

## Application Note

# Metabolic Phenotyping of Colorectal Tissue Samples by DESI-MSI for Clinical Research

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*For research use only. Not for use in diagnostic procedures.*

This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

This application brief demonstrates the spatial localization and lipid profile/distribution of healthy colorectal tissue compared to cancer to elucidate the metabolic changes associated with cancer progression and invasion in clinical research.

## Benefits

The examination of lipid profiles and distributions in healthy and cancerous colorectal tissue samples measured by desorption electrospray ionization coupled to mass spectrometry imaging (DESI-MSI) in clinical research.

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## Introduction

Over the past decade, mass spectrometry imaging (MSI) has been used increasingly by researchers to

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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), as 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 the potential to provide non-subjective information about biochemical distribution of molecules after just one measurement. This approach allows 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.

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## Results and Discussion

In order to perform a DESI-MSI analysis, fresh frozen tissue samples were cryo-sectioned at 10 µm and mounted on glass slides, which were then stored at -80°C prior to the measurements. Slides were placed onto the 2D linear moving stage and Waters High Definition Imaging (HDI) v1.4 Software was used to define the area to be imaged. The area was rastered line-by-line using the DESI sprayer, with mass spectra collected at predefined x and y coordinates using a 100 µm pixel size, at 4 scans/sec. Experiments were acquired in negative ionization mode with a  $m/z$  range of 50–1,000. Raw imaging data were processed and visualized using HDI software and tissue sections were subsequently stained with haematoxylin and eosin (H&E) to allow the overlay of digitalized H&E stained optical images with corresponding DESI-MSI molecular images from the same tissue sections (Figure 1).

