the cylindrical axis orientation, then the extraction of molecular parameters is quicker compared to fitting the PIPO SHG intensity surface plot with Eq. 2.93 [89].

After SHG signal is generated the SHG radiation propagating through the optical elements can be described by the classical $4 \times 4$ Mueller matrix (i.e. treated as linear) formalism, which is the case for analyses performed by Mazumder et al. [52]. In addition, the linear scattering effects can be modeled by adding a partial depolarizer Mueller matrix (defined as a square diagonal matrix: $M_{ij} = \delta_{ij} d_i$, where $i, j = 0...3$, $d_0 = 1$, and $0 < d_i = d < 1$ is the extent of depolarization of the SHG Stokes vector) in order to separate the polarized light from the unpolarized light [104].

In summary, a theoretical framework for the double Stokes-Mueller polarimetry for two photon-processes is provided in this section. The double Mueller matrix is composed of second-order susceptibilities for the second harmonic generation, which can also be used for the sum- (or difference-) frequency generation processes when the Kleinman symmetry applies. The derived equations relate the outgoing Stokes vector for the SHG signal to the polarization state of the incoming fundamental radiation beam and to the second-order susceptibility tensor values of the sample. The theory is provided in the context and in analogy with previous works on Stokes-Mueller formalism as well as nonlinear optics notations.
2.3 Three Photon Polarimetry

Three photon processes such as third-harmonic generation (THG) and coherent anti-Stokes Raman scattering (CARS) are important processes that reveal unique information about the sample under study. In general in a three-photon process, radiation with three distinct frequencies are incident on the material as shown in Fig. 2.3. The resulting outgoing radiation from the material is related to the incoming radiation electric fields and determined by the third-order susceptibility tensor $\chi^{(3)}$ of the material. Therefore, similar to the second-order processes, the polarimetric framework for three-photon processes can be developed using the general formalism presented at the beginning of this Chapter.

\[ P_{i}^{(3)} = \chi_{ijkl}^{(3)} E_{j} E_{k} E_{l} = \chi_{iA}^{(3)} \psi_{A}^{(3)} \]  
\[ (2.104) \]

For nonlinear polarimetry in the most general case, where three different frequencies are involved, the state vector can be written as:

\[ \psi^{(3)}(\omega_1, \omega_2, \omega_3) = \begin{pmatrix} \psi_{1}^{(3)} \\ \psi_{2}^{(3)} \\ \psi_{3}^{(3)} \\ \psi_{4}^{(3)} \end{pmatrix} \]  
\[ (2.105) \]
The corresponding coherency matrix for the three-photon process is:

\[
\rho^{(3)}(\omega_1, \omega_2, \omega_3) = \langle \psi^{(3)} \cdot \psi^{(3)*}\rangle = \begin{pmatrix}
\langle \psi_1^{(3)} \cdot \psi_1^{(3)*}\rangle & \langle \psi_1^{(3)} \cdot \psi_2^{(3)*}\rangle & \langle \psi_1^{(3)} \cdot \psi_3^{(3)*}\rangle & \langle \psi_1^{(3)} \cdot \psi_4^{(3)*}\rangle \\
\langle \psi_2^{(3)} \cdot \psi_1^{(3)*}\rangle & \langle \psi_2^{(3)} \cdot \psi_2^{(3)*}\rangle & \langle \psi_2^{(3)} \cdot \psi_3^{(3)*}\rangle & \langle \psi_2^{(3)} \cdot \psi_4^{(3)*}\rangle \\
\langle \psi_3^{(3)} \cdot \psi_1^{(3)*}\rangle & \langle \psi_3^{(3)} \cdot \psi_2^{(3)*}\rangle & \langle \psi_3^{(3)} \cdot \psi_3^{(3)*}\rangle & \langle \psi_3^{(3)} \cdot \psi_4^{(3)*}\rangle \\
\langle \psi_4^{(3)} \cdot \psi_1^{(3)*}\rangle & \langle \psi_4^{(3)} \cdot \psi_2^{(3)*}\rangle & \langle \psi_4^{(3)} \cdot \psi_3^{(3)*}\rangle & \langle \psi_4^{(3)} \cdot \psi_4^{(3)*}\rangle
\end{pmatrix}
\]

(2.106)

Therefore, the general triple Stokes vector can be found similar to the linear and two-photon processes according to:

\[
S^{(3)}(\omega_1, \omega_2, \omega_3) = \text{Tr}(\gamma \rho^{(3)})
\]

(2.107)

where the 4 × 4 \(\gamma\) matrices and the associated identity matrix are hermitian and obey the unique orthogonality relation \(\text{Tr}(\gamma_\mu \gamma_\nu) = 2\delta_{\mu\nu}\). As mentioned in Section 2.1.7, the matrices for three-photons are developed as following. Let \(\gamma\) represent the set of matrices for three-photon polarimetry where \(n + 1 = 4\). Then following the recipe developed in Section 2.1.7, first the set \(\gamma''\) matrices are obtained:

\[
\gamma''_{1,1} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{1,2} = \begin{pmatrix} 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{1,3} = \begin{pmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{1,4} = \begin{pmatrix} 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}
\]

\[
\gamma''_{2,1} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{2,2} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{2,3} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{2,4} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}
\]

\[
\gamma''_{3,1} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{3,2} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{3,3} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{3,4} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 \end{pmatrix}
\]

\[
\gamma''_{4,1} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{4,2} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{4,3} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \gamma''_{4,4} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}
\]

(2.108)

Note that \(\gamma''\) can also expand the coherency matrix \(\rho^{(3)}(\omega_1, \omega_2, \omega_3)\). However, the resulting vector for \(S(\omega_1, \omega_2, \omega_3)\) will be complex as well as the corresponding \(M^{(3)}\). Thus, following steps 1 and 2 of the recipe (see Eq. 2.32), the one-dimensional set of \(\gamma\) matrices can be
produced:

\[
\begin{align*}
\gamma_4 &= \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, & \gamma_5 &= \begin{pmatrix} 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, & \gamma_9 &= \begin{pmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, & \gamma_{10} &= \begin{pmatrix} 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix} \\
\gamma_{11} &= \begin{pmatrix} 0 & 0 & 0 & -i \\ 0 & 0 & 0 & 0 \\ i & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, & \gamma_3 &= \sqrt{\frac{3}{4}} \begin{pmatrix} 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, & \gamma_6 &= \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, & \gamma_8 &= \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix} \\
\gamma_{15} &= \begin{pmatrix} 0 & 0 & 0 & -i \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ i & 0 & 0 & 0 \end{pmatrix}, & \gamma_{12} &= \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & -i & 0 \\ 0 & i & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, & \gamma_2 &= \sqrt{\frac{6}{3}} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \end{pmatrix}, & \gamma_7 &= \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix} \\
\gamma_{16} &= \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -i \\ 0 & 0 & 0 & 0 \\ i & 0 & 0 & 0 \end{pmatrix}, & \gamma_{14} &= \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -i \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix}, & \gamma_{13} &= \sqrt{\frac{3}{3}} \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{pmatrix}, & \gamma_{1} &= \sqrt{\frac{2}{3}} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \end{pmatrix}
\end{align*}
\]

where the two dimensional $4 \times 4$ $\gamma'$ set is shown but labeled with the one-dimensional sixteen-element set $\gamma$ as described in Eq. 2.109. The presented order of $\gamma$ matrices in Eq. 2.109 is chosen to maintain the consistency with the order of the linear Stokes vector (determined by Pauli matrices in Eq. 1.16) as well as the second-order Stokes vector (determined by Gell-Mann matrices in Eq. 2.44).

Similar to the linear Stokes parameters the vector for third-order interaction obeys the following relation:

\[
3S_1^2 \geq \sum_{N=2}^{16} S_N^2
\]

where the equality is valid for the purely polarized state. Therefore, it is convenient to use the degree of polarization (DOP) parameter to characterize the fundamental radiation using this vector:

\[
DOP(\omega_1, \omega_2, \omega_3) = \sqrt{\sum_{N=2}^{16} S_N^2 / 3S_1^2}
\]

where $DOP$ ranges from 0 to 1 for unpolarized to fully polarized fundamental radiation, respectively.
Third Harmonic Generation

Polarimetric measurements of third-harmonic generation (THG) in crystalline material, ordered aggregates or isotropic media carry information about the nonlinear optical properties and organization of the structure in a material. In this section, the triple Stokes-Mueller formalism is developed for the THG polarimetry and convenient expressions are derived for the triple Mueller matrix in terms of the nonlinear susceptibilities. The polarization analysis is further developed for isotropic and cylindrically symmetric structures providing the method to calculate the characteristic susceptibility components and also orientations of molecules in the cylindrical structures. This information, for example, can be used to investigate lipid structures in the cell membrane or pigment aggregates within the cell.

The polarization state of incoming electric field radiation for THG is:

$$
\psi(\omega, \omega, \omega) = \begin{pmatrix}
E_1^3 \\
E_2^3 \\
3E_1^2E_2^* \\
3E_1E_2^2
\end{pmatrix}
$$

(2.112)

The corresponding third-order coherency matrix for THG is:

$$
\rho(\omega, \omega, \omega) =
\begin{pmatrix}
\langle E_1^3 E_1^{*3} \rangle & \langle E_1^3 E_2^{*3} \rangle & \langle 3E_1^3 E_1^2 E_2^* \rangle & \langle 3E_1^3 E_1 E_2^{*2} \rangle \\
\langle E_2^3 E_1^{*3} \rangle & \langle E_2^3 E_2^{*3} \rangle & \langle 3E_2^3 E_1^2 E_2^* \rangle & \langle 3E_2^3 E_1 E_2^{*2} \rangle \\
\langle 3E_1^2 E_2 E_1^{*3} \rangle & \langle 3E_1^2 E_2 E_2^{*3} \rangle & \langle 9E_1^2 E_2 E_1^2 E_2^* \rangle & \langle 9E_1^2 E_2 E_1 E_2^{*2} \rangle \\
\langle 3E_1 E_2^2 E_1^{*3} \rangle & \langle 3E_1 E_2^2 E_2^{*3} \rangle & \langle 9E_1 E_2^2 E_1^2 E_2^* \rangle & \langle 9E_1 E_2^2 E_1 E_2^{*2} \rangle
\end{pmatrix}
$$

(2.113)

where $C$ (defined in Eq. 1.12) is the coherency matrix for the fundamental wavelength $\omega$.

Therefore, the elements of the vector of polarization state for incoming radiation gener-
Chapter 2. Theory of Nonlinear Optical Polarimetry

Evaluating THG is \( S_N(\omega, \omega, \omega) = \text{Tr}(\rho \gamma_N) \):

\[
S_N(\omega, \omega, \omega) = \begin{pmatrix}
\frac{\sqrt{2}}{2} \left( \langle E_1^3 E_1^3 \rangle + \langle 9E_1^2 E_2 E_1^2 E_2^* \rangle + \langle 9E_1 E_2^2 E_1^2 E_2^* \rangle + \langle E_2^3 E_2^3 \rangle \right) \\
\frac{\sqrt{6}}{6} \left( \langle E_1^3 E_1^{*3} \rangle + \langle 9E_1^2 E_2 E_1^{*2} E_2^* \rangle - \langle 27E_1 E_2^2 E_1^{*2} E_2^* \rangle + \langle E_2^3 E_2^{*3} \rangle \right) \\
\frac{\sqrt{3}}{3} \left( \langle E_1^3 E_1^3 \rangle - \langle 18E_1^2 E_2 E_1^2 E_2^* \rangle + \langle E_2^3 E_2^3 \rangle \right)
\end{pmatrix}
\]

\[
= \frac{1}{4} \begin{pmatrix}
\sqrt{2}s_0(5s_0^2 - 3s_1^2) \\
\sqrt{6}(-\frac{2}{3}s_0^3 + 3s_0^2 s_1 + 2s_0 s_1^2 - 3s_1^3) \\
\sqrt{3}(-\frac{8}{3}s_0^3 - 3s_0^2 s_1 + 4s_0 s_1^2 + 3s_1^3) \\
s_1(3s_0^2 + s_1^2) \\
s_2(s_2^2 - 3s_3^2) \\
3(s_0 - s_1)(s_2^2 - s_3^2) \\
9s_2(s_2^2 + s_3^2) \\
3s_2(s_0 - s_1)^2 \\
3s_2(s_0 + s_1)^2 \\
3(s_0 + s_1)(s_2^2 - s_3^2) \\
s_3(s_3^2 - 3s_2^2) \\
6s_2 s_3(s_0 - s_1) \\
-9s_3(s_2^2 + s_3^2) \\
3s_3(s_0 - s_1)^2 \\
-3s_3(s_0 + s_1)^2 \\
-6s_2 s_3(s_0 + s_1)
\end{pmatrix}
\]

(2.114)
Note, the last six elements of the vector above vanish when \( s_3 \) is zero. This implies when the incoming radiation is linearly polarized, the triple Stokes vector for THG in the above equation will have at most the first 10 nonzero elements. This information can simplifies and helps with designing experiments involving triple Mueller matrix.

The incoming radiation in terms of Poincaré coordinates for THG is:

\[
S(\Psi, \Omega) = \frac{1}{4} \left( E_0^6 \right) \begin{pmatrix}
\sqrt{2}[5 - 3\cos(2\Omega)^2 \cos(2\Psi)^2] \\
\sqrt{6}[-3\cos(2\Omega)^3 \cos(2\Psi)^3 + 2\cos(2\Omega)^2 \cos(2\Psi)^2 + 3\cos(2\Omega) \cos(2\Psi) - \frac{4}{3}] \\
\sqrt{3}[3\cos(2\Omega)^3 \cos(2\Psi)^3 + 4\cos(2\Omega)^2 \cos(2\Psi)^2 - 3\cos(2\Omega) \cos(2\Psi) - \frac{8}{3}] \\
\cos(2\Omega)^3 \cos(2\Psi)^3 + 3 \cos(2\Omega) \cos(2\Psi) \\
\cos(2\Omega)^3 \sin(2\Psi)^3 - 3 \cos(2\Omega) \sin(2\Omega)^2 \sin(2\Psi) \\
3 (\cos(2\Omega) \cos(2\Psi) - 1) \left( \cos(2\Omega)^2 \left( \cos(2\Psi)^2 - 1 \right) - \cos(2\Omega)^2 + 1 \right) \\
-9 \cos(2\Omega) \sin(2\Psi) \left( \cos(2\Omega)^2 \cos(2\Psi)^2 - 1 \right) \\
3 \cos(2\Omega) \sin(2\Psi) \left( \cos(2\Omega) \cos(2\Psi) - 1 \right)^2 \\
3 \cos(2\Omega) \sin(2\Psi) \left( \cos(2\Omega) \cos(2\Psi) + 1 \right)^2 \\
-3 (\cos(2\Omega) \cos(2\Psi) + 1) \left( \cos(2\Omega)^2 \left( \cos(2\Psi)^2 - 1 \right) - \cos(2\Omega)^2 + 1 \right) \\
\sin(2\Omega)^3 + 3 \sin(2\Omega) \sin(2\Psi)^2 \left( \sin(2\Omega)^2 - 1 \right) \\
-6 \cos(2\Omega) \sin(2\Psi) \sin(2\Psi) \left( \cos(2\Omega) \cos(2\Psi) - 1 \right) \\
9 \sin(2\Omega) \left( \left( \sin(2\Omega)^2 - 1 \right) \left( \sin(2\Psi)^2 - 1 \right) - 1 \right) \\
3 \sin(2\Omega) \left( \cos(2\Omega) \cos(2\Psi) - 1 \right)^2 \\
-3 \sin(2\Omega) \left( \cos(2\Omega) \cos(2\Psi) + 1 \right)^2 \\
-6 \cos(2\Omega) \sin(2\Omega) \sin(2\Psi) \left( \cos(2\Omega) \cos(2\Psi) + 1 \right)
\end{pmatrix}
\]

\[(2.115)\]

Coherent Anti-Stokes Raman Scattering

The coherent anti-Stokes Raman scattering (CARS) is a nonlinear \( \chi^{(3)}(\omega_s; \omega_p, \omega_p, -\omega_s) \) effect, where a pump beam \( \omega_p \) and a Stokes beam \( \omega_s \) are used to generate the so-called anti-Stokes signal at frequency \( \omega_as \). The general state of the incoming radiations for the purpose of nonlinear CARS polarimetry can be written as:

\[
\psi'(\omega_p, \omega_p, -\omega_s) = \begin{pmatrix}
E_1(\omega_p)^2 E_1^*(\omega_s) + E_1^*(\omega_s)^2 E_1(\omega_p) \\
E_2(\omega_p)^2 E_2^*(\omega_s) + E_2^*(\omega_s)^2 E_2(\omega_p) \\
3 E_1(\omega_p)^2 E_2^*(\omega_s) + 3 E_1^*(\omega_s)^2 E_2(\omega_p) \\
3 E_2(\omega_p)^2 E_1^*(\omega_s) + 3 E_2^*(\omega_s)^2 E_1(\omega_p)
\end{pmatrix}
\]

\[(2.116)\]
where the first term describes the anti-Stokes for $\omega_p$ as the pump beam and $\omega_s$ as the Stokes beam, and the second term is the anti-Stokes for the $\omega_s$ as the pump beam and $\omega_p$ as the Stokes beam. This implies a two part contribution to the CARS signal due to two beams. Since no new physics is introduced by keeping the anti-Stokes too general, and for simplicity, each part can be written separately. Therefore, the state of electric fields for CARS due only to one pair of pump-Stokes beams is:

$$\psi(\omega_p, \omega_p, -\omega_s) = \begin{pmatrix} E_1(\omega_p)^2 E_1^*(\omega_s) \\ E_2(\omega_p)^2 E_2^*(\omega_s) \\ 3 E_1(\omega_p)^2 E_2^*(\omega_s) \\ 3 E_2(\omega_p)^2 E_1^*(\omega_s) \end{pmatrix}$$  \hspace{1cm} (2.117)

