Optical and Structural Characterization of Natural Nanostructures

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The picture on the front page shows the scarab beetle *Chrysina gloriosa* and the background is an optical microscopy image form its exoskeleton.

During the course of research underlying this thesis, Lía Fernández del Río was enrolled in Agora Materiae and Forum Scientium, multidisciplinary doctoral programs at Linköping University, Sweden.

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Ami familia

COLORS are the smile of Nature
Leigh Hunt

Abstract

The spectacular biodiversity of our planet is the result of millions of years of evolution. Over this time animals and plants have evolved and adapted to different environments, developing specific behavioral and physical adaptations to increase their chances of survival. During the last centuries human's curiosity has pushed us to study and understand the phenomena and mechanisms of the nature that surrounds us. This understanding has even led to the fields of biomimetics where we seek solutions to human challenges by emulating nature.

Scarab beetles (from the insect family *Scarabaeidae*) have fascinated humans for centuries due to the brilliant metallic shine of their chitin-rich exoskeletons and more recently for their ability to polarize reflected light. This doctoral thesis focuses on the optical characterization of the polarized reflected light from beetles in the *Chrysina* genus, although beetles from other genera also have been investigated. All the *Chrysina* beetles studied here share one characteristic, they all reflect left-handed near-circular polarized light. In some cases we also detect right-handed polarized light.

We have observed two different main behaviors among the studied *Chrysina* beetles. Those which are green-colored scatter the reflected polarized light, whereas those with metallic appearance are broadband specular reflectors. We present a detailed analysis of the optical properties with Mueller-matrix spectroscopic ellipsometry combined with optical- and electron-microscopy studies of the exoskeletons. This allow us to create a model that reproduces the optical properties of these structures. The model consists of a chiral (helicoidal) multilayer structure with a gradual change of the pitch and a constant rotation of the optic axis of the layers.

Beetles are not alone to have polarizing structures in nature and it is known that many birds and insects have the ability to detect linearly polarized light. This raises the question of whether the polarization properties of the beetles are the direct or indirect results of evolution or just pure coincidence. In order to get a better understanding of the possible reasons of this particular ability, we present a simulation study of different possible scenarios in nature where incoming light could be polarized or unpolarized, and where we consider detectors (eyes) sensitive to different states of polarized light. If the beetles are able to use this characteristic for camouflage, to confuse predators or for intraspecific communication is, however, still unknown and requires further investigation.

My research results provide deeper understanding of the properties of light reflected on the beetle's exoskeleton and the nanostructures responsible for the polarization of the reflected light. The developed model could be used as bio-inspiration for the fabrication of novel nano-optical devices. My results can also complement biological behavioral experiments aiming to understand the purposes of this specific optical characteristics in nature.

Populärvetenskaplig sammanfattning

Under de senaste århundradena har människans nyfikenhet drivit oss att studera och förstå biologiska fenomen och mekanismer. Detta har även lett till utvecklingen av så kallad biomimetik där vi söker lösningar på mänskliga problem genom att efterlikna naturen.

Skalbaggar från familjen bladhorningar (Scarabaeidae) har fascinerat människor i många år på grund av de ofta har metallglänsande skal och redan i forntida Egypten användes de som smycken och amuletter. Numera är deras förmåga att reflektera polariserat ljus en orsak till att många forskare visar ett stort intresse, framförallt på grund av att dessa optiska effekter inte är så vanliga i naturen.

Att ljuset är polariserat betyder att ljusvågor rör sig i samma plan. Polariserat ljus kan bli linjärt, elliptiskt eller cirkulärt. Å andra sidan, i opolariserat ljus, som solljus, är ljusvågor spridda och rör sig i många plan. Polariserat ljus används i, exempelvis, LCD-skärmar, 3D filmer och polariserade solglasögon.

Mina forskningsresultat ger en djupare förståelse av egenskaperna av ljuset som skalbaggar reflekterar. Dessutom ges en detaljerad beskrivning av den underliggande nanostruktur som ansvarar för polarisationsfenomenen. Mina resultat kan också komplettera biologisk beteendeforskning med syfte att förstå de specifika optiska egenskapernas roll i naturen.

Min forskning fokuserar på optisk karakterisering av det reflekterade ljuset från skalbaggar av släktet *Chrysina* från Centralamerika. Det reflekterade ljuset kan för vissa infallsvinklar och ljusvåglängder bli i det närmaste cirkulärpolariserat. Oftast är detta ljus vänsterpolariserat.

Skalbaggar är inte de enda djur som har polariserande strukturer i naturen. Fjärilar reflekterar till exempel linjär-polariserat ljus. Dessutom har många fåglar och insekter också förmågan att detektera linjärt polariserat ljus. Detta väcker frågan om skalbaggaras polarisationsegenskaper är ett resultat av evolution eller bara en ren tillfällighet. För att få en bättre förståelse för de möjliga orsakerna gjorde vi därför en simuleringsstudie av olika möjliga scenarier i naturen, där det inkommande ljuset var polariserat eller opolariserat, och detektorerna (ögonen) var känsliga för olika polarisationstillstånd.

I mina studier observerar jag två typer av reflektion hos de skalbaggar som studerats. Skalbaggar som är grönfärgade sprider det reflekterade ljuset medan skalbaggar med metalliskt utseende är mer spegelreflekterande. I forskningen används Mueller-matrisellipsometri, en avancerad teknik som ger en detaljerad analys av skalets optiska egenskaper. Denna teknik har kombinerats med mikroskopi. Detta tillåter oss att skapa en modell som återger de optiska egenskaperna hos strukturerna. Modellen består av en vriden flerskiktstruktur med en gradvis förändrad stigning. Studierna kan därmed användas som biomimetisk inspiration, t. ex. för tillverkning av nya nano-optiska komponenter.

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Part I

Introduction

Chapter 1

Background

Beetles from the *Scarabaeidae* family have fascinated humans for centuries due to the metal-like shine of their exoskeleton. Already in the ancient Egypt these, so called scarabs, were used as jewelry and amulets. However, it was not until the early 20th century that they become subject of interest for scientists when the Nobel laureate A. A. Michelson noticed the polarizing properties of these beetles. In particular he claimed that the jewel scarab *Chrysina* (then *Plusiotis*) *resplendens* reflected circularly polarized light [1].

The structural color and polarization properties observed in some beetles are due to a helicoidal multilayer structure in the beetles exoskeleton [2]. This discovery was possible with the invention of the electron microscope in the mid 1900's. The exoskeleton or cuticle consists of planes of microfibrils which rotate progressively to form helicoidal structures known as Bouligand structures [3, 4]. Recently, there has been a growing interest on the polarization properties of these helicoidal structures [5, 6]. This has lead to the creation of models of the structure that reproduce its optical response [7] and to biomimetic fabrication of similar structures [8].

Beetles are not the only animals which have polarizing structures in nature. Butterflies, shrimps and squids, to name some, also polarize light reflected on their bodies. However, there are rather few examples of the generation of circularly polarized light by animals and beetles are here an exception. Moreover, it is known that many birds and insects have the ability to detect polarized light. However, whether beetles use the polarized reflected light as intraspecific communication, as a defense mechanism, or if it has no purpose at all, is still under debate.

In this work we apply spectroscopic Mueller-matrix ellipsometry to present a detailed analysis of the polarization properties of light reflected on several beetles. Analysis of the ellipsometric data provide us with structural details such as thickness of the layers that are corroborated with electron microscopy. The measurements and images suggest that the helicoidal nano-structure and a gradient in the pitch are responsible for the polarization characteristics of the reflected light. This is confirmed by a model that we have developed that reproduces such structure and its optical response.

An analysis of the polarization properties of light reflected from such beetles when illuminated with polarized light is also presented. This analysis is helpful to understand how the beetles are seen in their environment by other animals with vision sensitive to polarized light. Together with behavioral experiments, this analysis could help to find out the evolutionary role of the polarization of the beetle's exoskeleton.

The results presented in this work can be used as inspiration for the fabrication of multi-functional materials [9]. A similar structure built with similar materials, like chitin or cellulose, would have long-lasting structural coloration, high reflectance and metal-like coloration. Besides, it would have the described complex polarization properties. Moreover, it could present interesting mechanical properties, super-hydrophobic and anti-adhesion characteristics, low weight and high strength [10].

Chapter 2

Theory

This chapter is a brief introduction to the theory of light and its interaction with matter as well as different representations of polarized light.

2.1 Optical parameters

In many studies of light propagation, a purely scalar approach is often sufficient. This work is, however, centered around the studies of materials with optically anisotropic properties and their relation to polarized light, that is, in this approach the vectorial nature of light has to be considered. Light is then described as an electromagnetic wave traveling in space and time and is a solution to Maxwell's equations [11]. Maxwell's equations may be written as

$$\nabla \cdot \mathbf{D} = \rho \quad (2.1)$$

$$\nabla \cdot \mathbf{B} = 0 \quad (2.2)$$

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t} \quad (2.3)$$

$$\nabla \times \mathbf{H} = \mathbf{J} + \frac{\partial \mathbf{D}}{\partial t} \quad (2.4)$$

where $\mathbf{D}$ and $\mathbf{B}$ are the electric and magnetic flux densities, respectively, and $\mathbf{E}$ and $\mathbf{H}$ are the electric and magnetic field intensities, respectively. ρ is the volume charge density and $\mathbf{J}$ is the current density [12].