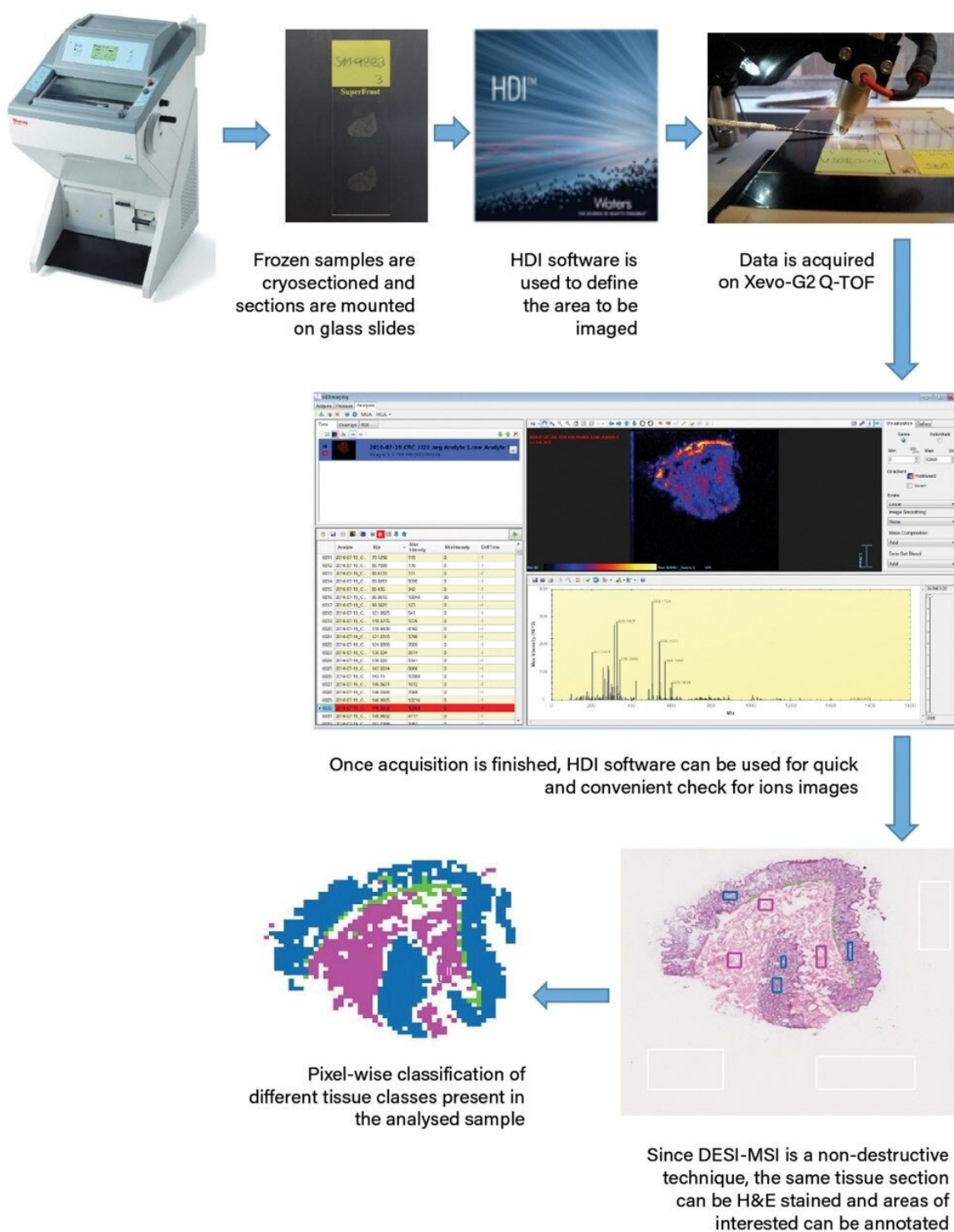


Figure 1. Workflow of DESI-MSI of colorectal sample.

DESI-MSI generates intense fatty acid and phospholipid signals in negative ionization mode, as shown in Figure 2. This figure also shows different metabolic profiles for different tissue types within the healthy colorectal tissue.

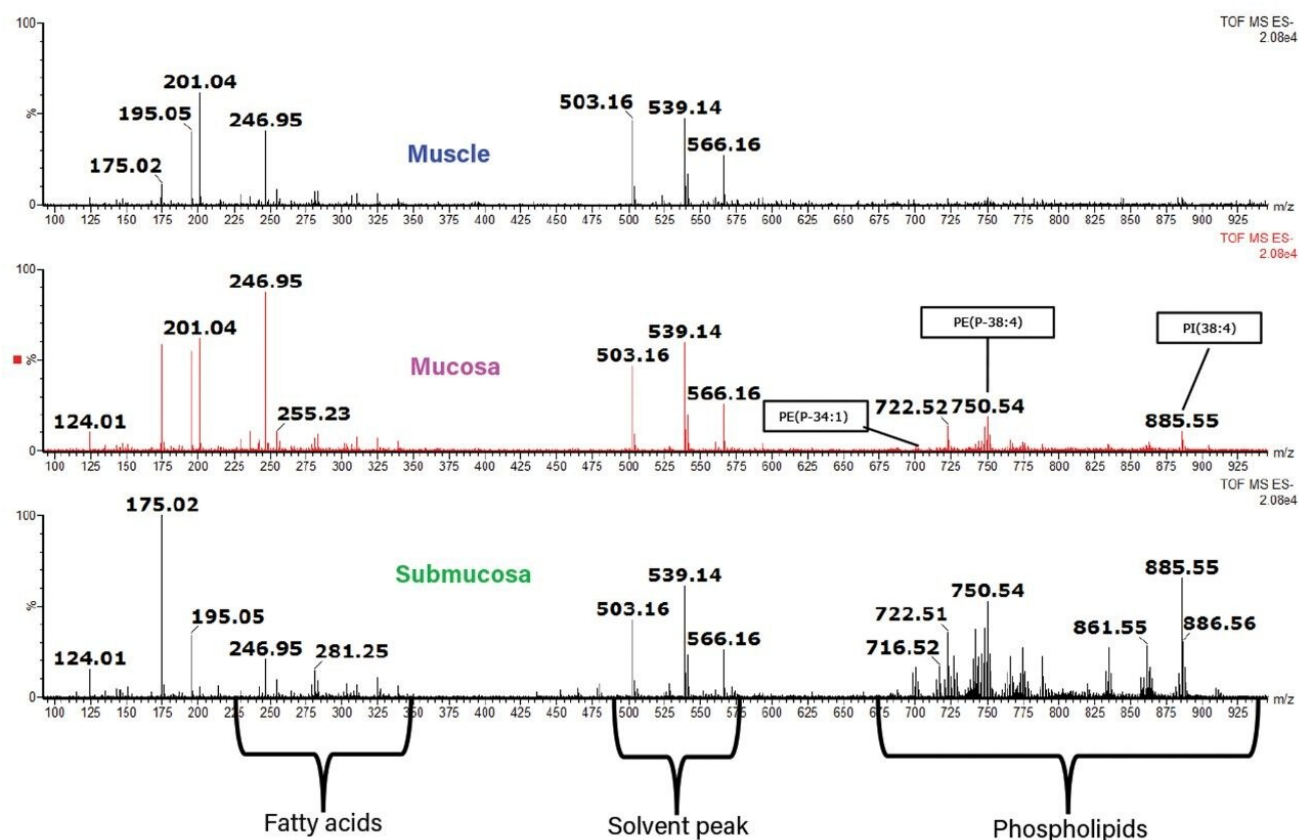


Figure 2. Averaged mass spectra acquired in negative ion mode from the different tissue types within a normal colorectal tissue sample with mass ranges corresponding to fatty acids and phospholipids with some putative IDs.

