

# **Morphological Analysis of Mass Spectrometry Imaging Data to Discover Heterogeneity Markers in Oncology**

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## **Introduction**

The dedicated to detect biomarkers within tissue are based on molecular intensities. A biomarker is a specific molecule that helps to describe tissue, disease and histology. It may change either intensity or morphology. We've developed a tool that quantify the morphological information of such as surface, coverage of each molecules. This has been used to identify characteristics of multiple samples, discover new markers defined by their tissue features and heterogeneity on-tissue instead of evaluating the intensity. As tumors are highly heterogeneous, we perform morphometry analysis of tissue and identify heterogenous markers that were interesting to understand the pathology. The work have been developed under Multimaging that also allows to share information and analysis the datasets into the cloud.

## **Material and methods**

10µm thick tissue sections of P815 tumor xenografts were thaw mounted onto ITO coated slides, and 1,5-diaminonaphtalene (1,5DAN) matrix was coated using TM-Sprayer device. MSI analysis of metabolites and lipids were performed using 7T MALDI FTI-ICR. MSI data were recorded in negative ion mode ( $m/z$  range 50 – 1000) at 30 µm pixel size with an on-line calibration. Data acquisition, processing, and visualization were performed using the Flex software suite and Multimaging. A new data processing workflow based on custom python scripts was applied, and plots made using matplotlib[14]/seaborn DOI:10.5281/zenodo.54844, objects having a surface lower than 4 pixels were deleted.

## **Conclusion:**

We've developed a tool based on morphological characteristics that allows the detection and selection of onco-metabolites (Tryptophan and Kynurenine) and other unknown markers from the tissue. This has been done through the determination of the noise and its standard deviation of the noise. Then segmentation into objects has been obtained with Otsu method. This shows a black and white color image in which the tool automatically calculates the features of its morphological parameters (surface, perimeter and percent of coverage). We've selected the 1,000 most intense molecules in order to detect heterogeneously distributed ions. After a visual comparison, we validate the workflow which permits the identification of ions with a particular distribution within the necrosis and proliferative regions. We then apply the same workflow to a mIDO tumor model which has an overexpression of (IDO) which converts Tryptophan into kynurenine. We investigated Tryptophan and Kynurenine morphological parameters and found

those markers are up and downregulated depending on the level of IDO expression. In the meantime, we found metabolites and lipids which also have heterogeneous distributions (less than 10% of tissue coverage) in specific regions (either necrotic, inflammation or proliferative sites). The interesting value of the software is the capacity to investigate not only the intensity variations, but also the morphological changes within the tissue. It can also be used as a tool to create statistical models of the tumors by integrating intensity with morphology parameters of the ions.

## Innovation

New tool for biomarker detection based on morphology characteristics of ions