The *constitutive equations* relate the flux densities and the field intensities in a material in terms of the electric permittivity tensor, ε , the electric permeability tensor, μ , and the permittivity and permeability of vacuum, ε_0 and μ_0 , respectively

$$\mathbf{B} = \mu_0 \mu \mathbf{H} \quad (2.5)$$

$$\mathbf{D} = \varepsilon_0 \varepsilon \mathbf{E}. \quad (2.6)$$

In this study $\mu = 1$ since, for most materials, it does not differ much from its vacuum value at optical frequencies [12]. The linear optical properties of a material can then be described by the permittivity tensor

$$\varepsilon = \begin{bmatrix} \varepsilon_{xx} & \varepsilon_{xy} & \varepsilon_{xz} \\ \varepsilon_{yx} & \varepsilon_{yy} & \varepsilon_{yz} \\ \varepsilon_{zx} & \varepsilon_{zy} & \varepsilon_{zz} \end{bmatrix} \quad (2.7)$$

where each component, ε_{ij} , is a complex-valued quantity that relates how the medium responds to an external electric field. In the absence of optical activity the permittivity tensor is symmetrical. In this case it is possible to use Euler rotation to transform ε into a diagonal tensor. The *constitutive equation* (Eq. 2.6) will then be

$$\begin{bmatrix} D_x \\ D_y \\ D_z \end{bmatrix} = \varepsilon_0 \begin{bmatrix} \varepsilon_x & 0 & 0 \\ 0 & \varepsilon_y & 0 \\ 0 & 0 & \varepsilon_z \end{bmatrix} \begin{bmatrix} E_x \\ E_y \\ E_z \end{bmatrix}. \quad (2.8)$$

In the case of no absorption the permittivities will be real-valued. Thus, only three coefficients, ε_x , ε_y and ε_z , are needed to describe a non-absorbing anisotropic medium. The permittivities are related to the refractive indices n_x , n_y and n_z by

$$\varepsilon_i = n_i^2 \quad \text{with } i = x, y \text{ or } z. \quad (2.9)$$

Depending on the values of these coefficients optical media can be classified as isotropic, uniaxial anisotropic or biaxial anisotropic as described in Table 2.1. In the case of an uniaxial anisotropic media n_o and n_e are the ordinary and the extraordinary refractive indices, respectively.

Media	Permittivity	Refractive index
Isotropic media $\varepsilon_x = \varepsilon_y = \varepsilon_z = \varepsilon$	$\begin{bmatrix} \varepsilon_x & 0 & 0 \\ 0 & \varepsilon_x & 0 \\ 0 & 0 & \varepsilon_x \end{bmatrix}$	$\begin{bmatrix} n_x^2 & 0 & 0 \\ 0 & n_x^2 & 0 \\ 0 & 0 & n_x^2 \end{bmatrix}$
Uniaxial anisotropic media $\varepsilon_x = \varepsilon_y \neq \varepsilon_z$	$\begin{bmatrix} \varepsilon_x & 0 & 0 \\ 0 & \varepsilon_x & 0 \\ 0 & 0 & \varepsilon_z \end{bmatrix}$	$\begin{bmatrix} n_o^2 & 0 & 0 \\ 0 & n_o^2 & 0 \\ 0 & 0 & n_e^2 \end{bmatrix}$
Biaxial anisotropic media $\varepsilon_x \neq \varepsilon_y \neq \varepsilon_z$	$\begin{bmatrix} \varepsilon_x & 0 & 0 \\ 0 & \varepsilon_y & 0 \\ 0 & 0 & \varepsilon_z \end{bmatrix}$	$\begin{bmatrix} n_x^2 & 0 & 0 \\ 0 & n_y^2 & 0 \\ 0 & 0 & n_z^2 \end{bmatrix}$

Table 2.1: Definitions of isotropic and anisotropic media in terms of permittivities and refractive indices.

The permittivity of absorbing media is described by a complex permittivity $\varepsilon = \varepsilon_1 + i\varepsilon_2$ from which also a complex refractive index N is defined as

$$\varepsilon = N^2 \quad \text{where} \quad N = n + ik \quad (2.10)$$

and k is the extinction coefficient [13]. The extinction coefficient relates to the absorption coefficient α and the wavelength λ as

$$k = \frac{\lambda}{4\pi} \alpha. \quad (2.11)$$

2.2 Polarized light

We have seen that light can be described by the electric field vector $\mathbf{E}$. The polarization of light is then described by analyzing the components of $\mathbf{E}$ in the plane perpendicular to the direction of propagation [14]. Considering a wave traveling in the z -direction of a *Cartesian coordinate system*, $\mathbf{E}$ varies in time and space according to

$$\mathbf{E}(z, t) = \mathbf{E}_x(z, t) + \mathbf{E}_y(z, t) = \begin{bmatrix} E_x e^{i(qz - \omega t + \delta_x)} \\ E_y e^{i(qz - \omega t + \delta_y)} \end{bmatrix} \quad (2.12)$$

where $\mathbf{E}_x$ and $\mathbf{E}_y$ are the complex-valued field components in the x - and y -directions, respectively. E_x and E_y are the amplitudes and δ_x and δ_y the phases of the components. ω is the angular frequency and $q = 2\pi N/\lambda$ is the propagation constant where N is the complex refractive index and λ the wavelength.

The state of polarization of light is determined only by the phase difference and relative amplitudes. Therefore the field representation can be simplified and written as

$$\mathbf{E} = \begin{bmatrix} \mathbf{E}_x \\ \mathbf{E}_y \end{bmatrix} = \begin{bmatrix} E_x e^{i\delta_x} \\ E_y e^{i\delta_y} \end{bmatrix}, \quad (2.13)$$

which is known as the *Jones vector* introduced by R. Clark Jones [15] for the purpose of simplifying calculations.

Let us determine some polarization states as an example. Figure 2.1a represents linearly polarized light traveling in the z -direction and with the electric field oscillating along the y -direction. In this case $E_x = 0$ and $E_y = A$, where A is a constant. In the absence of an E_x component the phase δ_y is set to zero for convenience. The corresponding Jones vector is then

$$\mathbf{E} = \begin{bmatrix} 0 \\ A \end{bmatrix} = A \begin{bmatrix} 0 \\ 1 \end{bmatrix}. \quad (2.14)$$

However, when only the polarization state is of interest, Jones vectors are represented in their normalized form. Therefore, with $A = 1$, the linear polarization oscillating along the y -direction is written

$$\mathbf{E} = \begin{bmatrix} 0 \\ 1 \end{bmatrix}. \quad (2.15)$$

Figure 2.1: Illustrations of different polarization states. The direction of the light propagation is along the z -axis (towards the reader).

Figure 2.1b represents linearly polarized light propagating along a plane inclined an angle α with respect to the x -axis. In this case E_x and E_y are in phase, i.e. $\delta_y - \delta_x = 0$. We also set the phases to zero, $\delta_x = \delta_y = 0$. The normalized Jones vector is in this case

$$\mathbf{E} = \begin{bmatrix} \cos \alpha \\ \sin \alpha \end{bmatrix} \quad (2.16)$$

where the inclination with respect to the x -axis depends on the value of the angle α . $\alpha = 0^\circ$ corresponds to linearly polarized light in the x - and $\alpha = 90^\circ$ to linearly polarized in the y -direction. Generally the light is linearly polarized when the phase difference $\delta_y - \delta_x$ is 0° or 180° .

For any phase difference other than 0° or 180° the light is elliptically polarized as in Fig. 2.1c and with Jones representations as in Eq. 2.13.

A special case is circular polarization when the phase difference is 90° and $E_x = E_y$, as shown in Fig. 2.1d. Right- and left-handed normalized circular polarization are represented by

$$\mathbf{E} = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ i \end{bmatrix} \quad \text{and} \quad \mathbf{E} = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ -i \end{bmatrix}, \quad (2.17)$$

respectively.

The proportion of light being polarized depends on the correlation between E_x and E_y . Light is said to be totally polarized when E_x and E_y are completely correlated and unpolarized when they are completely uncorrelated. Light can also be partially polarized depending on the degree of correlation. To characterize partially polarized light it is useful to introduce the concept of degree of polarization P

$$P = \frac{I_{pol}}{I_{tot}} \quad (2.18)$$

where I_{pol} is the irradiance of the polarized part of the wave and I_{tot} is the total irradiance.

2.3 The polarization ellipse

The polarization state of a plane wave of polarized light is characterized by four parameters, the amplitudes and phases of $\mathbf{E}_x$ and $\mathbf{E}_y$. These four parameters can be transformed into four new parameters in the polarization ellipse which is a good visualization of the different polarization states.

