Pipeline for acquisition and analysis of 3D MALDI imaging mass spectrometry data

3D MALDI imaging mass spectrometry (3D MALDI-IMS) is a label-free imaging technique[1] which reveals 3D spatial distribution of biomolecules in an organ or tissue specimen thus contributing to our understanding of biochemical and metabolic processes in a 3D spatially resolved manner. We present a pipeline for acquisition and analysis of 3D MALDI-IMS data established at the MALDI Imaging Lab, University of Bremen.

OBJECTIVE

Establishing a complete and robust 3D MALDI-IMS pipeline combined with efficient computational data analysis methods (FIG. 1).

METHOD

One of the critical points for successful 3D MALDI-IMS volume generation is the preservation of the tissue inherent morphological integrity. The commercially available PAXgene® Tissue Containers (PreAnalytiX, Germany) provide a fixation system that is compatible with MALDI-imaging. Paraffin embedding facilitates the slicing process which is of high importance for 3D-MALDI-IMS since sections of reproducible quality are required.

3D MALDI-IMS is performed by acquiring MALDI-IMS data of serial sections and merging them afterwards into a 3D dataset (FIG. 2). Therefore the reproducibility of acquired data is of high importance, which requires a homogenous matrix layer. The ImagePrep™ spray generator (Bruker Daltonik, Germany) uses the sensor controlled vibration vaporization technique that allowed us to achieve reliable and reproducible matrix coverage.

Data acquisition for 3D MALDI-IMS is still a time consuming process and measurement time depends mostly on the laser repetition rate. MALDI Imaging Lab is equipped with the autoflex speed™ LRF MALDI-TOF mass spectrometer (Bruker Daltonik) with smartbeam™ laser technology with 1 kHz repetition rate for improved measurement speed.

3D MALDI-IMS datasets are extremely large in size and very complex. Efficient data analysis methods for construction, processing and visualization of 3D volume data have to be applied in order to extract the relevant and valuable information. The spatial segmentation approach combined with *a priori* edge-preserving 3D image denoising to reduce MALDI-imaging characteristic spectrum-to-spectrum variation[2] is the ideal solution for such complex datasets.

