

**QUANTITATIVE CHARACTERIZATION OF MWHT INDUCED CHANGES IN  
MOUSE EXPERIMENTAL MELANOMA BY NON-INVASIVE *IN VIVO* STUDY  
BY COMBINATION OF CONFOCAL AND TWO-PHOTON MICROSCOPY**

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The second harmonic generation (SHG) imaging along with confocal laser scanning microscopy in reflectance mode [1] can be considered as a simple, fast, and yet powerful tool for non-invasive *in vivo* studies. When used simultaneously, these microscopic methods can improve their diagnostic value. To investigate these possibilities, we performed observations on cancer tissue *in vivo*, in totally anesthetized animals, as well as *ex vivo* on freshly harvested tumor specimens.

In this investigation, murine B16F10 melanoma cells were subcutaneously inoculated in syngeneic mice C57BL/6. The tumors were studied when developed into up to 15-20 mm in diameter. Comparisons were also done with experimental melanomas after microwave hyperthermia application. The microscopic images were acquired by three imaging modes using a Leica SP2 AOBS MP confocal laser scanning microscope: 1) 1-photon imaging in reflectance mode; 2) SHG imaging, using 2-photon (2P) excitation, to detect collagen morphology and distribution; 3) 2P imaging of tissue autofluorescence. In many cases, merging of images acquired from different channels proved to be more informative both on morphological and functional aspects than the analysis of separate channels.

Our previous studies showed the potential of confocal microscopy by means of SHG imaging combined with reflectance mode analysis for *in vivo* histopathological investigations. In this study, the evaluation of granularity of melanoma tissue was performed by measuring the maximum Euler-Poincaré characteristics of binary images obtained by thresholding of the grayscale images after pre-processing the acquired images by smoothing and application of Lipschitz filter. This parameter was suitable for analysis of melanoma morphological structure, reflecting tumor changes induced by microwave hyperthermia treatment.

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[1] Y. Li, S. Gonzalez, T.H. Terwey, J. Wolchok, Y. Li, I. Aranda, R. Toledo-Crow, A.C. Halpern, „Dual mode reflectance and fluorescence confocal laser scanning microscopy for *in vivo* imaging melanoma progression in murine skin“, *J Invest Dermatol* **125**, 798–804 (2005).