Considering the path traced out by the electric field $\mathbf{E}(\mathbf{r}, t)$ of an electromagnetic plane wave at a fixed xy -plane ($z = z_i$), the x - and y -components oscillate harmonically about the origin so the locus of $\mathbf{E}$ is in general an ellipse as in Fig. 2.2.

The polarization ellipse is characterized by the size and the shape. The size is specified by the total amplitude $A = (a^2 + b^2)^{1/2}$ where a and b are the major and the minor axes of the ellipse, respectively. The absolute phase δ is defined as the angle between the major axis of the ellipse and the direction of the electric field at $t = 0$. The shape of the ellipse is specified by the azimuth angle α , which defines the orientation of the ellipse, and the ellipticity e

$$e = \pm \frac{b}{a} = \pm \tan \epsilon \quad (2.19)$$

where ϵ is the ellipticity angle. The polarization is defined as right-handed with a positive ellipticity when the electric vector rotates clockwise when looking into the beam towards the source. On the other hand, it is said to be left-handed with a negative ellipticity, when the electric vector rotates counterclockwise [16].

Figure 2.2: *The polarization ellipse.*

2.4 The Stokes vector

The polarized light representations presented so far require knowledge of electric field amplitudes and phases. However, these parameters are not easy to acquire at optical frequencies because current measurement techniques, such as reflectometry and ellipsometry, measure irradiances.

Stokes realized that irradiances also could be used to describe polarization so he introduced four parameters that have the advantage of being able to represent partially polarized light [13, 16, 17]. These parameters were later introduced as elements of a column matrix called the Stokes vector. The Stokes vector is defined as

$$\mathbf{S} = \begin{bmatrix} I \\ Q \\ U \\ V \end{bmatrix} = \begin{bmatrix} I_x + I_y \\ I_x - I_y \\ I_{+45^\circ} - I_{-45^\circ} \\ I_r - I_l \end{bmatrix}. \quad (2.20)$$

The first parameter of the Stokes vector, I , represents the total irradiance of the light wave, where I_x and I_y are the irradiances for linear polarization in the x and y directions. The second parameter, Q , is the difference between the I_x and I_y irradiances. U represents the difference between the irradiances of the light wave in the $+45^\circ$ (I_{+45°) and -45° (I_{-45°) directions of the linear polarization. The last parameter V represents the difference between the irradiances of the right-circular state (I_r) and the left-circular state (I_l) of polarization.

The Stokes vectors are commonly normalized to $I = 1$. As an example, the Stokes vector of unpolarized and normalized light is

$$\mathbf{S} = \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \end{bmatrix}. \quad (2.21)$$

The Stokes vectors corresponding to the polarization states represented in Fig. 2.1 are

$$\mathbf{S}_{linear(y)} = \begin{bmatrix} 1 \\ -1 \\ 0 \\ 0 \end{bmatrix}, \quad \mathbf{S}_{linear(+45^\circ)} = \begin{bmatrix} 1 \\ 0 \\ 1 \\ 0 \end{bmatrix}, \quad \mathbf{S}_{circular} = \begin{bmatrix} 1 \\ 0 \\ 0 \\ 1 \end{bmatrix}. \quad (2.22)$$

Circular polarization is a special case in which $I_r - I_l = \pm 1$, being right-handed when V is positive and left-handed when it is negative. In the case of linear polarization the difference $I_r - I_l$ is equal to 0. If the difference $I_r - I_l$ is between 0 and ± 1 and Q and/or U are non-zero, the polarization is elliptical.

The degree of polarization can be determined from

$$P = \frac{\sqrt{Q^2 + U^2 + V^2}}{I}, \quad (2.23)$$

the degree of linear polarization as

$$P_{lin} = \frac{\sqrt{Q^2 + U^2}}{I}, \quad (2.24)$$

and finally, the degree of circular polarization as

$$P_{circ} = \frac{V}{I}. \quad (2.25)$$

2.5 The Mueller matrix

The use of Stokes vectors is a convenient way to represent partially or totally polarized light. In connection a 4×4 matrix, known as the Mueller matrix, $\mathbf{M}$, is introduced to describe optical components. The interaction of light with an optical component can then be expressed as a linear combination of the four Stokes parameters of the incident beam $\mathbf{S}_i$, and the Mueller matrix $\mathbf{M}$, expressed as $\mathbf{S}_o = \mathbf{M}\mathbf{S}_i$ or if expanded

$$\mathbf{S}_o = \begin{bmatrix} I_o \\ Q_o \\ U_o \\ V_o \end{bmatrix} = \begin{bmatrix} M_{11} & M_{12} & M_{13} & M_{14} \\ M_{21} & M_{22} & M_{23} & M_{24} \\ M_{31} & M_{32} & M_{33} & M_{34} \\ M_{41} & M_{42} & M_{43} & M_{44} \end{bmatrix} \begin{bmatrix} I_i \\ Q_i \\ U_i \\ V_i \end{bmatrix}. \quad (2.26)$$

The Mueller matrix is commonly normalized to the element M_{11} ($m_{ij} = \frac{M_{ij}}{M_{11}}$, $i, j = 1, 2, 3, 4$).

In the particular case of unpolarized incident light with $\mathbf{S}_i$ given by Eq. 2.21, the Stokes vector of the outgoing beam $\mathbf{S}_o$ corresponds to the first column of the Mueller matrix as seen from

$$\begin{bmatrix} I_0 \\ Q_0 \\ U_0 \\ V_0 \end{bmatrix} = \begin{bmatrix} 1 & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{bmatrix} \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \end{bmatrix} = \begin{bmatrix} 1 \\ m_{21} \\ m_{31} \\ m_{41} \end{bmatrix}. \quad (2.27)$$

The vector $\mathbf{P} = [m_{21}, m_{31}, m_{41}]^T$, where T indicates transpose, is called the polarizance vector and shows the capability of a system to polarize unpolarized light [16].

Finally, we give two examples of $\mathbf{M}$. The Mueller matrix representing an ideal reflecting surface like an ideal mirror is

$$\mathbf{M} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix} \quad (2.28)$$

and an ideal left-handed circular polarizer is represented by

$$\mathbf{M} = \frac{1}{2} \begin{bmatrix} 1 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 \end{bmatrix}. \quad (2.29)$$

Notice that $\mathbf{M}$ in Eq. 2.29 is not normalized as we have included a prefactor $\frac{1}{2}$ to indicate that 50% of unpolarized light is (reflected or transmitted). Optical systems can also be represented as a combination of Mueller matrices [13].

Chapter 3

Reflection and transmission characteristics

In this chapter the properties of light reflected and transmitted on isotropic and anisotropic materials, as well as some mechanisms for the generation of polarized light are described.

3.1 Basic reflection and transmission characteristics

3.1.1 Reflection at a plane isotropic surface

Lets assume that a wave traveling in an isotropic medium with complex refractive index N_0 is incident at an angle θ_0 to the surface normal of another isotropic medium of complex refractive index N_1 ¹. The angle of refraction θ_1 is obtained from Snell's law

$$N_0 \sin \theta_0 = N_1 \sin \theta_1. \quad (3.1)$$

An unpolarized incident electric field can be expressed as two vectors with complex-valued amplitudes E_p and E_s , being parallel and perpendicular to the plane of incidence, respectively. For an interface between two materials boundary conditions require a continuity of the tangential components of the amplitude of the electric field $E_{is} + E_{rs} = E_{ts}$, where the sub-indices i , r and t represent the incident, reflected and transmitted fields, respectively. In addition the tangential magnetic fields must be continuous. Based on these two requirements it is possible to derive reflection and transmission coefficients for the interface as a function of N_0 , N_1 , θ_0 and θ_1 . They are usually referred to as the *Fresnel equations* for

¹If the ambient medium is air, the refractive index is normally considered to be realvalued.

reflection and transmission. For s -polarized light we have

$$r_s = \frac{E_{rs}}{E_{is}} = \frac{N_0 \cos \theta_0 - N_1 \cos \theta_1}{N_0 \cos \theta_0 + N_1 \cos \theta_1} \quad (3.2)$$

$$t_s = \frac{E_{ts}}{E_{is}} = \frac{2N_0 \cos \theta_0}{N_0 \cos \theta_0 + N_1 \cos \theta_1}. \quad (3.3)$$

In a similar way, the reflection and transmission equations for p -polarized light are

$$r_p = \frac{E_{rp}}{E_{ip}} = \frac{N_1 \cos \theta_0 - N_0 \cos \theta_1}{N_1 \cos \theta_0 + N_0 \cos \theta_1} \quad (3.4)$$

$$t_p = \frac{E_{tp}}{E_{ip}} = \frac{2N_0 \cos \theta_0}{N_1 \cos \theta_0 + N_0 \cos \theta_1}. \quad (3.5)$$

The reflectance R and transmittance T for p - and s -polarized light are defined by

$$R_p = |r_p|^2 \quad T_p = \frac{n_1 \cos \theta_1}{n_0 \cos \theta_0} |t_p|^2 \quad (3.6)$$

$$R_s = |r_s|^2 \quad T_s = \frac{n_1 \cos \theta_1}{n_0 \cos \theta_0} |t_s|^2. \quad (3.7)$$

The Brewster angle

There is a particular angle of incidence where the reflectance in the p -direction has a minimum. For a non-absorbing material, this minimum value is zero, that is, the p -component of the electric field is not reflected at all. The reflected light is then linearly polarized in the s -direction as shown in Fig. 3.1. This angle is known as the Brewster angle

$$\theta_B = \arctan \frac{n_1}{n_0} \quad (3.8)$$

where n_0 is the refractive index of the ambient and n_1 the refractive index of the material [16].

