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Non-linear Optical Microscopy and Spectroscopy for Biomedical Studies

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Abstract

This thesis is based on the application of non-linear optical microscopy and spectroscopy techniques within biomedical research. Non-linear optical microscopy gives the possibility of exciting fluorophores using near infrared light. This is an advantage when working with biological tissue, which has low absorption in this wavelength area, making up an ”open window” for non-invasive three dimensional imaging. Of particular interest has been the study of fluorescent xenobiotics in human skin using two-photon fluorescence laser scanning microscopy. The background is the desire to develop new non-invasive tools to study topical drug delivery and improve the understanding of mechanisms involved in contact allergy. In addition, two-photon fluorescence microscopy is a potential tool for non-invasive skin cancer diagnostics, which also is a topic of this thesis.

In order to acquire quantitative data, two-photon fluorescence microscopy has been combined with fluorescence correlation spectroscopy (TPFCS). This is to the best of my knowledge the first time TPFCS has been applied to study the diffusion and distribution of fluorescent molecules in human skin. By the use of this method a reactive compound, acting as a contact allergen, has been demonstrated to bind to proteins in the top epidermal layers of the skin, resulting in a significantly slower diffusion.

It has been proposed that endogenously formed protoporphyrin IX (PpIX) can be applied to improve contrast when performing two-photon fluorescence microscopy for diagnostics of non-melanoma skin cancer. In this thesis, it is demonstrated that detection of two-photon excited fluorescence of endogenous PpIX in human skin is not possible. Instead, it is preferable to use a slightly shorter wavelength, i.e. 710 nm, to induce one-photon anti-Stokes fluorescence. This finding is of great importance for continued work in the field, bringing non-linear optical microscopy into the clinics.

Plasmonic noble metal nanoparticles, e.g. gold nanoparticles, have been proposed as contrast enhancers for several biomedical applications. In this thesis, gold nanoparticles have been explored with respect to their multiphoton induced luminescence when combined with non-linear optical microscopy. By investigating 10 nm gold nanoparticles deposited on glass plates, it is here demonstrated that aggregation and short inter-particle distances are prerequisites in order to detect multiphoton induced luminescence. Thus detection of single particles in a biological environment is unlikely, and future work should be undertaken to explore how the clustering can be controlled in a biological environment to, e.g., be used as a contrast mechanism.

Keywords: Two-Photon Excitation Microscopy, Fluorescence Correlation Spectroscopy, Multiphoton Luminescence, Human Skin, Gold nanoparticles