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Benchtop Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging of Human Tonsil Proteins using MALDI HiPLEX-IHC Probes

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1. Introduction

- MALDI mass spectrometry imaging (MSI) is a versatile technique capable of characterising lipid, polysaccharide, metabolite, pharmaceutical, protein and peptide distributions within a wide variety of samples. Recently, MSI has been combined with commercially available antibody probes containing photocleavable mass-tags (PC-MTs) which target specific proteins in what has been termed MALDI-immunohistochemistry (MALDI-IHC) MSI.
- In this study, we show imaging of FFPE human tonsil sections on an entry level benchtop MALDI-8020 using both a multiplex of 6 antibodies and a cancer marker screening panel consisting of a multiplex of 20 antibodies at a stage step size of 30µm, demonstrating a reliable, low-cost approach with good quality results.
- Analysis of a follicular hyperplasia tissue sample showed visible differences from normal human tonsil tissue relating to structural features and cell distributions (Fig. 1).

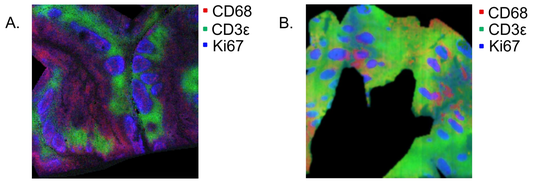

Fig. 1 MALDI-IHC images of PC-MT labelled antibodies from a 6 plex panel on (A) normal human tonsil and (B) human tonsil with follicular hyperplasia where an increased number of variably-sized germinal centres and different T-cell distribution are visible (IonView™, Shimadzu Corporation)

2. Methods

Prior to the mounting of tissue sections, FlexiVision-mini ITO slides were coated with 20µl 50:50 Poly-L-lysine solution: H2O containing 0.07% IGEPAL-CO63. Following deparaffinisation, the slides were immersed in a series of wash solutions (Ethanol: aqueous in varying concentrations) prior to antigen retrieval through exposure to an alkaline buffer. Following application of antibodies with novel photocleavable mass-tags (AmberGen, Billerica, MA) and subsequent coating with DHB using a sublimation device (iMLayer, Shimadzu Corporation), tonsil sections were imaged in linear mode (30µm spacing) on a MALDI-8020 benchtop MALDI-TOF mass spectrometer (Shimadzu Corporation). Imaging files were processed using IonView™ and IMAGEREVEAL™ MS software packages (Shimadzu Corporation).

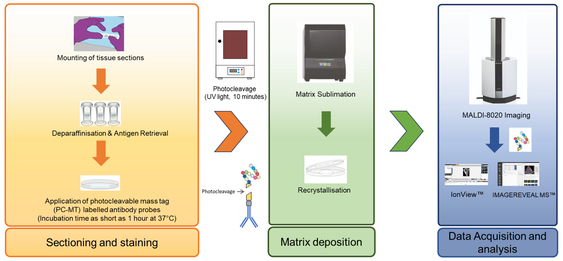

Fig. 2 MALDI-IHC workflow for the imaging of human tonsil.

3. Results

- 6-PLEX analysis

Optimal recrystallisation of the DHB matrix following sublimation was seen to be critical to produce good quality spectra for imaging. Recrystallisation was achieved through incubation at 55°C with 5% IPA. The time was optimised in house and was specific to the apparatus and sample preparation. Imaging analysis was performed with a stage step size of 30 µm. 60,201 profiles were accumulated in approximately 4 hours 8 minutes which produced a clean spectrum with isotopic resolution, yielding clearly visible structural organisation and immune cell distributions in the images generated. Overlays of different extracted ion images corresponding to different m/z produced good quality images which correspond with the features identified by traditional immunohistochemistry (Fig. 3).

- 20-PLEX oncology panel (Fig. 4)

Imaging analysis was performed with a stage step size of 30 µm. 27,300 profiles were accumulated in approximately 1 hour 52 mins Clear isotopic resolution was seen across the extended mass range of ~2kDa with detection possible of all 20 peptide mass tags (Fig. 5).