3.1.2 Transmission through anisotropic media

As mention in section 2.1 anisotropic materials can be uniaxial or biaxial. The material is uniaxial when $\varepsilon_x = \varepsilon_y \neq \varepsilon_z$ and has two complex refractive indices, N_e and N_o . The z -direction is called the optic axis and the complex refractive index in this direction is named the extraordinary index $N_e = n_e + ik_e$. The complex refractive indices in the x - and y -directions are the same and are called the ordinary index $N_o = n_o + ik_o$.

Figure 3.1: When unpolarized incoming light is incident at the Brewster angle, the reflected beam is linearly polarized in the s-direction.

Linear birefringence

When we consider the refractive indices n_o and n_e , materials are said to exhibit birefringence which can be quantified by $\Delta n = n_e - n_o$.

The velocity of light in a material depends on the refractive index as $v = c/n$, where c is the vacuum speed of light. Light propagating in an anisotropic material with $\mathbf{E}$ perpendicular to the optic axis propagates at a speed $v = c/n_o$ and light propagating with $\mathbf{E}$ parallel to the optic axis with a speed $v = c/n_e$. The axes are named the fast axis (the one with lower refractive index) and the slow axis (the one with higher refractive index). This effect can be observed in Fig. 3.2 that shows a birefringent crystal of calcite on top of a text. The text appears twice due to the double refraction which happens because of the two different indices of refraction. Linear birefringent materials can be used to generate circularly polarized light with $\lambda/4$ wave plates and are used in prism polarizers [16].

Linear dichroism

The previous cases of anisotropy considered non-absorbing media. In the case of anisotropic absorbing media the permittivity tensor has complex-valued elements with different absorption. This is due to the type of crystal or to the molecular structure of the material. This phenomenon is known as linear dichroism.

Linear dichroic media have different extinction coefficients for perpendicular directions and light transmitted through such materials becomes polarized. The extinction coefficients of the medium for light polarized parallel and perpendicular to the optic axis are k_e and k_o , respectively. The dichroism of the medium can be quantified by $\Delta k = k_e - k_o$. Such materials are used for the fabrication of sheet polarizers like Polaroids [16].

Figure 3.2: *Calcite is a uniaxial anisotropic material.*

Circular birefringence - Optical activity

Certain materials cause a rotation of the $\mathbf{E}$ -field of incident linear polarization. That means that linearly polarized light undergoes a rotation when propagating in such materials. The phenomenon is called circular birefringence. These materials are called optically active and are called dextrorotatory if the rotation is clockwise when looking into the beam (towards the source) or levorotatory if the rotation is counter-clockwise. In an optical active material the speed of propagation of circularly polarized light is different depending on if the light is right- or left-handed polarized. Therefore, the material can be described as having two indices of refraction, n_r and n_l , for the right- and left-handed polarization, respectively. Circular birefringence can be quantified by $\Delta n = n_r - n_l$. The angle of rotation of linearly polarized light transmitted through a circular birefringent material of thickness d is [16]

$$\theta = \frac{\pi d}{\lambda} (n_r - n_l). \quad (3.9)$$

Circular dichroism

Circular dichroism implies different absorption of right- and left-handed circularly polarized light. This phenomenon is due to chiral molecules or crystals in an absorbing anisotropic medium. The extinction coefficients for right- and left-handed polarized light of the medium are k_r and k_l , respectively. It can be detected in the absorption bands of optically active molecules and is observed in biological molecules due to their dextro- and levo-rotatory components. Circular dichroism gives a representative structural signature and is widely used in modern biochemistry [16].

3.2 Reflection and transmission from multilayer structures

In this section we will take a closer look at layered structures including the helicoidal structure under study in the main part of this work.

3.2.1 Isotropic multilayer structures

From Fresnel's reflection and transmission coefficients describing a single interface (Eqs. 3.2-3.5) it is possible to derive reflection and transmission coefficients for more complex structures having one or more layers with different optical properties and/or thicknesses.

In all the cases below we consider an ambient with refractive index $N_0 = n_0$, a number of layers of refractive index N_i ($i = 1, 2, 3, \dots, m$) and at the bottom, the substrate, with refractive index $N_{m+1} = n_{m+1} + ik_{m+1}$. A plane wave is incident at an angle of incidence θ_0 . Due to the interaction of the wave with the thin film structure there will be a reflected and a transmitted wave. The reflection coefficients for the p - and s -polarizations are denoted r_p and r_s , respectively, and the transmission coefficients for the p - and s -polarizations are denoted t_p and t_s , respectively. The Fresnel coefficients of the interface between media i and j are r_{ijp} , r_{ijs} , t_{ijp} and t_{ijs} .

A single isotropic layer of refractive index N_1 on a substrate can be shown [16] to have the reflection and transmission coefficients

$$r_p = \frac{r_{01p} + r_{12p}e^{i2\beta}}{1 + r_{01p}r_{12p}e^{i2\beta}} \quad t_p = \frac{t_{01p}t_{12p}e^{i2\beta}}{1 + r_{01p}r_{12p}e^{i2\beta}} \quad (3.10)$$

$$r_s = \frac{r_{01s} + r_{12s}e^{i2\beta}}{1 + r_{01s}r_{12s}e^{i2\beta}} \quad t_s = \frac{t_{01s}t_{12s}e^{i2\beta}}{1 + r_{01s}r_{12s}e^{i2\beta}} \quad (3.11)$$

where β is the film phase thickness and corresponds to the phase shift of a wave when it transverses a film with thickness d

$$\beta = \frac{2\pi d}{\lambda} N_1 \cos \theta_1 \quad (3.12)$$

where θ_1 is the angle of refraction determined by Snell's law.

The reflection and transmission coefficients for more layers can be derived through recursion but the procedure will be increasingly cumbersome with the addition of more layers.

More effective is to use matrix algebra to yield expressions for multilayer structures. A common approach is to describe each layer as well as each interface with a 2×2 matrix [18]. A scattering matrix $\mathbf{S}$ for the system is then obtained by a multiplication of layer matrices $\mathbf{L}$ and interface matrices $\mathbf{I}$ according to

$$\mathbf{S} = \mathbf{I}_{01}\mathbf{L}_1\mathbf{I}_{12}\dots\mathbf{L}_m\mathbf{I}_{m,m+1} = \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix} \quad (3.13)$$

where $\mathbf{I}_{ij}$ is the matrix for interface $i - j$ defined by

$$\mathbf{I}_{ij} = \begin{pmatrix} 1 \\ \frac{1}{t_{ij}} \end{pmatrix} = \begin{bmatrix} 1 & r_{ij} \\ r_{ij} & 1 \end{bmatrix} \quad (3.14)$$

and $\mathbf{L}_i$ is the matrix for layer i defined by

$$\mathbf{L}_i = \begin{bmatrix} e^{-i\beta} & 0 \\ 0 & e^{i\beta} \end{bmatrix}. \quad (3.15)$$

The formalism is applied independently on p - and s -polarization and the total reflection and transmission response is then finally obtained from the four elements of $\mathbf{S}$ as

$$r_p = \frac{S_{21p}}{S_{11p}} \quad t_p = \frac{1}{S_{11p}} \quad (3.16)$$

$$r_s = \frac{S_{21s}}{S_{11s}} \quad t_s = \frac{1}{S_{11s}}. \quad (3.17)$$

The technique is well suited for computer code and is implemented in the software used in this work.

A special case is a periodic multilayer where a sequence of layers (often two) are repeated several times through out the structure. In this way the multilayer with a period Λ forms a one-dimensional analogue to a crystal with a certain lattice constant. Thus, wave propagation in the periodic multilayer can be compared to electron motion in a crystal and can be treated using concepts from Bloch wave theory.

For instance, periodic multilayers of two transparent materials, A and B , with thicknesses d_A and d_B and two different refractive indices, n_A and n_B , respectively will give a first order reflectance peak with a center wavelength of approximately [19]

$$\lambda = 2(n_A d_A \cos \theta_A + n_B d_B \cos \theta_B) \quad (3.18)$$

where θ_A and θ_B are the angles of refraction in material A and material B , respectively. The period will in this case be $\Lambda = d_A + d_B$.

