NONLINEAR MICROSCOPY AND SPECTROSCOPY OF TISSUE AUTOFLUORESCENCE

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The diagnosis of superficial cancers can be carried out with fluorescence techniques 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. For instance, 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].

In this study, we combined a non-linear microscope with a sensitive prism-based spectrograph and employed it for auto-fluorescence imaging and spectroscopy of unstained skin tissues. The system has a sub-micron spatial resolution and a spectral resolution of better than 5 nm. The spectral images contain signals arising from two-photon excited fluorescence (TPEF) of endogenous fluorophores in the skin and from second harmonic generation (SHG) produced by the collagen fibers. We were able to spectrally image weak cellular autofluorescence as well as strong collagen SHG. The spectral images were analyzed by spectral unmixing and the results exhibit a clear spectral signature for the different skin layers.

[1] Sakcs, P.G., H.E. Savage, J. Levine, V.R. Kolli, R.R. Alfano, and S.P. Shantz, *Cancer Lett.*, **104**171-181 (1996).
[2] Shantz, S.P., V.R. Kolli, H.E. Savage, G. Yu, J.P. Shah, D.E. Harris, A. Katz, R.R. Alfano, and A.G. Huvos, *Clin. Cancer Res.*, **4**1177-1182 (1998).
[3] Kim, B.-M., J. Eichler, K.M. Reiser, A.M. Rubenchik, and L.B. Da Silva, *Lasers Surg. Med.*, **27**329-335 (2000).
[4] Zoumi, A., A. Yeh, and B.J. Tromberg, *Proc. Natl. Acad. Sci. USA*, **99**11014-11019 (2002).
[5] Guo, Y., H.E. Savage, F. Liu, S.P. Schantz, P.P. Ho, and R.R. Alfano, *Proc. Natl. Acad. Sci. USA*, **96**10854-10856 (1999).