Unravelling Tissue Complexity in Samples of Human Inflammatory Bowel Disease Using Imaging Mass Spectrometry

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1. Introduction

Inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) are chronic and relapsing inflammatory conditions of unknown aetiology. Current treatments are not universally effective and can have severe adverse effects. Therefore, early detection and precise identification of the type of disease is important in order to apply the correct treatment. Colon tissue is complex and contains different types of cells, and therefore a cell-specific analytical tool is required to improve diagnosis and prognosis. Here we present a mass spectrometry imaging (MSI) study at 10 micron (µm) spatial resolution of endoscopic biopsies obtained from patients with IBD. As lipid composition varies between cell types, the lipid fingerprint may serve as a signature of each type of lesion.

2. Methods

The sample collection for this study was specifically approved by the Ethics Research Committee of the Balearic Islands (IB 2118/13 PI). Excised human colon tissue was snap frozen in liquid nitrogen and cryo-sectioned at 10 µm thickness. Sections were covered with a suitable matrix for negative-ion detection (1,5-diaminonaphthalene, DAN) using a stainless-steel sublimator, and imaged using the Shimadzu MALDI-7090™ MALDI-TOF-TOF mass spectrometer (laser spot diameter 10 µm, stage step size 10 µm, scanning range 200-1200 Da; Figure 1).

Figure 1. MALDI-7090™.

3. Results

3-1. MALDI-MS imaging analyses (10 µm spatial resolution)

The anatomy of human colon poses an important challenge for a MALDI mass spectrometer, as the lining of the crypts are composed of single-cell thick layer outline. Therefore, a combination of carefully optimised sample preparation and a MALDI instrument capable of achieving high spatial resolution is fundamental to provide an accurate representation of the colon tissue.

Figure 2 shows the MALDI images of the *m/z* 861.550 and 885.550 species, alongside the corresponding H&E stained sections and segmentation images. The lamina propria, crypts and muscularis mucosae tissues are clearly distinguishable, thanks to the 10 µm spatial resolution.