In analogy with crystal reflections of x-rays such a multilayer is called a Bragg reflector and can be designed to give very low reflectance (anti-reflecting) or high reflectance, often using layers with an optical thickness equal to a quarter of the desired peak wavelength (the Bragg wavelength). There are several examples from nature where multilayer stacks of organic materials are highly reflecting [19].

3.2.2 Anisotropic multilayer structures

Reflection and transmission coefficients for structures with anisotropic materials require even more laborious derivations compared to the isotropic case. Even cases with few layers of uniaxial materials with the optic axis oriented to keep the reflection matrix diagonal (i.e. $r_{ps} = r_{sp} = 0$) give many possible cases, each demanding a thorough derivation. In relation to this work the case where the layers are uniaxial with the optic axis in the plane of the layers is of importance.

Just as in the isotropic case, reflection and transmission coefficients, can be derived for one, two or more anisotropic layers. Besides stacking layers with different materials, the anisotropy also allows for the possibility to use only one material but with a change of the orientation of the optic axis for adjacent layers. The optic axis can for instance rock back and forth or change in constant angular steps through the stack of layers. Both designs are used in the so called Šolc filters [20].

The latter case is represented in the chiral nematic (cholesteric) liquid crystals, the chiral version of the nematic phase. Rod-like molecules are here organized in parallel pseudo-planes defined by a director (optical axis) that rotates from layer to layer resulting in a helicoidal structure. The period of this rotation, i.e. the pitch Λ , is the distance along the rotation axis for which the director of the pseudo-planes has completed a 360° rotation (Fig. 3.3).

Figure 3.3: *Crystal organization in a cholesteric liquid crystal. Λ is the pitch.*

Light incident on such structures at normal incidence is reflected circularly polarized with the same handedness as the helicoidal structure and transmitted circularly polarized with the opposite handedness. The reflected and transmitted light at oblique incidence are elliptically polarized. The reflection occurs when circularly polarized modes cannot propagate inside the liquid crystal. This selective reflection is called circular Bragg reflection [21]. The center of the Bragg region can be approximately calculated by

$$\lambda = n_{av}\Lambda \cos \theta' \quad (3.19)$$

where n_{av} is the average refractive index of the ordinary and extraordinary directions and θ' is the angle of refraction.

The chiral twist can be right- or left-handed depending on the chiral elements of the molecule. However, the handedness of the macroscopic chirality is independent of that of the molecule itself. Depending on the direction of rotation of the director the helicoidal structure is right- or left-handed [22].

This structure has many resemblances with the natural chitin-proteins structures studied in this work [23] and is used as a starting configuration for modeling of cuticle structures. The exocuticle of the studied beetles is an anisotropic solid/solid multilayer structure where the rotation of the optic axis of the layers is the result of the birefringence of the chitin-protein nanofibrils that form the structure. The high reflectance observed in some beetles is due to both a large number of layers in their exoskeleton and a relative high birefringence. The latter property has a strong influence on the penetration depth of the selectively reflected mode [24].

Chapter 4

Ellipsometry

Ellipsometry proves to be a very appropriate technique for the study of the optical properties and the structure of the beetle's exoskeleton due to the fact that it is a non-destructive technique giving complete optical information. This is very important since some of the specimens studied are loans from museums and thus it is very important to preserve them. This chapter provides a description of the ellipsometry technique and the analysis of ellipsometric data.

4.1 Ellipsometry

Ellipsometry is a non-destructive technique used for optical and structural characterization of thin films, surfaces and interfaces. The technique is based on the analysis of the polarization changes in a light beam when reflected on (or transmitted through) a sample. The polarization state of the incident beam is known and by comparing this with the polarization of the reflected (or transmitted) light, it is possible to determine the optical properties of the sample [25]. We will focus on reflection-based ellipsometry since only reflection measurements have been performed in this work.

The basic property measured in ellipsometry is the ratio

$$\rho = \frac{\chi_r}{\chi_i} \quad (4.1)$$

where χ_r and χ_i are complex-number representations of the polarization states of the reflected and incident beam, respectively. χ_r and χ_i can be defined in a Cartesian coordinate system where the p - and s -directions are parallel and perpendicular to the plane of incidence, respectively. The definition of χ is

$$\chi = \frac{E_p}{E_s} \quad (4.2)$$

where E_p and E_s are the complex-valued representations of electric fields in the p - and s -directions, respectively. As mentioned in Sec. 3.1.1 the reflection coefficient for a sample is defined as the ratio between the reflected and incident fields including both amplitude and phase. In the general case there are four coefficients r_{ij} ($i = p, s$ and $j = p, s$). However, for light reflected from an optically isotropic sample or an uniaxial anisotropic sample with its optic axis normal to the surface, no coupling occurs between the orthogonal p - and s -polarizations ($r_{ps} = r_{sp} = 0$). The remaining reflection coefficients $r_{pp} = r_p$ and $r_{ss} = r_s$ are written

$$r_p = \frac{E_{rp}}{E_{ip}} \quad \text{and} \quad r_s = \frac{E_{rs}}{E_{is}}. \quad (4.3)$$

With $\chi_r = E_{rp}/E_{rs}$ and $\chi_i = E_{ip}/E_{is}$, Eq. 4.1 then expands to

$$\rho = \frac{E_{rp}}{E_{rs}} \frac{E_{is}}{E_{ip}} = \frac{r_p}{r_s} = \tan \Psi e^{i\Delta} \quad (4.4)$$

which gives the relation between the sample properties r_p and r_s and the experimental data Ψ and Δ . The measured parameters Ψ and Δ are known as the *ellipsometric parameters* [25].

The ellipsometric parameters Ψ and Δ depend on optical and structural properties of the sample and in addition on the wavelength and angle of incidence. In the case of a thin film with optical constants n , k and thickness d this can for each chosen wavelength and angle of incidence be written as

$$\rho = \tan \Psi e^{i\Delta} = f(n, k, d). \quad (4.5)$$

For anisotropic samples the Jones formalism can be applied. Then it is necessary to measure at least three polarization changes at three different polarizations of the incident beam to characterize the sample. However, depolarizing samples require a more advanced methodology. In this case the use of Mueller-matrix formalism in Section 2.5 is appropriate [13].

4.2 Mueller-matrix ellipsometry

In this work a Mueller-matrix ellipsometer has been used. The ellipsometer has dual rotating compensators which makes it possible to control the polarization state $\mathbf{S}_i$ of the incident light and to determine the polarization state $\mathbf{S}_o$ of the reflected light. In this way the 15 elements of the normalized Mueller matrix of the sample can be determined.

The configuration of this type of ellipsometer is shown in Fig. 4.1. Unpolarized light generated by the light source passes first through a polarization state generator, composed of a polarizer and a rotating compensator. This generates polarized light that can be expressed by the Stokes vector $\mathbf{S}_i = [I_i, Q_i, U_i, V_i]^T$. Light is then reflected on the sample at an angle of incidence θ and directed through a polarization state detector, composed of another rotating compensator and a polarizer, in this case called an analyzer. This detector determines the Stokes vector $\mathbf{S}_o = [I_o, Q_o, U_o, V_o]^T$ of the reflected beam and therefore the polarization state of the reflected light is known. The 15 elements of a normalized Mueller matrix can be determined from the relation $\mathbf{S}_o = \mathbf{M}\mathbf{S}_i$.

Figure 4.1: *Schematic illustration of the configuration of a dual rotating compensator ellipsometer.*

For a non-depolarizing sample the Mueller-matrix elements can be shown to be a function of the reflection coefficients r_{pp}, r_{ss}, r_{ps} and r_{sp} where r_{pp} and r_{ss} are the ordinary reflection coefficients for p - and s -polarization, respectively [16]. The coefficient r_{sp} is non zero if mode coupling occurs, i.e. incident s -polarization contributes to reflected p -polarization and similar for r_{ps} . Such mode coupling occurs for samples with in-plane anisotropy like a chiral beetle cuticle. For depolarizing samples the m_{ij} elements become more complex as they will be functions of several contributions to the change in state and degree of polarization.

4.3 Measurements

The instrument used in the study was a dual rotating-compensator ellipsometer (RC2, J. A. Woollam Co., Inc.). The measurements were performed on the scutellum, a triangular area between the head and the wing covers, or on the elytra which are the wing covers (Fig. 4.2). The use of focusing probes reduced the beam size to around $50\text{ }\mu\text{m}$. Measurements were performed in the spectral range 245 to 1000 nm. Two kind of measurements were performed where specular reflected or scattered light were measured. The specular reflected light was measured at angles of incidence from 20° to 75° , in steps of 5° . The scattered light was measured by fixing the angle of incidence at 45° and varying the detector position $\pm 15^\circ$ in steps of 3° . The acquisition time was 30 s for all the measurements.

Figure 4.2: Photograph of a beetle where the positions of the elytra and the scutellum are indicated. A magnified image of the scutellum shows the light beam seen as a white spot.

