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Identification of Lipid Biomarkers of Human Inflammatory Bowel Disease Using Imaging Mass Spectrometry

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Introduction

Inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) are chronic and relapsing inflammatory conditions of unknown etiology. 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. Here we present a mass spectrometry imaging (MSI) study of endoscopic biopsies obtained from the following patient cohorts, IBD, hereditary colorectal cancer (CRC-H) and control, at 10 micron (µm) spatial resolution. As lipid composition may vary between tissues, detecting several lipids could serve as specific biomarkers of each type of lesion.

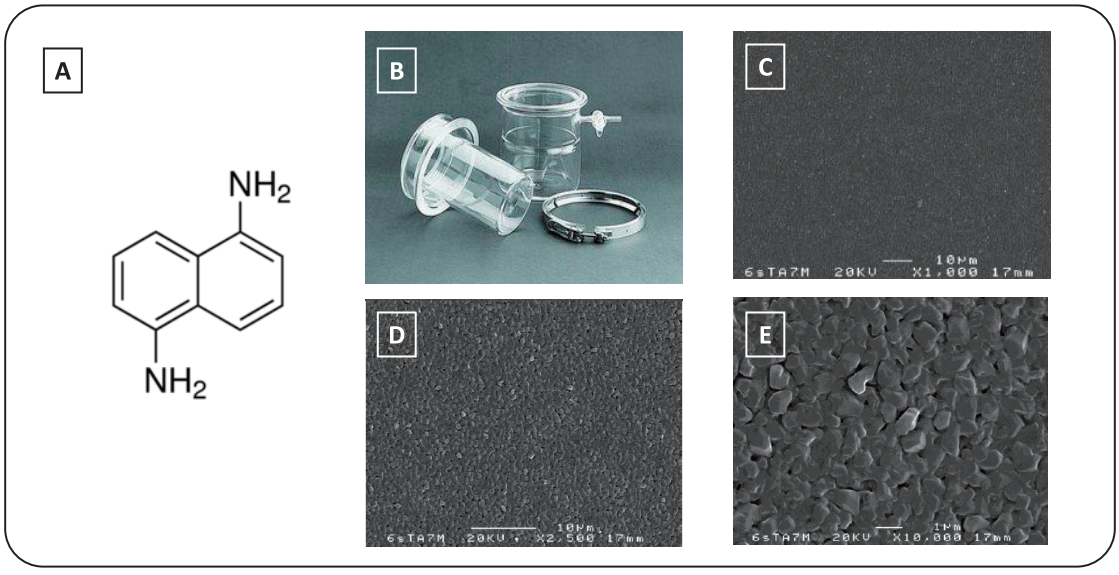

Figure 1 Structure of 1,5- Diaminonapthalene (1,5-DAN) (A), the Ace Glass Sublimation glassware (B) and SEM images of the DAN crystals (matrix density of 89 µg/cm^2) at x1,000 (C), x2,500 (D) and x10,000 (E) magnifications. The homogenous matrix deposition of 1,5-DAN via sublimation can be seen in the SEM images with an average crystal size of ~3µm

Methods and Materials

The sample collection for this study was specifically approved by the Ethics Research Committee of the Balearic Islands (IB 2118/13 PI and IB 2291/14). For initial method development, mouse colons were snap frozen and sectioned at 10 µm thickness. The five sections were coated with 1,5-DAN (Figure 1) at three different sample preparation times (8 mins, 10 mins and 12 mins) to assess which method produced the best MS images.

Following the initial experiments, excised human colon tissues were snap frozen in liquid nitrogen and subsequently cryo-sectioned at 10 µm thickness. Tissue was taken from three patient cohorts; IBD, CRC-H, and control patients.

Sections were covered with 1,5- diaminonapthalene (1,5-DAN), a suitable matrix for negative-ion detection, using a glass sublimator (Ace Glass 8023). A MALDI-7090™ TOF-TOF (Shimadzu) mass spectrometer was used to image the samples (laser spot diameter 10 µm, pixel size 10 µm, scanning range 200-2000 Da). Circular 10 µm laser spots facilitated imaging to be carried out without the need for oversampling; the resolution achieved allowed us to successfully distinguish between the lipid profiles of lamina propria, colonocytes and muscularis mucosae. MS images were processed using the IonViewTM software (Kratos Analytical Ltd.).