The corresponding coherency matrix for CARS can be written as:

$$\rho(\omega_p, \omega_p, -\omega_s) = \left\langle \psi(\omega_p, \omega_p, -\omega_s) \cdot \psi(\omega_p, \omega_p, -\omega_s)\right\rangle =$$

$$\begin{pmatrix} C(\omega_p)_{1,1}^2 C(\omega_s)_{1,1} & \ldots & C(\omega_p)_{1,2}^2 C(\omega_s)_{2,1} & \ldots & C(\omega_p)_{1,2}^2 C(\omega_s)_{1,1} \\ \ldots & \ldots & \ldots & \ldots & \ldots \\ C(\omega_p)_{2,1}^2 C(\omega_s)_{1,2} & \ldots & C(\omega_p)_{2,2}^2 C(\omega_s)_{2,2} & \ldots & C(\omega_p)_{2,2}^2 C(\omega_s)_{1,2} \\ \ldots & \ldots & \ldots & \ldots & \ldots \\ C(\omega_p)_{2,1}^2 C(\omega_s)_{1,1} & \ldots & C(\omega_p)_{2,2}^2 C(\omega_s)_{2,1} & \ldots & C(\omega_p)_{2,2}^2 C(\omega_s)_{1,1} \end{pmatrix}$$  \hspace{1cm} (2.118)

where $C(\omega_p)$ denote the coherency matrices for the beam $\omega_p$:

$$C(\omega_p) = \begin{pmatrix} \langle E_1^*(\omega_p) E_1(\omega_p) \rangle & \langle E_2^*(\omega_p) E_1(\omega_p) \rangle \\ \langle E_1^*(\omega_p) E_2(\omega_p) \rangle & \langle E_2^*(\omega_p) E_2(\omega_p) \rangle \end{pmatrix} = \frac{1}{2} \begin{pmatrix} s_0(\omega_p) + s_1(\omega_p) & s_2(\omega_p) + s_3(\omega_p) \, i \\ s_2(\omega_p) - s_3(\omega_p) \, i & s_0(\omega_p) - s_1(\omega_p) \end{pmatrix},$$  \hspace{1cm} (2.119)

and $C(\omega_s)$ for the $\omega_s$ beam:

$$C(\omega_s) = \begin{pmatrix} \langle E_1^*(\omega_s) E_1(\omega_s) \rangle & \langle E_2^*(\omega_s) E_1(\omega_s) \rangle \\ \langle E_1^*(\omega_s) E_2(\omega_s) \rangle & \langle E_2^*(\omega_s) E_2(\omega_s) \rangle \end{pmatrix} = \frac{1}{2} \begin{pmatrix} s_0(\omega_s) + s_1(\omega_s) & s_2(\omega_s) + s_3(\omega_s) \, i \\ s_2(\omega_s) - s_3(\omega_s) \, i & s_0(\omega_s) - s_1(\omega_s) \end{pmatrix}.$$  \hspace{1cm} (2.120)
Finally, the Stokes vector for CARS polarimetry is:

\[
S(\omega_p, \omega_p, -\omega_s) = \frac{1}{I} \langle E_0(\omega_p)^4 E_0(\omega_s)^2 \rangle \begin{pmatrix}
\frac{\pi}{2} [10 s_0(\omega_s) s_0(\omega_p)^2 - 16 s_1(\omega_s) s_0(\omega_p) s_1(\omega_p) + 10 s_0(\omega_s) s_1(\omega_p)^2] \\
\frac{\pi}{3} [36 s_0(\omega_p) s_1(\omega_p) s_0(\omega_s) - (s_0(\omega_p)^2 + s_1(\omega_p)^2)(18 s_1(\omega_s) + 8 s_0) + 20 s_0(\omega_p) s_1(\omega_p) s_1(\omega_s)] \\
\frac{\pi}{3} [s_0(\omega_s)(s_0(\omega_p)^2 + s_1(\omega_p)^2) - (9 s_0(\omega_s) - 9 s_1(\omega_s))(s_0(\omega_p) + s_1(\omega_p))^2 + 2 s_0(\omega_p) s_1(\omega_p) s_1(\omega_s)] \\
s_1(\omega_s) s_0(\omega_p)^2 + 2 s_0(\omega_s) s_0(\omega_p) s_1(\omega_p) + s_1(\omega_s) s_1(\omega_p)^2 \\
s_2(\omega_s) s_2(\omega_p)^2 + 2 s_1(\omega_s) s_2(\omega_p) s_3(\omega_p) - s_2(\omega_s) s_3(\omega_p)^2 \\
3 (s_2(\omega_p)^2 - s_3(\omega_p)^2) (s_0(\omega_s) - s_1(\omega_s)) \\
9 s_2(\omega_s) s_2(\omega_p)^2 - 18 s_3(\omega_s) s_2(\omega_p) s_3(\omega_p) - 9 s_2(\omega_s) s_3(\omega_p)^2 \\
3 (s_0(\omega_p) - s_1(\omega_p))^2 s_2(\omega_s) \\
3 (s_0(\omega_p) + s_1(\omega_p))^2 s_2(\omega_s) \\
9 s_2(\omega_s) s_2(\omega_p)^2 - 18 s_3(\omega_s) s_2(\omega_p) s_3(\omega_p) - 9 s_2(\omega_s) s_3(\omega_p)^2 \\
6 (s_0(\omega_s) - s_1(\omega_s)) s_2(\omega_p) s_3(\omega_p) \\
-9 s_3(\omega_s) s_2(\omega_p)^2 - 18 s_2(\omega_s) s_2(\omega_p) s_3(\omega_p) + 9 s_3(\omega_s) s_3(\omega_p)^2 \\
-3 (s_0(\omega_p) - s_1(\omega_p))^2 s_3(\omega_s) \\
3 (s_0(\omega_p) + s_1(\omega_p))^2 s_3(\omega_s) \\
-6 (s_0(\omega_s) + s_1(\omega_s)) s_2(\omega_p) s_3(\omega_p)
\end{pmatrix}
(2.121)
\]

Interesting predications can be made based on the polarization states of \(\omega_p\) and \(\omega_s\) beams. For example, like \(S\) for THG all last 6 elements of \(S(\omega_p, \omega_p, -\omega_s)\) vanish when both of the beams are linearly polarized. This means for a system with real susceptibilities, no elliptically polarized light is expected if both \(\omega_p\) and \(\omega_s\) beams are linearly polarized. Meanwhile, unlike its harmonic counterpart, the \(S\) for CARS is not necessarily zero in the last 6 elements when only one of the beam is linearly polarized, although that would make the last column of \(\mathcal{M}^{(3)}\) irrelevant since the last component of \(S\) would be zero. Meanwhile, if one of the beam has any circular component then some elliptically outgoing CARS signal is generated.\(^8\)

\(^8\)Usually CARS is measured at resonance of selected vibration. Therefore, there will be a phase dependence in the Mueller matrix.
\section*{2.3.2 Derivation of $\mathcal{M}^{(3)}$ Matrix for the Medium}

Consistent with the definition of electric field in the state function for THG $\psi(\omega, \omega, \omega)$ and CARS $\psi(\omega_p, \omega_p, -\omega_s)$, the general form of the third-order susceptibilities for polarimetry can be written in a contracted index notation as:

$$\chi^{(3)} = \begin{pmatrix} \chi^{(3)}_{xxxx} & \chi^{(3)}_{xzzz} & \chi^{(3)}_{xxxx} & \chi^{(3)}_{xzzz} \\ \chi^{(3)}_{zxzx} & \chi^{(3)}_{zzxx} & \chi^{(3)}_{zxzx} & \chi^{(3)}_{zzxx} \end{pmatrix}$$  \hspace{1cm} (2.122)

Here the matrix is constructed mainly with the THG process in mind, where the Klienmen symmetry is not required. In extending the matrix for non-THG processes it is required to ensure that the Klienmen symmetry is valid. Otherwise, an effective $\chi^{(3)}$ may be defined.

The contracted notation can be written more compactly by letting $\chi^{(3)}_{jkl} = \chi^{(3)}_{ijkl}$:

$$\chi^{(3)} = \begin{pmatrix} \chi^{(3)}_{111} & \chi^{(3)}_{122} & \chi^{(3)}_{133} & \chi^{(3)}_{144} \\ \chi^{(3)}_{211} & \chi^{(3)}_{222} & \chi^{(3)}_{233} & \chi^{(3)}_{244} \end{pmatrix}$$  \hspace{1cm} (2.123)

where

$$jkl : \hspace{0.5cm} xxx \hspace{0.5cm} zzz \hspace{0.5cm} xzz, xzx, zxx \hspace{0.5cm} xzz, zxx, zzz$$

$$A : \hspace{0.5cm} 1 \hspace{0.5cm} 2 \hspace{0.5cm} 3 \hspace{0.5cm} 4$$  \hspace{1cm} (2.124)

Therefore, the susceptibility product matrix is:

$$X^{(3)} = \langle \chi^{(3)} \otimes \chi^{(3)*} \rangle =$$

\[
\begin{pmatrix}
\chi_{1111} & \chi_{1112} & \chi_{1113} & \chi_{1114} \\
\chi_{1212} & \chi_{1213} & \chi_{1214} & \chi_{1214} \\
\chi_{1313} & \chi_{1314} & \chi_{1314} & \chi_{1314} \\
\chi_{1414} & \chi_{1414} & \chi_{1414} & \chi_{1414} \\
\chi_{2111} & \chi_{2112} & \chi_{2113} & \chi_{2114} \\
\chi_{2212} & \chi_{2213} & \chi_{2214} & \chi_{2214} \\
\chi_{2313} & \chi_{2314} & \chi_{2314} & \chi_{2314} \\
\chi_{2414} & \chi_{2414} & \chi_{2414} & \chi_{2414} \\
\chi_{3111} & \chi_{3112} & \chi_{3113} & \chi_{3114} \\
\chi_{3212} & \chi_{3213} & \chi_{3214} & \chi_{3214} \\
\chi_{3313} & \chi_{3314} & \chi_{3314} & \chi_{3314} \\
\chi_{3414} & \chi_{3414} & \chi_{3414} & \chi_{3414} \\
\chi_{4111} & \chi_{4112} & \chi_{4113} & \chi_{4114} \\
\chi_{4212} & \chi_{4213} & \chi_{4214} & \chi_{4214} \\
\chi_{4313} & \chi_{4314} & \chi_{4314} & \chi_{4314} \\
\chi_{4414} & \chi_{4414} & \chi_{4414} & \chi_{4414} \\
\end{pmatrix}
\]  \hspace{1cm} (2.125)

where an ensemble average for each element is assumed. The colour indicates similar attributes as in two-photon case: white for no relative phase; yellow for relative phase for incoming (i.e first) index; purple for outgoing (i.e second) index; and green for both indices being different. Therefore, following the derivation similar to the linear and two-photon processes, the third-order $\mathcal{M}^{(3)}$ matrix elements are:

$$M^{(3)}_{tg} = \frac{1}{4} \text{Tr}(\tau_t \chi^{(3)} \gamma_g \chi^{(3)*}) = \mathcal{T} X^{(3)} \Gamma^{-1}$$  \hspace{1cm} (2.126)
where the derivation is shown for a single source, and the ensemble average of generators can be included in derivation as was shown for the general case in Section 2.1.4. Γ is derived from γ matrices in Eq. 2.109, where each row of Γ is derived from the vectorization of the γ matrices and is given according to:

\[
\Gamma = \begin{pmatrix}
\frac{\sqrt{3}}{2} & 0 & 0 & 0 & 0 & \frac{\sqrt{3}}{2} & 0 & 0 & 0 & \frac{\sqrt{3}}{2} \\
\frac{\sqrt{6}}{6} & 0 & 0 & 0 & \frac{\sqrt{6}}{6} & 0 & 0 & 0 & \frac{\sqrt{6}}{6} & 0 & 0 & 0 & -\frac{\sqrt{6}}{6} \\
\frac{\sqrt{3}}{3} & 0 & 0 & 0 & \frac{\sqrt{3}}{3} & 0 & 0 & 0 & -\frac{2\sqrt{3}}{3} & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -i & 0 & 0 & i & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -i & 0 & 0 & i & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & i & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -i & 0 & 0 & 0 & i & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -i & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{pmatrix}
\]

(2.127)

Γ is invertible and obeys \(\Gamma^{-1} = \frac{1}{2} \Gamma^\dagger\).

Similar to the symbolic notation which was given for the second-order matrix \(\mathcal{M}^{(2)}\) (Eq. 2.67), the symbolic matrix for the third-order \(\mathcal{M}^{(3)}\) matrix becomes:

\[
\begin{pmatrix}
NP & NP & NP & NP \\
NP & NP & NP & NP \\
O^c & O^c & O^c & O^c \\
O^s & O^s & O^s & O^s \\
\end{pmatrix}
\begin{pmatrix}
I_0^c & I_\Delta^c & I_\nabla^c & I_\nabla^c & I_\Delta^c & I_\nabla^c & I_\nabla^c \\
O_0^s & O_\Delta^s & O_\nabla^s & O_\nabla^s & O_\Delta^s & O_\nabla^s & O_\nabla^s \\
O_0^s & O_\Delta^s & O_\nabla^s & O_\nabla^s & O_\Delta^s & O_\nabla^s & O_\nabla^s \\
\end{pmatrix}
\]

(2.128)

In the elemental form the susceptibility product can be found using \(X_{ij} = \frac{1}{2} T_{it}^N M_{tN} \Gamma_{Nj}\), where \(i = (a - 1)^2 + b\) and \(j = (A - 1)^4 + B\). Since, \(\chi_{aA} \chi_{bB}^* = |\chi_{aA}| \chi_{bB} |e^{i(\delta_{aA} - \delta_{bB})}\), then the relative phase between any two susceptibility elements \(\chi_{aA}\) and \(\chi_{bB}\) can be found.
according to:

\[
\delta_{aA} - \delta_{aA} = \Delta_{aA,bb} = \tan^{-1}\left(\frac{iX_{kl}X_{ij} - \chi_{bbA}^{*}}{\chi_{AA}^{*}X_{kl} + \chi_{bbA}X_{ij}}\right) = \tan^{-1}\left(\frac{iX_{kl} - X_{ij}}{X_{kl} + X_{ij}}\right)
\]

\[\frac{\Delta_{kl}M_{TN}T_{lN}}{\Gamma_{kN}M_{TN}T_{lN}T_{lN}}\] (2.129)

where \( k = (b-1)2 + a \) and \( l = (B-1)4 + A \), and summations is performed over repeated indices. Equation 2.129 shows that by measuring the three-photon polarimetry matrix, and using matrices \( T \) from Eq. 2.21 and \( \Gamma \) in Eq. 2.127, the relative phase between the susceptibility elements can be obtained.

### 2.3.3 Properties and Symmetries of \( \mathcal{M}^{(3)} \) Matrix

#### Triple Mueller Matrix for Real Susceptibilities

When Kleinman symmetry is valid for a third-order process, there are five unique nonzero susceptibilities. Additionally, the last six columns of the first three rows and the first ten elements of the last row in \( \mathcal{M}^{(3)} \) are zero:

\[
\mathcal{M}^{(3)}_{\text{Kleinman}} = \left(\chi_{XXXZ}\right)^2
\]

\[
\begin{pmatrix}
\frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} \\
\frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} \\
\frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} \\
\frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} \\
\frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} \\
\frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4} & \frac{\chi(3)^{2}}{4}
\end{pmatrix}
\]

\[\begin{pmatrix}
\delta_{aA} & \delta_{aA} & \delta_{aA} & \delta_{aA} & \delta_{aA} & \delta_{aA} \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}
\]

where \( a = \chi_{XXXZ}/\chi_{XXXZ}^{3} \), \( b = \chi_{ZZZZ}/\chi_{XXXZ}^{3} \), \( c = \chi_{ZZZZ}/\chi_{XXXZ}^{3} \), and \( d = \chi_{XXXZ}/\chi_{XXXZ}^{3} \).

#### Triple Mueller Matrix for Isotropic Media

For isotropic material where \( X \) and \( Z \) components are of interest, only three elements are nonzero, of which only one is independent: \( \chi_{XXXZ}^{3} = \chi_{ZZZZ}^{3} = 3\chi_{XXXZ}^{3} \) (see Box: *Spatial Symmetries Frequently Used in This Thesis* in Section 1.3.2). Substituting these
symmetry relations in Eq. 2.130, the matrix for isotropic material is as following:
\[
M^{(3)}_{\text{iso}} = (\chi^{(3)}_{XXZZ})^2 \begin{pmatrix}
5\sqrt{2} & \frac{4\sqrt{6}}{3} & \frac{8\sqrt{3}}{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & \frac{\sqrt{2}}{3} & -\frac{\sqrt{3}}{3} & -9 & 0 & 3 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 9 & 0 & 1 & 3 & 3 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -9 & 0 & 1 & 3 & -3 & 0
\end{pmatrix}
\]

As can be seen, the matrix elements for isotropic material is independent of susceptibility tensor components and only scale with the effective susceptibility value.

### Triple Mueller Matrix for Hexagonal Symmetry

For hexagonal material, where only X and Z components are of concern, three elements are independent: \(\chi^{(3)}_{XXXX}, \chi^{(3)}_{ZZZZ}\) and \(\chi^{(3)}_{XXZZ}\). Substituting them in Eq. 2.130, the hexagonal material matrix becomes:
\[
M^{(3)}_{\text{hex}} = (\chi^{(3)}_{XXZZ})^2 \begin{pmatrix}
\frac{\sqrt{2}}{2} (a^2+b^2+2) & \frac{\sqrt{2}}{3} (a^2+b^2-2) & \frac{\sqrt{3}}{3} (a^2+b^2-2) & (a^2-b^2) & 0 & 0 & 0 & 0 & a & 0 & 0 & 0 & 0 & 0 & 0 \\
\frac{\sqrt{2}}{2} (a^2-b^2) & \frac{\sqrt{2}}{3} (a^2+b^2+4) & \frac{\sqrt{3}}{3} (a^2-b^2+2) & -(a^2+b^2) & 0 & 0 & 0 & -a & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & a & b & 0 & 1 & b & a & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{pmatrix}
\]

where \(a = \chi^{(3)}_{XXXX}/\chi^{(3)}_{XXZZ}\) and \(b = \chi^{(3)}_{ZZZZ}/\chi^{(3)}_{XXZZ}\).

### 2.3.4 Third Harmonic Generation Polarimetry

For the most general case, in order to find each element of \(\mathcal{M}^{(3)}\) matrix independently, at least sixteen independent measurements of the outgoing Stokes vector with unique incoming polarization states are required. For each measurement \(Q\), all four component of the outgoing vector has to be recorded. The solution to the third-order matrix from polarimetry data is:
\[
\mathcal{M}^{(3)} = S^{-1} s'
\] (2.133)

A set of prepared polarization states composed of 16 different orientations for an incoming radiation that generates an invertible matrix \(S\) is shown on Fig. 1.1. Additionally the matrix is given in Eq. 2.136.
Measurements of Medium Matrix by THG Polarimetry

In terms of Poincaré coordinate the set for the triple Stokes is (also shown in Fig. 1.1):

\[
\begin{align*}
\Gamma = & \left\{ \left[ 0, 0, \frac{\pi}{4}, 0 \right], \left[ \frac{\pi}{4}, 0, \frac{\pi}{4}, 0 \right], \left[ 0, \frac{\pi}{4}, \frac{\pi}{4}, 0 \right], \left[ \frac{\pi}{4}, \frac{\pi}{4}, \frac{\pi}{4}, 0 \right], \left[ 0, 0, 0, 0 \right] \right\} \\
\end{align*}
\]

\[
\begin{align*}
\left( \begin{array}{cccccccccccccccc}
\Gamma & \Omega
\end{array} \right) = & \left( \begin{array}{cccccccccccccccc}
0 & 0 & \frac{\pi}{4} & 0 & \frac{\pi}{4} & 0 & \frac{\pi}{4} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{array} \right)
\end{align*}
\]

\[
\begin{align*}
\mathbf{s}_{t,Q} = & \left( \begin{array}{cccccccccccccccc}
\mathbf{s}_{t,1} & \mathbf{s}_{t,2} & \mathbf{s}_{t,3} & \mathbf{s}_{t,4} & \mathbf{s}_{t,5} & \mathbf{s}_{t,6} & \mathbf{s}_{t,7} & \mathbf{s}_{t,8} & \mathbf{s}_{t,9} & \mathbf{s}_{t,10} & \mathbf{s}_{t,11} & \mathbf{s}_{t,12} & \mathbf{s}_{t,13} & \mathbf{s}_{t,14} & \mathbf{s}_{t,15} & \mathbf{s}_{t,16}
\end{array} \right)
\end{align*}
\]

\[
\begin{align*}
\left( \begin{array}{cccccccccccccccc}
\mathbf{s}_{0,Q} & \mathbf{s}_{1,Q} & \mathbf{s}_{2,Q} & \mathbf{s}_{3,Q}
\end{array} \right) \propto \langle E_0^2 \rangle
\end{align*}
\]

\[
\begin{align*}
\left( \begin{array}{cccccccccccccccc}
\mathbf{S}_{1,Q} & \mathbf{S}_{2,Q} & \mathbf{S}_{3,Q} & \mathbf{S}_{4,Q} & \mathbf{S}_{5,Q} & \mathbf{S}_{6,Q} & \mathbf{S}_{7,Q} & \mathbf{S}_{8,Q} & \mathbf{S}_{9,Q} & \mathbf{S}_{10,Q} & \mathbf{S}_{11,Q} & \mathbf{S}_{12,Q} & \mathbf{S}_{13,Q} & \mathbf{S}_{14,Q} & \mathbf{S}_{15,Q} & \mathbf{S}_{16,Q}
\end{array} \right) \propto \langle E_0^6 \rangle
\end{align*}
\]
Chapter 2. Theory of Nonlinear Optical Polarimetry

THG Polarimetry for Linear PIPO Configuration

When the incoming electric field is linearly polarized at an angle \( \theta \) form Z-axis: \( E(\omega) = [E_1(\omega), E_1(\omega)]^T = [\sin \theta, \cos \theta]^T \). The vector for incoming electric fields for THG is:

\[
\psi(\theta) = \begin{pmatrix} E_1^3 \\ E_2^3 \\ 3E_1^2E_2 \\ 3E_1E_2^2 \end{pmatrix} = E_0^3 \begin{pmatrix} \sin(\theta)^3 \\ \cos(\theta)^3 \\ 3\cos(\theta)\sin(\theta)^2 \\ 3\cos(\theta)^2\sin(\theta) \end{pmatrix} \quad (2.137)
\]

