



Tissue Imaging on Mesoscopic Scale

Application Note

Introduction

Confocal imaging in scattering tissue is widely applied today. Many problems concerning optical quality of tissue, namely scattering, has been overcome by multiphoton excitation. This technique is known as two photon microscopy. Due to the nonlinear excitation process an extremely high excitation intensity is necessary. This is normally realized by ultra short pulses and high aperture focusing.

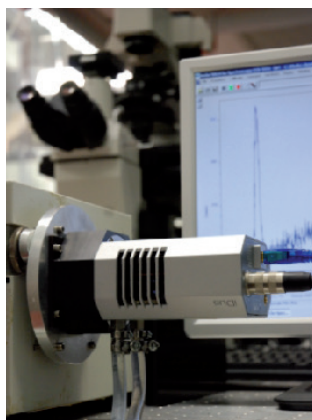
The aim of this project is to examine the metabolism within the tissue in vivo by the spectral response of the tissue without the use of marker substances.

As we are not interested in the metabolism of single cells but on collective response to injuries or diseases, the spatial resolution must average over $50 \times 50 \mu\text{m}^2$, the whole image should cover $1 \times 1 \text{cm}^2$, and the focal plane should lie 100 to $800 \mu\text{m}$ beneath the surface. Therefore a high aperture focusing is impossible. Moreover, due to the challenge of in vivo investigation, the excitation intensity is restricted.

The autofluorescence response, i.e. the fluorescence of intrinsic chromophores of the metabolism and tissue without added fluorescence markers, itself is very weak. The spectral characteristics of the emission, covering from 500 to 800 nm, are not pronounced. In order to extract some characteristic values from the emission spectra as e.g. spectral width, spectral center of mass, relative intensities, or edge steepness, a spectral resolution better than 5 nm is necessary.

In summary a very weak fluorescence signal must be detected over a broad spectral range with high spectral resolution.

Experimental Setup



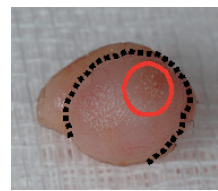
model DU420A-OE. Due to the temporal instability of the emission it is necessary to detect the whole spec-

Andor iDus CCD detector DU420A-OE attached to Acton 300i spectrograph

The tissue is excited by 500 fs laser pulses. We collect the tissue fluorescence by coupling it into a multimode optical fiber with a core diameter of $200 \mu\text{m}$. This was adapted to a spectrometer consisting of an Acton 300i Czerny Turner spectrograph with 3000 mm focal length and an iDus CCD detector from Andor Technology

trum at once with an optical multichannel analyzer instead of tuning the wavelength on a single channel detector. In the choice of the detector array the priority was put to high quantum efficiency, low noise, and low dark current, allowing long integration times. The requirements for the pixel numbers were at least 200×100 pixels. For the 'first light' experiment in the laboratory setup, a comfortable ROI and binning control as well as a rudimentary spectroscopic application tool was desired.

Results

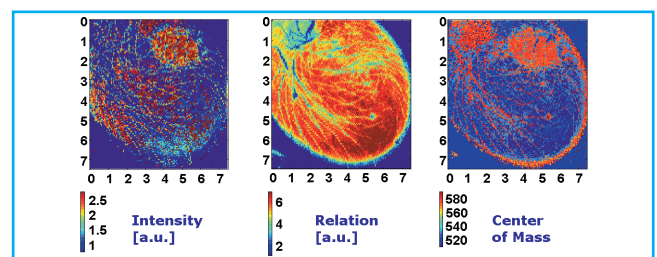


As a model system a freshly prepared human skin sample was exposed to a single erbium laser flash at $\lambda=2.94 \mu\text{m}$ and $5 \text{J}/\text{cm}^2$ in a spot of 2.5 mm in diameter. The injury was not visible to the naked eye.

Afterwards the sample was scanned in our microscope setup over an $8 \times 8 \text{mm}^2$ area and the autofluorescence spectra were measured in steps of $50 \mu\text{m}$ with an integration time of 100 ms each.

The resulting data set contains a full spectrum for each point of the sample. The spectral response can be deconvoluted to variety of different mathematical characteristics. Here are presented three different pseudocolored pictures which are all derived from the same spectroscopic data set.

This spectral technique of tissue imaging on the mesoscopic length scale allows to visualize the laser injury (left and right picture) as well as blood vessels (middle) depending on the selected characteristics.



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