MALDI-TOF/TOF
Linear & reflectron
PSD & High Energy-CID
High resolution MS/MS
355 nm ultra-fast solid state UV laser up to 2kHz laser repetition
Variable beam focus from 10 µm to >100 µm

Figure 2 MALDI-7090™ TOF/TOF Mass Spectrometer

Results

Part I

In agreement with previous literature1, the three basic tissues found in the colon mucosa were clearly identifiable in the MS images due to their unique lipid composition. The basic tissues include the epithelial layer, the lamina propria and the muscularis mucosae. Table 1 includes some of the lipid species detected in the following negative ion mode MS images.

Table 1. Negative ion mode species previously identified from the three main types of tissue in human colon (adapted from [1]Garate *et al*. 2015)

Theoretical m/z	Peak assignment
715.5750	Cer-PE 38:1-H-
700.5287	PE P-18:0/16:1 or PE P-16:0/18:1
726.5598	PE O-16:0/20:3
750.5443	PE P-18:0/20:4
766.5392	PE 18:0/20:4
788.5447	PS 18:0/18:1
794.5094	SFT d18:1/16:0-2OH
797.6665	TG 48:4
810.5291	PS 38:4
833.5186	PI 34:2-H-
835.5342	PI 16:0/18:1
861.5499	PI 36:2
885.5438	PI 18:0/20:4

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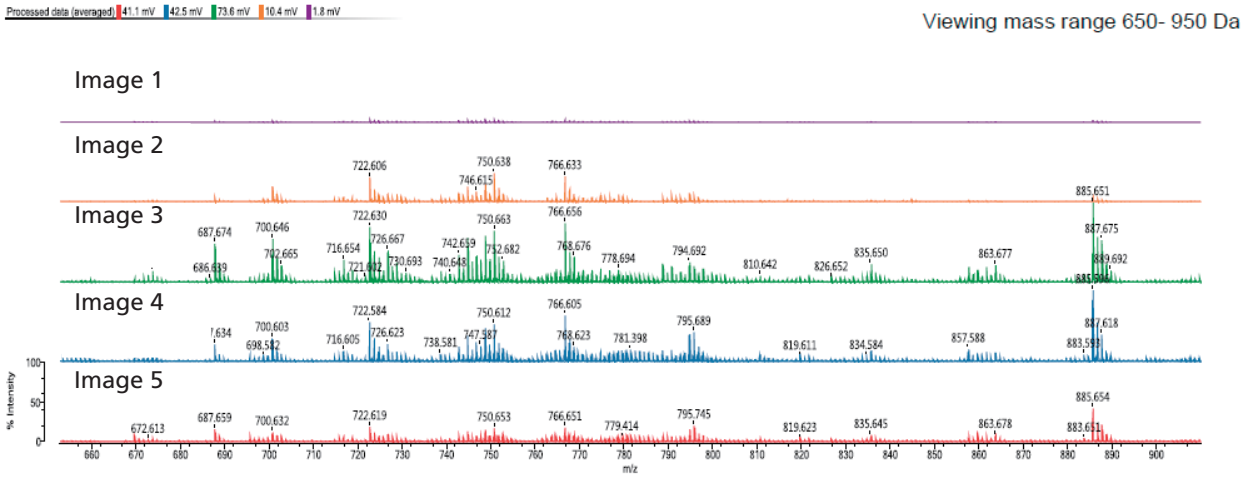

Figure 3 TIC spectra from the three sample preparation times are shown; 8 mins (Images 1 and 2), 10 mins (Images 3 and 4) and 12 mins (Image 5). Comparison of the TIC spectra shows that Image 3 (10 mins sublimation time) produced the overall highest signal intensities and the best MS images where the muscularis mucosae is clearly defined and the colonic crypts are visible (see corresponding three images in Figure 4).