The corresponding vector for linearly polarized incoming radiations is:

\[
S(\theta) = \langle E_0^6 \rangle = \begin{pmatrix} \frac{\sqrt{7}}{2} \cos(\theta)^6 + 9 \cos(\theta)^4 \sin(\theta)^2 + 9 \cos(\theta)^2 \sin(\theta)^4 + \sin(\theta)^6 \\ \frac{\sqrt{6}}{6} \cos(\theta)^6 - 27 \cos(\theta)^4 \sin(\theta)^2 + 9 \cos(\theta)^2 \sin(\theta)^4 + \sin(\theta)^6 \\ \frac{\sqrt{3}}{4} \cos(\theta)^6 - 18 \cos(\theta)^2 \sin(\theta)^4 + \sin(\theta)^6 \\ \sin(\theta)^6 - \cos(\theta)^6 \\ 2 \cos(\theta)^3 \sin(\theta)^3 \\ 6 \cos(\theta)^4 \sin(\theta)^2 \\ 18 \cos(\theta)^3 \sin(\theta)^3 \\ 6 \cos(\theta)^5 \sin(\theta) \\ 6 \cos(\theta) \sin(\theta)^5 \\ 6 \cos(\theta)^2 \sin(\theta)^4 \end{pmatrix} \quad (2.138)
\]

Multiplying Eq. 2.138 and Eq. 2.130, the THG Stokes vector is obtained:

\[
\begin{pmatrix} s'_0(3\omega) \\ s'_1(3\omega) \\ s'_2(3\omega) \\ s'_3(3\omega) \end{pmatrix} \propto \begin{pmatrix} \sigma_1^2 + \sigma_2^2 \\ \sigma_1^2 - \sigma_2^2 \\ 2\sigma_1\sigma_2 \\ 0 \end{pmatrix} \quad (2.139)
\]

where

\[
\sigma_2 = \chi^{(3)}_{ZZZZ} \cos(\theta)^3 + 3 \chi^{(3)}_{XZZZ} \cos(\theta)^2 \sin(\theta) + 3 \chi^{(3)}_{ZXXX} \cos(\theta) \sin(\theta)^2 + \chi^{(3)}_{XXZZ} \sin(\theta)^3, \\
\sigma_1 = \chi^{(3)}_{ZZZZ} \cos(\theta)^3 + 3 \chi^{(3)}_{ZXXZ} \cos(\theta)^2 \sin(\theta) + 3 \chi^{(3)}_{ZXXX} \cos(\theta) \sin(\theta)^2 + \chi^{(3)}_{ZXXX} \sin(\theta)^3 \quad (2.140)
\]
The resulting THG from the nonlinear medium passing through a linear analyzer is:

\[
s'(3\omega) = M_{\text{analyzer}} S(\omega)_{\phi=0} = \mathcal{L} \begin{pmatrix} (\sigma_1 \sin \varphi + \sigma_2 \cos \varphi)^2 \\ \cos(2\varphi)(\sigma_1 \sin \varphi + \sigma_2 \cos \varphi)^2 \\ \sin(2\varphi)(\sigma_1 \sin \varphi + \sigma_2 \cos \varphi)^2 \\ 0 \end{pmatrix}
\]

From Eq. 2.141 it follows that for real susceptibilities and linear incoming polarization \(s'_3 = 0\). Therefore, it is very informative to measure the \(s'_3\) component, and if the measured value is negligible, real susceptibilities may be assumed for the material. Additionally, the fundamental and THG radiations do not experience any birefringence. Such assumption applies often when measuring thin samples at the wavelength away from the fundamental and THG absorption bands. Moreover, the \(s'_0\) component is similar to the linear PIPO equation for THG. It can be used in nonlinear microscopy for fitting the THG PIPO imaging data, and more explicitly stated as follows:

\[
s'_0(3\omega) = I_{3\omega}(\theta, \varphi) = \mathcal{L} |\sigma_1 \sin \varphi + \sigma_2 \cos \varphi|^2
\]

For a sample with isotropic symmetry, there is only one independent susceptibility \((\chi^{(3)}_{XXX} = \chi^{(3)}_{ZZZZ} = 3\chi^{(3)}_{XZZX})\):

\[
s'_0(3\omega)_{\text{iso}} \propto |\chi^{(3)}_{XXXX}|^2 |\cos(\varphi) \cos(\theta) + \sin(\varphi) \sin(\theta)|^2 \propto |\cos(\varphi - \theta)|^2
\]

Thus, for isotropically symmetric material the outgoing intensity directly depends only on the direction of incoming radiation and scales according to an effective susceptibility. This predication is tested in Section 4.3 when THG from retinal molecules in the fruit fly eye is measured. For hexagonally symmetric samples, where Kleinman symmetry is also valid, there are three independent nonzero susceptibilities \((\chi^{(3)}_{XXXX}, \chi^{(3)}_{ZZZZ}, \chi^{(3)}_{XZZX})\):

\[
s'_0(3\omega)_{\text{hex}} \propto \left| \cos(\varphi) \left(\chi^{(3)}_{ZZZZ} \cos(\theta)^3 + 3\chi^{(3)}_{XZZX} \cos(\theta) \sin(\theta)^2 \right) + \sin(\varphi) \left(3\chi^{(3)}_{XZZX} \cos(\theta)^2 \sin(\theta) + \chi^{(3)}_{XXXX} \sin(\theta)^3 \right) \right|^2
\]

This relation can be used to fit the two-dimensional \((\theta, \varphi)\) intensity surface plot when performing polarimetry for a sample possessing hexagonal symmetry such as \(\beta\)-carotene aggregates in orange carrot root cells [128].
2.3.5 Discussion

The derivation of three-photon polarimetry, just like two-photon polarimetry, follows the general formalism. The dimensions of coherency matrix, the polarizations state vector and the material matrix are larger than the two-photon polarimetry. The $4 \times 4$ matrices expand the coherency matrix as well as the corresponding material matrix. The material matrix $\mathcal{M}^{(3)}$ and polarization state vector $S$ for three-photon polarimetry are $4 \times 16$ and $16 \times 1$, respectively. Similar to two-photon polarimetry, the susceptibility product matrix components and the material matrix components can be sorted into four groups with distinct phase relations between the incoming and outgoing retardance effects. Phase-independent ($NP$) elements form in the first two rows and four columns; those dependent on the incoming $I$ index situated in the first two rows and last twelve columns; elements dependent on the outgoing $O$ index located in the last two rows and first four columns; and those elements that depend on incoming as well outgoing $OI$ indices form the remaining elements. In addition, similar to two-photon polarimetry, the last six columns in the first three rows and the first ten elements in the last row vanish when susceptibilities are real (see the symbolic representation of the matrix in Eq. 2.128).

The set of incoming polarization states, composed of sixteen unique states, forms an invertible matrix that can be used in conjunction with the outgoing polarization states to determine uniquely all elements of the material matrix. For the material with real susceptibilities, and when Kleinman symmetry is valid, the matrix is composed of five independent elements, and it reduces to four ratios. Therefore, all 64 elements of matrix $\mathcal{M}^{(3)}$ are not independent. A reduced set of matrix components may also be employed to determine the susceptibility ratios for certain material symmetries.

Interestingly, the matrix for the isotropically symmetric susceptibilities forms matrix of pure numbers, and the THG signal scales only according to the effective susceptibility. Thus, the outgoing radiation has a simple dependence on the $\mathcal{M}^{(3)}$ matrix, and the verification of the material matrix component values in the isotropic media can give an easy verification for the derived equations. The matrix of hexagonally symmetric material depends on two ratios, and some elements in the matrix depend only on a single ratio. Therefore, in designing experiments for a material possessing hexagonal symmetry, a reduced polarimetry may be conducted to obtain the susceptibility component ratios, using only a few polarization states of incoming and outgoing radiation.
Similar to DFG, the CARS electric field has a negative frequency \( \omega_s \), and therefore, the matrix of incoming polarization states can be used with some considerations. The same incoming polarization states set (Eq. 2.134), which was used for the THG case, can be used for CARS. The difference is that the Stokes component \( s_3(\omega_s) = \sin(-2\Omega) \), because in the state function of incoming radiation \( \psi(\omega_p, \omega_p, -\omega_s) \) for CARS, the complex conjugate of electric field (i.e. negative frequency) is used for the \( \omega_s \) beam. Therefore, the \( \omega_s \) beam is negative in the \( \Omega \) coordinate (on the Poincaré sphere): \( \Omega_p = \Omega_{THG} = -\Omega_s \). Assuming this case, then the same matrix composed of sixteen polarization states (Eq. 2.136), which was used to determine the matrix for THG, can be used for CARS as well, where the prefactor should be scaled as \( \langle E_0(\omega_p)^4 E_0(\omega_s)^2 \rangle \) (rather than \( \langle E_0^6 \rangle \)).

2.4 Conclusion

The general formalism for nonlinear polarimetry is derived. The derivation stems from the basic nonlinear relationship between the polarization density and the resultant outgoing electric field from an intervening material due to the incoming radiation. In nonlinear polarimetry all three components of the expression including the incoming radiation, the material under study, as well as the outgoing radiation, are characterized by real-valued parameters. The state of the incoming radiations is characterized by \((n+1)^2 \times 1\) vector; the sample is represented by a \(4 \times (n+1)^2\) matrix; and the outgoing radiation is simply determined by a conventional \(4 \times 1\) Stokes vector. States are described in terms of electric fields, and the conventional Stokes vectors. The nonlinear matrix \( \mathcal{M}^{(n)} \) is derived in terms of nonlinear susceptibilities. The theoretical framework is comprehensive in that it encapsulates all aspects of the polarization state for outgoing radiation for a given material and incoming radiation. Previous successful nonlinear polarimetric studies such as polarization-in polarization-out (PIPO) equations are shown to be a particular case of nonlinear polarimetry, where linear polarizations are employed in non-birefringent and non-absorbing materials. The theory describes the polarimetry of important two-photon effects such as SHG, SFG and DFG, as well as three-photon effects including THG and CARS. For each case polarization states of incoming radiations as well as the nonlinear optical properties of the intervening material are described in terms of measurable polarimetric quantities.
The coherency matrix is constructed from a vector, which is composed of the electric fields of the incoming radiation. The expansion of the coherency matrix is facilitated by a set of matrices with unique properties forming the basis for the development of the polarization state vector as well as the susceptibility matrix. Elsewhere the $\eta$ matrices are shown to be the generalized matrices for group SU$(n+1)$, where an $(n+1)$-dimensional quantum system is described by $(n+1) \times (n+1)$ density matrix [136]. Therefore, these matrices may be used for quantum-mechanical derivation of nonlinear polarimetry.

The material matrices for SFG, DFG and SHG assume similar form. At the same time, the matrix for three-photon-polarimetry shares the same form for THG and CARS processes. It is conceivable that a similar approach can be taken to express the state for various other frequency mixing techniques including two-photon absorption, coherent Stokes Raman scattering (CSRS), stimulated Raman scattering (SRS). For each of these techniques the polarization states needs to be expressed in terms of the electric fields that nonlinearly interact and result in the nonlinear polarization density. For higher-order techniques, such as fourth- and fifth-harmonic, corresponding higher dimension $\eta$ matrices may be used.

In the next chapter the focus will shift to practical implementations of the nonlinear polarimetry measurements and experimental verifications of the nonlinear optical polarimetry.
Chapter 3

Nonlinear Polarimetric Microscopy

In this chapter the details of nonlinear optical polarimetric microscopy will be presented.\(^1\) Polarization measurements with a nonlinear microscope can be performed by sequentially imaging the sample at different polarizations, or by fast pulse-by-pulse polarization modulation. Various imaging modalities including multi-photon excitation fluorescence (MPF) \([62, 137, 138]\), second harmonic generation (SHG) \([59, 63, 139]\), and third harmonic generation (THG) \([61]\) have been used in nonlinear microscopy. The differential polarization technique has also been employed for circular dichroism, anisotropy of fluorescence, and the degree of polarization \([68–70]\). A novel nonlinear microscope system based on imaging with alternating polarization multiplexed laser beams will be demonstrated in Section 3.3. Images acquired during the same scan with the multiplexed beams can be recorded simultaneously, by detecting SHG as well as THG. Therefore, four different images can be recorded with one raster scan.

In this chapter, first details of implementation of the nonlinear optical polarimetry on a custom-built microscope is shown. Two operation modes of nonlinear polarimetry is described. Single beam polarimetric microscopy is discussed briefly, followed by details of a setup implementing parallel polarimetry. A custom-built nonlinear microscope system based on imaging with alternating polarization multiplexed laser beams is subsequently demonstrated. By combining the beams delayed by a half pulse-repetition period the

\(^{1}\)The following chapter was in part adapted from previously published work. Reprinted (adapted) with permission from Samim, M., D. Sandkuijl, I. Tretyakov, R. Cisek and V. Barzda (2013). “Differential polarization nonlinear optical microscopy with adaptive optics controlled multiplexed beams.” *International Journal of Molecular Sciences* 14(9): 18520-18534.
pulses are time-multiplexed, and the generated photons are demultiplexed by a field-programmable gate array (FPGA) chip. Forward SHG and THG images acquired during the same scan with the multiplexed beams are recorded. Multi-beam scanning is very beneficial for imaging biological samples that undergo rapid structural changes.

Afterwards, two unique modules, FPGA data acquisition and deformable membrane mirrors, both critical for implementation of differential polarization microscopy, are discussed. Data acquisition is entirely performed by a custom-programmed FPGA chip. Details of FPGA block diagram will be revealed in Section 3.3.3. As well, the beam overlap optimization using deformable membrane mirrors is demonstrated for a multi-beam laser-scanning microscope. A genetic-algorithm optimization routine was employed to optimize and overlap the beams. The multi-parameter fitness function of the algorithm depended on the distance between the focal volumes and the intensity of the nonlinear signal. The emphasis on the focal volume separation distance or signal intensity was controlled by a weighting-factor in the fitness function. Simultaneous imaging of ZnSe nanowires with two perpendicularly polarized beams was used for the beam overlap optimization. The algorithm required \( \sim 10^3 \) mirror shapes for the two beams to overlap with the separation distance reaching below the diffraction limit (\(< 30 \text{ nm})\). The differential polarization microscopy technique is scalable to many multiplexed beams with the possibility to obtain the molecular nonlinear polarimetric parameters in a single scan.

### 3.1 Multibeam Laser for Nonlinear Polarimetry

A femtosecond oscillator laser system is used for the nonlinear polarimetric microscopy. For all experiments in this thesis, a Yb:KGd(WO$_4$)$_2$ laser oscillator, which produces three synchronized pulsed beams, is used. The laser produced 430 fs pulses at 1028 nm wavelength with a repetition rate of 14.3 MHz [140,141]. Beams originated from opposite ends of the laser cavity. Two beams exited from one end of the cavity, while the third one was emitted from the opposite end. In addition, two of the three beams are orthogonally polarized with respect to the third one. The \( \sim 70 \text{ ns} \) inter-pulse time duration is sufficient for multiplexing of the beams, where alternating pulses from the two beams are placed half a period apart. The laser interpulse spacing also provide ample time for relaxations of excited molecules. Details of multiplexing is given in Section 3.3.
3.2 Single-beam Polarimetric Microscopy

In a single-beam setup, the incoming beam polarization state is sequentially prepared, and the outgoing radiation polarization state is serially measured. An example of this approach on a home-built microscope is shown in Fig. 3.1. For single-beam polarimetry experiments the polarization of the incoming radiation was prepared by passing the laser beam through a linear polarizer followed by a quarter-wave plate (QWP) and a half-wave plate (HWP) mounted on a rotation stage before the excitation objective. Light then passed through a 0.75 NA excitation objective (Zeiss, Germany) and focused onto the sample. The generated signal in the forward direction was collected by a custom-built UV-transmitting objective. The outgoing light was analyzed by a polarization state analyzer (PSA) also mounted on rotation stages. The outgoing signal was filtered from the fundamental wavelength by a BG39 filter (CVI Melles Griot) and an appropriate band-pass interference filter (SHG: F10 – 514.5 nm, CVI Melles Griot. THG: F10 – 340 nm, CVI Melles Griot.), and subsequently detected by photo-multiplier tubes (PMT).

Figure 3.1: Schematics of the of a sequential polarimetric microscope. The pulse trains of a beam is directed via scanning mirrors to the excitation objective, and the SHG is collected in forward direction by a custom-built objective and detected with a photomultiplier tube detector (PMT). The incoming beam polarization is prepared by a half-waveplate (HWP) and quarter-waveplate on motorized rotators, and the outgoing beam polarization is determined by a QWP and an analyzer. The components are not drawn to scale.
One advantage of the single-beam polarimetry is that it is quicker to build and requires fewer optoelectronic components compared to the multiplexed beam setup. When duration of the polarimetry measurements is not of immediate concern, polarization-in polarization-out (PIPO) experiments are typically performed on this setup.

3.3 Multiplexed-beam Polarimetric Microscopy

Fast biological processes such as a muscle contraction, an active transport of a cargo by nanomotors, and neuronal activities require high-speed imaging to capture events with at least a video-rate scanning (> 25 frames/sec) and at a microscopic resolution. The differential polarization nonlinear optical microscopy is an indispensable tool for structural investigations of the dynamic biological processes and provides time-resolved information about the structural changes. Moreover, any polarimetric technique involving large-area measurements such as whole-slide scanning requires quick and efficient measurement solutions to accomplish the complete specimen imaging in a reasonable time. The parallelized differential microscopy is a useful framework in order to increase the number of measured incoming and outgoing polarization states per unit time and to speed up the polarimetric data acquisition. One of the earliest multi-beam microscopes was realized with the Nipkow disk scanning [66]. The latest multi-beam scanning method uses time multiplexing of pulsed laser beams, where pulse trains are shifted with respect to each other by a fraction of the pulse repetition period [74]. Demultiplexing of the detected photon-counts is achieved with fast electronics by assigning the photons originating from different beams to separate arrays resulting in distinct images. The multiplexing technique can also be used to image samples with beams of different optical properties such as orthogonal polarization [142] or different wavelengths [77, 143]. Differential polarization microscopy sidesteps common issues such as sample movement during a sequential polarimetry measurement. Due to tens of megahertz alternating polarization of excitation pulses, fast data acquisition can easily measure changes in the nonlinear signal anisotropy in an in vivo experiment of dynamically changing structures. Several other applications of differential polarization microscopy, including imaging of cellulose fibers as well as fluorescence-detected nonlinear absorption anisotropy measurements of Congo Red-labeled cellulose are also demonstrated recently [80].
In this section, a multi-beam differential polarization microscopy setup, which incorporates the deformable membrane mirrors, is detailed. The operating principles of differential microscopy, particularly aspects that are useful for nonlinear polarimetry, are described. The optical design including the laser, DMMs, scanning mirrors, and detection is also revealed here. The detection scheme including the FPGA logic is illustrated and described under the data acquisition Section 3.3.3. Focal volumes from different beams in the multi-beam microscope can either have a precise spatial separation [83, 143], or must overlap in three dimensions (3D). A solution to overlapping multiplexed beams using deformable membrane mirrors (DMM) is presented the Section 3.4.

3.3.1 Principles of Differential Microscopy

A differential polarization microscopy setup can be divided into three functional parts: the laser-beam multiplexing unit, the microscope setup, and the signal-demultiplexing unit (Fig 3.4). While the microscope setup is very similar to other multi-photon laser scanning microscopes, the laser multiplexing and the signal demultiplexing units are novel techniques. The schematics in Fig. 3.2 show two synchronized pulsed laser beams that are combined so that their pulses are staggered in time. The sample is subjected to the combined excitation beam. The signal generated from the sample is detected by a photon-counting PMT (Fig 3.3). A photodiode is used to provide a synchronization signal according to the incoming beam for the FPGA to sort the signal from PMTs into separate channels. The signal from the sample can be generated with linear or nonlinear excitation, as long as the excitation is performed by a pulsed laser source.

The excitation beams can differ in many of their characteristics: for instance, they can have different polarizations, or different wavelengths. More than two beams can be combined as well, as long as the inter-pulse spacing time is longer than the time window that contains the signal from one of the beams. This limitation is mostly important in the case of fluorescence imaging where the signal is detected in a time window of many tens of nanoseconds.

