Benchtop Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI) of Human Tonsil Proteins using MALDI HiPLEX-IHC Probes

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1.Overview

- The MALDI-8020 and MALDI-8030 benchtop systems were used to perform protein imaging in tonsil tissue using MALDI-HiPLEX-IHC Probes.
- Images were produced at a stage step size of 50 µm.
- The benchtop systems produced clean spectra with isotopic resolution of the target mass reporter peptides.
- MALDI-IHC simplifies the analysis of large proteins in tissue by MALDI-MSI.
- These benchtop systems can offer a cost-effective entry into MALDI imaging or serve as a useful addition to more established imaging laboratories.

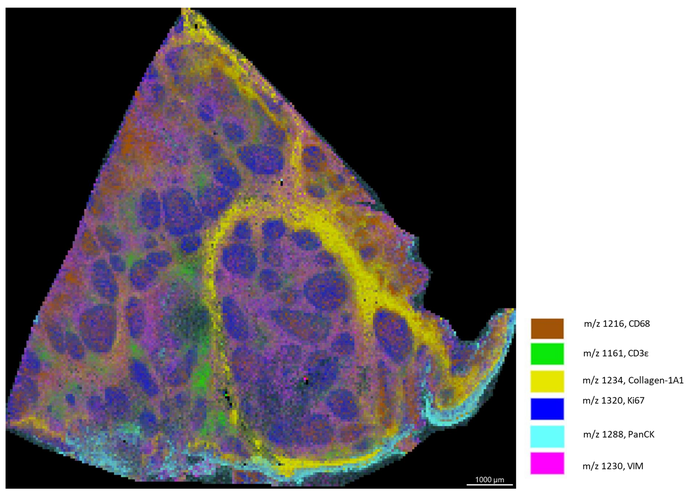

Figure 1. MALDI-IHC image of Human Tonsil section showing 6 separate PC-MT labelled antibodies (ion images generated using IMAGEREVEAL MS software (Shimadzu))

2. Introduction

As a secondary lymphatic organ, the human tonsil provides a rich site for the study of immune cell function with the potential to enhance research into autoimmune disease, food allergies, oncology and vaccination strategies, amongst others. Here, we demonstrate an entry level MALDI imaging solution to allow the precise mapping of proteins representative of immune cell populations and structural organisation within human tonsil tissue sections using Miralys™ MALDI HiPLEX-IHC probes (AmberGen, MA).

MALDI mass spectrometry imaging (MSI) is a versatile technique capable of revealing valuable information on lipid, polysaccharide, metabolite, pharmaceutical, protein and peptide distributions within a wide variety of samples. Requiring a minimal sample size, MALDI-MSI is proving itself to be a valuable tool for research and in combination with MALDI-IHC, we can further our knowledge of cellular interactions which is critical to the development new treatments and improving therapeutic outcomes. The Shimadzu benchtop MALDI instruments provide class-leading sensitivity and mass resolution. These robust systems are ideal platforms to begin exploring the worlds of MALDI-MSI and MALDI-IHC. In this study, we have been able to analyse FFPE human tonsil sections using a multiplex of 6 antibody probes (see Figure 1) at a stage step size of 50 µm, demonstrating a reliable low-cost approach with good quality results.

The photo-cleavable mass tag (PC-MT) labelled antibodies (See Figure 2) used in this study are designed to specifically bind to CD68 (cytoplasmic expression in macrophages, approximately 75-110 kDa), VIM[Vimentin](cytoplasmic & membranous expression in lymphoid tissue, approximately 57 kDa), Collagen-1A1(extracellular matrix, binds to the largest component of fibrillar collagen, approximately 138 kDa), Ki67(nuclear expression in germinal centre cells, two main isoforms of approximately 319 kDa and 359 kDa), CD3ε (cytoplasmic T-cell expression, binds to epsilon chain of the CD3 dimer, approximately 23 kDa) and Pan-CK (epithelial cell expression, binds to filament proteins approximately 40-68 kDa).

Following MALDI imaging, the sections underwent H&E staining, a traditional immunohistochemistry technique to compare with the images which were generated using both IonView™ and IMAGEREVEAL MS™ software (Shimadzu).

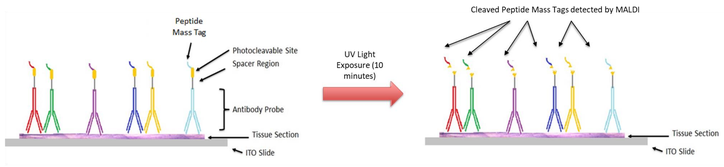

Figure 2. Schematic of PC-MT antibody probes in situ.