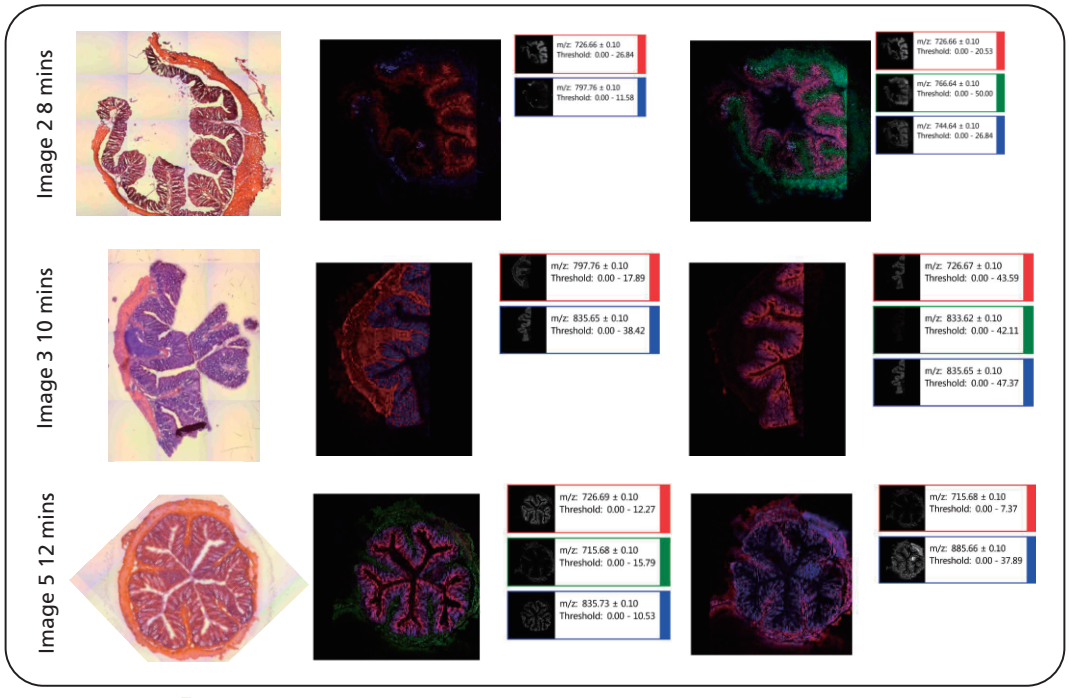

Figure 4 Microscope images of hematoxylin and eosin (H&E) stained consecutive sections are displayed alongside MS Images from the three sample preparation times. High spatial imaging enabled visualisation of the different regions within the colon tissue

