# *Histological Characterization of Melanoma Metastases by FTIR Imaging*

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In the last decades, incidence of melanoma has steadily increased worldwide. Once melanoma has metastasized, this advanced stage of disease is difficult to treat and does not respond to current therapies [1]. Numerous recent studies have emphasized the role of stromal cells in the tumor progression [2, 3]. Moreover, some particular treatments such as immunotherapies enable to act on these stromal cells to block tumorous progression. A precise identification of the different immune actors is a preliminary step to the choice of this kind of treatment. Histological analysis of biological tissues achieved by pathologists enables a visualization and quantification of the immune cell types found in the microenvironment. Yet, this step of vital importance is time-consuming, expensive and operator-dependant. In this study, we suggest to use FTIR imaging to characterize the immunologic microenvironment of tumor cells in metastatic lymph nodes, the first place of colonization of metastasis.

The spectroscopic imaging data on metastatic lymph nodes were acquired in transmission on deparaffinized 4 µm thick tissue slices deposited on 40x26 $mm^2$ $BaF_2$ slides using a Hyperion imaging system (Bruker) equipped with a 64*64 MCT (Mercury-Cadmium-Telluride) FPA (Focal Plane Array) detector. Every individual element of the array detector covers an area of 2.9*2.9 $\mu m^2$. One IR unit image results in 4,096 spectra (184*184 $\mu m^2$), each one being the average of 256 scans (ca 5 minutes recording).

16 lymph nodes from 8 patients were used in this study. 8 lymph nodes were invaded by melanoma metastases and we compared those with 8 tumor-free lymph nodes from the same 8 patients. A collection of spectra from the main cell types found in the tissue was recorded (melanoma cells, lymphocytes, erythrocytes and fibroblasts). A total of 26 000 spectra were acquired. Unsupervised analyses (PCA) were able to distinguish these main cell types on basis of their spectra. Supervised discriminant PLS-models were built for cell type identification with 2/3 of the database. The model was validated on the remaining spectra. Correct recognition percentages reached between 94 to 99% depending on the cell types, as assessed by a pathologist. In a second part of the study, we compared the spectral signatures from T lymphocytes infiltrating the tumor from metastatic lymph nodes with those from tumor-free lymph nodes. Using PCA (Principal Component Analysis), spectra clustered clearly in two groups.

We show here in an exploratory study that IR imaging can be applied on histological tissues to recognize the main cell types found in lymph node melanoma metastases. We also demonstrated that the spectral signature of lymphocytes from tumor-free lymph nodes and metastatic lymph nodes were different. This difference could arise from a difference in the activation state. As the presence of actors of the immune system in the tumor site appears to be crucial for the outcomes of the patients, FTIR imaging could become a method of choice for the analysis of clinical samples.

References:

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