The laser pulses can originate from the same laser source, or from several synchronized lasers. The most straightforward implementation uses a single laser, a beam splitter and a delay line to generate multiple synchronized excitation beams. This becomes cumbersome
when the repetition rate of the laser source is less than a few tens of megahertz. Multiple output couplers can be used in a long-cavity laser to achieve convenient pulse train delay and staggering of the excitation beams [144]. Previous implementations have shown up to six staggered output beams from a single laser cavity [75]. Combining two laser beams in a differential polarization setup is achieved with a polarizing cube beam splitter, which also ensures that the excitation polarizations are perpendicular to each other.

![Schematics of the differential polarimetry](image)

**Figure 3.2:** Schematics of the differential polarimetry. Two excitation beams are multiplexed, and a synchronization signal is derived from one of the beams. The sample is subjected to the multiplexed excitation, and the signal from the PMT is separated by a router containing fast electronics.

Previously, signal demultiplexing was performed using logic gates, while commonly used counting cards performed the routing on-board [142]. In this work, an FPGA-enabled board was used for demultiplexing, signal counting, and routing from PMTs (Fig. 3.3 and Section 3.3.3).

### 3.3.2 Optical Design

The design of the custom-built multi-beam nonlinear optical microscope is presented in Fig. 3.3. Two orthogonally polarized beams from the home-built laser were coupled into the setup (see description of the laser in Section 3.1). The two excitation beams were combined collinearly with interlaced pulses using a polarizing cube beam splitter. Hence, the multiplexed beam had alternating pulses with perpendicular linear polarizations and a temporal spacing of about 35 ns between them. Another polarizing cube split the multiplexed beam and directed the polarization components towards two 39-actuator de-
formable membrane mirrors (DMMs, Flexible Optical BV, Delft, The Netherlands). The electrostatically-driven actuators of DMMs controlled the wavefront of each beam independently with up to 1 kHz operating frequency. The high operating frequency is particularly important for fast steering of the beams and for rapid point-spread-function (PSF) optimization in the microscope. The nonlinear optical microscope was also equipped with a Shack-Hartmann wavefront sensor for dynamic wavefront sensing. The beams were recombined and directed to the resonant mirror operating at 15.5 kHz (EOPC, Fresh Meadows, NY, USA) and the galvanometric mirror operating at 60-400 Hz (Cambridge Technology, Bedford, MA) scanning in lateral X and Y directions, respectively. The multiplexed scanning beam was then relayed to the entrance of an air objective (0.75 NA, 20×, Zeiss, Jena, Germany). Combined, the scanning and deformable mirrors provided fast imaging in three dimensions 3D. Second- and third-harmonic generation signals generated at the focus of the excitation objective in the sample were collected in the forward direction by a custom-built UV-transmitting objective. The SHG and THG signals were separated by a dichroic mirror and detected with PMTs (Hamamatsu Model: H7421-40 (SHG), and PerkinElmer Model: MP1343 RS CPM (THG)).

The outgoing radiation can be analyzed by a polarization state analyzer (PSA). In the simplest case the PSA can be a linear polarizer; however, a complete PSA involves a quarter- and half-waveplate as well. Parallelizing the detection can be done by implementing a four-detector polarimeter [125,145].

### 3.3.3 Data Acquisition for Fast Nonlinear Polarimetry: FPGA

The main “brain” of the differential fast microscope is a custom-coded field-programmable gate array (FPGA) chip, depicted in Fig. 3.4. Data acquisition, signal demultiplexing, and processing are performed by a X5-210M FPGA board (Innovative Integration, Semi Valley, CA, USA). Analog signals from galvanometric, resonant and deformable mirrors, each corresponding respectively to a spatial coordinate X, Y, and Z of the focal volume in the sample, are captured and digitized continuously. The continuous stream of input voltages are saved, transferred, and converted to spatial coordinates by the card, only after a trigger is detected from one of the detectors (TTL signals from PMTs). The system initiates other triggers as well for saving the coordinates, for example, to mark special events including end-of-a-line-scan maker. Upon the detection of the trigger signal the
Figure 3.3: Schematics of the multi-modal differential nonlinear microscope. Two Yb:KGd(WO$_4$)$_2$ laser beams are coupled into the microscope for multiplexed imaging, and deformable membrane mirrors are used to shape the wavefronts of each beam. The multiplexed beam is reflected from scanning mirrors and relayed to the excitation objective. SHG and THG signals are collected in the forward direction via custom-built UV-transmitting objective, and the fluorescence signal can be detected in the epi-direction using PMT detectors. The scanning mirrors enable lateral scanning. The wavefronts are arbitrarily shaped by deformable membrane mirrors. The converging/diverging beams are coupled into the microscope objective and can be focused at the different depths using DMMs. A/4 - quarter-waveplate, BS - beam splitter, DC - dichroics, DMM - deformable membrane mirror, GSM - galvo scanning mirror, ISO - optical isolator, L - lens, LED - light emitting diode, LP - linear polarizer, M - mirror, MPF - multi-photon fluorescence filter, PMT - photomultiplier tube, RSM - resonant scanning mirror, SHG - second harmonic generation filter, THG - third harmonic generation filter. SH - Shack-Hartmann wavefront sensor. The components are not drawn to scale.
synchronous values of the mirror positions are retained in the memory, buffered, and then sent as packages through the PCIe interface to the operating system (Microsoft Windows) environment. For an oscillating variable a null array is initialized (corresponding to spatial coordinates), and each element in the array is incremented synchronously and tagged according to the state (true or false) of the photo-diode indicating the source (beam 1 or 2, respectively) as well as detectors (e.g. SHG or THG). All FPGA programming as well as data analyses are performed with custom-written programs using Xilinx libraries in MATLAB Simulink environment (The MathWorks, Natick, MA, USA) and LabVIEW (National Instruments, Austin, TX, USA). The front end control is programmed in C++.

At the host end, three-dimensional histograms corresponding to the spatial coordinates are initialized for each beam-detector pair. Each package is then sorted according to XYZ positions: for example, the datum $X_1, Y_1, Z_1$, tagged by beam 1 and SHG detector, added a value of +1 to the histogram at position $X_1, Y_1, Z_1$, and is assigned to the corresponding beam-detector pair. Subsequently, histograms are interpreted as the intensity images and used for display and analyses. The maximum scanning range is about $270 \times 180 \times 40 \mu m^3$ with a 0.75 NA 20× air objective.
Chapter 3. Nonlinear Polarimetric Microscopy

3.4 Adaptive Optics for Nonlinear Optical Microscopy

Adaptive optics (AO) techniques help to correct optical aberrations in a microscope as well as to overlap beams, which is usually required for precise nonlinear optical polarimetry using a multi-beam laser system. AO compensate for optical aberrations due to a microscope optical components, and thereby, improve its optical resolution [78, 146, 147]. The spatial resolution of a microscope is also improved by optimizing the point-spread-function (PSF) of the focal volume. The PSF improvement is especially effective for multi-photon processes, as the signal depends nonlinearly on the incident photon density, and increases with tighter focusing. Deformable membrane mirrors (DMMs) have been successfully employed to optimize the PSF for SHG and THG in microscopes with single beam excitation [78, 148]. The signal optimization technique commonly exploits evolutionary genetic algorithms (GA) that link the generated nonlinear signal strength to the shape of the DMM in an iterative process [78, 148, 149].

In addition to an optimized PSF, precise beam overlapping is also required for differential polarimetry. Overlapping two beams in 3D with sub-micrometer precision presents a challenging task, and an automated alignment aid is highly desired. For non-multiplexed multi-beam systems such as coherent anti-Stokes Raman scattering (CARS) and stimulated emission depletion (STED) microscopy, the overlapped beams interact [58, 147, 150], and therefore, the extent of overlapping can be optimized by monitoring the response signal. In contrast, for the multiplexed beams the pulses remain temporally separated. Thus, overlapping the multiplexed beams presents a dissimilar challenge. Deformable membrane mirrors (DMM) can be used to simultaneously optimize and overlap beams using genetic algorithms. The beam overlap with DMMs can be achieved with better accuracy than diffraction-limited PSF (see Section 3.4.4). As a result, pixel-by-pixel polarization-resolved measurements are enabled by such overlap precisions, which is crucial for fast polarization microscopy.

In this section, in the context of nonlinear polarimetry, applications of DMMs for multi-beam nonlinear microscopy is shown. Using AO and GA high-intensity harmonics signals were generated (∼10 million counts/sec from air-glass interface with ∼45 mW input power); PSFs were minimized (a minimum of 2 µm axial PSF for THG); and beams were steered to very precise positions (< 30 nm overlapping accuracy). All of these optimizations in a differential multi-beam microscopy setup ensured that the same voxel volume
is excited in the sample by two beams such that the corresponding nonlinear signal can be analyzed appropriately for a polarimetry study.

### 3.4.1 Point Spread Function Optimization

Point spread function is a measure of optical resolution of a microscope and a critical parameter for polarization microscopy. Typically, for a nonlinear optical microscopy third harmonic generation is measured across an air-glass interface to obtain the PSF of the microscope. THG is sensitive to differences in the refractive indexes of the materials at an interface [56, 60, 61], and therefore, any interface separating two media can be used to obtain THG. An air-glass interface of the microscope coverslip was used to generate THG signal. For a given focal-spot position, the glass coverslip was translated through the focal volume with the piezo-driven translation stage in the axial direction (Z-axis). Emitted THG photons were counted by a PMT and recorded on a computer as a function of axial position of the interface. The acquired curve represented the THG PSF of the focal-volume for the microscope.

Using Eq. 1.55 the THG intensity at an interface can be approximated to:

\[
I(b) \propto \left(\frac{1}{\iota}\right)^2 \left| \int_{-\infty}^{z_0} e^{i\Delta k z'} \frac{e^{i\Delta k z'}}{(1 + 2i z'/\iota)^{2}} dz' \right|^2
\]  

(3.1)

where \( z' \) is the axial coordinate, \( z_0 \) is the position of the interface, \( \iota \) is the confocal parameter to be optimized and \( \Delta k \) is the wavevector mismatch due to dispersion. The full-width-at-half-maximum (FWHM) of the THG intensity profile as a function of the axial coordinate of the interface \( z_0 \) is taken as the axial THG PSF [151], and is directly related to the confocal parameter \( \iota \) of the excitation beam. Since Eq. 3.1 is based on a simple Gaussian distribution for excitation as well as nonlinear signals, it is only an approximation when using an excitation objective with a high numerical aperture and arbitrary wavefront beam.

The PSF for a beam can be easily optimized using THG signal intensity. In addition, optimizing the THG intensity results in a minimized PSF. Initially, a standard optimization routine was employed where the fitness function \( F \) depended only on the intensity of THG from an air-glass interface. The genetic algorithm (see Section 3.4.2) searched
for the mirror shapes that provided the highest possible intensity of the THG signal. Once the algorithm converged, PSF had to be measured for each optimized intensity. The intensity profile across the interface was fit with a Gaussian curve, and the confocal parameter \( \iota \) was determined (see Eq. 3.1). The relationship between the total intensity generated from the interface and the corresponding PSF was shown to be consistent with the reciprocal relation described by Eq. 3.1, as shown in Fig. 3.5. A minimum PSF \( = 2.01 \pm 0.2 \, \mu m \), which is close to the theoretical diffraction limit \( 1.8 \, \mu m \) [151],\(^2\) was achieved in the nonlinear microscope (0.75-NA air objective; \( \lambda = 1028 \, nm \)). Thus, the search for the maximum THG intensity also achieved the minimum axial PSF. Since the axial and the lateral PSF are related (Eq. 3.1) [151], it can be deduced that a minimized 3D PSF was also achieved at the highest THG intensity.

![Figure 3.5](image)

**Figure 3.5:** THG signal intensity dependence on the axial point-spread-function. THG signal is generated across a glass-air interface of a microscope coverslip (open circles) and the FWHM of the axial PSF follows the theoretical curve (Eq. 3.1, solid line; \( R^2 = 0.82 \)). For the excitation objective (0.75 NA, \( \lambda = 1028 \, nm \)), the theoretical minimum limit of the axial THG PSF is \( 1.8 \, \mu m \) (dashed line).

### 3.4.2 Genetic Algorithm

Genetic algorithm is an evolutionary process whereby individuals’ genes are mutated (and/or recombined and crossed over), and their relative fitness are computed and ranked. The gene combinations with the highest fitness are then selected as a basis for the next generation. Previously, GAs were developed for improving imaging conditions in a single-

\(^2\)Note the FWHM defined in the reference is in fact half-width at half maximum.
beam microscope [152]. Usually, the nonlinear signal intensity from the sample is chosen as the fitness function or the parameter to be optimized during the evolution [152,153].

For the differential polarization microscope, at the beginning of optimization process, twenty unique and random shapes of DMM (i.e. individuals) were formed and ranked according to their relative fitness value based on the nonlinear signal intensity that they generate. In the next iteration, top ten mirror shapes were crossed over among themselves to produce twenty progenies for the next generation. In other words, crossing over was performed by randomly selecting two mirror shapes from the surviving individuals, and their voltage arrays of the actuators were split the same way for each mirror into two randomly chosen lengths. The complementary subsets were then swapped between the two mirror shapes to produce two new progenies. The resultant ten progenies and their parents were subjected to a constant 1.5 % mutation rate. Mutation was introduced by randomly selecting one of the actuators voltage array and replacing its current voltage value with a random value. The use of GA with a population size of twenty individuals per generation and the mutation rate of 1.5% yielded a reasonable convergence time (5-8 minutes or ~1600-2400 mirrors shapes).

3.4.3 Aberration Correction and PSF optimization

In order to improve the PSF for better imaging conditions, the following iterative procedure was carried out. The shape of the mirror was changed by applying voltages to the actuators. Any wavefront requested from the deformable mirror was represented by the sum of appropriate Zernike modes and their respective amplitudes (details of Zernike modes in Appendix C). The wavefront was actively monitored by the Shack-Hartmann wavefront sensor. If the observed wavefront deviated significantly from the requested wavefront, a closed-loop feedback was activated to minimize the root-mean-square error between the two wavefronts. The shaped laser beam was directed to the objective and focused at glass air interface to generate the THG signal. The change in the signal intensity determined the fitness function. Up to and including the fifth order Zernike modes were considered for the THG signal maximization.

Fig. 3.6 shows the optimized PSF for the entire range of the DMM. There is a one-to-one correspondence between the position of the focal spot placed by the DMM and
Algorithm for Nonlinear Signal Optimization using Adaptive Optics Deformable Mirror: An Iterative Solution

1. Manipulate the optical wavefront by increasing or decreasing the amplitude of the Zernike modes on a 39 channel deformable mirror.
2. Monitor the wavefront by a Shack-Hartmann wavefront sensor.
3. Direct the resultant beam to the microscope.
4. Detect THG signal by a PMT.
5. Has the signal improved by desired range?
6. Change the amplitudes of Zernike polynomials according to THG intensity increase. If the convergence condition is met then break the algorithm. Else repeat steps 1-3.

the position of glass-air interface determined by a calibrated piezoelectric stage. These results were obtained by using a closed-loop feedback between THG intensity and DMM shapes. Additionally, when THG signal increased, narrower PSF was achieved. The results demonstrate that polarimetry and imaging of the samples can be carried out very precisely with optimal PSF and high signal intensity by using DMMs. The axial depth range of the focal volume positions at which PSF can be optimized was about 30 µm.

Figure 3.6: Axial scanning and point-spread-function optimization by deformable membrane mirrors. The improvement of the focusing conditions and THG intensity was achieved using deformable mirrors and a closed-loop feedback mechanism. Using convergence of the incoming beam, the beam was focused at different depth and PSF as well as the THG intensity was optimized. The axial depth was determined by a calibrated piezoelectric stage and a position sensor.
3.4.4 Beam Overlapping for Nonlinear Polarization Microscopy

Precise beam positioning, in addition to optimizing the intensity and PSF, is important for multi-beam differential microscopy. Key components of overlapping procedure including the samples used, the genetic algorithm for beam overlapping, and parameters crucial for achieving the precise beam overlapping for the nonlinear polarimetry measurements are discussed below.

Image overlapping and signal optimization studies were performed using ZnSe nanowires (NWs). The ZnSe NW sample was ideal for the study because of high SHG intensity and its polarization-dependent response, convenient size with diameter ranging 60-100 nm and length ranging from a few to tens of micrometers. ZnSe NWs were grown on a silicon substrate by the vapor-liquid-solid mechanism. NWs were dispersed on a microscope coverslip by depositing a drop of NWs suspended in methanol and allowing methanol to evaporate. The sample was then covered with another coverslip [154, 155]. The NWs were imaged with SHG microscopy continuously during the beam positioning procedure, and 512 × 512-pixel images were recorded at 120 frames/sec scanning rate. Twenty consecutively-imaged frames were averaged to improve the signal-to-noise ratio. The averaging did not affect the positioning kinetics, which were much slower than the frame scanning rate. A target position was arbitrarily chosen in the imaged field of view. The same initial position and the target position were kept for the positioning experiments with different weighting-factor values shown in Fig 3.8. During the positioning procedure, the distance $D$ between the target point and the centroid of the SHG intensity of the NW image was calculated for each mirror shape in every generation. Additionally, the total signal intensity from the whole NW image was obtained by summing signal intensities of all pixels in the image for each DMM shape. Each positioning experiment started from the optimized PSF at the initial location of the beam.

Genetic algorithm for Beam Overlap

In addition to optimizing the PSF, it is also possible to steer a beam using a deformable mirror. Complete beam overlap allows for pixel-by-pixel comparison of the experimental data and ensures similar excitation of a voxel by each beam. Both goals - PSF optimiza-

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3 The sample was kindly provided by Professor Ruda group, University of Toronto.
tion and precise overlapping - can be achieved simultaneously using a genetic algorithm (GA) optimization routine.

The fitness function proportional to the nonlinear signal intensity, described in Section 3.4.2 works well for optimizing a single-beam microscope setup. For the polarimetric multi-beam microscope, in order to steer a beam towards a target point, or to overlap the beams, the fitness function needed to be redefined by including the separation distance. The separation distance was defined by the norm of a vector (in 2D for an image and 3D for a volume) connecting two positions. The position was either predetermined, or it was calculated using the intensity-weighted average position (centroid) of the image produced by a reference beam. In order to overlap the beams for the two-beam polarimetric microscope, the separation distance $D$ was minimized. The fitness function $F_{\text{2beams}}$ was defined as:

$$F_{\text{2beams}} = w f_D + (1 - w) f_I$$

(3.2)

where $f_I$, and $f_D$ is the normalized intensity and distance fitness parameters:

$$f_D = \frac{D_{\text{max}} - D}{D_{\text{max}} - D_{\text{min}}} \quad \text{and} \quad f_I = \frac{I}{I_{\text{max}}}$$

(3.3)

$D_{\text{max}}$ and $D_{\text{min}}$ are the maximum and minimum distances obtained within each generation. $f_I$ is the normalized intensity contribution to the overall fitness function, and it depends on the intensity $I$ generated from the sample by the steered beam. $I_{\text{max}}$ is the maximum intensity obtained for the generation in question. The weighting-factor $w$ ranges from 0 to 1 and determines the contribution to the fitness function of the distance parameter relative to the intensity parameter. Practically, the value of $w$ expresses the relative preference of the GA for maximizing the intensity vs. minimizing the distance during the optimization procedure.

The distance parameter $D$ has an uncertainty due to the error $\Delta D$ in localization of the centroid of the object in the field of view. The localization error in distance between the centroid and the target position can be approximated using the equation [156]:

$$\langle (\Delta D)^2 \rangle = \frac{\kappa^2 N}{\kappa_x^2 + \frac{8\pi\kappa^2 B^2}{N\xi^2}}$$

(3.4)

where $\kappa_x^2 = \kappa^2 + \xi^2/12$, and $\kappa$ is the standard deviation of the lateral PSF (or 0.37 of the axial PSF for the SHG signal at 0.75 NA objective), $\xi$ is the pixel size, $N$ is the
total number of photons from the sample, and $B$ is the background noise. Thus, the centroid location and the distance $D$ can be determined and optimized more accurately using smaller pixel sizes. Similarly, a high intensity and small background noise (i.e. large signal-to-noise ratio (SNR)) also reduces the error in determining the separation distance. Note that an optimal SNR ($> 5$) can be achieved by averaging over multiple image frames, which determines more precisely the fitness value, but it also increases the convergence time. Nonetheless, Eq. 3.4 shows that beams can be overlapped with better precision than diffraction-limited resolution. Therefore, depending on the application, the target value of the convergence criteria, $D$, for the separation distance can be set below the diffraction-limited resolution at the Nyquist sampling theorem limit, or the target can be set to a value determined by the Eq. 3.4 for the super-resolution experiments.