Figure 3 displays examples of DESI images representing four endogenous molecules for a healthy colorectal (3A) and tumor colorectal (3B) tissue sections, alongside the corresponding H&E stained images of the same samples. Colorectal tumor samples are more homogeneous, presenting generally just one tissue type, as shown in Figure 3B.

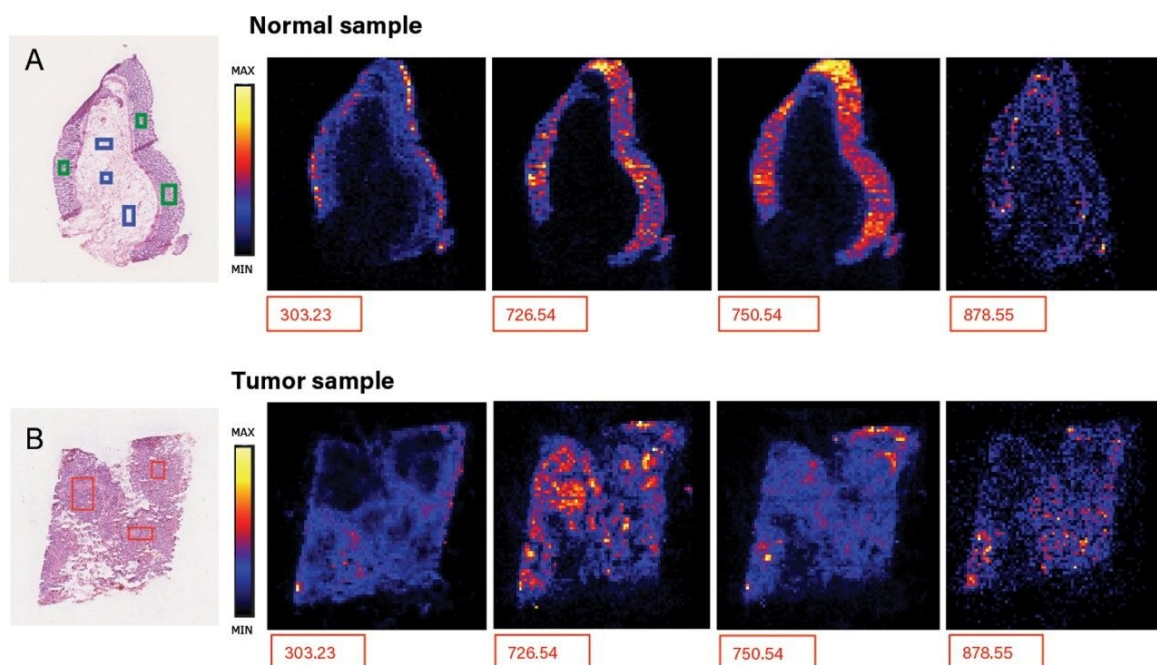


Figure 3. Colorectal tumor and healthy tissue sample analyzed in negative ion mode at 4 scans/second. The same molecules can be found in these two tissue types and due the nature of the DESI-MS imaging it is also possible to visualize their distribution in analyzed tissue sections. Putative IDs:  $m/z$  303.23 FA(20:4);  $m/z$  726.54-PE(P-36:2);  $m/z$  750.54-PE(P-38:4);  $m/z$  878.55-PE(44:8).