- Follicular Hyperplasia sample

Follicular hyperplasia is a benign condition which is characterized by an increased number of germinal centres with variably-sized mantle zones. The tissue was stained using the 6-plex probe panel. Imaging analysis was performed with a stage step size of 30 µm. 29,650 profiles were accumulated in approximately 2 hours 2 minutes. As shown in Fig. 1, the distributions of key markers (Ki67 and CD3ε) are visibly different in normal tonsil tissue when compared with tonsil tissue with follicular hyperplasia, showing the potential for this technology to develop into clinical applications.

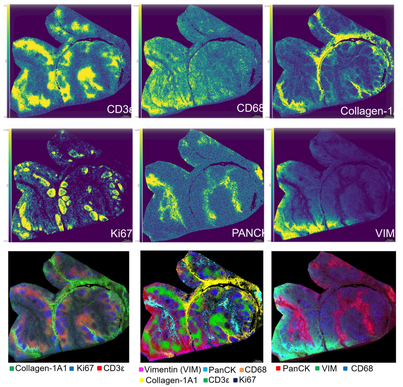

Fig. 3 Individual and overlaid extracted ion images from a 6-plex probe panel.

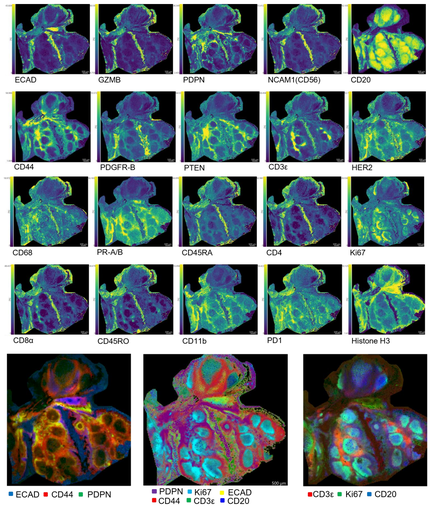

Fig. 4 Individual and overlaid extracted ion images from a 20-plex probe panel.

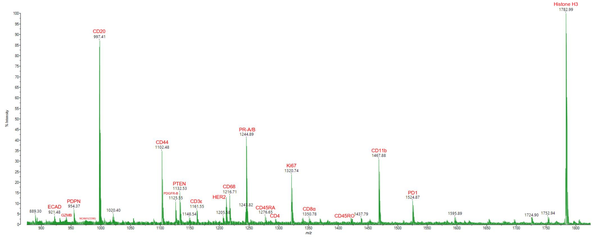

Fig. 5 Accumulated MALDI-TOF mass spectrum following imaging analysis of the 20-plex tonsil section.

- H&E Staining

Following MSI, matrix was removed using acetone and the sections underwent H&E staining. Comparison of the H&E stained images with the extracted ion images from the 6-plex probe panel showed corresponding structures which aligned closely when overlaid (Fig. 6).

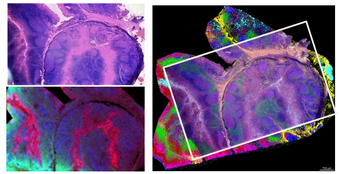

Fig. 6 H&E stained tissue showing comparable results to MALDI-IHC

4. Conclusion

- We have successfully mapped up to 20 biomarkers with a stage step size of 30µm using a low cost, linear benchtop MALDI-TOF mass spectrometer in conjunction with the Miralys™ probe kit.
- The spectra were well-resolved allowing for clear and specific imaging in both normal and clinically significant samples.
- The Shimadzu MALDI-8020 and MALDI-8030 MALDI-TOF mass spectrometers are robust systems with class leading sensitivity and resolution capable of a wide variety of applications including, as demonstrated here, MALDI imaging. These systems can provide an invaluable contribution to any research laboratory.

5. Acknowledgements

We would like to thank AmberGen™ for the customer support provided to us during the course of this project.