Site-to-site reproducibility of Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging from Formalin-Fixed Paraffin-Embedded samples

S Deininger[1], R Longuespée[2], R Casadonte[3], P Wandernoth[3], K Schwamborn[4], C Bollwein[4], C Marsching[5], C Hopf[5], W Weichert[4], J Kriegsmann[3], P Schirmacher[2], M Kriegsmann[2], and A Ly[1]
[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, [3]Proteopath GmbH, Trier, Germany; [4] Institute of Pathology, Technical University Munich, Munich, Germany, [5]Hochschule Mannheim, Mannheim, Germany;

IMSS/OurCon 2018

Introduction

Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) allows extraction of molecular information from tissue sections and can be used to create clinically relevant classification models, also known as tissue typing.[1] For widespread clinical application, MALDI-MSI must be performed on FFPE tissues. We present an integrated MALDI-MSI workflow for proteomic analysis of FFPE samples which is capable of discriminating between fine histological structures, which can be reproduced when carried out at different sites at multiple timepoints.

Methods

FFPE tissues (mouse intestine, TMA constructed from six different human tumours collected at three sites) were prepared for MALDI-MSI as outlined in Figure 1. Samples were sectioned onto conductive glass slides (Bruker Daltonik GmbH), and underwent deparaffinisation and antigen retrieval. Samples were sprayed with 0.025 µg/µl trypsin dissolved in an optimised buffer, and digested at 50°C using a saturated potassium sulphate salt solution to maintain 97% humidity. After digestion, samples were coated with 10mg/ml alpha-cyano-4-hydroxycinnamic acid in 70% acetonitrile with 1% trifluoracetic acid. A TM Sprayer (HTX Technologies, USA) was used for spraying both trypsin and matrix. Samples were measured at 50 µm step size with a rapifleX MALDI Tissuetyper TOF mass spectrometer (Bruker). Following acquisition, the tissues were stained with H&E, annotated by a pathologist, and co-registered to the MALDI-MSI datasets. Data were analysed using flexImaging (Bruker), SCiLS Lab Pro (Bruker) and open source software.

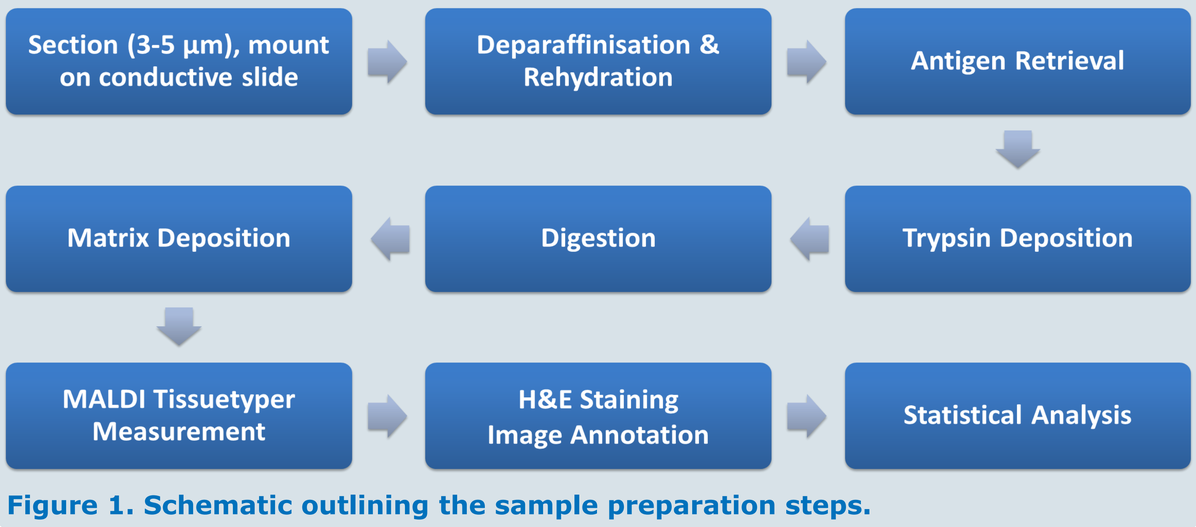

Figure 1. Schematic outlining the sample preparation steps.

