Fully automated chemical imaging with LA-REIMS™

Gabriel Stefan Horkovics-Kovats[1,2], Richard Schäffer[1], Csaba Hajdu[1], Gitta Schlosser[2], Julia Balog[1]; Budapest
[1]Waters Research Center Kft., [2]Eötvös Loránd University

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Introduction

Mass spectrometry chemical imaging is an emerging technology, which can be used for multiple applications in different scientific fields, for example chemical imaging based histological assessment or drug monitoring. The Laser Assisted – Rapid Evaporative Ionization Mass Spectrometry (LA-REIMS™) is an ambient technique requiring no sample preparation of the target tissue. The technique combined with an imaging setup developed for this purpose is suitable for a micro-scale resolution metabolic profiling through point-by-point laser ablation of the tissue section.

Goal

Creating a functional and fully automated LA-REIMS™ based imaging platform, testing its usability and compare to other imaging modalities (e.g. DESI™).

Instrumentation

- Imaging setup is linked to a Xevo™ G2-QTof-MS with REIMS interface. All experiments and testing procedures were performed in sensitivity and negative ion mode of the mass spectrometer.
- For this purpose, an Opolette™ 2940 from Opotek is used which, due to its applied wavelength of 2940 nm, selectively disrupts hydrogen bonds and thus releases other constituents for MS - analysis.
- To handle microscope slides automatically, the Pannoramic™ Scan II - automatic slide sorter from 3DHISTECH has been integrated into the setup.
- A Masslynx™ compatible home made software allows the control of all components of the setup and enables easy to use ROI selection and acquisition handling

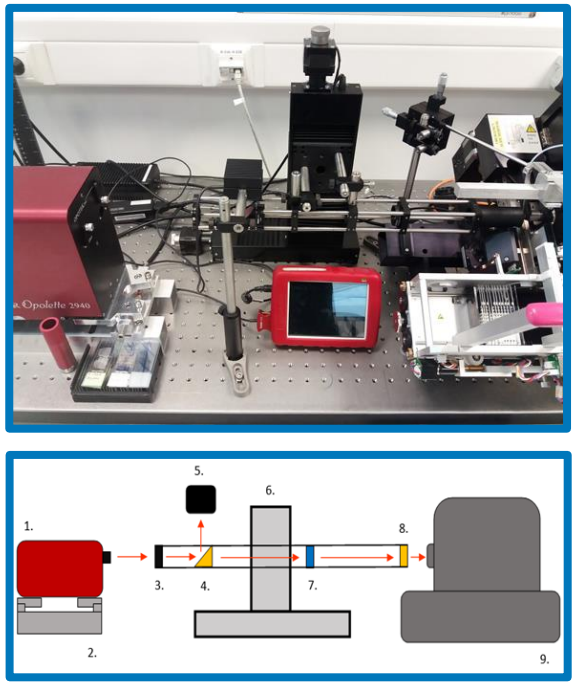


Figure 1. Current LA-REIMS™ Imaging setup and schematic hardware structure

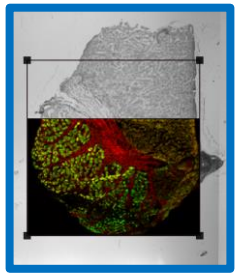
Creation and Challenges

- Combine laser, optics and Pannoramic Scan II into an integrated system
- Correct laser beam alignment to achieve round spot size
- Testing of different lenses to obtain the best possible spot size and spectra
- Camera adjustment through hardware and software to avoid radial and tangential torsion as much as possible
- Creation of a suitable image preview

Setup components (Figure 1):

1. Opolette 2940
2. Adjustment plates
3. Cage System Iris Diaphragm (Ø0.8 - Ø20 mm)
4. CaF_2 Plate Beamsplitter
5. Possible mount for energy measurement
6. 2x motorized linear translation stage (stepper motor)
7. mounted neutral density filter
8. ZnSe aspheric lens (f=12.7mm)
9. Pannoramic Scan II – automatic slide sorter

Results: Proof of Concept



- Successful sampling and image generation (Figure 2)
- First direct comparisons between LA-REIMS™ and DESI™ results (Figure 3)
- Correct classification of brain sectors through PCA and LDA (Figure 4 and 5)

Figure 2. Created image on a specific ROI of a cancerous dog testicle section, sampled on 50 microns resolution that visualizes separation of tissue types

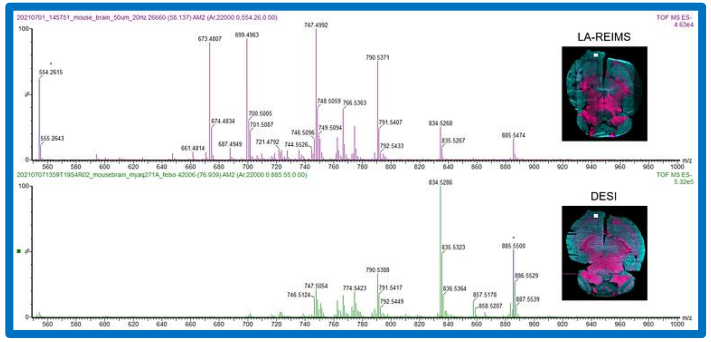


Figure 3. LA-REIMS™ VS DESI™ imaging comparison on mouse brain sections on 50 microns sampling resolution (500-1000 m/z, single scan comparison from the same cortex region). Showcasing of complementary spectra: LA-REIMS™ spectra featuring more abundant PEs with ammonia loss; DESI™ spectra featuring more abundant PIs

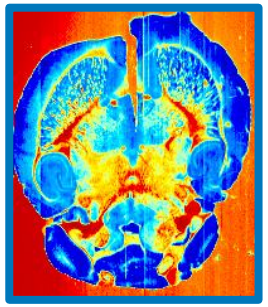


Figure 4. PCA 1 of mouse brain using custom built software, AMX

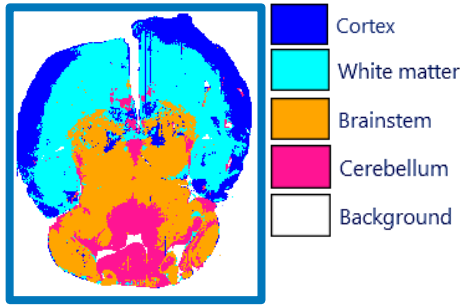


Figure 5. Classification of mouse brain section based on previously calculated LDA classifier

Conclusion

We have created a fully automated, integrated, high throughput (up to 150 slides), LA-REIMS™ based imaging platform and demonstrated its feasibility to achieve 50 um resolution clear chemical images from tissue sections.

PREPARED WITH THE PROFESSIONAL SUPPORT OF THE DOCTORAL STUDENT SCHOLARSHIP PROGRAM OF THE CO-OPERATIVE DOCTORAL PROGRAM OF THE MINISTRY OF INNOVATION AND TECHNOLOGY FINANCED FROM THE NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION FUND.