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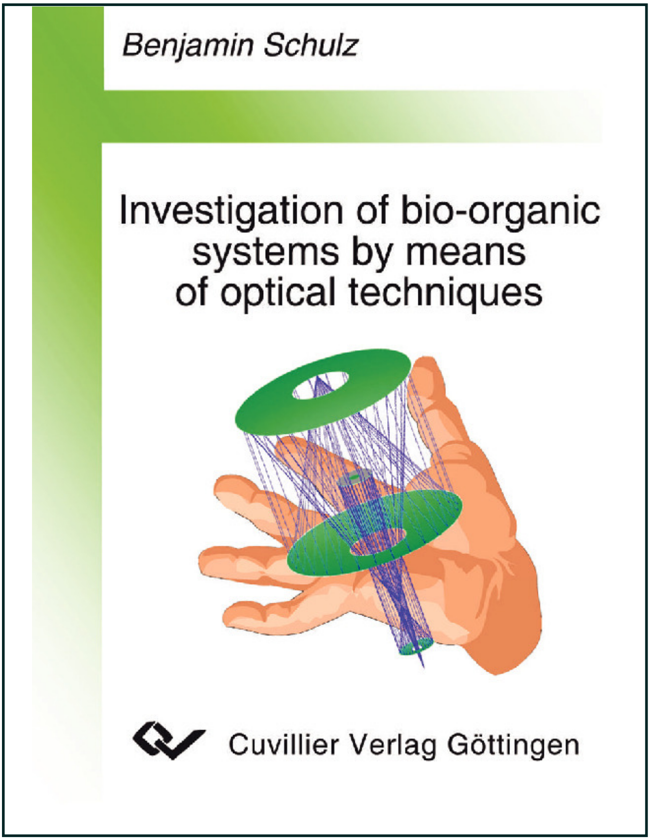
Investigation of bio-organic materials by means of optical techniques



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Chapter 1

Introduction

In the present work, bio-organic materials like nails, skin, liver tissue, and hairs are studied by means of spectroscopic ellipsometry and deep-UV resonance Raman spectroscopy. Apart from studying the basic applications of these optical methods for investigating bio-organic samples, ellipsometry and Raman spectroscopy were applied to conduct different studies, especially regarding the role of water in keratin-based materials.

In contrast to condensed matter, biological samples display a larger variation when investigating the same sample on different spots; these variations are referred to as biological (or intraindividual) variations. Another focus of this work was to determine if the applied optical techniques have the ability to draw a distinction between the biological variations and the signal variations between different samples or changing of parameters in one sample, referred to as individual (or interindividual) variations.

During this work, a novel Raman spectrometer was put into operation featuring a fully reflective design with a large entrance objective with a numerical aperture of 0.5. The system was supplemented with light sources, optical beam path, CCD cameras and cryostat. Calibrations and quality control measurements were performed, measurement procedures and handling routines were developed. This work is summarized in publication P1. Publications P5 and P6 present resonance Raman studies showing the versatility and power of the setup. These studies were performed on $LaMnO_3$ and on $GaP_{1-x}N_x$, respectively.

The biophysical studies were performed mainly using spectroscopic ellipsometry. Keratin-based bio-organic materials and the interplay of water with the keratin matrix were investigated. The ability of spectroscopic ellipsometry to differentiate between individual and biological variations and resolve time-dependent processes like hydration dynamics has been successfully explored. A model for the hydration dynamics in the human fingernails was developed, which explains the acquired data very well.

The Raman experiments carried out on bio-organic materials include fluorescence tests at various wavelengths as well as a resonance study on pure water. The obtained results motivated to investigate keratin-based bio-organic materials in the deep-UV wavelength range around 244 nm. Nails, hair and skin were compared as well as a study on hair damages was conducted. Also a study with a various skin punch biopsy samples was performed to investigate the ability of Raman spectroscopy to differentiate between individual and biological variations.

This thesis essentially consists of four publications, cited as P1 to P4. Publications P5 and P6, containing the first resonance Raman studies carried out at the UT-3 setup, are reprinted in the appendix. To put P1 to P4 into a larger context, an overview of the used experimental techniques and the biological background of keratin-based bio-organic materials together with a introduction about the role of water in these systems is given.

P1 Fully Reflective DUV-NIR Raman Spectrometer and Entrance Optics for Resonance Raman Spectroscopy
M. Rübhausen, B. Schulz, J. Bäckström, D. Budelmann, M. V. Klein, E. Schoeffel, A. Mihill, and S. Yoon
Manuscript(2004)

P2 Hydration dynamics in human fingernails: An ellipsometric study
B. Schulz, D. Chan, J. Bäckström, M. Rübhausen, K. P. Wittern, S. Wessel, R. Wepf, and S. Williams
Physical Review E, **65**, 061913 (2002)

P3 Spectroscopic ellipsometry on biological materials - investigation of hydration dynamics and structural properties
B. Schulz, D. Chan, J. Bäckström, and M. Rübhausen
Thin Solid Films, **455-456**, 731-734 (2004)

P4 In-vivo spectroscopic ellipsometry measurements of human skin
Danny Chan, Benjamin Schulz, Kathrin Gloystein, Heike Müller, and Michael Rübhausen
submitted to Biophysical Journal (2004)

P5 Orbital ordering in $LaMnO_3$ Investigated by Resonance Raman Spectroscopy
R. Krüger, B. Schulz, S. Naler, R. Rauer, D. Budelmann, J. Bäckström, K. H. Kim, S-W. Cheong, V. Perebeinos, and M. Rübhausen
Physical Review Letters **92**, 097203 (2004)

P6 Resonant Raman scattering spectroscopy studies of $GaP_{1-x}N_x$ and $GaAs_{1-x}N_x$ in the ultra-violet energy range
S. Yoon, J. F. Geisz, Sung-Ho Han, A. Mascarenhas, M. Rübhausen, and B. Schulz
submitted to Physical Review B (2004)

Chapter 2

Experimental techniques

This section describes the optical techniques used to investigate bio-organic samples. Apart from standard techniques to characterize or document the samples like microscopy, two more advanced techniques were used, spectroscopic ellipsometry and resonance Raman spectroscopy. Spectroscopic ellipsometry is a self-normalizing technique detecting changes in light polarization due to the interaction with the sample. In Raman spectroscopy, incident light is inelastically scattered by, e. g., vibrational or electronic modes.

2.1 Ellipsometry

In ellipsometry, the polarization change of light upon reflection on a sample is investigated.[2] A basic scheme of an ellipsometer is shown in Fig. 2.1. The light emerging from the light source is polarized when passing polarizer 1, and is incident on the sample under an angle ϕ_0. At the sample, the polarization of the light is changed due to the refractive index mismatch of the sample against its surrounding (air, matrix, etc.). This change is described with the Fresnel coefficients (r_x,r_y), which are deduced from the Maxwell equations. The reflected light passes an analyzer (polarizer 2) and hits then a detector.

The change of the polarization ellipse is quantified with the ellipsometric parameters Ψ and Δ. They are connected with the Fresnel coefficients:

$$\tan \Psi e^{i\Delta} = \frac{r_x}{r_y} = \rho, \tag{2.1}$$