Results

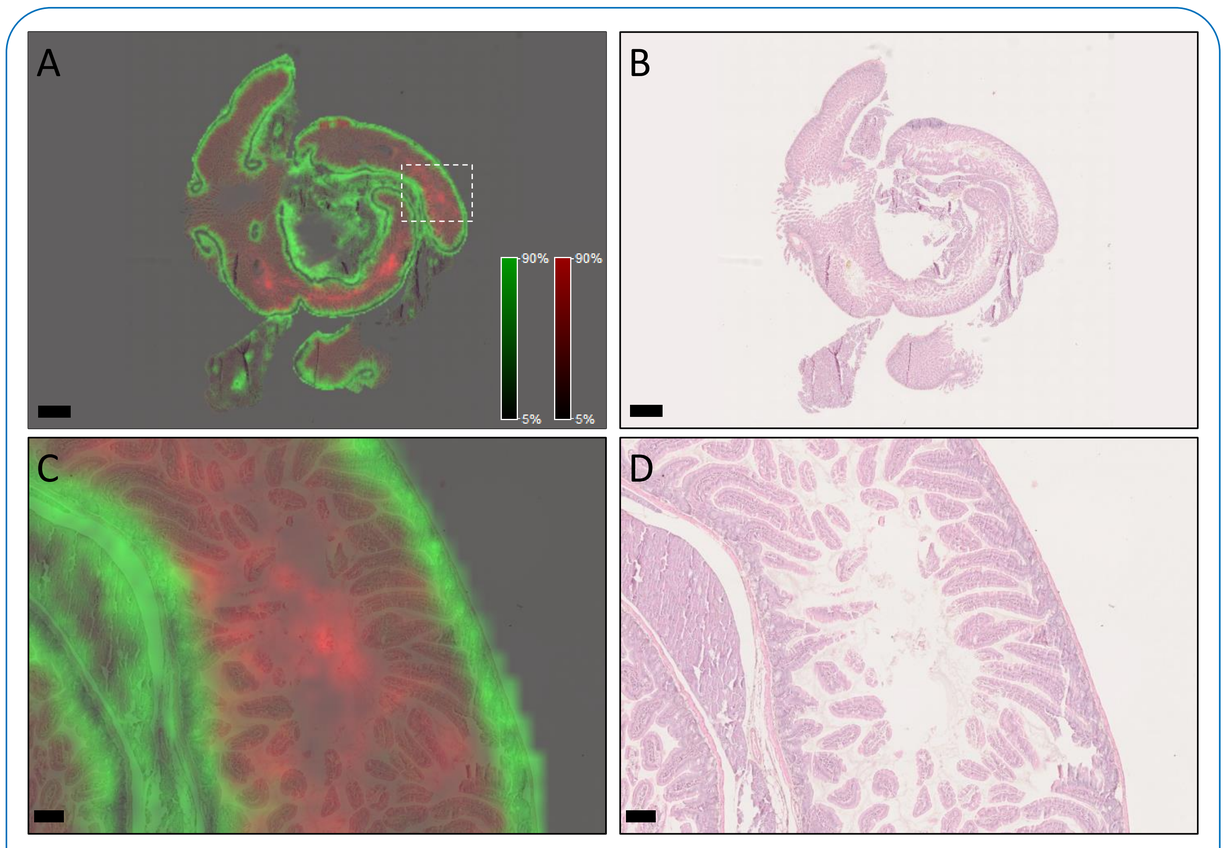

Figure 2. Conservation of *m/z* species to distinct regions in mouse jejunum fixed and processed according to pathology protocols.
A: MALDI image of whole mouse jejunum showing distribution of *m/z* 944.6 (red) limited to the villi and lumen, while *m/z* 1105.6 (green) is primarily in the crypts and muscle layers. **B:** Post-measurement H&E stained sample. Scale bars in **A** and **B** indicate 1 mm. **C & D:** Higher magnification view of the white-dotted box location in **A** confirms the distribution. Scale bars indicate 100 µm.

