Early Detection of Melanoma with the Combined Use of Acoustic Microscopy, Infrared Reflectance and Raman Spectroscopy

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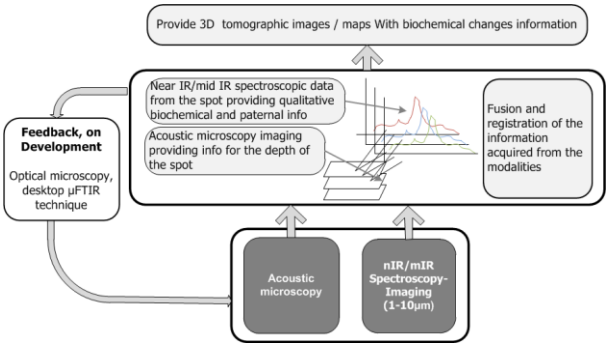
The objective of this study is the application of a combination of the non-invasive imaging techniques acoustic microscopy, infrared (IR) and Raman spectroscopies, in conjunction with histological and immunohistochemical analysis, for the detection, mapping and identification of developing tumors, in a novel animal model of melanoma. Acoustic microscopy provides information about the 3D structure of the tumor, whereas, both spectroscopic modalities give qualitative insight of biochemical changes during melanoma development. Growth of actual tumors in an animal melanoma model, with the use of human malignant melanoma cells was achieved. Acoustic microscopy with central operating frequencies of 75MHz, 110MHz and 175MHz was used, resulting to the tomographic imaging of the tumor, while using the infrared (IR) spectroscopic modality in reflectance mode differences among spectra of normal and tumor sites were identified in skin depth. The development of tumors at different time points was displayed using acoustic microscopy. Moreover, the changes of the IR spectra were studied between the melanoma tumors and adjacent healthy skin. The most significant changes between healthy skin and the melanoma area were observed in the range of 900-1800cm-1 and 350-2000cm-1, respectively.

Proposed systems – methods

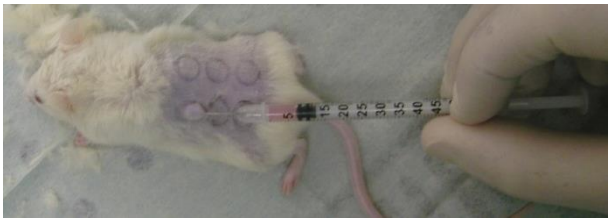


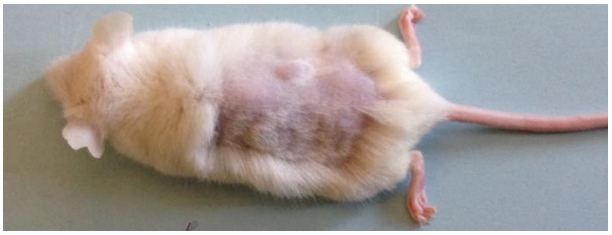
Acoustic microscope combined with the customised IR reflectance spectroscope.

Animal model of human melanoma

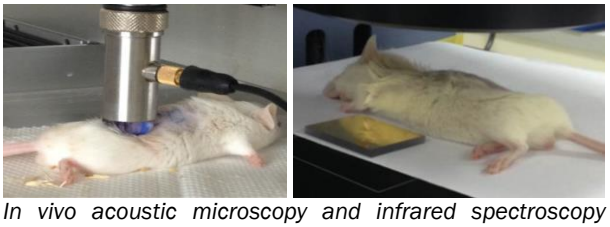
An experimental model for cutaneous melanoma – induction by endermal injection of a human cell line was developed as follows:

Under deep anesthesia, SK-MEL-28 cells (6×105 cells/injection in DMEM) were endermally injected to seven NOD SCID male mice with one week interval between each site, at three equally spaced sites of the back skin. Three, bilaterally symmetrical sites served as controls of unaffected tissue. Five weeks after the first injection normal skin and tumor sites were visualized using acoustic microscopy and analyzed using infrared reflectance spectroscopy, in vivo, under deep anesthesia. Mice were sacrificed with transcardial perfusion of 4% paraformaldehyde, the skin of the back was removed and the same diagnostic techniques were applied ex vivo. Sections of normal skin and skin including the tumor sites were examined using histological and immunohistochemical techniques.



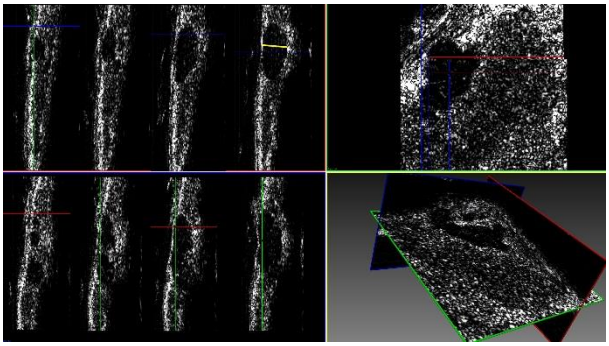


Endermal injection of SK-MEL-28 cells in NOD SCID mouse back skin and respective tumors developed.