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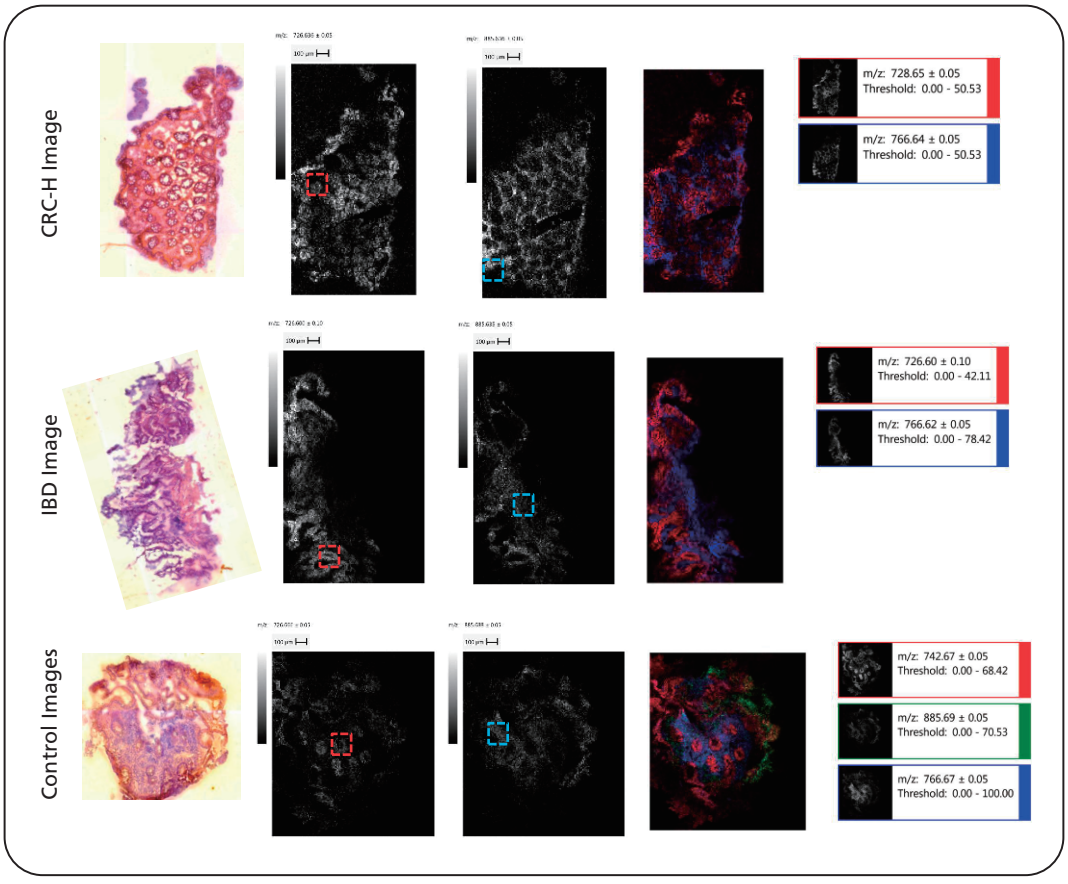

Figure 5 Microscope images of H&E stained consecutive sections are displayed alongside the MALDI MS images. Subsequent multiplexing of the ion distributions demonstrate that ions are specific to either the epithelium or lamina propria. The red box indicates the areas selected to generate the region of interest (ROI) spectra from colonocytes and the blue box for the lamina propria (see ROI spectra in Figure 6)

Part II

The results clearly indicate that each tissue type presents a unique lipid fingerprint. Changes in the relative intensities of the lipid species can also be observed between samples. Whilst the exact correlation between intensity ratios is yet to be investigated it is clear that these cell-specific profiles are most likely to be related to their physiological function within tissue1. Data suggests that only certain location specific biomarkers are able to distinguish between IBD and neoplastic lesions. Such observations highlight the complexity of the disease and the importance of using an imaging technique to isolate ROIs for the search of biomarkers. Spectra from the colonic crypts and lamina propria of the three sample types were extracted as ROIs and compared.