3. Methods and Materials

Prior to the mounting of formalin-fixed paraffin-embedded (FFPE) tissues, ITO slides were coated with 20 µL 50:50 Poly-L-lysine solution: H2O containing 0.07% IGEPAL-CO63. Slides were then mounted with 5µm thick sections of normal human tonsil sections (AMSBio, Abingdon, UK). To prepare the slides for application of the probes, the slides were deparaffinised, rehydrated by immersion in a series of wash solutions (Ethanol: aqueous in varying concentrations) and washed in a buffer solution. The samples then underwent antigen retrieval through exposure to an alkaline buffer. Antibody probes (AmberGen, Billerica, MA) were then applied to the tissue and incubated. The slides were then dried and stored at 4 ℃ prior to analysis. Immediately before analysis, the slides were exposed to UV light by placing in a light box (AmberGen, Billerica, MA) for 10 minutes. The slides were then coated with DHB using a sublimation device (iMLayer™, Shimadzu Corporation) and deposited matrix was recrystallised (5% IPA, 55 ℃). The tissues were imaged in linear mode (50 µm stage step size) on a MALDI- 8020 benchtop MALDI-TOF mass spectrometer (Shimadzu Corporation). Imaging files were processed using IonView™ and IMAGEREVEAL MS™ software packages (Shimadzu Corporation).

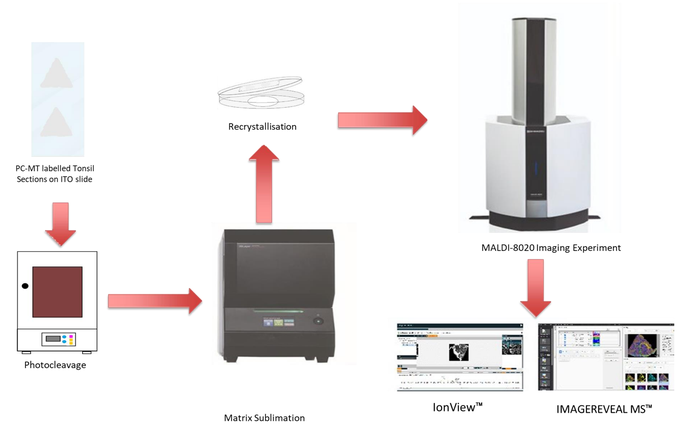

Figure 3. MALDI-IHC workflow for the imaging of immune cell distributions in human tonsil.

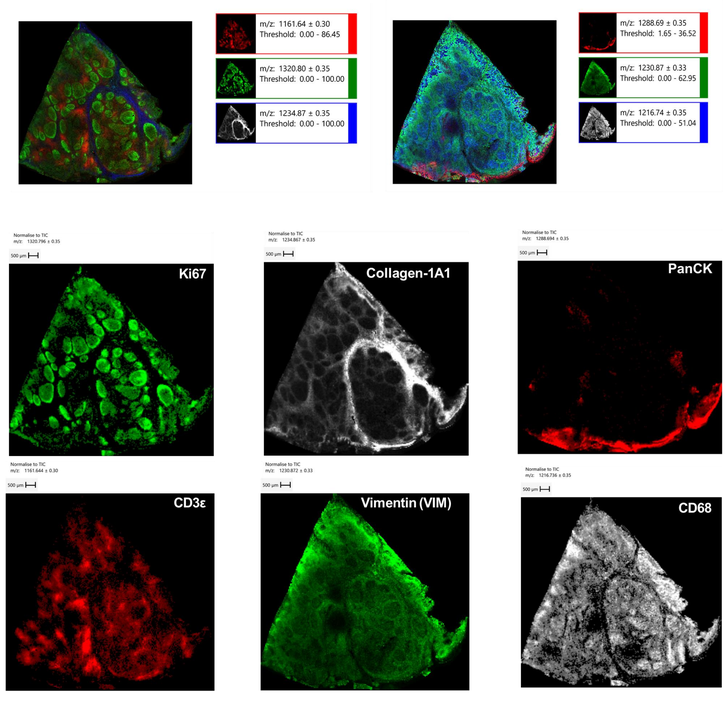

Figure 4. Individual and overlaid ion images show distinct patterns corresponding with the known cellular distributions in the human tonsil (ion images generated using IonView software (Shimadzu)).