**Beam Steering and Overlapping: Parameter Space**

Two criteria are important for positioning the beams in nonlinear optical microscope: (i) the speed of positioning, and (ii) the nonlinear signal intensity at the final position. The distance and the SHG intensity evolution during the GA iterations are presented for three weighting-factor values between 0.30 and 0.70 in Fig. 3.8. Outside of this range either the intensity or the distance did not always converge. Fig. 3.8c summarizes how the positioning convergence time and the final SHG intensity at the target position depend on the weighting-factor value. Given the distance convergence time and the relative intensity recovered, the optimum $w$ factor is $\sim 0.5$.

Fig. 3.8d shows that the convergence time is linearly dependent on the initial separation distance $D$. As deduced from the linear fit of the graph (Fig. 3.8d), the speed with
Figure 3.8: Beam positioning with deformable mirrors in the nonlinear microscope. Time trajectories of a separation distance (a) and SHG intensity (b) of the steering beam at different weighting-factor values. (c) recovered intensity (red squares, right vertical axis) and convergence time (blue diamonds, left vertical axis) for a range of weighting-factor values. The initial separation distance was 6.3 ± 0.8 pixels (1.9 ± 0.2 µm); (d) scatter plot of the distance convergence time vs. the separation distance for the weighting-factor value \( w = 0.55 \). The speed of convergence 0.09 ± 0.01 µm/sec (0.12 ± 0.01 pixel/gen) is deduced from the linear fit (solid line, \( R^2 = 0.9485 \)).

which the convergence occurs was 0.09 ± 0.01 µm/sec (0.12 ± 0.01 pixel/gen). Therefore, with optimum weighting-factor value a separation distance of 2 µm was minimized in 220 seconds by the GA-assisted overlapping procedure. Fig. 3.8a,b show that when the beam reached its approximate target position the SHG intensity also reached to its minimum value. Afterwards, the intensity recovered; however, the intensity recovery was slow. The intensity could be increased faster while keeping a minimum separation distance by changing the value of \( w \) during the optimization procedure. Fig. 3.9 shows a time-dependent evolution of separation distance and the SHG intensity of the image during a typical beam overlapping procedure with varying weighting-factor values.

The maximum distance that can be minimized by the positioning process is limited by the stroke range of the DMM electrostatic actuators. If at the beginning of the overlapping process the distance between focal volumes is too large, then the extreme limits of the actuators are reached during the optimization procedure. In the differential polarization microscope, up to 4 µm separation distance between the centroids of the images of the two beams was successfully minimized with one DMM (NA = 0.75 air objective). Employing the second DMM doubled the initial separation distance that could be compensated.

High overlapping precision (< 30 nm) becomes effortlessly accessible by using DMMs. In addition, DMMs driven by the GA correct aberrations introduced by the overlapping
The overlapping procedure above is a necessary step for differential microscopy, where it must be ensured that the images from the multiplexed beam can be contrasted on a pixel-by-pixel basis. Such an overlap provides confidence in ratiometric studies of intensities from different polarizations as demonstrated in Section 4.2.4. The high-accuracy overlapping procedure may also help in using the multiplexed beam differential polarization microscopy for super-resolution imaging applications, such as stimulated emission depletion (STED) microscopy [157].

3.5 Conclusions and Outlook

Nonlinear optical polarimetry is an indispensable technique for biomedical applications particularly for microscopy of biological tissue. However, the implementation of nonlinear polarimetry also require advanced and unique approaches in order to fully capture the polarization response from the specimen, including precise optimization of the incoming radiations. For a selected number of studies, sequential polarimetric microscopy is relatively easy to implement. The main reason is that sequential polarimetry can be performed with a single beam, and a few optoelectronic components are required to con-
struct a polarization state generator (PSG) and polarization state analyzer (PSA). Static structural studies using SHG and THG can be conducted with sequential polarimetry technique. However, dynamical studies such as a muscle contraction, as well as important two-beam techniques such as SFG or CARS, require multi-beam setups as well as rapid PSG, PSA and detection schemes. Differential polarimetry is an important technological advance in this regard, which can be successfully employed for fast polarimetry measurements.

Differential polarization nonlinear optical microscopy is an imaging tool capable of measuring anisotropy of nonlinear signals with photon counting sensitivity. The technique helps with common issues associated with slow imaging such as sample movement, and allows for scanning of biological samples with multiple nonlinear image contrast mechanisms simultaneously. The technique can be used in many challenging applications, where weak polarization signals have to be measured or polarization anisotropy has to be obtained from a moving sample. Additionally, the novel microscope is equipped with deformable mirrors that enable fast differential polarimetry in 3D [79].

An automated beam positioning procedure in multi-beam multiplexed microscopy was demonstrated using deformable membrane mirrors and the genetic algorithm optimization procedure. The multi-parameter fitness function of the genetic algorithm optimized concurrently the signal intensity and the overlap distance between two parallel images. The weighting-factor in the newly-defined multi-parameter fitness function provides the option to emphasize the beam overlap or the PSF during optimization. Different optimization strategies can be developed by changing the weighting-factor $w$ during the course of the procedure. In the presented example, weighting-factor value of $\sim 0.5$ rendered optimal results for overlap optimization, which also provided almost full recovery of the SHG signal. The genetic optimization algorithm uses normalized images and thus allows overlapping of the beams with different polarizations, wavelengths or other differences in the beam properties that result in slightly dissimilar images from each beam. The process of optimization and overlapping can be extended to setups comprising of more than two beams. The technique gives a quick and convenient way to overlap automatically the multiplexed beams, which is a tedious task especially if the separation distance has to be much smaller than the diffraction limit.

To achieve video-rate scanning, the axial position of the focal volume was translated by
changing the wavefront with the deformable mirrors. The use of deformable mirrors also
corrects for optical aberrations at each focusing depth. With the 39-channel deformable
mirrors the range of axial scanning is about 30 µm. Additionally, the focal volume can
be dynamically altered to increase the THG signal intensity. For various shapes of the
mirror, the intensity of THG increased 2-8 folds, while the width of the PSF narrowed.
These results enable significantly higher contrast in the images and better resolution
during rapid 3D scanning. The combination of fast axial focusing and video-rate lateral
scanning of the laser enables 3D volumetric imaging in the dynamically changing biolog-
ical samples. Deep-tissue imaging of biological samples and visualization of fast moving
cellular organelles become possible. Therefore, the methodology is an important advance
in the field of 3D microscopy as well as in the fast nonlinear polarimetric imaging.

Data acquisition and signal routing were performed with a custom-programmed board
and a FPGA chip. The unique signal routing mechanism, where the position of the
scanning mirrors are tagged for each photon detected from a PMT assigned to particular
contrast mechanism (i.e SHG or THG) as well as to the beam responsible for generating
the signal, ensures efficient use of resources including logic gates, memory and high-speed
transfer-rates of 3D data. In addition, the approach is scalable to multiple beams and
detectors. The signal processing including visualizing images from multiple-depths were
easily achieved on a PC in real-time. The methodology gives real-time feedback to the
user about the status of a differential polarization microscopy experiment.

The newest advancements in the nonlinear polarimetric microscopy have been focused
on the imaging of time varying structural conformations in the biological samples and
increasing the imaging area to the whole microscopy slide. For example, recent efforts in
Professor Barzda laboratory are directed towards the whole-slide polarimetric imaging of
clinical histopathology samples, where approximately an inch-squared microscopy slides
of various types of cancerous tissues are studied at sub-diffraction-limited resolution in
order to characterize protein organization in the extracellular matrix of the tissue. The
fast differential polarization microscopy described in this chapter is also an important
step in developing the high-speed parallelized nonlinear polarimetric microscopy scanner.
Chapter 4

Nonlinear Polarimetric Microscopy of Biological Tissue

In this chapter analyses and polarimetric measurements of biological samples will be presented. Biological samples often contain highly ordered molecular structures and microcrystalline aggregates that play important structural and physiological roles. At the same time, these structures often exhibit signal anisotropy in the linear and nonlinear optical regimes. Examples of these structures are ordered protein assemblies, such as myosin filaments and collagen fibers in striated muscle, and polysaccharide structures in starch granules and cellulose fibers. Therefore, measurement of polarization parameters can elucidate the organization of molecules in the microcrystalline aggregates. In this Chapter, nonlinear optical polarimetric microscopy measurements from biologically important tissue is provided in the interest of verification of the nonlinear Stokes-Mueller polarimetry theory as well as giving further insight into the structure of the biological samples.

Many important biological structures including muscle, collagen and starch provide generation of second harmonic. On the other hand, structures such as retinal in the eye and lipid bilayers of the cells membrane generate third harmonic. Revealing investigations of hierarchical structure of these samples have been conducted using different aspects of nonlinear polarimetry. A unifying account of these investigations using nonlinear Stokes-

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1The following chapter was in part adapted from previously published work. Reprinted (adapted) with permission from Samim, M., N. Prent, D. Dicenzo, B. Stewart and V. Barzda (2014). “Second harmonic generation polarization properties of myofilaments.” Journal of Biomedical Optics 19(5): 056005-056005.
Mueller polarimetry theory will be shown in this chapter. First, the general overview of the acquired data from the nonlinear polarimetric microscopy is discussed. Then, SHG polarimetry of the muscle myosin, rat-tail tendon collagen, and starch are presented. Measurements with linearly polarized incident radiation and linear polarimetry of SHG are presented for these tissues. The polarimetric microscopy is applied for the study of hierarchical structure of myosin motors within sarcomeres (contractility units) of muscle in indirect flight muscles from the fruit fly. The section on SHG polarimetry is concluded with a study of dynamics of muscle contraction. The final section is dedicated to THG polarimetric data from retinal, a light sensing molecule responsible for the vision in fruit fly eyes.

Before delving deeper into the experimental investigations of different tissues, a primer to the nature of data obtained from the nonlinear polarimetric microscopy is discussed, which is insightful for better understanding of data analysis presented in the subsequent sections.

### 4.1 Analyses of Polarimetric Nonlinear Microscopy Images

Nonlinear polarimetric microscopy measurements are performed by obtaining images at various incoming and outgoing polarization states. In general, the relation between incoming polarization state, the sample, and the measured radiation polarization is formalized by the equation $s' = M S$. For a two-photon process the full Stokes-Mueller polarimetry require $4 \times 9$ images at particular incoming and outgoing polarization orientations to obtain all components of Mueller matrix independently. For the second order processes the whole Mueller matrix is recovered by obtaining $4 \times 9$ images. In other words, the outgoing SHG (or THG) Stokes vector is measured for 9 (or 16) incoming polarization states, as was shown in Section 2.2.4, and consequently, the corresponding Mueller matrix is obtained using the relation $M = s' S^{-1}$.

In a low photon-counting regime, which is often the case in nonlinear microscopy, where signal to noise ratio can be low, repeated or extra polarization states are acquired to extract reliable nonlinear susceptibility values. Therefore, the system is overdetermined.
In this case, the solution is obtained by using the pseudo-inverse: \( \mathcal{M} \cong s' S_p^{-1} \), where \( p \) indicates the pseudo-inverse, defined as \( S_p^{-1} = (S^T S)^{-1} S^T \).

Since each pixel in the polarization images is independently measured, the nonlinear matrix \( \mathcal{M} \) can be determined for each pixel separately. The Mueller matrix component values are comprised of susceptibility tensor component values, which are usually used to characterize a sample. Therefore, the full nonlinear Stokes-Mueller polarimetry measurement can be used to characterize fully the measured sample in terms of nonlinear susceptibility tensor. The susceptibility products can be obtained using Eq. 2.23. Alternatively, since the elements of double Mueller matrix (as well as the susceptibility product matrix) are made of only a few independent susceptibility values, a fitting routine can be devised to obtain the best estimate of the susceptibilities.

### Analysis of Linear Polarization Polarimetry

As mentioned before, the linear polarization microscopy can be understood as part of the full-set of nonlinear polarimetry where the first component of the outgoing Stokes vector is the total intensity of the polarization-in polarization-out equation (see Section 2.1.8). However, as the theory of nonlinear polarimetry in Chapter 2 showed there are additional information in the Stokes vector that can be revealed from the remaining three components. For a setup measuring the Stokes vector of the outgoing radiation as a function of a linearly polarized incoming light, and a linear analyzer orientation, the following relation can be stated:

\[
s'(\theta, \varphi) = M(\varphi)MS(\theta)
\]  

(4.1)

where \( S(\theta) \) indicates that the incoming light is linearly polarized along angle \( \theta \), and \( M(\varphi) \) is the linear analyzer oriented at angle \( \varphi \). If there are \( Q \) measurements in total, where \( q = 1 \cdots Q \) is the pair of \( \theta \) and \( \varphi \) angles, then in the elemental form \( s'_t, q = M_{t,q,v} M_{v',N} S_{N,q} \). Therefore, for example, for each element of measured SHG Stokes vector, there is a two-dimensional surface. Let for each element \( t \) of SHG Stokes vector \( A = M_t \); that is, for \( s'_t, A \) represents the row \( t \) from the analyzer matrix \( M \). If the elements of \( A_{q,v} \) and the elements of \( S_{N,q} \) each make a square and invertible matrix, then the double matrix \( \mathcal{M}^{(2)} \) can be uniquely determined: \( \mathcal{M}^{(2)} = A^{-1} s' S^{-1} \). However, since the incoming radiation is linearly polarized, its last three components are zero (see Eq. 2.94). Thus, the matrix
$S$ cannot be invertible. Therefore, only first six columns of $\mathcal{M}^{(2)}$ can be determined. As well, for robust results, the outgoing Stokes vector is measured for a few extra linearly incoming polarizations. Consequently, the system of equations is overdetermined, and the solution is obtained by using pseudo-inverses: $\mathcal{M}^{(2)} = A^{-1}_p s' S^{-1}_p$, where $p$ indicates the pseudo-inverse, defined as $A^{-1}_p = (A^T A)^{-1} A^T$; an identical relation is used for $S^{-1}_p$.

After determining the Mueller matrix components, subsequent steps have to be performed to extract the corresponding susceptibility tensor components that characterize the structure. The susceptibility products can be obtained using Eq. 2.23. Alternatively, since the elements of double Mueller matrix (as well as the susceptibility product matrix) are made of only a few independent susceptibility values, a fitting routine can be devised to obtain the best estimate of the susceptibilities. In such a case, for example the observed susceptibility ratio, determined from different SHG Stokes vector elements can be directly compared to previous studies that use fitting procedures of PIPO surface for second-order susceptibility ratios [87–90].

Fig. 4.1 shows all components of the outgoing $s'(2\omega)$ as a function of linear polarization of the incoming beam vs. the analyzer orientation angle for the SHG radiation. In addition to PIPO surface plot shown in the first row, three more surfaces also provide means by which the data can be analyzed. The first column depict the Stokes vector components for a cylindrically symmetric structure resembling a myosin molecule in the muscle. Similarly, the second column in Fig. 4.1 shows the surface plots for the SHG Stokes vector from collagen-like samples that have real-valued susceptibilities, while in the third column the SHG Stokes parameters are plotted for collagen displaying complex susceptibilities to the signal. The fourth column of Fig. 4.1 shows the Stokes vector from a starch-like sample with complex-value susceptibilities. Noteworthy is the $s'_3(2\omega)$ component where the circular component of SHG is measured. The difference between the linear and elliptical SHG beam can be distinguished based on the features in this surface plot. For linearly polarized outgoing SHG signal, the value of $s'_3(2\omega) = 0$. Therefore, the presence of a feature in $s'_3$ is an exhibit of optical activity in SHG due to the sample, since the incoming beam is linearly polarized.

Note, the $s'_3(2\omega)$ component surface plot changes high- and low-value pattern due to the

\[^2\text{The matrix } \mathcal{M}^{(2)} \text{ as well as the matrix obtained for each of SHG Stokes vector elements can be vectorized, where the } \mathcal{M}^{(2)}_t = W^{-1} s'_t, \text{ and the matrix } W \text{ is constructed from elements of } s' \text{ and } S: W_{q,m} = A_{q,t} S_{N,q} \text{ where } m = (t' - 1)9 + N [104].\]
Chapter 4. **Nonlinear Polarimetric Microscopy of Biological Tissue**

Figure 4.1: Stokes parameters as a function of incoming polarization angle and a linear analyzer angle. First column shows simulations of Stokes parameters as a function of incoming polarization and analyzer angles for SHG from a muscle-myosin-like: $\chi_{ZZZ}/\chi_{ZXX} = 0.5$. Second column shows simulations of Stokes parameters as a function of incoming polarization and analyzer angles for collagen-like sample: $\chi_{ZZZ}/\chi_{ZXX} = 1.3$. Third column shows the same as the second column, but for a collagen-like sample with complex second-order susceptibility components: $\chi_{ZZZ}/\chi_{ZXX} = 1.3 + 0.6i$. The fourth column shows the simulation of SHG Stokes vector as a function of incoming polarization and analyzer angles for a starch-like sample with complex second-order susceptibility components: $\chi_{ZZZ}/\chi_{ZXX} = 4 - 0.6i$. The color in the first row shows the total intensity from the sample (normalized to the maximum intensity), while in the second, third, and fourth row indicates the normalized Stokes vector components value (normalized to the maximum intensity).
sign of the complex component in the susceptibility (compare the $s'_3(2\omega)$ components in Fig. 4.1 and Fig. 4.1). This may indicate a preference for measuring all Stokes components, including $s'_3$; however, some differences are distinguishable among the $s'_0(2\omega)$ plots for the various values of susceptibilities. The optical activity due to an elliptically incoming polarization, complex susceptibilities, and/or birefringence can give rise to changes in the $s'_0$ surface plot as well. For instance, changes in $s'_0$, between the second and third column in Fig. 4.1, due to the presence of complex susceptibilities in the collagen-like sample, is noticeable. In principle, for each case a fitting routine can be implemented, and each component of measured SHG Stokes vector can provide the structural information regarding the sample independently.

The measured Stokes vector includes polarized and unpolarized light: $s' = s'_u + s'_p$. Moreover, it is the first component $s'_0$ that has this information. Therefore, the total light intensity encapsulated by the $s_0$ parameter can be written as (see Eq. 1.22):

$$s_0 = (1 - dop)s_0 + s_0dop$$

(4.2)

The polarized component is mainly the coherent SHG from the sample. Thus for data with background signal this needs to be taken into account during fitting. Typical sources of unpolarized signal are detector dark counts, ambient light, and or depolarization of the polarized components.

### 4.2 SHG Polarimetry of Biological Tissue

SHG polarization studies of biological tissue have revealed important structural information for myosin, collagen, and starch molecules. Myosin proteins in a sarcomere of a muscle are organized in a non-centrosymmetric helical structures and become the source of second-harmonics. Thus, SHG from myosin provides a great opportunity to probe myosin physiological states during a muscle contraction. Collagen is another example where strong SHG is observed. Collagen is one of the main constituents of the connective tissue. It is responsible for a variety of functions in an organism. For example, in a human body collagen is present in most organs including bone, cartilage, skin and heart. Its property is exploited for various functions such as shock absorber in bones and cartilaginous tissues and for light focusing media such as in the cornea of an eye. Cancer-
cells growth, function and treatment are all intimately linked to their micro-environment through the surrounding collagen extracellular matrix [158–162]. The nonlinear microscopy can provide valuable insight into the structural changes of collagen tissue in these samples. Similarly, starch exhibits strong SHG signal revealing radial structure of the granules. A linear polarization microscopy has been used to observe structures of starch granules since the mid-19th century [163, 164]. Recently, the organization of carbohydrates within starch granules have been studied with linearly polarized incident laser radiation and measuring the outgoing SHG with a linear analyzer [90]. Therefore, Stokes-Mueller polarimetric SHG microscopy has the potential to provide comprehensive structural information about these ordered biological structures. In the following, polarization measurements in muscle fiber, collagen and starch granule are presented.