4.4 Derived parameters

The Mueller-matrix elements are used to derive different parameters that describe the polarization state of the reflected beam and are described in Paper I. These parameters are the ellipticity angle ε , that describes the shape of the polarization ellipse, the absolute value of the azimuth angle α , that indicates the position of the major axis of the ellipse with respect to the x -axis and the degree of polarization P , that quantifies the proportion of light that has been polarized.

Another important parameter is the degree of circular polarization, P_{circ} . In the case of incoming unpolarized light this is the same as the m_{41} -element of a normalized Mueller matrix, and can also be written as a function of the ellipticity and the degree of polarization [16]

$$P_{circ} = m_{41} = P \sin(2\varepsilon) = P \sin(2 \arctan(e)). \quad (4.6)$$

4.5 Dispersion relation analysis

Structural information can also be extracted from analysis of the interference oscillations observed in the polarizance vector elements. The spectroscopic Mueller-matrix ellipsometry data show strong interference oscillations for beetles having low-absorbing cuticle. The analysis of the spectral dependence of the maxima and minima provides information of the thickness and pitch distribution of the structure. The dispersion relation analysis reveals a gradient pitch profile responsible for the broad band reflection. The results of such analysis are presented in paper V.

4.6 Modeling

Ellipsometry is an indirect technique that does not provide direct information on the sample properties. Instead, a model-based analysis must be performed to extract information such as layer thickness, optical constants or sample components. An optical model is built reproducing the structural characteristics of the sample and the data generated from such model is compared with the experimental data. The parameters in the model are then modified to minimize the difference between the model and experimental data. A non-linear regression analysis is performed using the Levenberg-Marquardt algorithm in the commercial software CompleteEASE (J. A. Woollam Co., Inc.) [26] in order to find the best fit, by minimizing the mean square error

$$MSE = \frac{1000}{L - M} \sum_{l=1}^L \sum_{i,j=1}^4 \left[\left(m_{ij,l}^{exp} - m_{ij,l}^{mod}(\mathbf{x}) \right)^2 \right] \quad (4.7)$$

where $L = N_\lambda N_\theta$ is the total number of measured Mueller matrices, i.e. the product of the number of wavelengths λ and angles of incidence. M is the number of fit parameters in the vector $\mathbf{x}$. When modeling a beetle's cuticle typical fit parameters are the pitch, the thickness of the layers and the Cauchy parameters for the refractive indices of the layers. $m_{ij,l}^{exp}$ and $m_{ij,l}^{mod}$ are the experimental and model generated Mueller-matrix elements, respectively.

Two models were generated in the studies, one for the beetle *Cetonia aurata* and other for the beetle *Chrysina chrysargyrea*. Both models consider a stack of twisted biaxial layers that represent the helicoidal structure in the exocuticle which is the optically active structure in the beetles exoskeleton. The effects of the interference oscillations are not taken into account in the model for *C. aurata* and therefore the model-generated spectra do not show the maxima and minima observed in the experimental data. On the other hand, the model generated for *C. chrysargyrea* considers a graded pitch profile that reproduces such interference. Both models are described in detail in papers IV and V, respectively.

Chapter 5

Microscopy

Microscopy is a very useful technique to observe the structure of the surface and the cross-section of the beetles exoskeleton. This chapter describes the different microscopy techniques used in this work.

5.1 Optical microscopy

Optical microscopy has been used in all beetles to observe the surface and in some cases even the cross-section where it is possible to distinguish some of the layers of the structure (Fig. 5.1). The microscope used is an Olympus BX60 with an Olympus E410 system camera. Some of the studied images can be seen in papers I and II.

Figure 5.1: *Optical microscopy image of the cross-section of a *Cetonia aurata* cuticle. The surface of the cuticle and the optically active layer are on top of the image.*

5.2 Scanning electron microscopy

To observe the micro- and nano-structure, scanning electron microscopy (SEM) was used. The SEM instrument used in this work is a Leo 1550 with a Gemini field emission column. The accelerating voltage has an operating range from 200 V to 30 kV [27]. 2 kV voltage was used to study the beetle's exoskeleton allowing us to obtain good resolution without hardly damaging the samples.

The main drawback of electron microscopy is the sample preparation. Samples must be vacuum compatible and electrically conductive, so the exoskeleton samples were coated with a 3.6 nm thick platinum film.

The samples observed with SEM were from the beetles *C. gloriosa*, *C. chrysargyrea* and *C. woodi*, the images can be seen in Papers I and II. The beetle *C. aurata* was also observed with SEM and the images can be seen in Paper IV.

5.3 Transmission electron microscopy

To observe even smaller details in the structure a higher magnification is necessary. This is possible with the use of transmission electron microscopy (TEM). However, sample preparation becomes much more complicated.

Specimens were fixed in a mixture of glutaraldehyde - formaldehyde according to a modified Karnovsky's solution [28], washed and post fixed in 2% osmium tetroxide, washed and dehydrated in a series of ascending concentration of ethanol. Infiltration took place in four steps and finally they were embedded in Spurr embedding medium at 65° for 24 hrs.

Blocks were trimmed and sectioned at 70-80 nm thickness by using a REICHERT ULTRACUT S ultramicrotome. Sections were collected onto formvar-coated, slot grids and contrasted with uranyl acetate and lead citrate. The observation and examination of the sections took place on a 100 kV Jeol JEM1230 transmission electron microscope.

Current work on TEM sample preparation has provided us with images of the cross-section of the beetle cuticle where it is possible to observe the different pitches at two different depths. Figure 5.2 top shows an image from the outer exocuticle and Fig. 5.2 bottom shows an image from the inner exocuticle. The images reveal arched structures typical of a cholesteric-like arrangement. This structure is known as the Bouligand structure and is explained in Sec. 6.3.2. Future studies of such images will aid in the modeling and analysis of the layering and the orientation of the fibers.

Figure 5.2: *Transmission electron microscopy images of the cross-section of the outer exocuticle (top) and the inner exocuticle (bottom) of C. gloriosa showing the typical Bouligand archs.*

5.4 Atomic force microscopy

Atomic Force Microscopy (AFM) was used to analyze the topography of the surface of the exoskeleton. The small tip used in this technique to scan the surface provides a measure of the depth of the cracks observed on the surface of the green *Chrysina* beetles studied. An example can be found on Paper II that reproduces one of the star-like shaped cracks observed on the green areas of *C. gloriosa*.

The instrument used was a Dimension 3100 SPM from Veeco Instruments Inc. operated in tapping mode. The scanned areas were $5 \times 5 \mu\text{m}^2$ for the gold-colored areas with a lateral resolution of about 10 nm.

Chapter 6

Polarization in nature

This chapter covers the role of polarization in nature. First, different natural sources of polarized light are presented. Detectors sensitive to polarized light are discussed next. Finally, the focus is on beetles and their structures responsible for polarization of reflected light.

6.1 Polarized light in nature

Polarized light, specially linearly polarized, is quite common in nature [29]. This is due to the fact that light undergoes transformations when reflected, scattered or transmitted when interacting with different materials. For example, sunlight reflected on a water surface or scattered from the sky can result in linearly polarized light [30,31]. As introduced in subsection 3.1.1, light incident on dielectric surfaces at the Brewster angle reflects as linearly polarized. Since biological materials have refractive indices around $n = 1.5$, they have a Brewster angle in the range 50 to 60° in air ($n_0 = 1$) and reflected light can be highly polarized [32].

Plants

Different orientations of surfaces on plants lead to differences in the polarization characteristics of the reflected light from such surfaces. This can be of special importance for flying animals, in particular during landing. It can also enhance contrast among different surfaces. In Fig. 6.1 we see how leaves, that might have similar coloration and brightness, can have a clear contrast difference due to polarized light reflected from the edges and surfaces at different orientations [33, 34].

Light reflected on some fruits is also polarized and this can be used as an indicator of the level of maturation. As the fruit matures its level of glucose increases changing the polarization properties [36]. This can be an advantage for animals with vision sensitive to polarization to identify mature fruits. A special case is the *Pollia* fruit which, even when dry, reflects circularly polarized light. This

Figure 6.1: Representation of the polarization of light reflected or transmitted on a leaf or canopy and observed by a cockchafer, a beetle that is able to detect polarization in the green region of the electromagnetic spectra¹ [35].

is due to the helicoidal arrangement of the cellulose microfibril layers that compose the outermost tissue layer [37].

Animals

Among animals there are a few examples of generation of polarized light. Two examples are the larvae of fireflies *Photuris lucicrescens* and *Photuris versicolor* which have two lanterns emitting light with a high degree of circular polarization. An interesting fact is that the two lanterns emit light with opposite handedness [38].

More common is the reflection of polarized light from arthropods like moths [39, 40], and butterflies such as *Parides sesostris* [41] and *Heliconius cydno* [42]. There are also several marine species that reflect polarized light and use it for intraspecific communication. Some squids reflect light that is almost 100% polarized in the blue and ultraviolet spectral range [43]. The mantis shrimps have red and blue polarization reflectors that are well adapted to the underwater environment [44]. The cuttlefish *Sepia officinalis* shown in Fig. 6.2, exhibits areas that reflect polarized light along the arms and in the forehead. However, this pattern disappears during specific situations such as aggression display or when the individual is camouflaged [45, 46].