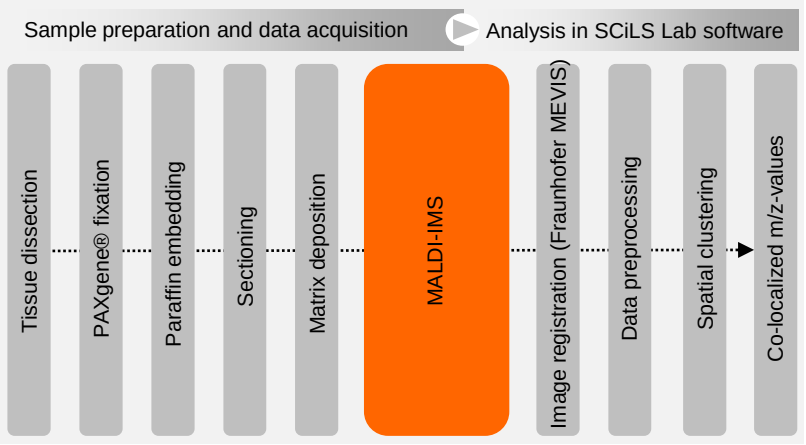

FIG. 1 Scheme of the pipeline

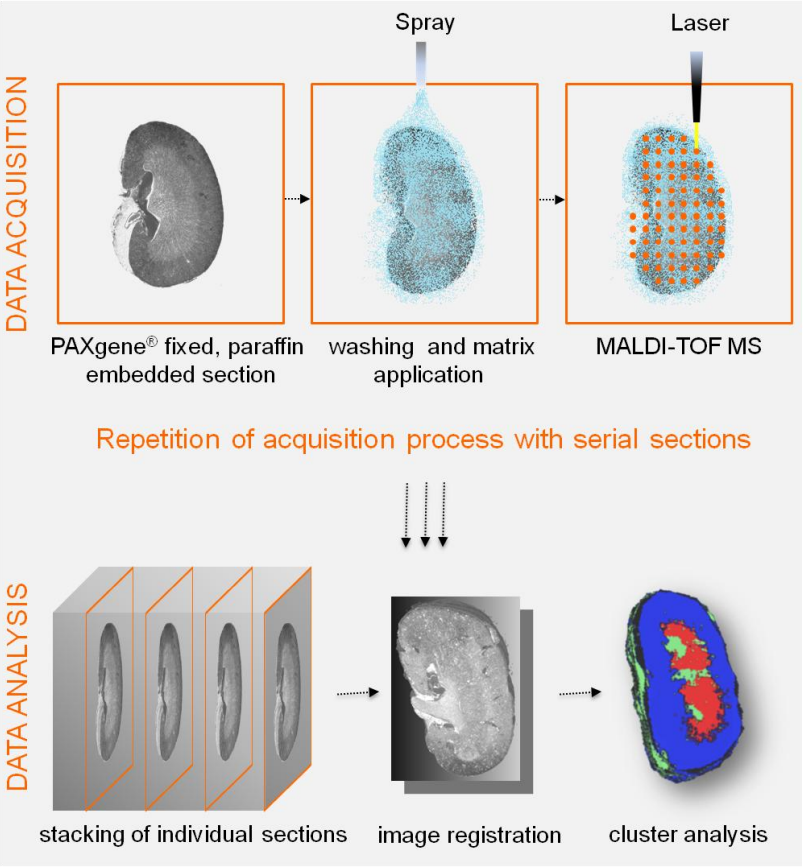

FIG. 2 Experimental and computational pipeline for 3D MALDI-IMS of a mouse kindey

RESULTS

Using the established pipeline exploiting 3D spatial segmentation, a complete reconstruction of the 3D-morphology of the mouse kidney was generated based only on the mass spectrometric information in form of m/z-values corresponding to peptide and protein signals. The 3D anatomy of organs is shown to be reflected by the segmentation map. Anatomical structures as renal cortex, pelvis, sinus and ureter with its calyces could be depicted and the respective clusters are shown independently and combined in FIG. 3. Virtual sections in all three dimensions were applied to visualize the cluster arrangement inside the 3D volume (FIG. 3).

Of biological importance is the 3D spatial distribution of proteins and peptides within an organ or biomedical specimen. We were able to detect m/z-values co-localized with the spatial mask filter generated through the segmentation algorithm. Several m/z-values co-localized with the anatomical structures were detected and their 3D volume distribution visualized. FIG. 3 shows the distribution of representative m/z-value for the anatomical structures renal sinus, pelvis and cortex. The homogeneity of the distribution verifies our experimental and bioinformatical setup.

CONCLUSIONS

With our experimental 3D MALDI-IMS pipeline combined with efficient and effective data analysis methods we were able to reproducibly measure individual serial sections with the MALDI-IMS technique and to reconstruct the 3D volume of an organ. This study shows the feasibility of the process and can in future be applied to biomedical or clinical applications.

KEYWORDS

3D MALDI imaging mass spectrometry, mouse kidney, data analysis, spatial segmentation

SUMMARY

- Data acquisition workflow for 3D MALDI-IMS was set up
- Reproducible quality of serial slices, sample preparation and acquired data are of high importance
- Edge-preserving image denoising and the spatial segmentation approach provide good algorithms for data analysis of large 3D MALDI-IMS datasets
- Reconstruction of 3D anatomy of a mouse kidney was achieved using only the mass spectrometric information