4.2.1 SHG Polarimetry Images of Biological Tissue

Muscle myosin, collagen, and starch can be studied with linearly polarized light and measuring the outgoing SHG Stokes vector. That is because the elements of the nonlinear $\mathcal{M}^{(2)}$ matrix are interdependent. Therefore, for the sake of minimizing the measurements time, the linearly incoming polarized light is used and the first six columns of matrix provide the necessary information. In addition, in this thesis, the laser excitation wavelength ($\sim 1028$ nm) are away from the absorption bands. Thus, only real susceptibility tensor components are used in the $\mathcal{M}^{(2)}$ matrix. The circular component of the outgoing SHG signal is expected to be low due to the reality of susceptibility values. Furthermore, very thin sections are used for imaging, where birefringence of the sample is negligible [87–89]. Therefore, for such imaging conditions the circular component $s'_3$ of outgoing radiation is small, and only linear polarization components of the outgoing signals are sufficient to characterize the sample. In order to test whether these conditions are valid, it is a good practice to calculate the degree of linear polarization, and if the dolp is large the circular component measurement can be neglected.

Fig. 4.2 shows the SHG Stokes components from myosins of a muscle sarcomeres in a fruit fly larva. Alternating bright A- and dark isotropic (I-) bands are clearly visualized with SHG microscopy due to the nonzero second-order susceptibility of myosin filaments located in A-bands. The $s'_3$ component appears small relative to the first three Stokes components. Quantitative analyses confirm that the degree of polarization
\[
dop = \sqrt{s_1'^2 + s_2'^2 + s_3'^2 / s_0'^2}
\]
from the thin sample is very close to the degree of linear polarization \( \text{dolp} = \sqrt{s_1'^2 + s_2'^2 / s_0'^2} \), as shown in Fig. 4.3. The difference between the two components for the muscle sample is on average about \( \sim 2.5\% \). Therefore, for thin samples the first three components of Stokes vector and the degree of linear polarization is enough to decipher structural information from the polarimetry study. On the other hand, if the sample has appreciable thickness, the degree of linear polarization can be low, indicating that \( s_3' \) component has to be measured.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fig4.png}
\caption{SHG polarimetry of a fruit fly muscle sarcomeres. The \( S_1 \) to \( S_4 \) at the top correspond to the incoming radiation polarization states of 0\(^\circ\), 90\(^\circ\), 45\(^\circ\) and \(-45\(^\circ\)\), respectively. The \( s_0' \) to \( s_3' \) indicated on the left correspond to the measured Stokes vector of SHG radiation from the sample. The color in the first row shows the total intensity per pixel, while in the second third, and fourth row indicates the Stokes vector components. Each Stokes image was obtained from the 31 x 14 \( \mu \text{m}^2 \) intensity images, acquired at \( \sim 22 \) mW incoming laser radiation, before excitation objective, for 10 seconds.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fig4.png}
\caption{Degree of polarization of SHG from muscle of a fruit fly in Fig. 4.2. The degree of polarization (dop) and degree of linear polarization (dolp) is calculated for muscle from a fruit fly. On average the difference between two parameters \( \text{dop} - \text{dolp} \approx 2.5\% \), which implies that the signal is mostly linearly polarized.}
\end{figure}

Fig. 4.4 shows the reduced polarimetry SHG measurements of collagen from a rat-tail tendon. For each incoming polarization, the outgoing linear components of SHG Stokes
vector are visualized, as well as the respective degree of linear polarization of second-harmonic signal. The first row shows the $s_0'$ component (i.e. total SHG) intensity from the sample. The collagen fibers are aligned in the tendon and in the image all point to the same direction. The second row shows the excess of light from $0^\circ$ vs. $90^\circ$, and the third row shows the excess of light from $45^\circ$ vs. $-45^\circ$. The pattern show an anisotropy of signal due to contribution of different molecular second-order susceptibility tensor components. The structure can be resolved by determining the relative contribution of the susceptibility component ratio (see Section 4.2.2).

![Figure 4.4: Reduced polarimetry of collagen tissue from a rat-tail tendon. Stokes parameters and the degree of linear polarization for four linearly polarized incoming radiation is shown. High degree of linear polarization, $<\text{dolp}> = 0.88 \pm 0.14$, is typical from thin samples of collagen. The color in the first row shows the total intensity per pixel, while in the second and third row indicates the normalized Stokes vector components (normalized to the maximum intensity for each incoming polarization state). Each Stokes image was obtained from the $25 \times 25 \ \mu m^2$ intensity images, acquired at $\sim 50 \ mW$ incoming laser radiation before excitation objective for 5 sec.](image)

Similarly the reduced set of measurements for starch granules is shown in Fig. 4.5. As in figures for muscle and collagen, the first row show the total SHG intensity and the components of the SHG Stokes vector. Here, due to larger contribution of one molecular
susceptibility over the other, the pattern is more “dipolar-like” and radiates away from center, which is consistent with the radial ultra-structure of amylose and amylopectin in starch granules. The outgoing signal, for example, more easily can be followed with visualizing the polarization of light relative to the image. However, more structural information can be obtained by determining the molecular susceptibility ratio and the orientation of the molecular axis. Nonetheless, visualization of these images are informative and some quick conclusions can be drawn from them including calculating the degree of linear polarization which is shown in the last row for both collagen and starch.

![Stokes parameters for starch granules](image)

**Figure 4.5:** Reduced Stokes-Mueller polarimetry of starch granules. The Stokes parameters for starch is shown for four linearly polarized incoming radiation. The incoming polarization angles are indicated at the top. On the left, the Stokes parameters for SHG is shown as well as the degree of linear polarization of the outgoing SHG signal. The color in the first row shows the total intensity per pixel, while in the second and third row indicates the normalized Stokes vector components (normalized to the maximum intensity for each incoming polarization state). Each Stokes image was obtained from the $40 \times 40 \, \mu m^2$ intensity images, acquired at $\sim50 \, mW$ incoming laser radiation before excitation objective for 5 sec.

Fig. 4.6 shows that the average degree of linear polarizations for myosin, collagen and
starch are high. It is clear from Fig. 4.6 that the signals from these specimen are linearly polarized to a good extent, where the average degree of linear polarization is $dolp = 0.9 \pm 0.1$. Therefore, the next step in the image analysis is to obtain structural information about the specimen by extracting susceptibility tensor components from the polarization images. From the $\mathcal{M}^{(2)}$ matrix the laboratory-coordinate susceptibility tensor elements can be computed. In addition, for muscle, collagen, and starch their molecular susceptibilities and orientation of the fibers in the image plane can be calculated because their structures are known to exhibit a cylindrical symmetry [87,89,90].

![Figure 4.6: Average degree of linear polarization of SHG from fruit fly muscle (left: 31 x 14 \mu m^2) rat-tail tendon collagen (left: 25 x 25 \mu m^2), and starch granules (left: 40 x 40 \mu m^2) over reduced polarimetry data sets shown in Fig. 4.2, Fig. 4.4 and Fig. 4.5, respectively. The dolp of SHG signal is calculated for each pixel, and then, the mean SHG dolp values over four incoming polarization states, namely 0°, 45°, 90° and −45°, are displayed. The samples show high dolp indicating highly linearly polarized SHG.](image)

### 4.2.2 Structural Information in SHG Polarimetry Images

Biological structures such as myofibrils, collagen fibers and starch granules are organized into cylindrical structures, which can be characterized by the molecular susceptibility ratio $\chi^{(2)}_{zz}/\chi^{(2)}_{xx}$, and the orientation of the cylindrical axis in each pixel of the image can be determined from the polarization data. Nonlinear Stokes-Mueller formalism provides the possibility to obtain the ratio and orientation from a minimal number of the polarization images. It requires 12 images at the following SHG polarization states: $s_{1,0}$, $s_{2,0}$, $s_{1,\pi/2}$, $s_{2,\pi/2}$, $s_{2,\pi/4}$ and $s_{2,-\pi/4}$, as was shown in the Section 2.2.3. However, in the photon-counting regime low signal to noise ratio conditions often occur; therefore, more than minimum measurements are required to determine the susceptibility component ratio with high precision. Typically, the entire range of linear polarization states (0° to 180°) are prepared for fundamental beam, and for each incoming state the entire range of linear analyzer orientation angles is rotated to obtain the SHG signal and to create the PIPO surface plot for each pixel of the image. Subsequently, a surface fitting algorithm is used to extract the orientation and the susceptibility ratio [87–89]. In
this section, a comparative analysis of extracting the susceptibility component ratio and cylindrical axis orientation is conducted between the fitting of PIPO surfaces and the reduced Stokes-Mueller polarimetry of the images.

For PIPO analysis, images are recorded incrementally every 22.5° from 0°-180° for both incoming and outgoing radiation linear polarization orientations (θ, ϕ), while the reduced SHG polarimetry employs a subset of the linearly polarized polarimetry images obtained at angles 0°, 90°, 45° and −45° for both incoming and outgoing linear polarization orientations. Fig. 4.7 shows the SHG image, and molecular susceptibility ratio distributions for muscle myosin, collagen and starch calculated according to PIPO measurements. For each pixel in the image, the ratios of the molecular susceptibility $\chi^{(2)}_{zzz}/\chi^{(2)}_{zxx}$ were obtained by fitting the surface plot to the Eq. 2.98. The mean value of the ratio for myosin (0.6), collagen (1.37), and starch (4.1) are in the expected range and consistent with previously reported values [87–90,121].

Figure 4.7: Second-harmonic linear-polarized Stokes-Mueller polarimetry of myosin, collagen, and starch. First row: SHG $s''_0$ is measured after passing through a linear analyzer from the myosin in indirect flight muscles of Mhc\textsuperscript{10};Y97 fruit fly (left), collagen from rat-tail tendon (middle), and starch granules (right). Second row: the molecular susceptibility ratio $\chi^{(2)}_{zzz}/\chi^{(2)}_{zxx}$ is calculated for each pixel. Final row: histograms showing the distribution of the molecular susceptibility components ratio are shown. The muscle, collagen and starch images are 25 × 25 μm$^2$, 25 × 25 μm$^2$ and 40 × 40 μm$^2$, respectively. Intensity images for muscle were acquired at ~150 mW of laser radiation, before excitation objective, for 5 seconds. For collagen and starch see Fig. 4.4 and Fig. 4.5, respectively.

Fig. 4.8 shows the SHG polarimetry analyses of the fruit fly indirect flight muscles, rat-
tail tendon, and starch as well as their organizational structure in the tissue using the reduced polarimetry set. For each pixel, the orientation of the fibril and the molecular susceptibility ratio was calculated using Eq. 2.80 in Section 2.2.3. The calculation using the reduced polarimetry is, however, much quicker when compared to fitting, as it entails simple algebraic calculations with images and no per-pixel fitting. The first column in the Fig. 4.8 shows the reduced polarimetry analyses of the fruit fly indirect flight muscles. The distribution and mean value of the molecular susceptibility ratio is consistent with the previously report values for myosin and PIPO analyses [87, 121]. The measurements and analyses, presented in the second column of Fig. 4.8, show the orientation map of the cylindrically symmetric collagen as well as the susceptibility component ratio distribution across the image. The mean and the distribution of the susceptibility ratio as measured by the reduced SHG polarimetry correspond very well with the data in Fig. 4.7 as well as with previously reported values for collagen [89]. The distribution of angles is narrow and consistent with the overall direction of fibers. In the last column the reduced polarimetry of starch granules are shown. As in muscle and collagen, the ratio and distribution of data are very similar to the results obtained from the PIPO polarimetry set.

**Figure 4.8:** Reduced second-harmonic Stokes-Mueller polarimetry of myosin, collagen, and starch. First row: molecular orientations are obtained from the reduced SHG polarimetry for the myosin in indirect flight muscles of a Mhc^{10};Y97 fruit fly (left), collagen from a rat-tail tendon (middle), and starch granules (right). Second row: the molecular susceptibility ratio $\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}$ is calculated for each pixel using reduced SHG polarimetry. Final row: histograms showing the distribution of the molecular susceptibility component ratio are shown. Image areas are the same as in Fig. 4.7.
The distributions of the susceptibility ratios are generally a bit broader in the reduced polarimetry set, when compared to the PIPO set, which is expected since fewer polarization measurements were used to extract the data. The comparison of two methods gives confidence in the reduced polarimetry method, which has the advantage of shorter measurement time as well as calculation time; however, when high precision in the value of the susceptibility component ratio is required, extra measurements should be made and a fitting routine of PIPO surface for each pixel might be required. To illustrate that more detailed structural information can be obtained with SHG polarimetry for myosin inside a muscle, a wild-type and a mutant of indirect fly muscles are investigated in the next section.

4.2.3 Structure of Myofilaments

The structure of muscle myosin within a sarcomere can be characterized with the second harmonic generation (SHG) microscopy [81, 121, 133, 165, 166]. SHG is generated in myofilaments that contain myosin molecules organized in a cylindrically symmetric structure [167, 168]. Various conformational states of the myosin molecules influence the SHG polarization properties during muscle contraction. Therefore, understanding the structural basis of SHG signal is of paramount importance in order to employ the SHG polarization microscopy for muscle contractility studies.

The ab initio calculations can be performed to model polarization properties of SHG generated from fibrillar proteins [169, 170]. The calculated susceptibility values have to be compared with the experimental results, which requires measurements of SHG polarization properties of structural domains constituting the protein. The myosin molecules are comprised of the light meromyosin (LMM) domains that constitute the core of the myofilaments, whereas, the heavy meromyosin (HMM) regions act as mobile parts to produce power stroke when attached to actin filaments during muscle contraction (see Fig. 4.9). The LMM domains can be studied in the central region of anisotropic bands (A-bands) of sarcomeres, where the myofilaments do not contain myosin heads, or in mutants lacking myosin head domains [87]. By understanding polarization properties of LMM domains, the SHG polarization contribution of myosin heads and S2 domains during myocyte contraction can be estimated.
The investigation of $\chi_{zzz}^{(2)}/\chi_{zzx}^{(2)}$ for different parts of the myosin molecule was performed in wild-type and mutants of indirect flight muscle (IMF) samples from fruit flies (*Drosophila melanogaster*). IFMs from two mutants, Mhc$^{10};Y97$ (containing LMM and S2 domains) and Mhc$^{10}$ (without myosin) [171], and a wild-type (containing all components of myosin) were imaged with SHG polarimetric microscopy. The Mhc$^{10}$ mutants lack myosin and therefore show very weak SHG signal. Thus, Mhc$^{10}$ was used only as a reference measurement to verify SHG from myosin. An adult fruit fly was held in place by pins and dissected under the dissection microscope to extract indirect flight muscles. The IFMs were then fixed in a mixture of 4% formaldehyde and saline solution for three minutes [166]. Thin slices were cut and placed between two glass coverslips in the saline buffer for SHG microscopy.

SHG polarization measurements can be performed by measuring the signal intensity at multiple laser polarization orientations [121,133,167]. When small variations in polarization properties are of interest, or high precision measurements are required, an analyzer can be added to perform the linear polarization-in polarization-out (PIPO) SHG experiments [88,89]. Linear SHG polarization measurements render a two-dimensional surface, which can be fit with the theoretical model, to obtain the susceptibility ratio values and the orientation of the cylindrical axis of the myofibril [88,89]. The fitting, therefore, reduces the uncertainty with the orientation of the polarizer with respect to the myofilament axis during measurements. The SHG intensity images at different incoming beam
polarization angles $\theta$ and analyzer angles $\varphi$ were recorded to obtain PIPO plots. For the fruit fly imaging, the HWP rotated the incoming field polarization 10 times at equal increments from 0° to 180°. At each angle $\theta$, the analyzer was rotated by $\varphi = 18^\circ$ increments from 0° to 180°, and images were recorded for each combination of polarizer and analyzer angles. Incident laser intensities on the samples were optimized to keep signal degradation below 10% variation during PIPO measurements. The signal degradation was monitored at every 11th scan of the measurement, which were performed at $\theta = 0^\circ$ and $\varphi = 180^\circ$ of polarizer and analyzer positions, respectively. The acquired images were fit globally for each pixel using a custom MATLAB scripts (MathWorks, Natick, MA). These fits determined the $\chi^{(2)}_{zzz}/\chi^{(2)}_{xxx}$ values for all pixels in the image. Since not all pixels in the images contain enough signal for fitting, only pixels with well fitted data of $R^2 > 0.95$ are considered. Histograms of the $\chi^{(2)}_{zzz}/\chi^{(2)}_{xxx}$ values were then generated from the data that had $R^2 > 0.95$.

The fruit fly mutant Mhc$^{10};Y97$ [166, 171] was imaged with SHG-PIPO microscopy and compared with data from intact sarcomeres of the wild-type IFMs (Fig. 4.10). The mutant Mhc$^{10}$ does not contain myosin, and therefore, the SHG signal was absent from the sarcomeres (data not shown). The myosin of IFM in the Mhc$^{10};Y97$ fly is mutated twice, once to eliminate the myosins and then to add headless myosin filaments. A previous study of the mutants had found that the mutant Mhc$^{10};Y97$ retained the myosin heavy chain, the rod, and the regulatory light chain (located on the neck of the S1 region) [171]. The previous results also showed that the Mhc$^{10};Y97$ mutants were only missing the globular heads [166,171].

Figure 4.10a and 4.10e show the SHG images of the wild-type and Mhc$^{10};Y97$ IFMs that were acquired for the purpose of PIPO analyses, respectively. The wild-type myocyte structures generated the highest SHG signal and also demonstrated the familiar striation patterns with easily distinguishable A- and I-bands. In contrast, Mhc$^{10};Y97$ mutant tissue generated less SHG intensity compared to the wild-type, and also had irregular sarcomere structures that appear like splotches. The irregular SHG intensity patterns from Mhc$^{10};Y97$ samples highlight the organizational role of myosin heads in the structure of a sarcomere. While the A-bands are visible, the striated patterns are not obvious in the image of the mutant. Moreover, the I-bands are difficult to visualize within the image. The lack of myosin heads disrupt the typical sarcomere formation and results in distorted SHG image. The SHG imaging is consistent with the previous study of the
mutants, which showed that as the level of mutation increases, the crystalline structure of the sarcomeric myosin becomes more affected [166, 171].

Susceptibility component ratio maps provide further details about the structure of A-bands (Fig. 4.10b and 4.10f). SHG polarimetry analyses of the wild-type muscle structure revealed an average $\chi^{2}_{zzz}/\chi^{2}_{zxx}$ value of 0.60±0.10 and a relatively broad distribution that extends to the higher ratio values (Fig. 4.10c). In contrast, the mutant Mhc$^{10}$;Y97 showed an average $\chi^{2}_{zzz}/\chi^{2}_{zxx}$ value of 0.45±0.05, and the distribution of the ratio was narrower and significantly different than the wild type sample (t(5546) = -62.88, $p < 0.0001$; Fig. 4.10c and 4.10g). The narrow ratio distribution in the muscle mutant is surprising since the muscle fiber structure in SHG images appears much more distorted compared to the wild type. This is in contrast to findings of collagen fiber distributions in various tissue. In a rat-tail tendon with regular and straight collagen fibers a narrow distribution and small average $\chi^{2}_{zzz}/\chi^{2}_{zxx}$ ratio value is observed. In comparison, in dermis, cornea or bone with a relatively irregular distribution of collagen fibers a broader distribution and larger $\chi^{2}_{zzz}/\chi^{2}_{zxx}$ ratio values are found [89]. The narrow distribution indicates a uniform myosin structure along myofilaments in the mutated sarcomeres, which is expected from a homogeneous myosin rod domains.
Wild-type muscles have fully intact myosin proteins, which allow for the myosin to attach to the actin filaments. The attachment alters the myosin head and S2 domain deflection angles from the myofilament axis and leads to an increase in the susceptibility component ratio [87, 114]. The observed $\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}$ values represent the ratio of second harmonic generated from myosin molecules that are embraced in a single focal volume. The ratio is determined by several factors including the ratios of different parts of myosin molecule, the conformational states of myosin (attached, detached or intermediate state of myosin) and the tilt of a myofibril with respect to the image plane. From the ratio image (Fig. 4.10) it is visible that heterogeneity is present on a single sarcomere level and also between sarcomeres, as well as in different areas of the myocyte. The distribution of the ratio in the wild-type sample is large, ranging from 0.3 to 0.9, indicating that the sample contains myosins in attached and detached states. The lower ratio found in the Mhc$^{10;Y97}$ sample could be due to both, the lack of myosin head domains, and reduced variation in the deflection angle of the S2 region. Most probably, the S2 domain has significant influence on the ratio due to ordered helical structure rendering larger contribution to the SHG signal. In Mhc$^{10;Y97}$, due to the inability of the myosin to attach to actin, the S2 region may have a lower deflection angle from the myofilament axes resulting in lower measured $\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}$ values. This also explains the narrow ratio distribution in the histogram (Fig. 4.10g) pointing to a uniform orientation of the S2 domains [89]. The significant influence of S2 domains on the measured $\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}$ values can be helpful for studies of myosin nanomotor conformational changes during muscle contractions. The rapid SHG polarization changes occurring during muscle contraction can be probed with advanced orthogonally polarized multi-beam microscopy (see Section 4.2.4). The low $\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}$ values for the mutant Mhc$^{10;Y97}$ and the M-line of the wild-type myocyte indicates that LMM with detached S2 domain has the susceptibility ratio of $0.45 < \chi_{zzz}^{(2)}/\chi_{zxx}^{(2)} < 0.49$. This ratio can be used as an experimental value for \textit{ab initio} calculations of the nonlinear susceptibility of the myosin helical domains.