¹Reprinted from Journal of Theoretical Biology, Vol 238, Ramón Hegedüs, Ákos Horváth, Gábor Horváth, Why do dusk-active cockchafers detect polarization in the green? The polarization vision in *Melolontha melolontha* is tuned to the high polarized intensity of downwelling light under canopies during sunset, Pages 230–244, Copyright (2006), with permission from Elsevier.

Figure 6.2: Full color (left) and false color polarization image (right) of the cuttlefish *Sepia officinalis*. The false color image shows a pattern of red stripes reflecting horizontally polarized light on the arms, forehead and around the eyes² [45].

Many beetles also reflect polarized light. Linear polarization is most common and has been observed in *Coptomia laevis* [47], *Chrysochroa raja* [48] and many other beetles [49,50]. Even the white beetle *Cyphochilus insulanus*, which is highly scattering, exhibits high degree of linear polarization for specular reflections [51]. There are also interesting observations of reflection of near-circular polarized light from some beetles [49,52–55] which will be described in more detail in section 6.3.

6.2 Polarization detectors in animal vision

Animal vision is very complex and specialized according to the habitat and necessities of the animal. In some cases the detector can discriminate a wide range of wavelengths in the visible similar to the human eye, but can also be more sensitive to low intensity environments as in nocturnal animals. Some animals have developed a special sensitivity to polarized light and are able to benefit from this [29,56–59]. Numerous investigations have been carried out on vision of locusts and crickets [60], cephalopods [61], and other animals [62] in order to understand the significance of the ability to detect polarized light.

Sensitivity to linear polarization is quite common and widely used for orientation in air and water, for navigation, and to identify polarizing objects for predation, signaling and recognition [57]. Certain terrestrial animals use the polarization pattern of the sky, like the desert locusts (grasshoppers) for long-range navigation during migrations [63], or the honeybees, *Apis mellifera*, to fly to a food source [64]. Other insects like *Papilio* butterflies [65] and ants [66] have also polarization sensitive vision. In the case of the *H. cydno* butterfly, whose females reflect polarized light, it has been proven that polarization assists males to find females in the shadows of the tropical rainforest [42].

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Underwater polarization can also be used for navigation under restricted conditions as proven by Lerner et al. [67] and Shashar et al. [68]. Specialized ultraviolet receptors in the retina allow fishes to see a polarized pattern [69]. Furthermore, the orthogonal orientation of neighboring photoreceptors in the eyes of *cephalopods* makes them sensitive to the orientation of polarized light. As explained before, specimens of squid, octopus and cuttlefish can control their display of linearly polarized light, which suggest that they use polarization for intraspecific communication [45].

We have seen that linear polarization is very common in nature and both sources and detectors have been widely studied. Sources of circular polarization have also been investigated for many years. The first one noticing circularly polarized reflections in the beetle *Plusiotis resplendens*, now *Chrysina resplendens*, was A. A. Michelson back in 1911 [1]. However, sensitivity to circular polarization is not so widely studied. In 2008, Chiou was the first to report a system able to detect and analyze circularly polarized light, in the *Stomatopod crustaceans* [70]. Their ability to differentiate the handedness of circularly polarized light might assist them in sexual signaling and enhancing contrast in turbid environments as suggested by Chiou. Later that year, the ability of the shrimp *Gonodactylus smithii* to combine the detection of linear and circular polarization was reported [56].

Some scarab beetles are also sensitive to linear polarization and use it mainly for navigation [59]. Dung beetles, for example, rely on the polarization of the sky to move away from the dung pile even at low light intensity [71, 72]. The flying beetle *Lethrus* has an ultraviolet polarization sensitive photoreceptor and the beetle uses the sky polarization to orientate when it exits the nest to find food [73]. However, whether some beetles have vision sensitive to circular polarization or not is a controversial topic and will be discussed in the next section.

6.3 Beetles

As mentioned above, linear polarization and its detection is very common in nature. However, circular polarization is more rare and only a few species are known to generate or reflect circularly polarized light. Interestingly, several beetles in the Scarabaeoidea superfamily reflect light with a high degree of circular polarization. Several specimens of this superfamily, in particular from the *Chrysina* and *Cetonia* genus, showing left-handed near-circular polarized reflections were selected for the study.

Most of the specimens were borrowed from museum collections and therefore only non-destructive techniques, such as ellipsometry and optical microscopy, could be used to study them. Other specimens like *Chrysina gloriosa*, *Chrysina chrysargyrea* and *Chrysina woodi* and *Cetonia aurata* were kindly provided by Dr. Parrish Brady (University of Texas at Austin) and Proff. Emeritus Jan Landin (Linköping University). We were able to analyze these specimens by electron microscopy and thus observe the internal structure of the exoskeleton.

The results of the work on the *Chrysina* genus can be found on papers I, II, III, V, VI. In addition studies on *Cetonia aurata* are also presented paper on paper IV and VI.

6.3.1 *Chrysina* genus

The *Chrysina* (formerly *Plusiotis*) beetles are found in different regions extending from southern United States to Ecuador. They live in juniper, primary pine and pine-oak forest at an altitude between 50 and 3800 m. The adults feed on the foliage whereas the larvae feeds on rotting logs from various tree species such as *Arbutus*, *Juniperus* and *Quercus* to name some [74]. The *Chrysina* genus has more than 100 species and for this work eight of them have been selected. The studied species can be seen at their approximate sizes in Fig. 6.3 where *C. woodi* (Horn, 1885) is approximately 3.6 cm large. They are *C. woodi*, *C. macropus* (Francillon, 1795), *C. peruviana* (Kirby, 1828), *C. gloriosa* (LeConte, 1854), *C. chrysargyrea* (Salle, 1874), *C. argenteola* (Bates, 1888) and *C. resplendens* (Boucard, 1875). Most of the *Chrysina* beetles are green or metallic colored but other variants can also be found, for example the beetle *C. adelaida* (Hope, 1840) with brown colored stripes as shown in Fig. 6.3h.

Figure 6.3: (a-g) *Chrysina* beetles included in the study, (f) the green-brown *C. adelaida* beetle. Adapted from Paper I

These beetles were selected because they all reflect near-circularly polarized light. This effect is easily observable using simple circularly polarizing filters with different handedness. Three of them, *C. woodi*, *C. macropus* and *C. peruviana*, are green. *C. chrysargyrea*, *C. argenteola* and *C. resplendens*, have a bright silverish or goldish metallic glance whereas *C. gloriosa* is green with gold-colored stripes along its elytras. We have investigated the polarization properties of these beetles, finding two main behaviors among those green colored and those with the metallic glance. The microscopy analysis also shows two different structures. The complete analysis and classification can be found in paper I. The particular case of *C. gloriosa* which has both structures, the green and the metallic-colored is analyzed in detail in paper II.

Since many scarab beetles reflect light with a high degree of circular polarization, it might be obvious to argue that they should be able to detect this kind of light. If the beetles would have vision sensitive to circularly polarized light, they could easily identify their conspecifics while they would remain hidden to predators [75]. From the evolutionary point of view their coloration and polarization of the reflected light could have become a camouflage mechanism. The green colors would help the beetles hide among leaves while the metallic like colors would make them imitate dappled sunlight or reflect light from the surroundings [34].

Behavioral experiments have been performed by several groups in order to find out whether the beetles are sensitive to circularly polarized light or not. Brady and Cummings (2010) showed that the beetle *C. gloriosa* actually can detect circularly polarized light [76,77]. This would prove that the reflection of circularly polarized light from their cuticles has a function and could be used for recognition. However, this result is controversial due to some issues with the set up and the number of studied specimens [34]. Other experiments performed on the *Rutelinae* beetles *Anomala dubia* (Scopoli 1763) and *Anomala vitis* (Fabricius 1775), the *Cetoniinae* beetles *C. aurata* (Linnaeus 1761) and *Protaetia* (formerly *Potosia*) *cuprea* (Fabricius 1775), that also reflect circularly polarized light, did not show any evidence of sensitivity to circularly polarized light [78]. This indicates that the reflection of circularly polarized light has minor biological relevance and that it would just be a byproduct of the helicoidal structure of the cuticles [78,79].

Paper III shows the characteristics of reflected light on the beetles exoskeleton when illuminated with different polarization states of the incoming light, simulating the possible conditions the beetles could find in their habitats. The signal acquired from detectors sensitive to polarized light is also derived in order to study whether the polarization of the reflected light could have any specific impact on a possible predator or conspecific.

6.3.2 Cuticle

The metallic coloration and polarization of the reflected light is caused by the micro- and nano-structure in the exoskeleton of the beetle. The exoskeleton, also known as cuticle, is divided in layers according to Fig. 6.4.