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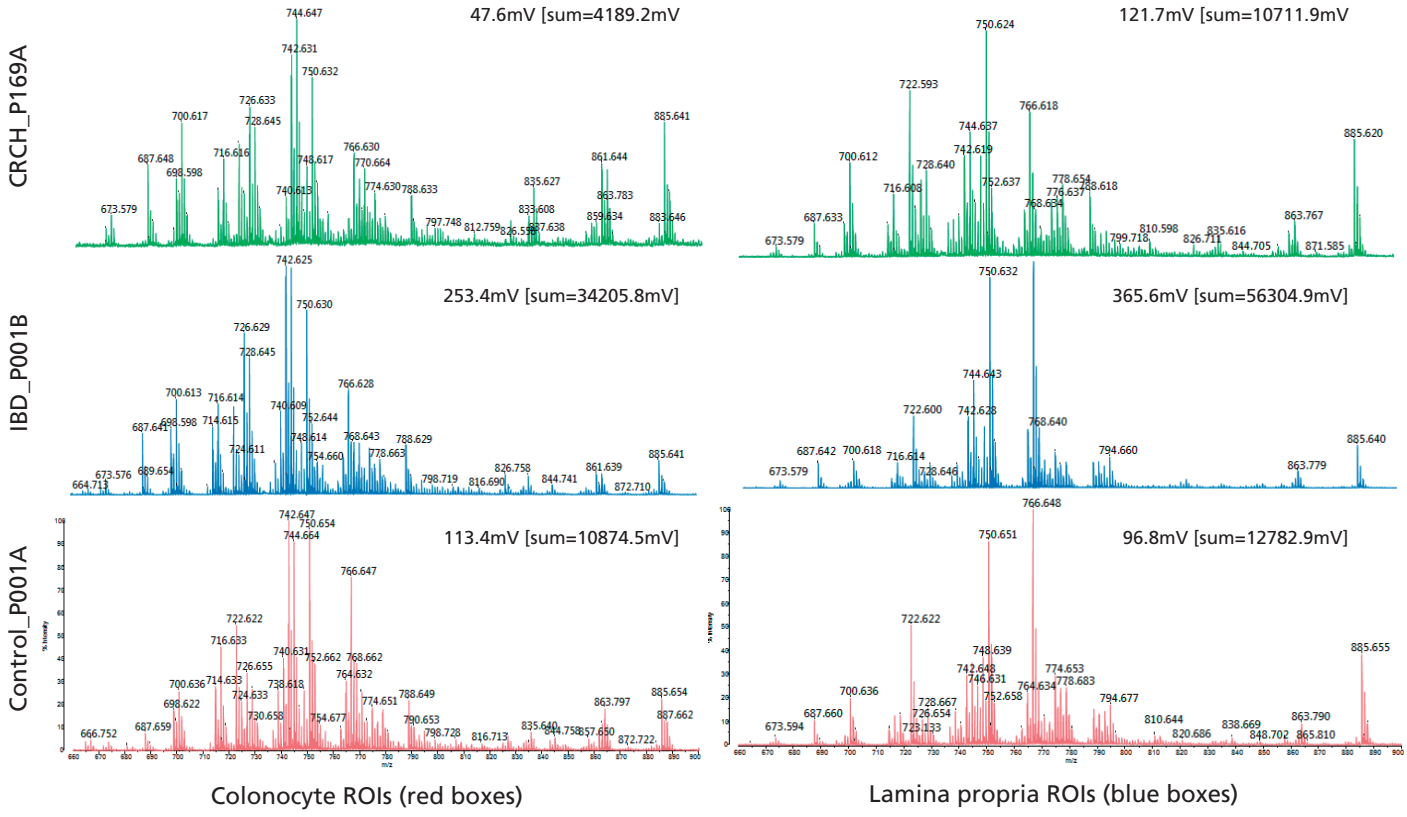

Figure 6 Lipid profiles comparing the colonocytes and lamina propria regions of the three tissue types; CRC-H, IBD and Control. In agreement with previous literature, PI 16:0/18:1 (*m/z* 835.5) and PI 36:2 (*m/z* 861.5) were more specific for colonocytes whereas PI 18:0/20:4 (*m/z* 885.5) produced a stronger signal in lamina propria and muscularis mucosae. PI 18:0/20:4 is the main reservoir of arachidonic acid; a signaling lipid, involved in inflammation and colonocyte maturation

Conclusions

This study demonstrates that with the correct matrix and sample preparation method, high resolution imaging can be achieved. Key advantages were demonstrated in comparison to data previously acquired on the LTQ-Orbitrap XL (ThermoFisher)1; data were acquired at higher laser repetition rates therefore reducing sample acquisition times and enabling the whole biopsy to be scanned at 10 µm resolution. Unlike the LTQ-Orbitrap XL, the MALDI-7090™ 10 µm laser spot also eliminates the need to oversample.

Our preliminary results show that differences between the lipid fingerprints of the three sample cohorts exist, both in the lamina propria and in the colonocytes. Interestingly, the lamina propria spectra of the control and CRC-H are similar when compared to that of IBD. Theoretically, the only difference between the control and CRC-H mucosa is that the latter has a mutation to make the mucosal lining prone to developing polyps. Future experiments will focus in the precise identification of the species that experience the largest changes using MS/MS directly from the tissue followed by statistical analyses.

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