MULTIPLEXED TARGETED IMAGING OF INTACT PROTEINS IN TISSUE BY MULTI REFLECTING TIME OF FLIGHT (MRT) MALDI-IHC

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INTRODUCTION

Multiplexed Matrix Assisted Laser Desorption ionsation immunohistochemistry (MALDI-IHC), is a technique where photo-cleavable peptide tags attached to antibodies are bound to proteins within tissue and released via UV cleavage prior to MALDI imaging and provide localisation information for the original protein. Typically, these proteins will have a molecular weight outside of the instrument mass range and would traditionally have required on-tissue digestion prior to analysis which can reduce localisation and specificity.

MALDI-IHC is shown to yield results similar to traditional fluorescence immunohistochemistry (1), Traditional immunofluorescence histochemistry is typically limited to 2-5 markers per tissue section, this is due to spectral overlap in excitation and emission wavelengths, in contrast MALDI-IHC is able to image >40 markers per tissue section and is only limited by the number of available masses and availability of tags for all proteins of interest.

Here we demonstrate the compatibility and advantages of this technique on a SELECT SERIES™ MALDI MRT mass spectrometer. The high mass accuracy (>500 ppb) allows for unambiguous identification of released tags, easily identified from endogenous signals which is coupled with the high mass resolution (>200,000 FWHM) which significantly reduces potential signal overlap with endogenous signals of similar mass aiding low background signal.

METHODS

Sections of Human Tonsil (Ambergen) and Human Kidney (ccRCC ISUP grade 3) FFPE tissues were prepared in accordance with the AmberGen MALDI HiPLEX-IHC Miralys™ Imaging laboratory Workflow user guide (Control number v173(J)) protocol. The tonsil control sections were purchased pre stained, the Kidney sections were stained in-house. The samples were analysed using a SELECT SERIES MRT MALDI, in positive ionization mode, with a mass range of 50-2400. Due to the tags small mass distribution, a fixed quad setting of 1000 Da was set. The laser repetition rate was 2 KHz with a scan speed of 10 s/s. Images were acquired at 50 µm pixel size and 20 µm pixel size with a laser focus setting of 4.0 mm and 5.8 mm respectively.

The protocol for tissue processing comprises of, deparaffinization, rehydration, antigen retrieval, tissue blocking and then staining. The pre stained tonsil tissue sections were stained with the following antibody probes, CD3ε, CD68, VIM, Collagen-1A1, PanCK and Ki67 The kidney tissue sections were stained with the probes for VIM, Actin-aSM, PanCK and Ki67. Before matrix application the samples were exposed to UV light to cleave the peptides. CHCA Matrix was applied to the tissue sections prior to analysis using a HTX™ M5 sprayer following the Miralys protocol.

Data were processed and visualised using Waters™ High definition imaging software (HDI™ 1.7)

RESULTS

The MALDI-IHC imaging results from the Human Tonsil FFPE tissue section can be seen in figure 1(50 µm pixel size). The resulting distributions match those previously reported in literature using an alternative MALDI system (1) and Ambergen Miralys validation kit documentation.

Figure 2 shows distributions for the Human Kidney section images (50 µm), Actin-aSM highlights the smooth muscle of the blood vessels, VIM highlights the vimentin intermediate filament protein in endothelial cells of blood vessels smooth vascular musculature. PanCK localizes pancytokeratin within the epithelium and finally Ki67 shows the location of nuclei in cells undergoing proliferation, increased prescence within the tumor is as expected.
Figure 3 shows the results of a 20 µm pixel experiment performed on the left hand tumor lobule seen in figure 2. The general distributions observed match those for figure 2. However, Ki67 signal can be seen to be isolated to individual separate pixels. Also, much more detail can be seen in terms of micro structures within the tumor with the Actin-aSM probe, this is highlighted in figure 4.

Figure 4 shows the distribution of Actin-aSM (A) with an expansion of one of the blood vessels (B) a signal cross section for the Actin-aSM through the blood vessel can be seen in C demonstrating the lack of background observed by the SELECT SERIES MRT MALDI system. The mass resolution >200,000 FWHM is shown in D.