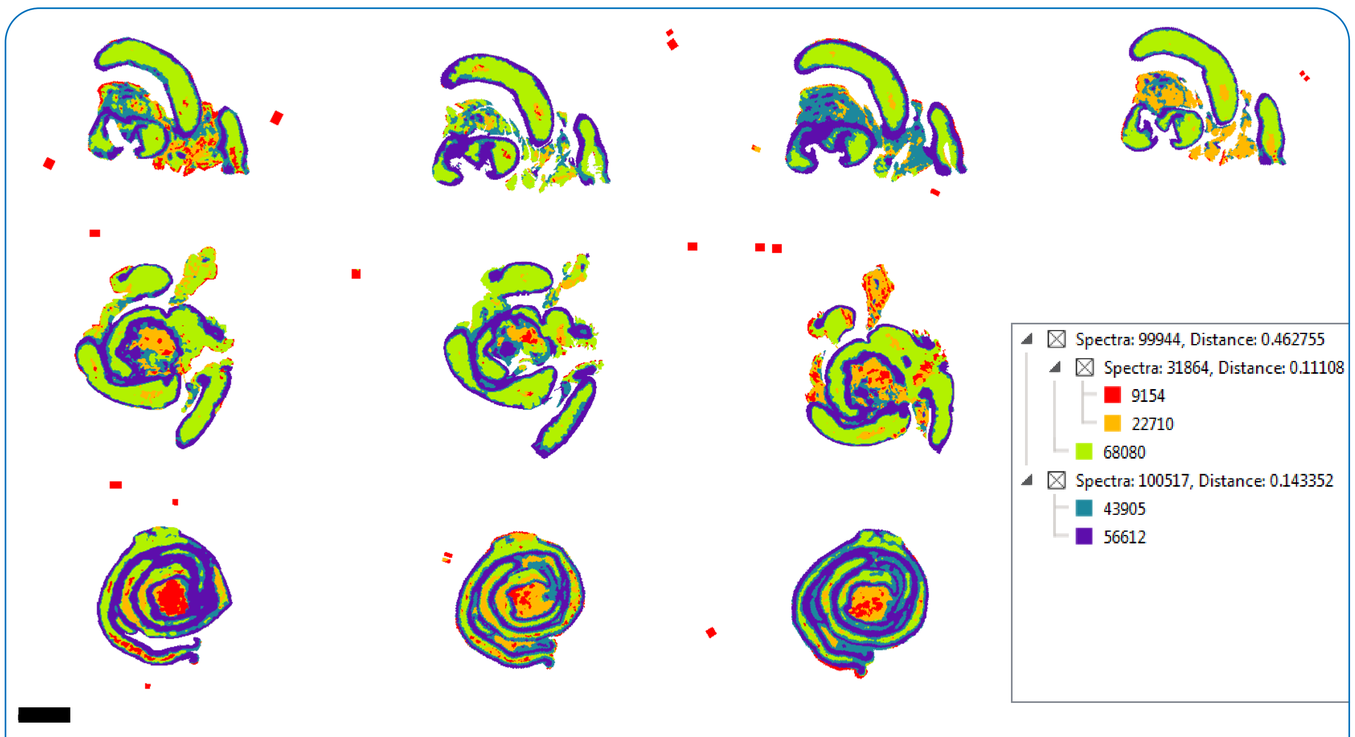

Figure 3. Segmentation analysis of mouse intestine samples measured at five sites over three time points. The clustering separates the spectra based on biological differences within the tissue (villi vs muscle – compare with Figure 2), rather than by location or time point. Scale bars indicate 3mm.