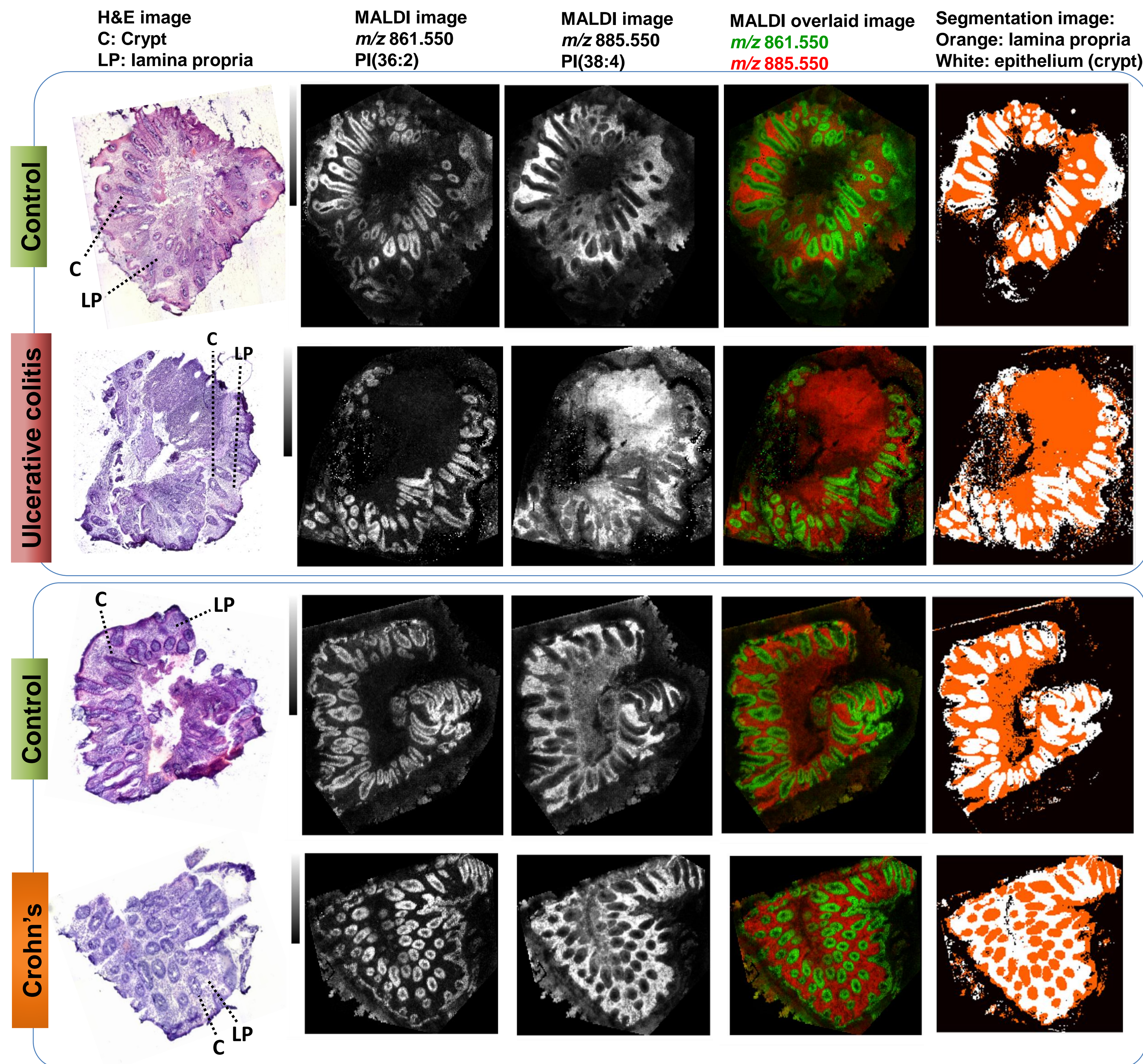


Figure 2. Microscope images of H&E stained consecutive sections (left) alongside the MALDI MS images. Multiplexing of the ion distributions demonstrate that the ion at *m/z* 861.550 is specific to the crypt units, while the ion at *m/z* 885.550 is specific to the lamina propria. Segmentation images (right) show a clear separation between the epithelium (white) and lamina propria (orange).

3-2. Truly exploiting high spatial resolution (Beyond traditional histology)

Six ulcerative colitis, six controls, seven Crohn's disease and seven controls were used for the data analysis. The excellent S/N ratio achieved even at single pixel level, enabled the analysis at (sub)cellular level. Figure 3 shows the results of the PCA classification. Segmentation of the MALDI images using a correlation-dependent algorithm exposed the true complexity of the lipid composition. Each cell population exhibits a characteristic lipid signature, enabling identification of colonocytes, pericryptal cells, lymphocytes or macrophages.