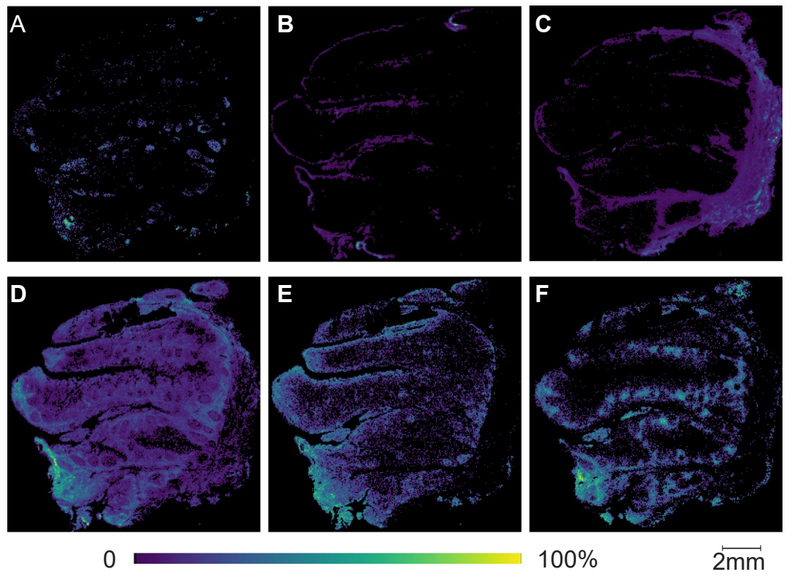

Figure 1. Results of MALDI analysis of pre-stained human tonsil FFPE section at 50µm pixel size. A) m/z 1320.7 Ki67, B) m/z 1288.7 PanCk, C) m/z 1234.8 Collagen-1A1, D) m/z 1230.8 VIM, E) m/z 1216.7 CD68, F) m/z 1161.65 CD3ε.

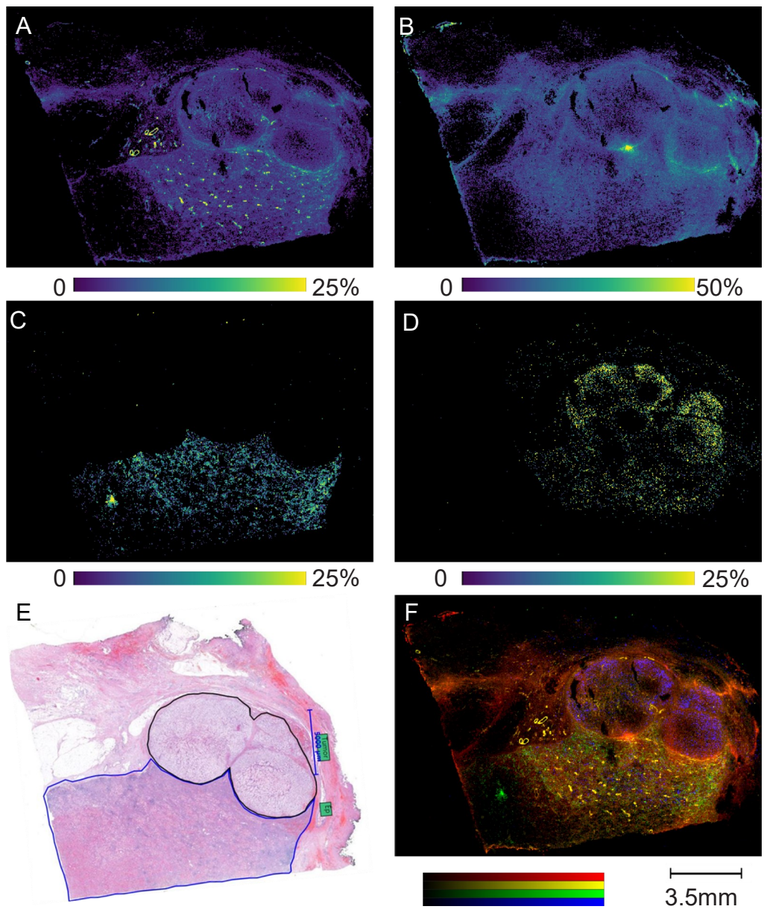

Figure 2. Results of MALDI analysis of stained human ccRCC ISUP grade 3) FFPE section with a 50 µm pixel size. A) m/z 1251.7 Actin-aSM, B) m/z 1230.8 VIM, C) 1288.72 PanCK, D) m/z 1320.7 Ki67, E) H&E stained consecutive section blue border region—epidermis, black bordered region—Tumor. F) 4 Colour overlay of A-D, yellow A, red B, green C, blue B.

Image intensity ranges have been adjusted to improve visualisation only, no lower cutoff is applied.

