MULTIDIMENSIONAL NON-LINEAR IMAGING OF HUMAN SKIN AND BASAL CELL CARCINOMA

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1. INTRODUCTION
Basal Cell Carcinoma (BCC) is the most common skin cancer in caucasian population. The diagnosis of BCC is typically built on a clinical basis and supported by tissue biopsy. An imaging technique with high sensitivity and high specificity, integrating the common optical dermoscopy, would be suitable for BCC diagnostics. Recently, non-invasive laser scanning based diagnostic methods also enabling the in-vivo study of skin tumors and lesions at a nearly histological resolution are becoming increasingly reliable [1].

2. RESULTS
We combined, in a multidimensional approach, multiple non-linear laser scanning imaging techniques, including two photon microscopy, second harmonic generation microscopy, fluorescence lifetime imaging microscopy, and multispectral two photon imaging. We performed a morphological and spectroscopic analysis of human healthy skin and basal cell carcinoma by using all the microscopy techniques described above. Basal cell carcinoma ex-vivo samples, excised during dermatological surgery, were layer-by-layer optically sectioned, characterized, and compared to corresponding healthy skin ex-vivo samples. Morphological and spectroscopic differences were found between malignant skin and corresponding healthy skin tissue. In comparison with normal healthy skin, cancer tissue showed a different morphology, a blue-shifted fluorescence emission, a high fluorescence response at 800 nm excitation wavelength, and a mean fluorescence lifetime distribution slightly shifted towards higher values. We also demonstrated that an enhancement of the fluorescence signal arising from malignant tissue, helping the morphological discrimination of the tumor, can be obtained by topical application of delta-aminolevulinic acid to the skin lesion three-four hours before excision. Contrast enhancement of the tumor borders was observed in both two photon fluorescence microscopy and fluorescence lifetime imaging. Fluorescence-based images showed a good correlation with conventional histological images arising from the same sample, confirming the diagnostic accuracy of our method. Multidimensional imaging enabled the discrimination between benign and malignant tissues in ex-vivo human skin samples, thus offering a non-invasive tool for the in-vivo skin diagnostic.