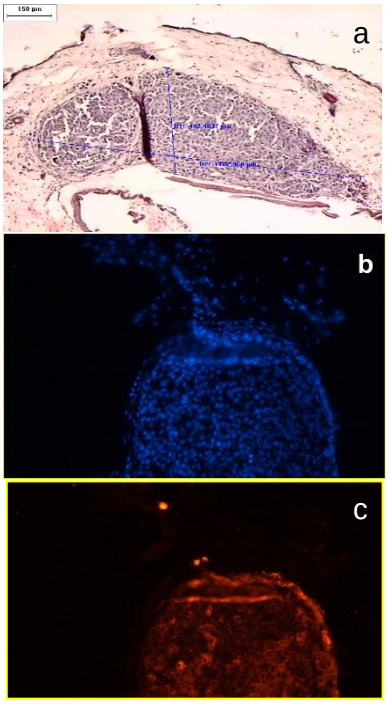


In vivo acoustic microscopy and infrared spectroscopy examination.

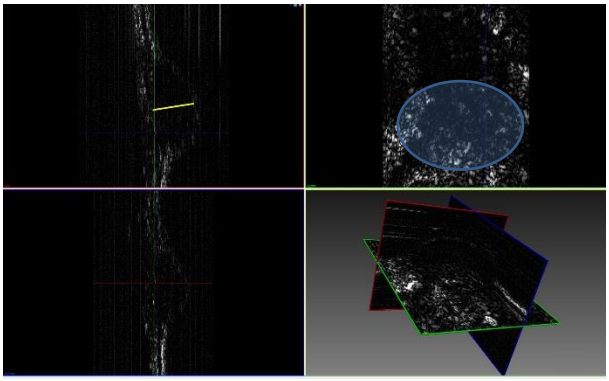
Measurements



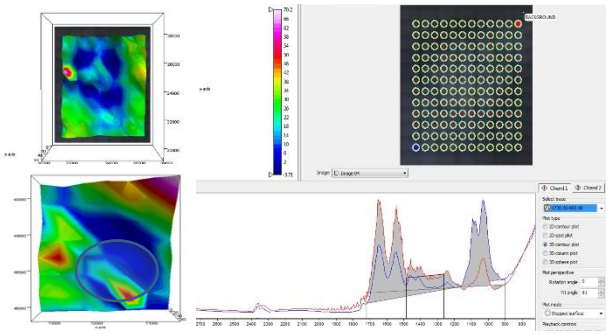
Acoustic Microscopy scan (75MHz) a) axial B-scan b) sagittal B-scan c) coronal B-scan and d) C-scan of mouse back skin including tumor. The yellow line represents a length of 725μm.



Photomicrograph of histological section of melanoma tumor developed within the epidermis of mouse 2, melanoma site C1 back skin stained with
a) Hematoxylin-Eosin or immunostained for
b) DAPI revealing the nuclei of all cells and
c) monoclonal Mouse Anti-Human Melan-A which marks melanocytic cells.



Scanning the ROI of the melanoma B2 of mouse 3 and the mapping image based on the IR spectroscopic technique, Corresponding acoustic microscopy tomographic image (175MHz). The yellow line represents a length of 695,6μm.



Selecting the spectral area between 900-1700cm-1 (14.2-5.8μm), spectroscopic mapping images are generated in the scanned ROI detecting the melanoma area.

Results

- Six out of seven animals developed tumors in the injection sites endermally, appearing 2-3 weeks after the injection. Tumor shape and size were visualized and identified using acoustic microscopy and infrared spectroscopy.
- The probes used for the acoustic microscopy scannings have a -3db bandwidth from 56 to 200MHz, and resulted to the detection of thin endermally developed structures, extending from a few hundred micrometers to more than 1mm in some tumor cases. The penetration depth is of the order of 1-1.x mm in 175MHz and even more using the 75MHz. The sensitivity of the developed methodology provides detailed mapping of the melanoma tumor. This finding gives the possibility for the *in vivo* detection of infiltration of these malignant cells.
- The resolution of the images is of the order of 5μm in the coronal and axial plane and of 15-20μm of in the sagittal plane.
- The spectroscopic mapping image of the melanoma is generated using the spectral area between 1386-1766cm-1 (7.2 - 5.6μm) and 900-1700cm-1 (14.2-5.8μm). The blue colored area corresponds to the detected melanoma and matches perfectly to the area detected using acoustic microscopy.
- Histological and immunohistochemical techniques revealed the structure and cytoarchitecture of tumors and confirmed the development of skin melanoma cancer.