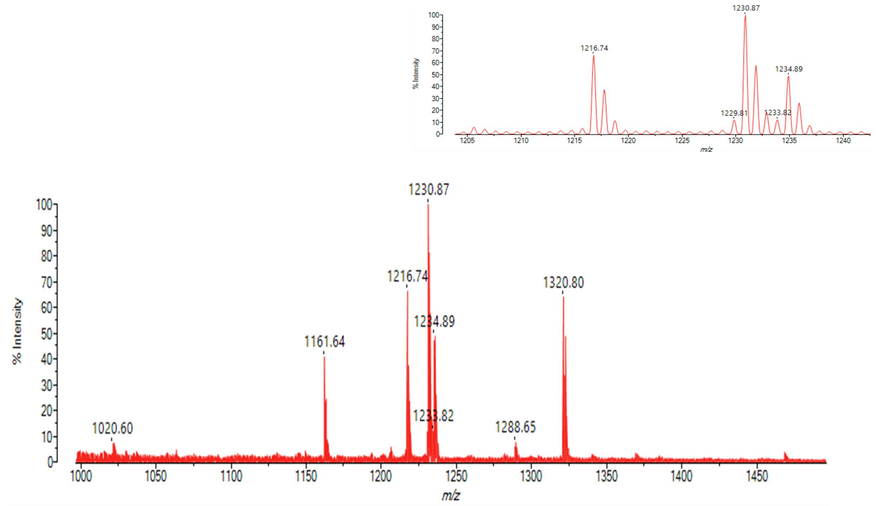

Figure 5. MALDI-MS spectrum following imaging analysis of human tonsil stained with a 6-plex probe mix. The probe mix contains mass tags detected at 1161, 1216, 1230, 1234, 1288 & 1320 m/z. Inset: Close up view of spectrum between m/z 1200-1250 showing isotopic resolution of peaks at 1216, 1230 and 1234.

4. Results

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℃ with 5% IPA. The time was optimised in house and was found to be dependent on the specific apparatus used.

Spectra were generated using DHB matrix and were optimised to achieve good isotopic resolution (See Figure 5). The commercially available PC-MTs (AmberGen, Ma) differ by more than 2 Da and the benchtop MALDI-8020 is more than capable of resolving the different mass tags. Optimisation of both the system and the procedure will be dependent on tissue type, matrix and apparatus available.

Imaging analysis was performed with a stage step size of 50 µm, which produced clearly visible structural organisation and immune cell distributions in the images generated (See Figure 4). Overlays of different m/z produced good quality images which correspond with the features identified by traditional immunohistochemistry staining and provide further detail on specific cell distributions not possible using traditional techniques.

Comparison with H&E staining of the slides following MALDI-MSI showed comparable structure to that seen in the generated images (See Figure 6). The use of specific antibody probes gives additional information not available from simple staining. Typical IHC staining can utilise 4-5 markers at one time. Given the wide number of PC-MT antibodies now available and with the possibility of additional custom-made probes, the number of probes that can be analysed in one analysis far exceeds that possible by traditional IHC staining. All available PC-MT antibodies have different mass tags that can be easily resolved by the benchtop system. Further experiments will be required to determine an upper limit on what is achievable in a single analysis.

The molecular weights of the proteins visualised in the experiment range from 20 kDa up to 359 kDa. Molecules of this size would be difficult, if not impossible to analyse by direct MALDI-MSI. This technique greatly increases the range of proteins that can be imaged through the implementation of a relatively straight forward workflow.

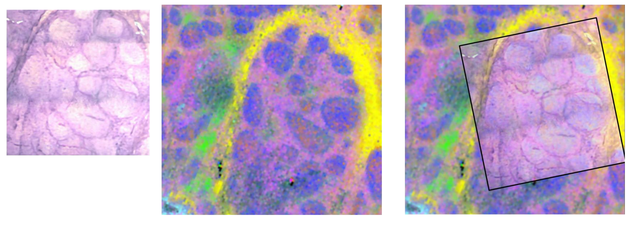

Figure 6. H & E staining of tissue following MALDI-MSI. The structural patterns show good alignment with collagen-1A1 (extracellular matrix) and Ki67 (nuclear expression in germinal centres) distributions in the MS image generated.

5. Conclusions

We have successfully mapped a multiplex of 6 protein biomarkers by MALDI-MSI on a low cost MALDI-TOF instrument. This was possible in conjunction with the Miralys photo-cleavable mass-tag antibody probes which are designed to specifically target proteins of interest. In this study, we have analysed FFPE tonsil tissue sections following deparaffinisation and treatment with the antibody probes. The resulting MS spectra were well-resolved, allowing for clear and specific ion image analysis (See inset, Figure 5). FF sample imaging analysis on higher performance MS instrumentation has been previously shown to achieve comparable results. The Shimadzu benchtop MALDI-8020 and MALDI-8030 are robust systems with class leading sensitivity, capable of a wide variety of applications. Following the introduction of the benchtop MALDI-TOF imaging starter kit, researchers are able to access a wide range of applications including MALDI imaging and, as shown here, MALDI-IHC. The instrument can serve as an entry into MALDI imaging or as an addition to more established imaging laboratories e.g., for method optimisation and to free up higher resolution/higher performance systems for studies requiring more detailed imaging analysis.

6. Acknowledgments

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