Fig. 4.11 shows that the measured ratio can be explained potentially by very different ratios of the myosin molecule. Two different hypotheses can be suggested. One is based on C-N backbone bond (in the peptide bond of the protein) as the SHG scatters which give low helix susceptibility component ratio (~0.4) [121], and the observed ratio increases as the S2 region and myosin heads change angle. On the other hand, very high ratio (50 to $\infty$) is expected from the \textit{ab initio} calculation that include all bonds in the amino
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Figure 4.11: Muscle myosin susceptibility ratio as a function of LMM and S2 angles from the main fiber axis. The measured values of the ratio (between 0.4-0.9) can be explained by an entire range of helix ratio. Low values of myosin helix is suggested by Nucciotti et al. [121]; while high helix ratio is based on ab initio calculations. The difference in the helix ratio calculations depends on which bond is expected to generate SHG. C-N bond is hypothesized by Nucciotti et al. [121]; however, all bonds, and more specifically the methylene bonds are suggested as the source of SHG by others [114, 172]. The latter hypothesis is consistent with other measurements such as SHG and SFG from collagen. Note, there is no change in observed ratio for helix $\chi_{zzz}/\chi_{zxx} = 3$ as expected from theory of tensor rotations. All values above and including 5 are colored the same.

acids, and suggests the observed ratio is mainly from LMM and S2 regions because the neck and globular heads produce little SHG. This latter suggestion is also consistent with the ab initio calculation and measurements of SHG and SFG from other materials with cylindrically symmetric structures such as collagen and starch [90, 114, 172]. The first hypothesis requires the LMM to be in line and parallel with the main axis of muscle sarcomere. While the second suggests that the LMM can be at an angle with respect of the main axis, and resides along a “pitch-like” angle within the bundle of myosins. The current SHG polarimetry cannot distinguish between these two hypotheses. Further studies including crystallographic techniques can verify one or the other hypothesis. Nonetheless, it is clear that the structural changes during muscle contraction can be observed.
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by SHG polarimetry. In the following section an example of fast differential polarization microscopy is presented where the susceptibility tensor component ratio changes in A-bands of sarcomeres are measured during a muscle contraction.

4.2.4 Differential Polarimetric SHG Microscopy for Structural Dynamics of Moving Samples

Differential polarization microscopy can be applied for imaging with various nonlinear contrast mechanisms. Polarization SHG from collagen fibers, an important biological structure, is regularly used to extract components of the second order polarizability tensor \([80]\). Commonly, \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}\) is determined by measuring the SHG intensity for many input polarization angles sequentially and fitting the polarization-dependent SHG intensity \([89]\). Instead, differential microscopy can be used to determine \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}\) in a single measurement by using multiplexed excitation beams polarized parallel and perpendicular to the collagen fiber orientation. The nonlinear susceptibility ratio \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}\) can then be immediately extracted using the relation \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)} = \sum s_{0,0}'/s_{0,\pi/2}'\), where \(s_{0,0}'\) and \(s_{0,\pi/2}'\) are the total SHG intensities obtained by parallel and perpendicular laser polarizations with respect to the fiber, respectively.

Figure 4.12 shows SHG signal from the stretched rat-tail tendon collagen sample used for this experiment, with the multiplexed excitation polarizations parallel and perpendicular to the fiber orientation (Fig. 4.12). The collagen fibers are well aligned, which indicate that their susceptibility ratio should be close to 1.4 \([89]\). Fig. 4.12 shows \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}\) for each pixel in the sample image. The extracted \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}\) in Fig. 4.12 is 1.41 ± 0.12, which is calculated directly from the near-simultaneous excitation with differential polarization nonlinear microscope. The only requirement is to keep the orientation of collagen fibers parallel to the polarization of one of the two beams. If the orientation of the fiber deviates from the polarization orientation, then other nonlinear susceptibility tensor components start contributing to the \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}\) ratio.

This experiment shows that SHG differential polarization microscopy can be used to extract quantitative information about the molecular orientation in the ordered tissue sample. Rapid changes of \(\chi_{zzz}^{(2)}/\chi_{zxx}^{(2)}\) can be monitored, for example, in a specimen where collagen fibers are dynamically stretched during the experiment or when a muscle is
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Figure 4.12: SHG polarization images from the stretched rat-tail tendon collagen sample used to determine $\chi^{(2)}_{zzz}/\chi^{(2)}_{zxx}$ (area size is $50 \times 50 \mu m^2$). The ratio value for each pixel is calculated as $\chi^{(2)}_{zzz}/\chi^{(2)}_{zxx} = \sqrt{s'_0(0^\circ)/s'_0(90^\circ)}$. The distribution of the ratio shows an average value of $\chi^{(2)}_{zzz}/\chi^{(2)}_{zxx} = 1.41 \pm 0.12$.

contracting. Figure 4.13 shows in vivo contractions of a kissing bug (*Rhodnius prolixus*) oviduct muscle cells. The observed ratio $\chi^{(2)}_{ZZZ}/\chi^{(2)}_{ZXX} = \sqrt{s_{0,1}(2\omega)/s_{0,2}(2\omega)}$ is recorded during contractions using the differential polarization microscopy.

Figure 4.13: Real-time anisotropy of contracting muscles imaged by the differential polarization microscope. Left panel: The change in $\chi^{(2)}_{ZZZ}/\chi^{(2)}_{ZXX}$, calculated from the sarcomeres of the muscle in the black box of right panel, increases due change in the myosin head orientation during contraction. Right panel: The pattern of high intensity (red) and low intensity (blue) are typical for the anisotropic (A-) band and isotropic (I) band in a sarcomere, respectively. The incident power in each of the orthogonal polarization beam from the laser was $\sim 50$ mW at the entrance aperture of the excitation objective.

A full polarimetry of the sample may be obtained by acquiring a full SHG Stokes-Mueller polarimetry to calculate the microscopic $\chi^{(2)}_{zzz}/\chi^{(2)}_{zxx}$, and therefore, to speculate about the range of angle changes that each domain of the myosin motor protein may undergo during contraction. Still, the reduced differential polarimetry of the kissing bug oviduct shows that dynamical changes of myosin motor can be observed during contraction, and it is the first demonstration of the live differential polarization microscopy for capturing dynamics in the biological systems.

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3The sample was kindly provided by Luara Sedra from Professor Angela Lange lab, University of Toronto.
4.3 THG Polarimetry of the Fruit Fly Eye

THG microscopy can be used to image pigmented structures in biological samples. The ultrastructure of the specimens can be investigated with third harmonic polarimetry in a similar way as in the second harmonic polarimetry, which was presented in the previous sections. For THG, the incoming beam is characterized by a 16 element Stokes vector and the outgoing third harmonic radiation is characterized by a 4 element Stokes vector. Therefore, the outgoing vector have to be measured for at least 16 measurements with distinct polarizations to obtain the $\mathcal{M}^{(3)}$ matrix that describes the nonlinear optical properties of the structure. If $s_3'(3\omega)$ component is small, the reduced nonlinear Mueller Stokes polarimetry can be applied, where only linearly polarized components are utilized. In the following, an application of THG polarization microscopy to investigate the retinal molecules within pigmented cells of a fruit fly eye is presented.

Vision is an important characteristic of living organisms and dates back to the early life on earth. Light sensitive molecules were utilized by organisms early in the evolutionary tree when green algae used the $\beta$-carotene molecules to sense light. In animals, such as in a fruit fly eye, $\beta$-carotene is converted to retinal and vitamin A to enable the sense of sight and vision [173]. Retinal accumulates in the eye, and is also a source of THG. Therefore, THG polarimetry can reveal organization of the molecules in the eye retina.

In this study, a fruit fly eye was imaged with third harmonics generation using the single-beam polarization microscope. Fig. 4.14 shows THG images recorded at four different incoming polarization states ($0^\circ$, $90^\circ$, $45^\circ$ and $-45^\circ$ linear polarization orientations) and the obtained $s_0(3\omega)$, $s_1(3\omega)$ and $s_2(3\omega)$ Stokes vector components of the outgoing radiation. The calculated degree of linear polarization for each incoming polarization state is also presented in the last row. By inspection, two quick and important observations can be made from the data in Fig. 4.14. First, the signal is linearly polarized to a great extent: $dolp > 0.9$. Therefore polarimetry can be conducted by using linearly polarized states of incoming radiation and a linear analyzer. Second, the maximum signal is obtained when the orientation of incoming and outgoing linear analyzer is the same. This pattern is expected from an isotropically symmetric sample.

Further analyses of the THG Stokes vector confirmed that the retinal third-order susceptibility in the fruit fly eye indeed has an isotropic symmetry. Fig. 4.15 shows the $s'_0$
Figure 4.14: Third-harmonic generation Stokes polarimetry of the fruit fly eye. The incoming polarization states are indicated at the top of images. The outgoing THG Stokes vector component is indicated on the left side at each row of the images. The color in the first row shows the total intensity per pixel, while in the second and third row indicates the normalized Stokes vector components (normalized to the maximum intensity for each incoming polarization state). The degree of linear polarization is presented in the last row. The pattern of signal is characteristic of an isotropic symmetry for third-order susceptibilities. Intensity images (40 × 40 µm²) were acquired at ~155 mW of laser radiation before excitation objective for 6 seconds.
component as function of linear incoming polarization and the outgoing linear analyzer orientation. The theoretical simulation using Eq. 2.143 is presented in the left panel and the experimental measurements are shown in the right panel. By comparing the images it can be concluded that the signal pattern is consistent with the isotropic symmetry of third order susceptibilities.

Figure 4.15: THG polarization-in polarization-out surface plot measured from the fruit fly eye. The incoming linear polarization state at the fundamental wavelength are denoted by $\theta$ and the outgoing linear analyzer orientation is denoted by $\varphi$. The color shows the minimum (blue) to maximum intensity (red). The first panel shows the $s'_0$ for THG according to theory in Eq. 2.143. The panel on the right shows the experimental data from the fruity fly eye. The sample shows isotropic symmetry.

The triple Mueller matrix also appears close to the isotropic case. Since only linear polarizations are concerned, the last six column of the triple Mueller matrix $\mathcal{M}^{(3)}$ can be ignored as well as its last row. The remaining $3 \times 10$ reduced matrix for isotropically symmetric susceptibilities is compared to the experimental data. The following two matrices contain values for the isotropically symmetric sample according to the theory, and the calculated matrix observed from the fruit fly eye.

$$\mathcal{M}^{(3)}_{\text{Reduced, Theory}} = \begin{pmatrix} 7.1 & 2.3 & 3.3 & 0 & 0 & 0.71 & 0 & 0 & 0 & 0.71 \\ 0 & -0.58 & 0.41 & -7.9 & 0 & 0.24 & 0 & 0 & 0 & -0.24 \\ 0 & 0 & 0 & 0 & 0.19 & 0 & 1.7 & 2.6 & 2.6 & 0 \end{pmatrix} \quad (4.3)$$

$$\mathcal{M}^{(3)}_{\text{Reduced, Obs.}} = \begin{pmatrix} 7.1 & 2.3 & 3.3 & -0.093 & 0 & 0.72 & 0.049 & 0.11 & 0.091 & 0.71 \\ 0.07 & -0.45 & 0.39 & -7.1 & 0.058 & 0.2 & 0.52 & 0.73 & 0.86 & -0.2 \\ 0.38 & 0.26 & 0.034 & 2.2 & 0.18 & -0.017 & 1.6 & 2.3 & 2.4 & 0.11 \end{pmatrix} \quad (4.4)$$

The observed values are very close to the expected theoretical values with $R^2 = 0.9442$, when all elements of two matrices are compared.
4.4 Conclusion

There are numerous ways to obtain structural information from a sample using nonlinear polarimetry. A full polarimetry measurement set is composed of the outgoing Stokes vector measurements for unique \((n + 1)^2\) incoming radiation polarization states of the \(n\)th-order process. For example, the incoming states for two-photon processes form a \(9 \times 9\) matrix, and for three-photon processes form a \(16 \times 16\) matrix that are invertible, similar to Eq. 2.92 and Eq. 2.136, respectively. The corresponding \(M^{(2)}\) and \(M^{(3)}\) elements can be uniquely determined by multiplying the inverted matrix with the outgoing measurement set. However, often structures have certain symmetry restrictions, and therefore, simplifications can be made to reduce the number of measurements.

In certain cases a reduced polarimetry may suffice to obtain the SHG molecular parameters. For a thin non-absorbing sample, where optical activity is negligible, the last component of the outgoing Stokes vector \(s_3'\) can be ignored. In this case, only linear polarized incoming beams can be employed to probe the sample and a linear analyzer can be used to obtain the first three components of the Stokes vector. For samples with cylindrically symmetric susceptibility tensor elements a reduced linear polarization polarimetry can be performed to obtain the susceptibility component ratio \(\chi^{(2)}_{zzz}/\chi^{(2)}_{zzz}\). In total 12 measurements are required for generating one set of solutions for reduced two-photon polarimetry. If the outgoing light is completely polarized, and there is no preference to use the differential components of the Stokes vector (i.e. \(s_1(2\omega), s_2(2\omega), s_3(2\omega)\)) over the first component \(s_0(2\omega)\), in other words the difference between intensities are not important, then only ten measurements for two sets of solution or eight for one set of solution are required to obtain the ratio.

The polarimetric microscopy can be used for obtaining structural and dynamic informa-
tion about the biological samples. Muscle cell imaging with second harmonic generation polarization microscopy provides evidences that the susceptibility component ratio value of headless myosins arranged in the myofilament is $0.45 < \frac{\chi^{(2)}_{zzz}}{\chi^{(2)}_{zxx}} < 0.49$. In contrast, the head containing regions of myofilaments give higher values of the susceptibility component ratio. The broad distribution of the ratio shows that conformational states of attached and detached myosin heads and the variation in the deflection angle of S2 domain influence the susceptibility component ratio. Thus, the ratio can be used for dynamic studies of contracting myocytes. The determined ratio of LMM with S2 domain can be potentially used for *ab initio* calculations to model nonlinear properties of myosin as well. Thus, SHG linear polarization imaging technique provides a robust and precise basis for studies of conformational dynamics of myosins within the A-bands of sarcomeres during a muscle contraction.

The applications of SHG imaging and nonlinear polarimetry study on collagen examples show that orientational and structural properties of the fibers can be quickly and accurately obtained with a single measurement using differential polarization microscopy. This capability is highly desired for probing the rapid dynamics in the sample such as contraction of a muscle. Observed susceptibilities dynamics could provide a clue about the underlying structural changes of the myosin domains during contraction.

Similarly, three-photon processes such as THG can take advantage of various polarimetry approaches to characterize a sample that generates the signal. The study of retinal distribution in the fruit fly eye reveals an isotropic distribution of THG generators, as was demonstrated by the symmetry of third-order susceptibility tensor components. The results are useful in interpreting the developmental aspects of the retina structure and organization of the light-sensing molecule in the eye.

In summary, the results in this chapter provide an experimental verification of the nonlinear Stokes-Mueller polarimetry theory, which was developed in Chapter 2. This chapter also presented several examples of the second and third harmonic polarimetric microscopy applications for biomedical research. The scope of the nonlinear polarimetry is not limited to the biomedical applications, however, and only time will tell what other fields can take advantage of this framework.
Chapter 5

Conclusion

Linear Stokes polarimetry and nonlinear optics each have had impressive developments, yet separate from each other. A key motivation for undertaking the study and developing the nonlinear optical polarimetry was to bring them together. Consequently, there is a three-fold implication: First, the generalized Stokes polarimetry broadens its scope to incorporate nonlinear optical phenomena, and therefore, can capitalize on existing linear polarimetry framework and expand to nonlinear interactions; second, the generalized nonlinear Stokes polarimetry can characterize more comprehensively the investigated material; and third, nonlinear polarimetry can express the state of incoming and outgoing light more comprehensively by incorporating coherent and non-coherent part of the radiation as well as linear and circular components.

The first realization will enable researchers in linear polarimetry field to incorporate important nonlinear optical techniques such as second-harmonic generation (SHG), third-harmonic generation (THG), coherent anti-Stokes Raman scattering (CARS), and others, into the polarimetric framework. The second realization will enable researchers to characterize the material with several parameters from linear and various nonlinear interactions. The nonlinear optical properties of the material can be explained in terms of observable Mueller matrix parameters that are comprehensive and are more intuitively understood. Finally, the nonlinear optical measurements in biological samples, which are highly scattering, can be better characterized by separating coherent and incoherent contributions of the radiation as well as potential sources of chirality and optical activity.
A key finding in this study is the generalized framework for optical polarimetry theory. In Chapter 1, the background theory for linear polarimetry and nonlinear optics was presented in order to develop the generalized nonlinear polarimetry. It was shown how from the general definition of polarization density both linear and nonlinear terms result in the generation of a response from a medium. Linear polarimetry has successfully uncovered rich light-matter interactions in linear regimes. The electric field notation and its transformation by Jones matrices, together with the coherency matrix and Stokes notations for polarization state, provided a phenomenological description of light-matter interaction using the Mueller matrix. Of course, since their initial introduction in the 1940s the fields of matrix optics have grown far beyond the characterization of the polarization state of light. The Stokes-Mueller formalism has been used in many fields from astronomy to biology and biomedical applications, particularly for microscopy techniques. Similarly, nonlinear optics has revealed far-reaching optical phenomena and characterized media through its own unique approaches. Although many aspects of two fields overlap to a great extent and their polarimetric approaches have complimented and extended the scope of the other technique, the unified theory has largely been evasive and exclusive to working with complex-valued notations of electric fields and susceptibilities. Experimentally observables and comprehensive description of light polarization are a key feature of the theory, developed in Chapter 2. The general form of the nonlinear optical polarimetry, resembling the linear Stokes-Mueller formalism, was presented to show that the nonlinear SHG and THG polarimetry could be performed to obtain unique structural information using nonlinear susceptibilities. More specifically, for example in the context of microscopy, the linear polarimetric measurements of biological tissue was further studied by incorporating SHG and THG to compliment or provide better insight into the tissue structure. Nonlinear microscopy can incorporate intuitive and geometrical means for the Stokes vector and Mueller matrix to describe the state and the degree of polarization of light as well as the extent to which a nonlinear susceptibility contributes to the outgoing radiation.