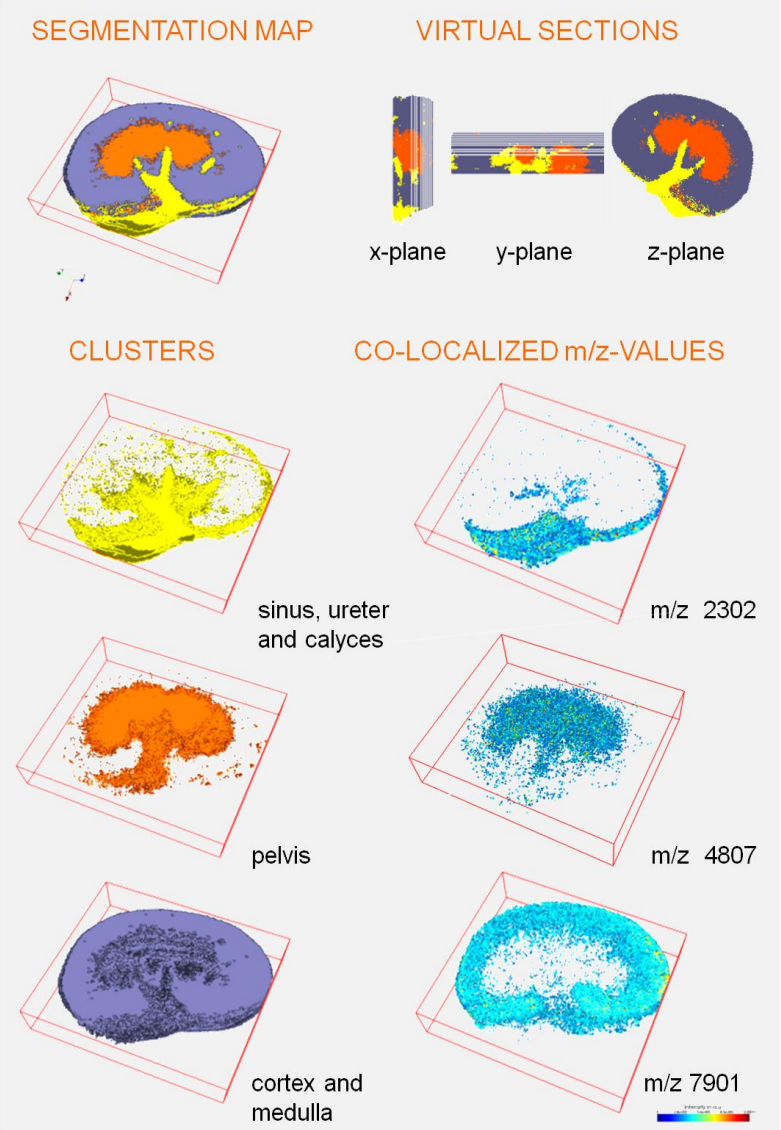

FIG. 3 Segmentation map with representative co-localized m/z-values visualized in the SCiLS Lab software

REFERENCES

1. M. Andersson et al. (2008) Imaging mass spectrometry of proteins and peptides: 3D volume reconstruction. *Nat. Methods*, 5(1):101–108
2. T. Alexandrov et al. (2010) Spatial segmentation of imaging mass spectrometry data with edge-preserving image denoising and clustering. *J. Proteome Res.*, 9(12):6535–6546

AUTHORS

J. Oetjen, K. Maedler, P. Maass, T. Alexandrov; MALDI Imaging Lab, University of Bremen

ACKNOWLEDGMENTS

We thank Dennis Trede and Stefan Schiffler (SCiLS) for special developments of the SCiLS Lab software for data analysis, Stefan Wirtz, Jan Strehlow, Judith Berger, and Stefan Heldmann (Fraunhofer MEVIS Institute for Medical Image Computing) for image registration, as well as Michaela Aichler and Axel Walch (Helmholtz Center Munich) for providing samples and the help in establishing the data acquisition workflow.

MALDI Imaging Lab SCiLS Universität Bremen

Address:
Leobener Str.
NW2 B2210
28359, Bremen
Germany

T: +49 (421) 218 63 227
F: +49 (421) 218 42 79

E: joetjen@uni-bremen.de
W: www.maldi.uni-bremen.de