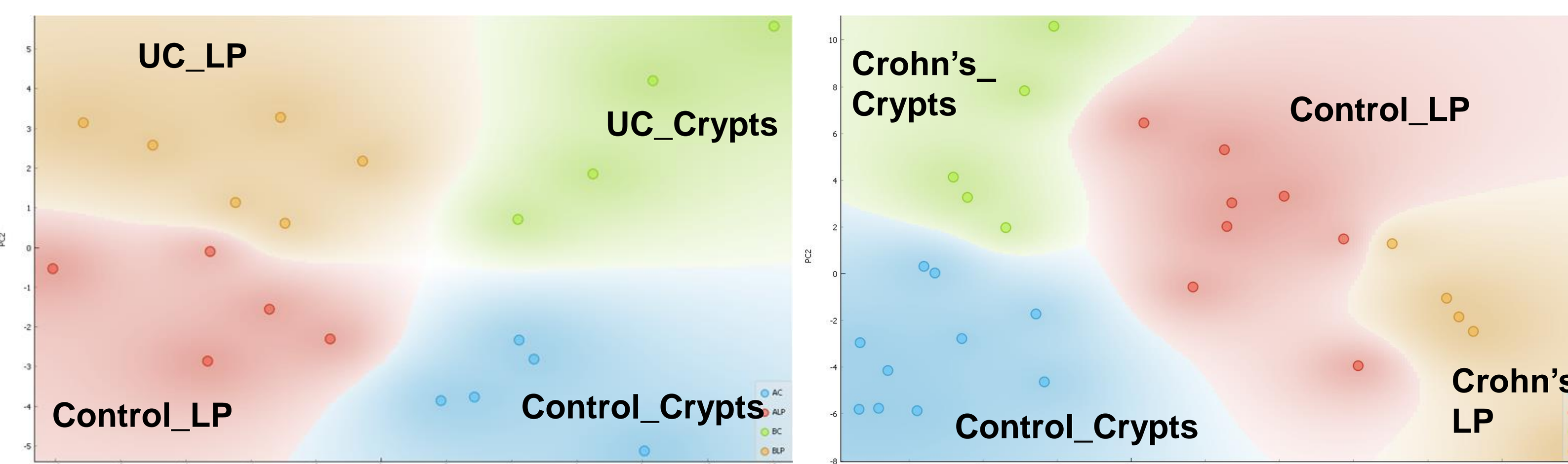
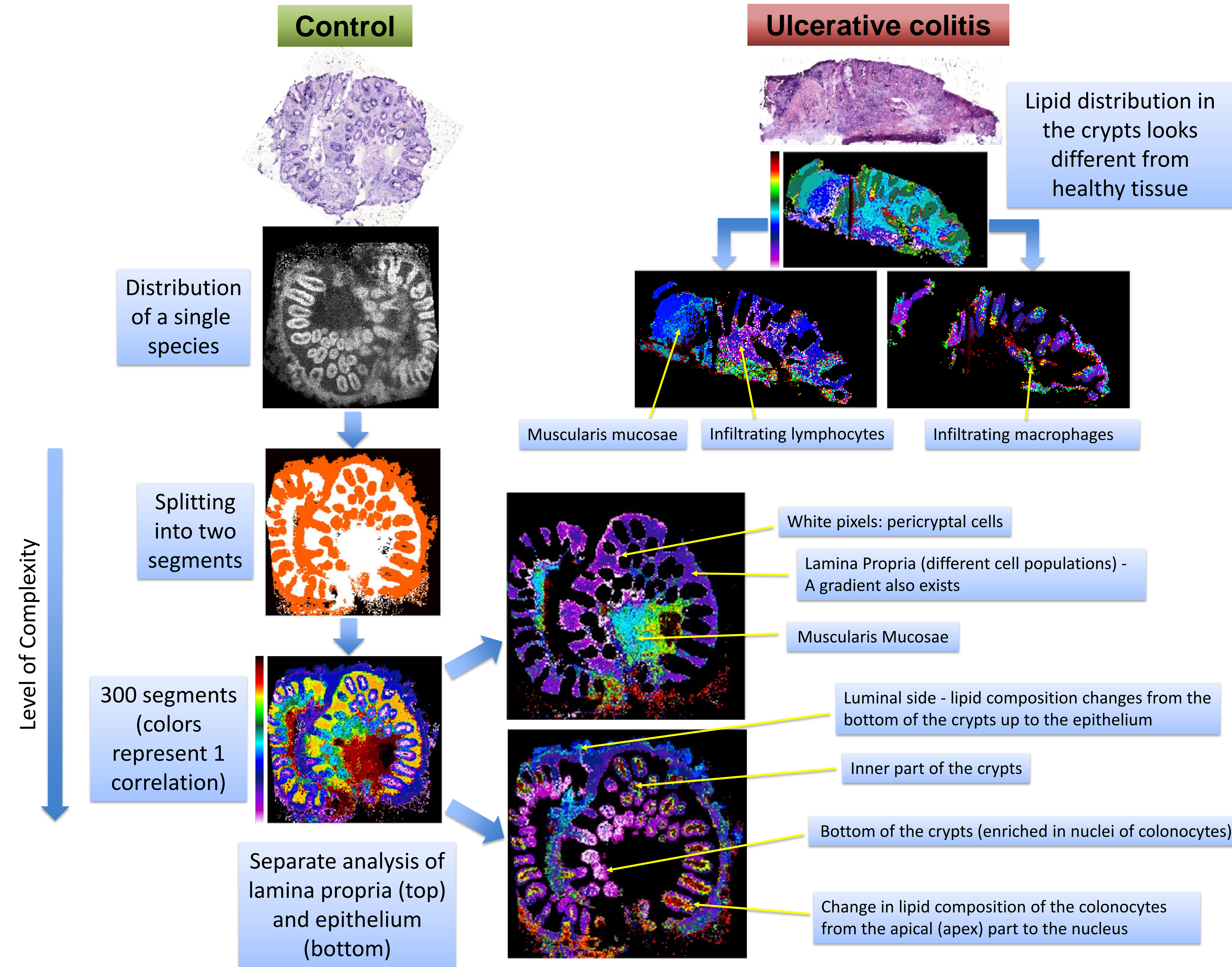


Figure 3. Analysis of the lipid signatures of LP and epithelium (crypts). There is a clear difference in lipid composition between control and disease but also between LP and epithelium.



Conclusions

Using matrix sublimation and the MALDI-7090 we were able to determine the lipid distribution in sections of biopsies of Crohn's disease and ulcerative colitis.

The comparative analysis between lipid distribution in lamina propria and crypts highlights important differences in lipid expression.

Deeper analysis unveils a complex structure, in which each cellular type has a characteristic lipid fingerprint.

Part of the differences in lipid expression may be due to the infiltrating cells of the immune system, reinforcing the need for spatially-sensitive techniques in the analysis of these complex samples.

Acknowledgments

The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.