DESI-MSI has been shown to provide good differentiation between distinct tissue types within one tissue section. In Figure 4, lipid species characteristic of the different tissue types found in this healthy colorectal sample are shown. Putative IDs for these tissue classes are as follows: mucosa (green)- $m/z$  750.54-PE (P-38:4); muscle (pink)- $m/z$  302.07-  $C_{10}H_{13}N_5O_4$ ; and submucosa (blue)- $m/z$  236.02-  $C_{10}H_9NO_7$



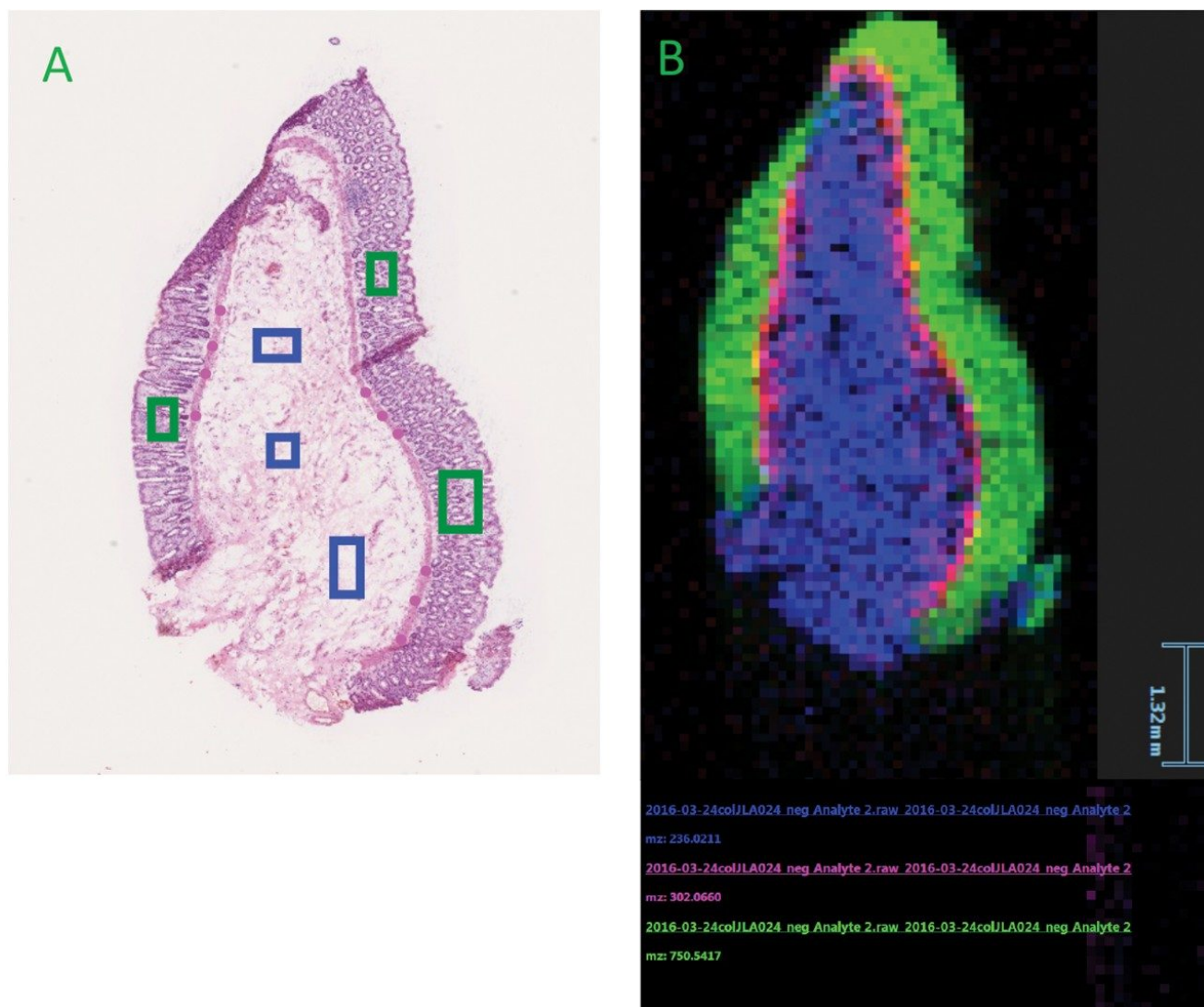


Figure 4. Healthy colorectal tissue sample analyzed in negative ion mode at 4 scans/second. Different lipid species can be overlaid (B) to create a matching ion image corresponding to the H&E stained tissue section (A). *M/z* values corresponding to different tissue classes are listed (mucosa (green)–750.54, muscle (pink)–302.07, submucosa (blue)–236.02). Putative IDs: *m/z* 750.54–PE(*P*-38:4), *m/z* 302.07–  $C_{10}H_{13}N_5O_4$ , *m/z* 236.02–  $C_{10}H_9NO_7$ .