The details of polarimetric nonlinear optical microscopy setups were described in Chapter 3. The basics of single-beam nonlinear polarimetric microscope was presented. Due to a large number of polarization states that have to be used for the complete polarimetry measurement, the measurement time extends to tens of minutes. Therefore, a fast measuring polarimeter employing two beams was presented in the Chapter 3. The multi-
beam microscopy also enabled fast imaging with different polarizations that were employed in differential mode to obtain Stokes parameters with high precision. Precise beam overlapping is important for differential microscopy. Additionally, adaptive optics technology were used for steering the excitation beams. This point is also important if the multiplexed beam differential polarization microscopy is to be used for super-resolution investigations. Coupling a multi-beam laser system to a nonlinear optical microscope required a precise control over beams properties, and therefore, needed to be considered in the design of the setup. Nonlinear polarimetry required that the beam wavefront and the location of the beam to be very precisely monitored and changed. Adaptive optics, using deformable membrane mirrors (DMM), as a unique technique helped in this regard. DDMs were employed to steer a beam to a desired position or to overlap with another beam. In the process a genetic algorithm (GA) optimization routine was conducted to optimize the shapes of DMMs for the highest nonlinear signal intensity and the best focal volume overlap in 3D. The GA optimization was achieved by defining a multi-parameter fitness function accounting for intensity as well the distance between the beams. The presented data demonstrated that focal volumes from two beams can overlap with better precision than a tenth of the diffraction-limited spatial resolution, and thus rendering the technique highly desirable for many potential super-resolution applications as well. The implementation of adaptive optics ensured high precision overlap of the two beams and allowed pixel-by-pixel calculation of the measured SHG anisotropy. Structural and accompanying polarization changes were determined in dynamic samples such as in contracting muscle cells. High-speed data acquisition and processing were performed by a custom-programmed field-programmable gate-array (FPGA) chip. A novel logic was implemented, which enabled a highly efficient mechanism for processing, transferring, and recording the multidimensional data. Therefore, time-multiplexed differential microscopy with photon counting detection and adaptive optics for beam shaping is an attractive tool for dynamic polarimetry applications.

Chapter 4 of the thesis provided experimental verification of the nonlinear polarimetric microscopy theory. For a class of biologically important structures including muscle myosin, connective tissue collagen and carbohydrate-rich starch, as well as retinal in the eye, structural conformation and molecular orientations are probed and determined. Previous studies, such as polarization-in/polarization-out techniques, where linearly incoming polarized light and a linear analyzer for outgoing radiations are used to study
samples, were shown to fit nicely within the new theoretical nonlinear polarimetry framework. In addition, experiments that required a reduced polarimetry were relatively easily designed and studied. For instance, it was shown that only 12 measurement were needed to capture the SHG molecular parameters for cylindrically symmetric material in any orientation within the imaging plane. Furthermore, real-time muscle myosin dynamics were captured with parallel measurements using the differential polarization microscopy.

The nonlinear polarimetry presents a few short-term technological challenges to overcome in order to expand the current experimental limitations. A key impediment to the growth of nonlinear optical polarimetric microscopy is the scarcity of more powerful pulsed lasers to generate high count-rates from a biological sample at an optimized wavelength. Multi-beam pulsed laser systems can be multiplexed, and therefore, a more powerful system will further improve real-time polarimetric studies and can capture more rapidly the multidimensional polarization response from a specimen. Of course, other hardware improvements need to go hand-in-hand. Sensitive detectors capable of detecting high count-rates (> 100 MHz) are also needed to overcome the limitation of count-rate-limited data acquisition. MEMS (micro-electro-mechanical systems) mirrors may help in high-speed scanning in excess of 100 kHz. Gigahertz sampling-rate and more resourceful FPGAs when combined with advanced 3D rendering techniques using graphics processor units (GPU) will bring a new era of visualization for nonlinear polarimetric data.

On the theoretical side, the thesis may lead to some fruitful future studies. Incorporating non-coherent nonlinear optical effects into the theory is a key step. Understanding how the nonlinear outgoing signals depend on these effects and combine with coherent signals will help in deducing the sources of background signal after quantifying them with the current nonlinear polarimetry theory. Importing potentially useful approaches from linear polarimetry may be a good starting point. However, due to the nonlinear relationships, between physical parameters as well as the underlying algebra, new mathematical tools may be experimented. Other nonlinear studies such as investigations into the chiral response of a material possessing hierarchical structures, can use the nonlinear polarimetry theory to predict multiple polarization responses from the media. Furthermore, new approaches that more precisely estimate errors in measurements and characterize the sample may also need to be investigated. Some of these challenges are actively studied, and the nonlinear optical polarimetry theory developed in this thesis can provide a broader perspective for these exciting research investigations.
Most work in this thesis was achieved collaboratively with the guidance from Professor Virginijus Barzda. I developed the generalized theory of nonlinear polarimetry. Sergeui Krouglov and I developed the double Stokes-Mueller formalism initially for second-harmonics generation, and I worked out the details for sum- and difference-frequency generation. I developed the formalism for nonlinear polarimetry of three-photon processes as well as its variations for third-harmonic generation and coherent anti-Stokes Raman scattering.

I built, programmed and optimized the fast differential polarization microscope. Sergey Musikhin and Richard Cisek had purchased the opto-mechanics and electronics for the microscope. I programmed the FPGA and the LabVIEW interface for the microscope. All custom-written MATLAB scripts for nonlinear polarimetry analyses were my own. I aligned the two beams with deformable membrane mirrors and implemented the adaptive optics on the fast differential microscope. I developed and implemented the multiparameter genetic algorithm for beams optimization and overlap on the differential microscope.

I acquired the differential polarization data from the muscle and analyzed the data. Muscle dissection for kissing bug oviducts were prepared kindly by Laura Sedra from Professor Angela Lange lab. Collagen data were obtained by Daaf Sandkuijl, and I analyzed the data in the context of Stokes Mueller formalism. Starch data was acquired by Richard Cisek, and I analyzed the data and performed all polarimetry analyses interpretations. The fruit fly muscle data was kindly provided by Professor Brian Stewart. I analyzed the myosin data from the mutant and wild-type fruit fly, and performed the interpretations including developing the model. Lukas Kontenis acquired the polarimetry data for larva muscle on the fast differential microscope. I performed all the relevant polarimetry anal-
yses. The fruit fly eye was prepared kindly by Abiramy Karunendiran from Professor Brian Stewart lab; I performed the THG polarimetry and analyses.

Publications Pertinent to Thesis

List of Refereed Publications


Selected List of Conference Presentations


Samim, M., I. Tretyakov, N. Prent and V. Barzda (2012). Three-dimensional structural dynamics in contracting myocytes of beating chick embryo hearts imaged with nonlinear
microscopy. 56th Biophysical Society Meeting, San Diego, California, USA.


List of Conference Publications


Bibliography


Appendix A

Abbreviations

AO - Adaptive Optics
CARS - Coherent Anti-stokes Raman Scattering
DFG - Difference Frequency Generation
DMM - Deformable Membrane Mirror
dop - Degree of Polarization
dolp - Degree of Linear Polarization
FPGA - Field-Programmable Gate Array
GA - Genetic Algorithm
HWP - Half Wave Plate
NLOM - NonLinear Optical Microscopy
NW - Nanowire
PIPO - Polarization-in Polarization-out
PMT - Photo-Multiplier Tube
PSA - Polarization State Analyzer
PSF - Point Spread Function
PSG - Polarization State Generator
QWP - Quarter Wave Plate
SFG - Sum Frequency Harmonic Generation
SHG - Second Harmonic Generation
THG - Third Harmonic Generation
List of Notations

Ψ - Azimuthal Poincaré coordinate
Ω - Latitude Poincaré coordinate
Φ - Outgoing electric field state function
ψ - Nonlinear electric field state function
ω - Electric field oscillation frequency
ε - permittivity of free space
χ - Susceptibility
P - Polarization density
E - Electric field
η - General basis for expanding coherency matrix
H - Vectorized form of general bases for expanding coherency matrix
λ - Wavelength
λ_N - General basis set for expanding two-photon coherency matrix
Λ - Vectorized form of general bases for two-photon expanding coherency matrix
γ - General basis for expanding three-photon coherency matrix
Γ - Vectorized form of general bases for expanding three-photon coherency matrix
C - Coherency matrix for the outgoing radiation
ρ - Coherency matrix for the incoming radiations
τ - Pauli matrices
\mathcal{T} - Vectorized form of Pauli matrices
† - Complex conjugate transpose
M - Conventional $4 \times 4$ Mueller matrix
\mathcal{M} - Matrix representing the sample showing nonlinear response
τ - Denotes transposition
θ - Polarization angle of linearly polarized light
φ - Linear analyzer orientation
dop - Degree of polarization
dolp - Degree of linear polarization
s - $4 \times 1$ Conventional Stokes vector
S - Nonlinear $(n + 1)^2 \times 1$ vector representing $n^{th}$ order electric field for polarization state of incoming radiations
F - Fitness function for (genetic algorithm) optimization routine
$J$ - $2 \times 2$ Jones matrix
$L$ - Normalization constant for intensity or $4 \times 1$ Stokes vector
$Q$ - Incoming polarization measurement set
$R$ - Molecular susceptibility component ratio for cylindrical symmetry
$w$ - Weighting factor for overlapping procedure
$xyz$ - Molecular coordinate system
$XYZ$ - Laboratory coordinate system
$\alpha$ - Ascension angle of the fiber from the imaging plane
$\delta$ - Azimuthal angle of the fiber in the imaging plane
$\zeta$ - Distance along the propagation direction
$\mu_0$ - Permeability of free space
$\iota$ - Confocal parameter
$\kappa$ - Standard deviation of point spread function
$\xi$ - Pixel size
$B$ - Background noise
$\hat{n}$ - Imaginary part of the refractive index
$n$ - Refractive index
$n$ - Order of nonlinear interaction
$c$ - Speed of light
Appendix B

Alternative Derivations

B.1 Product of Susceptibilities

The nonlinear Stokes Mueller formalism can be stated in different forms. Below two-photon process SHG is used as an example to show that the Mueller matrix and the susceptibility product matrices can be written in the vector as well square matrix forms. These forms are useful for analyzing experimental data and decompositions of matrices.

If in Eq. 2.64 the product of Pauli and Gell-Mann matrices are separated from the product of $2 \times 3$ susceptibility matrix $\chi^{(2)}$ and its conjugate, then the following is arrived:

$$M_{\gamma N}^{(2)} = \frac{1}{2} \text{Tr} \left( (\tau_\gamma \otimes \lambda_N^T)X^{(2)} \right)$$

(B.1)

where $X^{(2)}$ is a $6 \times 6$ "coherency" type matrix of $\chi^{(2)}$ which is defined as: $X^{(2)} = (\text{vec}(\chi^T))(\text{vec}(\chi^T))^\dagger$, where $\text{vec}(A) = \vec{A} = [a_{1,1}, ..., a_{s,1}, a_{1,2}, ..., a_{s,2}, ..., a_{1,t}, ..., a_{s,t}]^T$ is the vectorization of a $s \times t$ matrix $A$. Since $\text{Tr}(AB) = \text{vec}(A^T)^T \text{vec}(B)$, then:

$$M_{\gamma N}^{(2)} = \frac{1}{2} \left( \text{vec} \left( (\tau_\gamma \otimes \lambda_N^T)^T \right) \right)^T \text{vec}(X^{(2)}) = \frac{1}{2} \left( \text{vec} \left( \tau_\gamma^T \otimes \lambda_N \right) \right)^T \text{vec}(X^{(2)})$$

(B.2)

Let $\vec{M}^{(2)} = \text{vec}(M^{(2)T})$ be the vectorization of the double Mueller matrix $M^{(2)}$ with indices $\gamma N$, then $\vec{M}^{(2)}$ is a 36-element column vector indexed according to $m = (\gamma - 1)9 + N$. Similarly, let $\vec{X}^{(2)} = \text{vec}(X^{(2)})$ be the vectorization of the coherency matrix $X^{(2)}$, then $\vec{X}^{(2)}$ is a 36-element column vector indexed as $x = (t - 1)6 + s$. By letting the row
Appendix B. Alternative Derivations

vector $\vec{T}_m = \frac{1}{2} \left( \text{vec} \left( \tau^T \otimes \lambda_N \right) \right)^T$, then the Mueller vector element is:

$$\mathcal{M}_m^{(2)} = T_{mx} X_x^{(2)}$$

(B.3)

where the transformation matrix $T = [\vec{T}_1, ..., \vec{T}_1]^T$. Therefore, the desired susceptibility tensor component products can be calculated using the transformation matrix as:

$$\vec{X}^{(2)} = T^{-1} \vec{M}^{(2)}$$

(B.4)

The explicit form of the matrix $\mathcal{M}^{(2)}$ was provided in the Box: Elements of the Material Matrix, and the order there is representative of vector $\vec{M}^{(2)}$. Its expanded form is given below.

If the complex susceptibility tensor components are expressed in the form of absolute values with phase delays, $\chi^{(2)}_{ijk} = |\chi^{(2)}_{ijk}| e^{i\delta_{ijk}}$, where the first index refers to the outgoing polarization orientation, while the latter two refer to the incoming polarization orientation, interesting expressions relating to the elements of double Mueller matrix arise. For example, the double Mueller elements that have the imaginary number $i$ scale with sin function, while those without it scale with cos function, as shown in the symbolic matrix of Eq. 2.67. In addition, some of the double Mueller matrix components are phase independent. The explicit expressions for the first row of double Mueller matrix elements are:

$$\mathcal{M}_{01} = \frac{\sqrt{6}}{6} \left( (|x_{zxx}|^2 + |x_{zzz}|^2 + |x_{xzz}|^2) + (|x_{xxx}|^2 + |x_{zzz}|^2 + |x_{xzz}|^2) \right)$$

$$\mathcal{M}_{02} = \frac{\sqrt{3}}{6} \left( (|x_{zxx}|^2 + |x_{zzz}|^2 - 2|x_{xzz}|^2) + (|x_{xxx}|^2 + |x_{zzz}|^2 - 2|x_{xzz}|^2) \right)$$

$$\mathcal{M}_{03} = \frac{1}{2} \left( (|x_{xxx}|^2 - |x_{zzz}|^2) + (|x_{xxx}|^2 - |x_{xzz}|^2) \right)$$

$$\mathcal{M}_{04} = (|x_{xzz}| |x_{zzz}| \cos \Delta_{xzz,xzz}) + (|x_{xxx}| |x_{zzz}| \cos \Delta_{xxx,xzz})$$

$$\mathcal{M}_{05} = (|x_{zzz}| |x_{xzz}| \cos \Delta_{xxx,xzz}) + (|x_{zzz}| |x_{xxx}| \cos \Delta_{xzz,xzz})$$

$$\mathcal{M}_{06} = (|x_{xzz}| |x_{zzz}| \cos \Delta_{xxx,xzz}) + (|x_{xxx}| |x_{zzz}| \cos \Delta_{xxx,xzz})$$

$$\mathcal{M}_{07} = (|x_{zzz}| |x_{zzz}| \sin \Delta_{zzz,xzz}) + (|x_{xxx}| |x_{zzz}| \sin \Delta_{xxx,xzz})$$

$$\mathcal{M}_{08} = (|x_{zzz}| |x_{xzz}| \sin \Delta_{zzz,xzz}) + (|x_{zzz}| |x_{xzz}| \sin \Delta_{zzz,xzz})$$

$$\mathcal{M}_{09} = (|x_{zzz}| |x_{xzz}| \sin \Delta_{zzz,xzz}) + (|x_{zzz}| |x_{xzz}| \sin \Delta_{xxx,xzz})$$

(B.5)

The explicit expression for the second row elements are:
\[ M_{11} = \frac{\sqrt{2}}{3} \left( (|xzx||xxx| + |xxx||xzz| + |xzz||xxz| - (|xzx||xxx| + |xzz||xxz|) \right) \\
M_{12} = \frac{\sqrt{2}}{3} \left( (|xzx||xxx| + |xxx||xzz| + |xzz||xxz| - 2|xzx||xxx| + |xxz||xzz|) \right) \\
M_{13} = \left( (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \right) \\
M_{14} = (|xzx||xzz| + |xxx||xxz| + |xzz||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{15} = (|xzx||xzz| + |xxx||xxz| + |xzz||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{16} = (|xzx||xzz| + |xxx||xxz| + |xzz||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{17} = (|xzx||xzz| + |xxx||xxz| + |xzz||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{18} = (|xzx||xzz| + |xxx||xxz| + |xzz||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{19} = (|xzx||xzz| + |xxx||xxz| + |xzz||xxx| + |xxx||xzz| + |xzz||xxz|) \\

The explicit expression for the third row elements are:

\[ M_{21} = \frac{\sqrt{2}}{3} \left( (|xzx||xxx| \cos \Delta_{xxx} + |xxx||xzz| \cos \Delta_{xxx} + |xzz||xxz| \cos \Delta_{xxx} + |xxx||xzz| \cos \Delta_{xxx} + |xzz||xxz| \cos \Delta_{xxx} \right) \\
M_{22} = \frac{\sqrt{2}}{3} \left( (|xzx||xxx| + |xxx||xzz| + |xzz||xxz| - 2|xzx||xxx| + |xxz||xzz|) \right) \\
M_{23} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{24} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{25} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{26} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{27} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{28} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{29} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\

The explicit expression for the fourth row elements are:

\[ M_{31} = \frac{\sqrt{2}}{3} \left( (|xzx||xxx| \sin \Delta_{xxx} + |xxx||xzz| \sin \Delta_{xxx} + |xzz||xxz| \sin \Delta_{xxx} + |xxx||xzz| \sin \Delta_{xxx} + |xzz||xxz| \sin \Delta_{xxx} \right) \\
M_{32} = \frac{\sqrt{2}}{3} \left( (|xzx||xxx| + |xxx||xzz| + |xzz||xxz| - 2|xzx||xxx| + |xxz||xzz|) \right) \\
M_{33} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{34} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{35} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{36} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{37} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{38} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\
M_{39} = (|xzx||xxx| + |xxx||xzz| + |xzz||xxz|) \\

The explicit matrices T and X^{(2)} are give on the next page.
X matrix is:

\[
\begin{pmatrix}
\delta e & \delta e & \delta e & \delta e & \delta e & \delta e \\
\delta e & \delta e & \delta e & \delta e & \delta e & \delta e \\
\delta e & \delta e & \delta e & \delta e & \delta e & \delta e \\
\delta e & \delta e & \delta e & \delta e & \delta e & \delta e \\
\delta e & \delta e & \delta e & \delta e & \delta e & \delta e \\
\delta e & \delta e & \delta e & \delta e & \delta e & \delta e \\
\end{pmatrix}
\]

And the T matrix is:

\[
\begin{pmatrix}
\chi e & \chi e & \chi e & \chi e & \chi e & \chi e \\
\chi e & \chi e & \chi e & \chi e & \chi e & \chi e \\
\chi e & \chi e & \chi e & \chi e & \chi e & \chi e \\
\chi e & \chi e & \chi e & \chi e & \chi e & \chi e \\
\chi e & \chi e & \chi e & \chi e & \chi e & \chi e \\
\chi e & \chi e & \chi e & \chi e & \chi e & \chi e \\
\end{pmatrix}
\]

(B.9)
Appendix C

Zernike Modes

Optical wavefront can be represented by a 2D surface over the aperture. The deviation from a flat surface is the wavefront error sensed by the Shack-Hartmann wavefront sensor. The infinite-series representation of the wavefront by Zernike polynomials is given by:

$$\Phi(r, \theta) = A_{00} + \frac{1}{\sqrt{2}} \sum_{n=2}^{\infty} A_{n0} \mathcal{R}_n^0 \left( \frac{r}{R} \right) + \sum_{n=1}^{\infty} \sum_{m=1}^{n} (A_{nm} \cos(m\theta) + B_{nm} \sin(m\theta)) \mathcal{R}_n^m \left( \frac{r}{R} \right)$$

(C.1)

where $A_{nm}$ and $B_{nm}$ are the coefficients for the radial polynomials and azimuthal polynomials describing the wavefront. The radial polynomials $\mathcal{R}$ as the function of radial variable $r$ over a circle of radius $R$ are:

$$\mathcal{R}_n^m \left( \frac{r}{R} \right) = \sum_{s=0}^{n-m} (-1)^s \frac{(n-s)!}{s! \left( \frac{n+m}{2} - s \right)! \left( \frac{n-m}{2} - s \right)!} \left( \frac{r}{R} \right)^{n-2s}$$

(C.2)

In practice, wavefront reconstruction can be performed based on the array of tilts with respect to a reference flat surface. In the setup, wavefronts of the laser beam were manipulated by the deformable membrane mirrors, which in turn translated into distinct axial focusing.

The use of deformable mirrors permits corrections to the potential aberrations, including coma, astigmatism, and tilt that may limit the full range of axial focusing. By actively controlling the form of the mirrors to remove such optical aberrations, the multimodal nonlinear responses were recorded with the aid of photomultiplier tubes and photon-counting electronics to determine the relative fitness of a mirror shape.