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Integration of Raman spectroscopy to multiphoton microscopy for label-free optical diagnostics of biological tissue

Lucas Kreiß[1,2], Pascal Longequeue[1], Jay Patankar[3], Ingo Ganzleben[3], Moritz Leppkes[3], Martin Hohmann[4], Lena Götz[1], Oliver Friedrich[1,2], Maximilian Waldner[2,3], Sebastian Schürmann[1,2]

[1]Institute of Medical Biotechnology, Friedrich-Alexander-Universität, Erlangen-Nürnberg (FAU), Paul-Gordan-Str. 3, 91052 Erlangen, Germany.
[2]Erlangen Graduate School in Advanced Optical Technologies, FAU, Paul-Gordan-Str. 7, 91052 Erlangen
[3]Department of Medicine 1, University Hospital Erlangen, Friedrich-Alexander-Universität, Erlangen-Nürnberg*, Ulmenweg 18, 91054 Erlangen*
[4]Institute of Photonic Technologies, FAU, Konrad-Zuse-Straße 3/5, 91052 Erlangen
lucas-kreiss@fau.de

Optical technologies play a central role in clinical diagnostics of many diseases. In the case of gastrointestinal diseases, high-definition gastrointestinal endoscopy currently represents the primary optical technology for the initial diagnosis and follow-up of inflammatory bowel diseases (IBD) or colon cancer. In addition to the structural analysis via histology, the bio-chemical composition of the tissue is gaining importance for the diagnosis of several diseases. In this regard, multimodal concepts that combine different experimental setups have proven to be the most promising approach. Here, we present a hybrid setup consisting of Raman spectroscopy incorporated into a multiphoton microscope, allowing contact-free access to information regarding structure *and* composition that can be used for an all-optical, label-free diagnosis of diseases.

Multiphoton microscopy is based on cellular autofluorescence (AF) of endogenous molecules such as nicotinamide adenine dinucleotide (NAD) or flavin adenine dinucleotide (FAD) and second harmonic generation (SHG) from collagen I or myosin II. It provides detailed information about the tissue morphology *in situ* without any staining procedures [1]. In addition to the evaluation of tissue morphology alone, label-free multiphoton microscopy has also been shown to allow characterization of individual cells based on the AF spectra [2-3]. The SHG intensity is linked to the second order polarization properties of the sample, which has been used for a noninvasive differentiation between active states in muscular motor-proteins [4] or the determination of the orientation angles of organic fiber structures [5]. Raman spectroscopy on the other hand, relies on inelastic scattering by molecules with rotational and vibrational energy bonds [6]. It is molecule-specific and can measure the chemical composition of a tissue, which already allowed the identification of various types of cancer in several studies [7-8]. This novel combination of multiphoton imaging with Raman spectroscopy synergizes information regarding microstructure, autofluorescence, polarization properties and relative Raman intensities from a given sample in a contact-free experimental setup without the need for biochemical staining. Until now, the value of these advanced optical technologies for the evaluation of inflammatory diseases, autoimmune responses and their link to tumor development has not yet been determined.

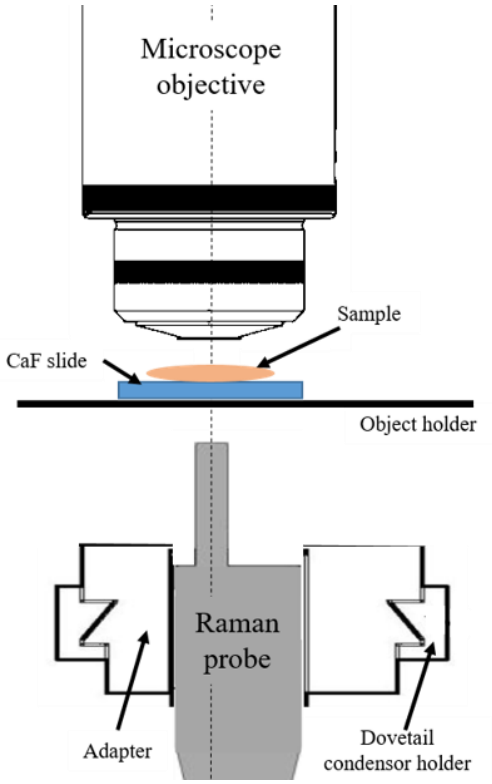

Figure 1 Experimental setup. The Raman probe is inside the adapter that is mounted on the dovetail condenser holder of the microscope. The microscope objective and the Raman probe are aligned and the respective focal positions are in the same plane within the sample

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