To investigate the variation in the lipid profile between tissue types, statistical analysis was performed with HDI 1.4/SIMCA (Umetrics) and in-house MATLAB algorithms.

Regions of interest (ROIs) were defined for the different tissue types within the healthy colorectal DESI-MSI images in HDI based on histopathological annotations. The differences between the tissue types were assessed

using Partial Least Squares Discriminant Analysis (PLS-DA) with soft independent modeling of class analogies (SIMCA). Figure 5 shows the results of statistical analysis, with clear definition of the different regions.

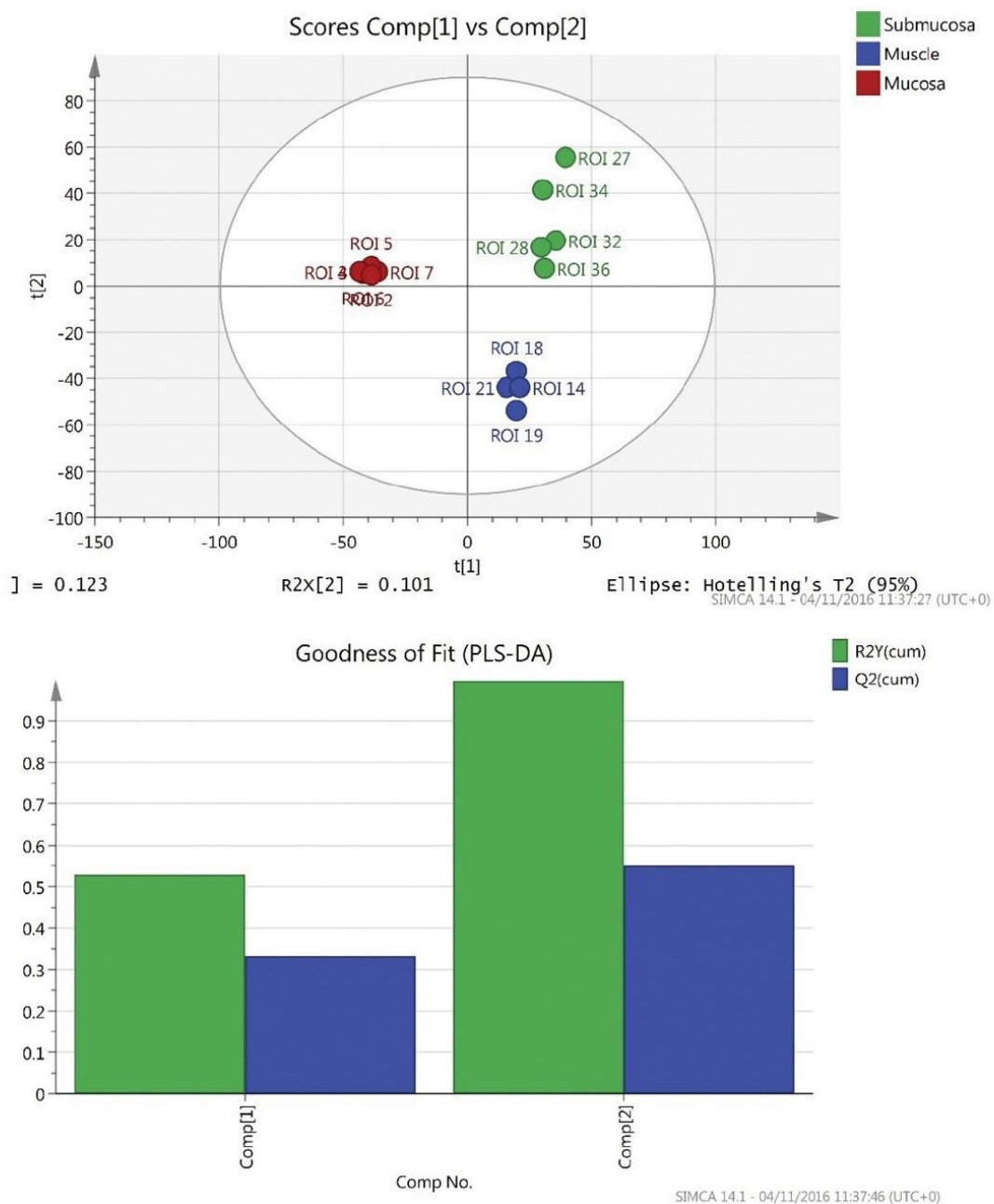


Figure 5. Separation of three tissue classes in healthy colorectal tissue sample using soft independent modelling of class (SIMCA) packages. Partial Least Squares Discriminant Analysis (PLS-DA) score plot and goodness of fit for model are shown.