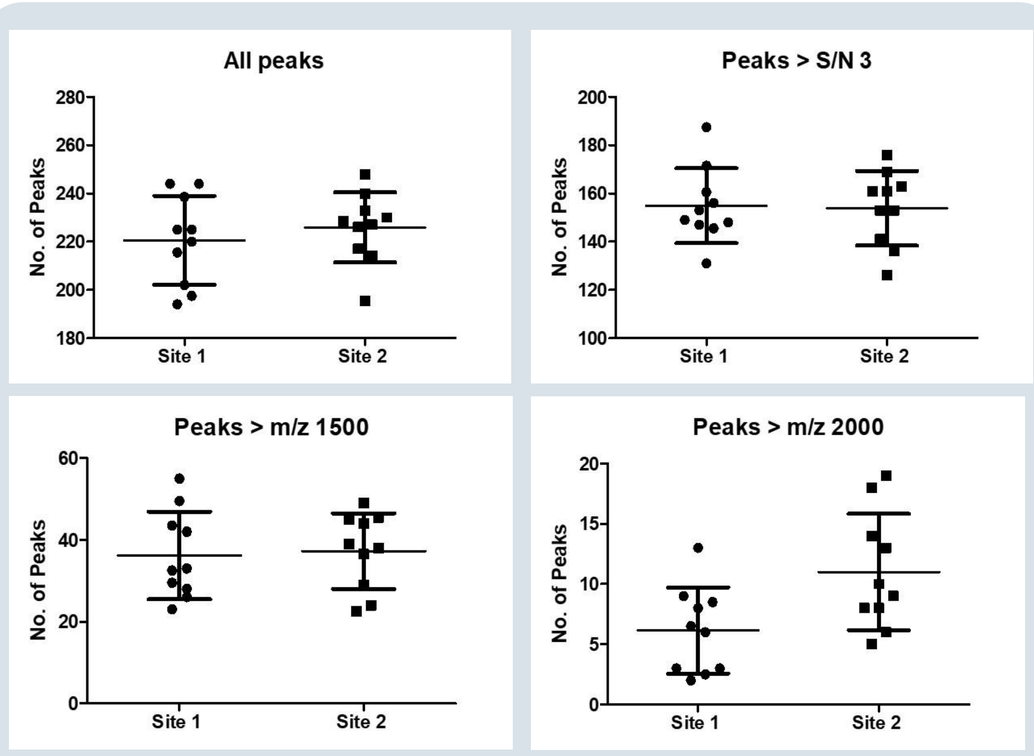

Figure 4. Inter-site reproducibility.
Twenty measurements of two different mouse intestine blocks from two sites (10 measurements per site) were compared for the median total number of peaks per spectrum, peaks with a signal to noise ratio above 3, peaks over *m/z* 1500 and over *m/z* 2000. An overlap of the confidence intervals is observed for all parameters, indicating reproducibility despite the differences in the tissues.

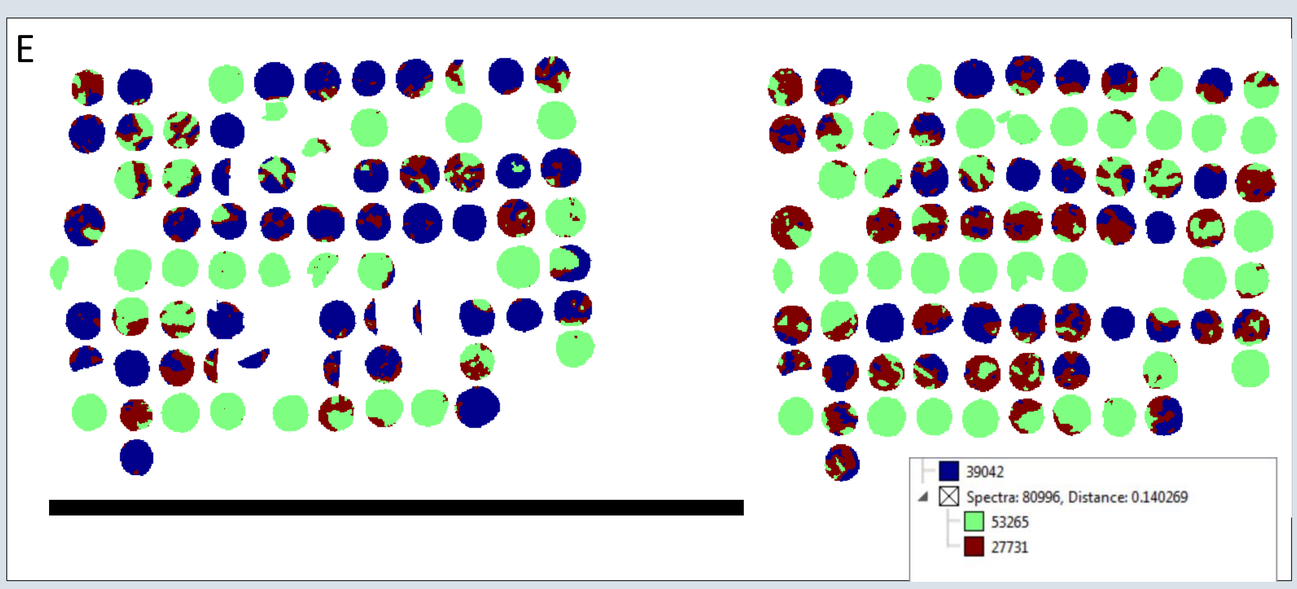

Figure 5. TMA composed of different tumours measured with MALDI-MSI.
Segmentation analysis of two TMA measurements (containing 6 different tumor entities sampled from three different sites) from different sites shows that the initial clusters (represented in blue, brown and green) do not separate in a way that reflects the measurement location or the sampling site. Clusters are instead formed based on non-tissue regions or regions high in stroma. Scale bar indicates 3 cm.

Conclusion

A protocol for the MALDI-MSI of clinical FFPE samples was published in 2011.[2] We present an improved workflow with updated instrumentation and modified sample preparation steps.

This workflow produces reproducible peak numbers and spatial resolution when conducted by different users, at different sites and time points.

Summary

- MALDI-MSI allows visualisation of multiple molecules in a single tissue section
- Improved workflow can discriminate histological regions
- Spatial resolution maintained in research and clinical samples

References and Acknowledgements

1. Longuespée et al., *Proteomics*, 2018
2. Casadonte and Caprioli, *Nature Protocols*, 2011

We would like to thank Christiane Zgorzelski from the Institute of Pathology, University Hospital Heidelberg, Germany and Susanne Hecht from Bruker Daltonik GmbH for expert help in sample preparation. We also thank Alain Creissen and HTX for the loan of a TM-sprayer and valuable input in protocol development.

MALDI Tissuetyper