

# **Ovarian Cancerous Tissue Identification by DESI Imaging for Clinical Research**

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## GOAL

Introduction of DESI imaging to tissue identification and characterization in ovarian cancer research using Waters® Xevo® G2-XS QTof Mass Spectrometer and lipidomic analysis.

# BACKGROUND

Over the past decade, mass spectrometry imaging (MSI) has been used increasingly by researchers to investigate the distribution of metabolites, drugs, peptides, and proteins in tissue surfaces. The potential for the application of MSI to unambiguously map hundreds of biomolecules in a single analysis has led to this approach being used in research studies of cancer. Recently, there has been a significant increase in the application of desorption electrospray ionization (DESI) because this soft ionization technique can be performed under ambient environmental conditions. Furthermore, it requires little to no sample preparation and is minimally invasive, making it suitable for direct tissue analysis. DESI-MSI has potential to provide non-subjective information about biochemical distribution of molecules after just one measurement.

The use of DESI can help distinguish between several different tissue types in ovarian cancer samples during clinical research.



Figure 1. DESI imaging workflow.

Therefore, this technique enables robust tissue recognition and identification of tissue-specific lipid ion patterns, which could in the future be useful in cancer diagnosis and prognosis at a histology-level. DESI-MSI is compatible with both the Waters SYNAPT® G2-S*i* and Xevo G2-XS Mass Spectrometers.

#### THE SOLUTION

Sample preparation for DESI analysis is much simpler than other MSI techniques. The fresh frozen tissue samples (in this case, serous ovarian carcinoma) were cryosectioned at a 10 µm thickness and then mounted on glass slides. The slides were stored at -80 °C prior to DESI measurements. For the DESI analysis, glass slides were placed onto the 2D linear moving stage and High Definition Imaging® (HDI®) 1.4 Software (Waters Corporation) was used to define the area to be imaged. The DESI sprayer was guided through this defined area with mass spectra collected at predefined x and y coordinates using a resolution of 100 µm. These DESI-MSI experiments were carried out in negative ionization mode with a mass range of m/z 50–1,000. After the measurements were complete, raw imaging data were processed and visualized using the HDI 1.4 Software. The tissue sections were then stained with haematoxylin and eosin (H&E) to allow the overlay of digitalized H&E-stained optical images with corresponding DESI molecular images from the same tissue sections. The overall MS spectrum was found to be rich in lipids, especially in phospholipids and fatty acids. Figure 2 shows an example of the tumor tissue average spectrum with ions relating to different lipid species. The majority of phospholipids identified were in the phosphatidylethanolamine (PE) class. Different ions produce different images as they have different spatial distributions across the sample surface, as shown in the Figure 3.

The optical image was then evaluated by a qualified histopathologist, and the different tissue types found in the tissue section were annotated. The RGB overlay image of different ion images (Figure 3) is comparable to the histological image. Statistical analysis was performed from HDI-processed DESI-MSI data by firstly defining the Regions of Interest (ROIs) on the MSI ion images based on histopathological annotations. Differences between the two tissue types identified by the histopathologist (stroma and tumor) were evaluated.



Figure 2. Average DESI-MS spectrum from tumor tissue acquired in negative ion mode. Fatty acids (FA), phosphatidylethanolamines (PE), phosphatidylinositol (PI) can be observed in spectrum.



Figure 3. Ion images of specific fatty acids and phospholipids from serous ovarian carcinoma sample together with optical H&E image and RGB image.



Figure 4. Tissue type differentiations using SIMCA statistical software tool where the ROIs on the tumor-associated stroma and serous carcinoma are separated by Partial Least Squares Discriminant Analysis (PLS-DA). The goodness of fit plots indicated a good regression and predictive model.



Figure 5. Serous carcinoma samples with two tissue types (tumor-associated stroma and serous carcinoma). Principle component analysis (PCA) and maximum margin criteria analysis (RMMC) cross validated with respective leave one pixel out cross validation accuracy.

Figure 4 shows Partial Least Squares Discriminant Analysis (PLS-DA) using SIMCA® Software (MKS Data Analytics Solutions), which demonstrates a good fit for the model generated with a high R2 (regression fitness) and Q2 (predictive fitness). Using a SIMCA infomatics workflow, it is possible to link the ion images of the dataset with the loading plot in HDI. A range of commercial statistical software packages are available.

In an alternative approach, an in-house imaging toolbox developed at Imperial College London was used for unsupervised and supervised statistical analysis. Principal Component Analysis (PCA) shows a clear separation between surrounding tumor-associated stroma and the tumor tissue with the first principle component (Figure 5). Using this in-house imaging toolbox, the supervised analysis shows an overall cross validation accuracy of 100% in classification between these two tissue types (Figure 5).

### SUMMARY

These results demonstrate robust tissue recognition and identification of tissue-specific molecular ion patterns for an ovarian cancer sample using mass spectrometry imaging for clinical research. Tumor and tumor-associated stroma present distinct lipidomic profiles rich in phosphatidylethanolamines (PE) in negative ion mode.

The advantages of this technique include:

- Minimum sample preparation required prior to DESI acquisition
- A non-destructive ambient technique that allows additional analyses of the same tissue section, e.g. H&E staining
- Lipidomic profiles can be achieved, and differences between normal and tumor tissue observed – making it an ideal technique to complement and supplement histology in clinical research

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