An in-house imaging toolbox, developed by Imperial College London, was also used to analyze the samples.



Based on their lipid composition, discrimination was possible between mucosa, muscle and submucosa in the colorectal tissue sample. Furthermore, it was found that one component was sufficient to separate these three tissue types in unsupervised principal component analysis (PCA) (Figure 6A). Supervised analysis using maximum margin criteria (MMC) (Figure 6B) was followed by leave-one-region-out Mahalanobis cross validation giving an accuracy of 98% (mucosa and muscle) and 94% (submucosa) (Figure 6C).

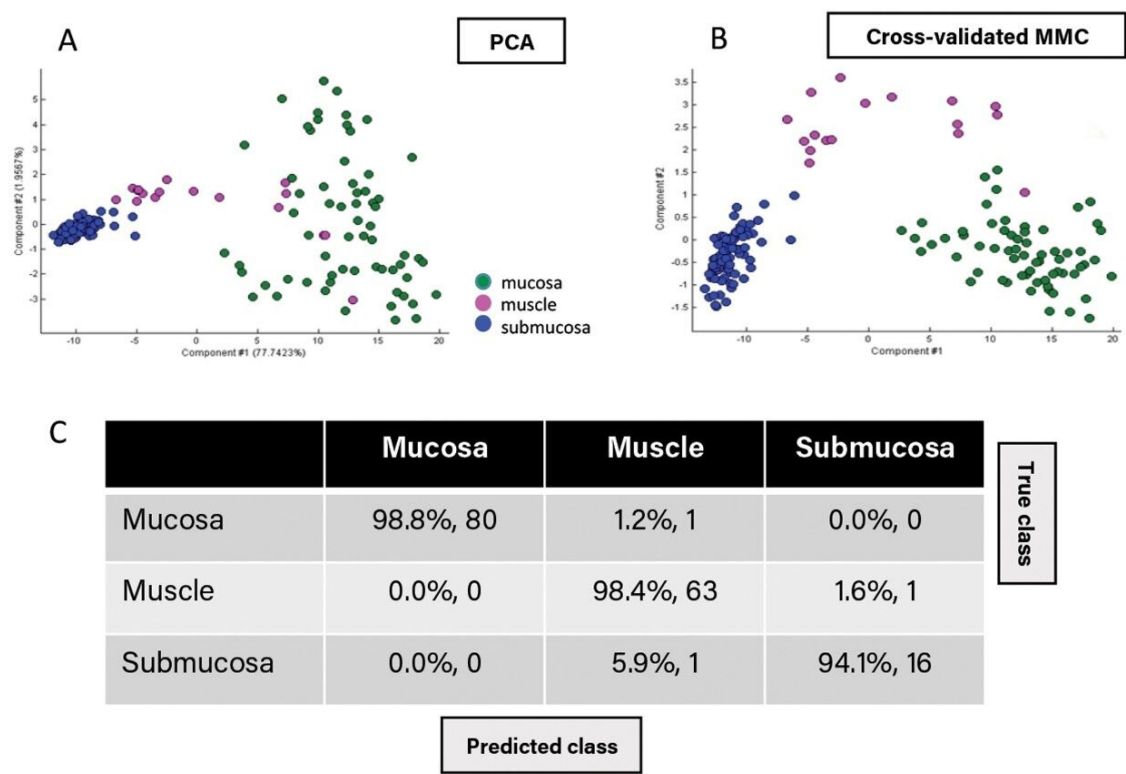


Figure 6. Statistical analysis using an in house imaging toolbox. Separation of three tissue classes in healthy colorectal tissue sample using (A) principal component analysis (PCA) and (B) recursive maximum margin criteria (MMC) analysis. (C) The results of Leave One Out cross validation accuracy are also shown.

## Conclusion

DESI-MSI is a very effective technique for use in clinical research to determine the spatial localization and distribution of molecules within a variety of samples under ambient conditions.

The advantages of DESI-MSI include:

- Minimum sample preparation required prior to DESI-MSI analysis, with no need to apply matrix (as required for example in MALDI imaging)
- Measurements can be performed under ambient environmental conditions
- DESI-MSI is a non-destructive technique, which allows additional analyses of the same tissue section (in this case H&E staining)
- Lipid distributions can differentiate various tissue types within one tissue section
- Good analytical sensitivity for a wide range of molecules

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