Figure 6.4: Sketch showing some of the most important layers of the beetle's cuticle³.

The outermost layer, the epicuticle, is a very thin layer, less than $2\text{ }\mu\text{m}$ thick, mainly composed of proteins, lipids, lipoproteins and dihydroxyphenols. Next is the procuticle, which is divided in the exocuticle and the endocuticle, composed of chitin and proteins. The exocuticle is the optically active layer and in most of the studied cases it is divided in an outer exocuticle and an inner exocuticle [79].

Chitin is a polysaccharide with similar structure as the vegetal cellulose (Fig. 6.5a). The chitin molecule is a long chain of N-acetylglucosamine monomers. The molecules group in two or three rows forming the microfibrils which are almost crystalline rods. The fibrils are coated with proteins and ordered parallel to each other forming a lamella, the direction of the lamellae often changes from layer to layer [32,79]. This helicoidal multilayer structure, shown in Fig. 6.5b is known as the Bouligand structure [4,80].

The Bouligand structure is optically active and reflects polarized light which is left-handed near-circular in most of the studied cases. The handedness of the polarized reflected light depends on the sense of rotation of the helicoidal structure. This selective reflection is analogous to that of cholesteric liquid crystals [2,3,81,82]. A rotation of 360° of the director of the layers defines the pitch which corresponds to two lamellar periods. Different pitch profiles have been observed in the cuticle of beetles [24,83].

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Figure 6.5: (a) Chitin molecule and (b) model of the chitin fibrils showing the Bouligand arcs⁴.

The graded pitch profile plays an important role in the polarization properties of the reflected light. For that reason we analyzed the experimental data from *C. chrysargyrea*. From the information extracted from the analysis of the interference oscillations observed we built a model simulating the features of the experimental data. These results are presented in Paper V.

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Chapter 7

Outlook

The results presented in this thesis provide a deeper understanding of the optical properties of light reflected from polarizing biological structures. At the same time the structure responsible for these effects is analyzed. Hopefully this work will stimulate further investigations.

Some of the measurement parameters, such as angle of incidence or the size of the incident beam, are limited by the equipment. In the future it would be desirable to extend the measurements to angles of incidence from 0 to 20° and from 75 to 90° to cover the hole range from 0 to nearly 90°. The size of the light spot is larger than 50 μm . When measuring the green beetles showing cusp-like structures and a polygonal pattern on the surface, this is comparable to the size of 5 polygonal cells. A smaller beam of around 10 μm in diameter would allow us to measure on a single cell to obtain more accurate data.

In order to find out the evolutionary role of the polarization effects on the beetles exoskeleton, more and improved behavioral experiments are needed as well as studies on the anatomy of the eyes of the beetles. The results presented here are a good complement to such behavioral experiments. It would be interesting to find out from those experiments whether the studied beetles are sensitive to circularly polarized light or not, to which kind of polarization and in which wavelength range. In addition, it is known that some insects, like honeybees, are sensitive to ultraviolet light, others reflect it, as some butterflies. In other cases they are sensitive to the infrared, like the beetle *Melanophila acuminata*. Therefore, it would be interesting to extend the measured wavelength range to the ultraviolet and infrared regions of the spectra in order to complement the studies.

To expand the study on *Chrysina* beetles and be able to generalize our findings to the hole family it would be necessary to study more *Chrysina* species and other color variants of the species studied here. However, it is not easy to find specimens for such studies. They are not easy to breed in captivity and some of them are protected species in some countries. However, since beetles are not the only polarizing structure in nature, it would be interesting to apply the techniques used in this thesis to other polarizing animals. One of the species that I suggest to study in the future is the polarizing *mantis shrimp*.

Scanning electron microscopy has proved to be an important complement to the optical studies. Transmission electron microscopy will be a valuable addition to further resolve structural details. Initial studies of sample preparation schemes has shown very promising results as already shown in section 5.3. Sharma et al. [84] already studied the beetle *C. gloriosa* with confocal microscopy by using a 488 nm laser line of an Argon ion laser. Therefore the use of super-resolution fluorescence microscopy techniques such as Stimulated Emission Depletion (STED) or Reversible Saturable Optical Linear Fluorescence Transitions (RESOLFT) might also be useful to visualize the micro-structure.

By measuring the Mueller matrix on different parts (scutellum, elytra, head, abdomen, etc) of the beetle, variations in the graded pitch profile could be explored. This could be important for a better understanding of cuticle morphogenesis and mechanical functionality. Also, determining the graded pitch profile in different species of the *Chrysina* genus, specific characteristics could be extracted.

Future work also includes improvement of the model to expand it to other beetles and to be able to generalize the result and fully control the optical properties of the modeled structure. The perspective is that such model will inspire the fabrication of similar structures which could, for instance, substitute the traditional metallic paintings and coatings. Nowadays these metallic paintings and coatings contain metals which makes them better electric and thermal conductors and very detrimental to the environment. Chitin would be an environmentally friendly alternative since it is biodegradable and can be obtained from the shell-fish industry waste products. These structures would provide more brilliant and long-lasting colors and due to their dielectric nature they would be electrically and thermally insulating. These new metal-like structures could be used in a broad range of applications from everyday products to automotive or communication devices.

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Part II

List of Publications

Included publications

Paper I

Polarizing properties and structure of the cuticle of scarab beetles from the *Chrysina* genus

Lía Fernández del Río, Hans Arwin and Kenneth Järrendahl

Phys. Rev. E 94 (2016) 012409.

Author's contribution

I loaned some of the studied samples from the Natural History Museum in Madrid. I performed Mueller-matrix spectroscopic ellipsometry (MMSE), analyzed the data and plotted the figures with input from the co-authors. I did the sample preparation for microscopy and took the pictures and microscopy images. I wrote the paper with input from the co-authors and submitted the paper.

Paper II

Polarizing properties and structural characteristics of the cuticle of the scarab Beetle *Chrysina gloriosa*

Lía Fernández del Río, Hans Arwin and Kenneth Järrendahl

Thin Solid Films 571 (2014) 410.

Author's contribution

I was responsible for the planning of the project. I performed the MMSE experiments. I analyzed the data and plotted the figures with the help of co-authors. I did the sample preparation for microscopy and took images of the sample with optical microscope, scanning electron microscope and atomic force microscope. I was the main author during the writing process and submitted the paper.

Paper III

Polarization of light reflected from *Chrysina gloriosa* under various illuminations

Lía Fernández del Río, Hans Arwin and Kenneth Järrendahl

Materials Today: Proceedings 1S (2014) 107.

Author's contribution

I planned this project with the help of co-authors, I carried out and executed the experiments. I analyzed the data and plotted the figures. I wrote the manuscript and submitted the paper.

Paper IV

Comparison and analysis of Mueller-matrix spectra from exoskeletons of blue, green and red *Cetonia aurata*

Hans Arwin, **Lía Fernández del Río** and Kenneth Järrendahl

Thin Solid Films 571 (2014) 739

Author's contribution

For this work, I took part in the MMSE experiments. I was involved in the discussions and the writing process.

Paper V

Graded pitch profile for the helicoidal broadband reflector and left-handed circularly polarizing cuticle of the scarab beetle *Chrysina chrysargyrea*

Arturo Mendoza-Galván, **Lía Fernández del Río**, K. Järrendahl and Hans Arwin

In manuscript

Author's contribution

I planned and performed the MMSE experiments. I analyzed the data and performed the calculations. I participated in the evaluation and interpretation of the results. I wrote the parts of the manuscript related to the acquisition of data.

Paper VI

On the polarization of light reflected from beetle cuticle

Hans Arwin, **Lía Fernández del Río**, Christina Åkerlind, Sergiy Valyukh, Arturo Mendoza-Galván, Roger Magnusson, Jan Landin, Kenneth Järrendahl
Materials Today: Proceedings 00 (2016) 000-000.

Author's contribution

For this work, I planned and executed the experiments related to *Chrysina gloriosa*.
I participated in the writing process after the first draft was written.

Related but not included publications

Paper VII

Exploring optics of beetle cuticles with Mueller-matrix ellipsometry

Hans Arwin, Roger Magnusson, **Lía Fernández del Río**, Christina Åkerlind, Eloy Muñoz-Pineda, Jan Landin, Arturo Mendoza-Galván, Kenneth Järrendahl
Materials Today: Proceedings 1 (2014) 155

Author's contribution

I planned and executed some of the experiments, analyzed the data and plotted the figures. I participated in the writing process after the first draft was written.

Paper VIII

Exploring polarization features in light reflection from beetles with structural colors

Hans Arwin, Roger Magnusson, **Lía Fernández del Río**, Jan Landin, Arturo Mendoza-Galván, Kenneth Järrendahl
Proc. SPIE, Bioinspiration, Biomimetics, and Bioreplication 9429 (2015)

Author's contribution

I planned and performed some of the experiments. I analyzed the data and plotted the figures. I contributed to the writing process after the first draft was written.

Publications

The articles associated with this thesis have been removed for copyright reasons. For more details about these see:

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