Imaging of immune cell dynamics in small intestine and the eye by 2-photon microcopy

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Autofluorescence based 2-photon microscopy was investigated as a tool for studying the dynamics of immunological processes in vivo. Nearly all tissue components were simultaneously visible by autofluorescence and a nearly complete visualization of tissue architecture was possible. Within the tissue, immune competent cells like lymphocytes, macrophages and dendritic cells were visualized in their dynamic interaction with other cells or surrounding tissues.

Immunological processes were studied in the small intestine and at the surface of the eye. Excitation and emission spectra of the different mucosal tissue components were quantitatively determined and a compared to the respective spectra of endogenous chromophores. It was shown, that by using only two excitation wavelengths within the tuning range of a Ti:Saphire laser enterocytes, antigen presenting cells and lysosomes of the small intestine could be discriminated based on the excitation and emission properties. By additionally using an intravital nuclear stain, motion of lymphocytes in the lamina propria and the epithelium of small intestine villi was quantitatively analyzed.

2-photon microscopy was also a powerful tool for studying the conjunctiva and cornea of the eye. Lymphocyte dynamics and uptake of microspheres or fluorescing heat-inactivated E. coli was followed over time in the conjunctiva-associated lymphoid tissue of mice. In a different mouse model of suture induced corneal hem- and lymphangiogenesis, immune cell migration into lymphatic vessels and luminal transport of individual cells was observed *in vivo*.

Autofluorescence based 2-photon intravital microscopy is a valuable tool to study dynamic immunological processes. While tissue autofluorescence gives a good overview over all relevant structures and allows for discriminating different cell types by spectral analysis, the additional combination of specific staining with external dyes or fluorescent proteins is easily possible and enhances the potential of the technique further.