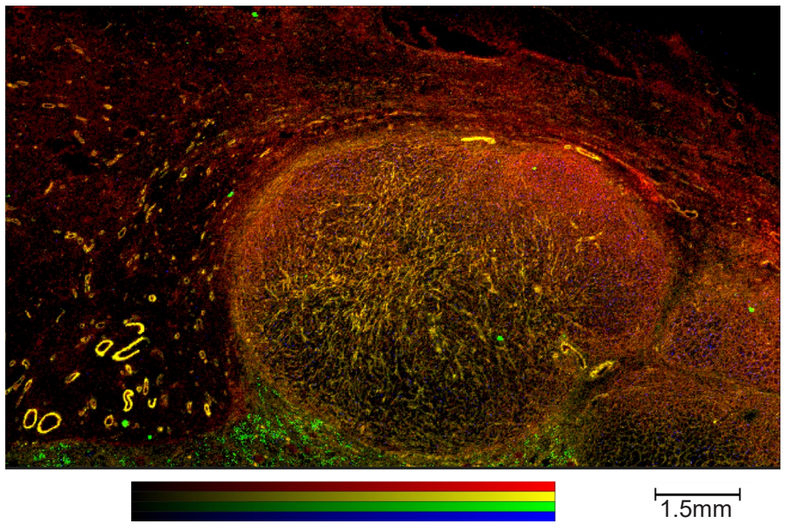

Figure 3. 20 µm pixel size image 4 color overlay of the left side tumor lobule, maximum intensity has been scaled for visualization no minimum cut off has been applied, yellow; Actin-aSM, max intensity 25%, red; Vim, max intensity 50%, green; PanCK, max intensity 25%, blue; Ki67, max intensity 10%.

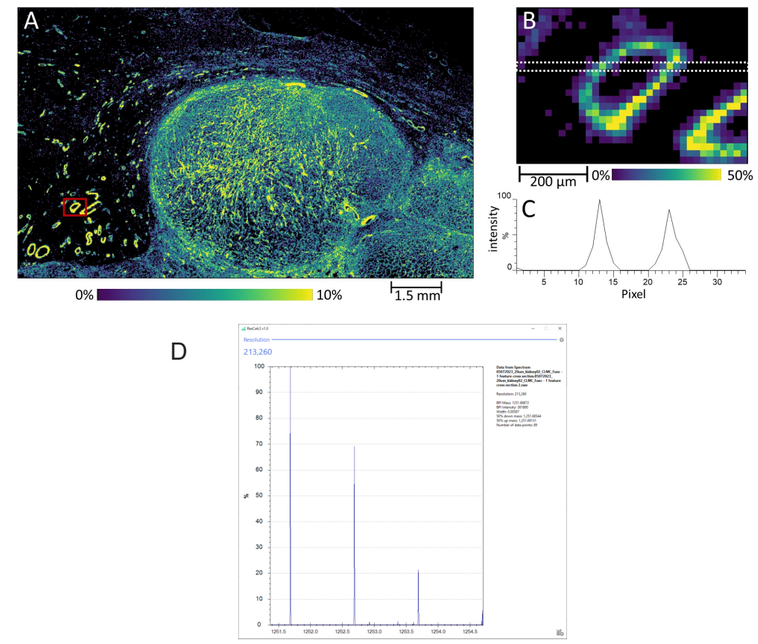

Figure 4. A) Distribution of released peptide tag from Actin aSM protein probe - m/z 1251.68 (20 µm Pixel size) in Human Kidney (ccRCC ISUP grade 3) FFPE tissue section, red box denotes area shown in expansion (Panel B). B) expansion of area indicated in panel A, dotted region denotes pixel line intensity profile extracted and shown in panel C. C) extracted intensity profile for line indicated in panel B extracted mass window = 0.0055 Da. D) Mass resolution calculation for m/z 1251.68 using combined spectra from C, mass resolution is calculated to be 213,260 FWHM

CONCLUSION

- **MALDI IHC analysis was successfully performed on a SELECT SERIES MRT MALDI mass spectrometer**
- **Results were validated with kit provided information**
- **Images were acquired at 20 µm with low to non existent background noise and a mass resolution >200,00 FWHM**
- **Successful MALDI-IHC paves the way for multiOMIC spatially resolved experiments on the same mass spectrometer**

References

1. G. Yagnik *et al* ;*J. Am. Soc. Mass Spectrom.* 2021, 32, 4, 977−988

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