NON-LINEAR MICROSCOPY FOR NON-INVASIVE DIAGNOSIS OF SUPERFICIAL CANCER

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Non-linear microscopy is employed in a study on the non-invasive early detection of cancer in superficial tissues. We applied both two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) simultaneously to image unstained skin tissues. The combined TPEF/SHG technique is expected to allow a more precise localization of tumors and tumor boundaries, which is of major importance for surgical treatment of cancer.

The diagnosis of superficial cancers can be carried out with optical techniques by using excitation wavelengths in the UV and measuring the auto-fluorescence of tissues [1, 2]. However, the very limited penetration depth of UV light in the tissues has so far prevented the clinical application of such an approach. The use of NIR (which has a large penetration depth in tissues) and multi-photon excitation fluorescence enables the excitation of molecules having absorption bands in the UV at a high penetration depth.

Second harmonic generation is another promising contrast mechanism for microscopy on superficial tissues. Collagen fibers are the predominant structural component of superficial tissues and exhibit a strong second harmonic signal [3, 4]. The growing of a tumor leads to modifications of the connective tissues (mainly collagen), which results in a change of the SHG signal [5].

Two-photon excitation fluorescence (TPEF) and SHG can be recorded simultaneously in a two-photon excitation microscope [4]. Previously, we employed the difference in time behavior between the two signals to separate the TPEF from the SHG image [6]. The introduction of a spectrograph into the imaging setup has provided us with spectrally-resolved images. Aside from being able to spectrally separate the SHG and the TPEF images, the present imaging setup also has the potential to spectrally resolve different fluorophores in the unstained skin tissue.

We present TPEF and SHG images of healthy human skin sections and those containing tumors. Differences in spectra and intensity profiles between healthy and tumorous skin samples are also presented. Comparing the SHG and TPEF images with histology, the images clearly show the areas where the collagen fiber is denatured and invaded by tumor cells and the bounding healthy collagen fibers. Our results show that the nonlinear imaging of skin through SHG and TPEF is a very promising tool for early diagnostic of superficial cancer.

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