DESI AND/OR LA-REIMS? ADJACENT AUTOMATED AMBIENT TECHNIQUES FOR THE PRECISE IDENTIFICATION OF CANCER TISSUE

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

INTRODUCTION

Recently created Automated Desorption Electrospray Ionization (AutoDESI) and Laser assisted Rapid Evaporative Ionization Mass spectrometry (LA-REIMS) are ambient techniques requiring no specific sample preparation. The imaging setups created for both technologies enable metabolic step by step profiling of target tissue from microscope slides. AutoDESI (***Figure 1.***) uses an angulated charged solvent stream for sampling, while LA-REIMS (***Figure 2.***) allows sampling through laser ablation. In surgical environments, databases can already be generated by collecting samples with CO_2 LA-REIMS. To support surgical environment with high resolution and annotated images, the imaging technologies should also be compared with CO_2 LA-REIMS for comparability testing of the data bases.

AIMS

Investigate advantages and disadvantages of LA-REIMS Imaging/AutoDESI:
1. Regarding comparability of imaging with surgical CO_2 LA-REIMS sampling
2. Regarding sampling resolution, tissue handling, spectral difference (quality and content) and application possibilities

METHODS

AutoDESI and LA-REIMS for imaging and surgical CO_2 LA-REIMS for ex vivo sampling as applied technologies with the following samples:
- Tumorous and healthy tissue on veterinary sample
- Fresh frozen tumorous veterinary sample for application test and spectrum comparison
- Deparaffinized FFPE tumorous human sample for application test and spectrum comparison
- Fresh frozen mouse brain sample for sampling resolution and method invasiveness comparison

Extracted solvent (AutoDESI) and generated aerosol (LA-REIMS) are introduced into a Xevo™ G2-XS ToF MS (Waters Corporation) equipped corresponding DESI autoloader and REIMS™ source. Multivariate statistics including Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) was used for point-by-point pixel classification and model generation.

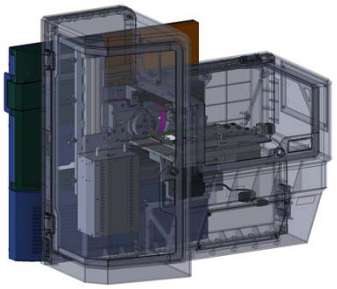


Figure 1. AutoDESI
- *DESI autoloader*
- *High performance sprayer*
- *Heated transfer line*
- *Auto measurement of up to 80 slides*

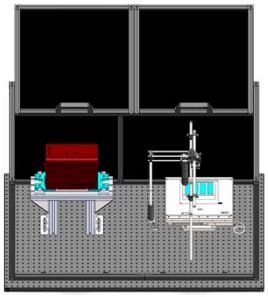


Figure 2. Boxed LA-REIMS
- *Optical parametric oscillator*
- *Self made optical path focusing*
- *Commercial motorized x-y-z stage*
- *Laser-safe box cover*

RESULTS

1. Imaging method comparison with CO_2 LA-REIMS

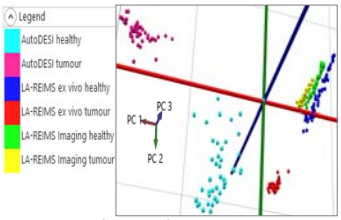


Figure 3. *PCA model of a veterinary dog sample (AutoDESI, CO_2 laser REIMS sampled ex vivo and LA-REIMS Imaging comparison)*

- Significant separation of techniques and healthy/tumorous tissue through LA-REIMS (CO_2), LA-REIMS Imaging and AutoDESI (***Figure 3.***)
- Denser accumulation of spectra for LA-REIMS, and greater variance for AutoDESI (***Figure 3.***)

Table 1. *Classification of LA-REIMS Imaging data* set of ***Figure 3***

	AutoDESI	LA-REIMS (CO_2)
LA-REIMS Imaging	0	90

Table 2. *Classification of CO_2 LA-REIMS sampled ex vivo data* set of ***Figure 3*** *without AutoDESI*

	LA-REIMS Imaging healthy	LA-REIMS Imaging tumour
LA-REIMS ex vivo healthy	37	14
LA-REIMS ex vivo tumour	0	31

- LA-REIMS Imaging data 100% classified into CO_2 LA-REIMS group, so different LA-REIMS applications can be compared with each other (***Table 1.***): Consequently, better comparison to surgery environment of CO_2 laser sampling compared to AutoDESI
- Differentiation of healthy and diseased tissue resulted in 83% correct classification of CO_2 LA-REIMS data on LA-REIMS Imaging database (***Table 2.***)
- Further investigation of the differences of the two laser technologies for more precise comparability is necessary

2. Detailed imaging technology comparison (spectra, resolution, application)

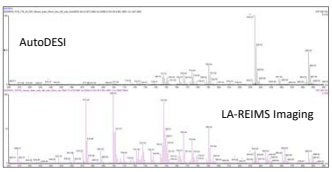


Figure 4. *Combined and lock massed spectra of 50 scans from mouse brain cortex sampled with AutoDESI (black) and LA-REIMS Imaging (Purple)*

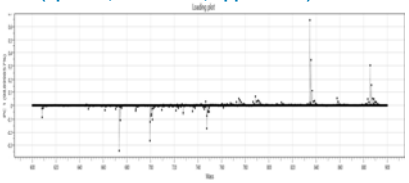


Figure 5. *PC1 Loading plot of mouse brain cortex from **Figure 4.** showing most important peak differences between LA-REIMS and AutoDESI sampling*

Identified peak list significant for LA-REIMS Imaging and AutoDESI sampling from ***Figure 5.***:

LA-REIMS peaks:
647.45 : PA(32:0)
661.45 : LPA(34:0) and/or PA(34:7)
673.45 : PA(34:1)
687.45 : PS(30:1) and/or PA(35:1) and/or DG(42:10)
699.55 : PE(34:1)
701.55 : PE(34:0) and/or PA(36:1)
709.45 : PA(P-38:3) and/or PE(P-36:2)
721.45 : PE(36:4)
727.55 : PE(36:1)
735.45 : PI(P-28:1)
747.55 : PE(38:5) or PG(34:1)

AutoDESI peaks:
774.55 : PE(P-40:6)
788.55 : PS(36:1)
834.55 : PS(40:6)
857.55 : PI(36:4)
885.55 : PI(38:4)

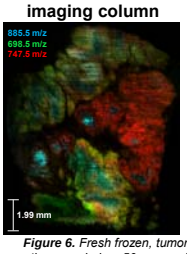
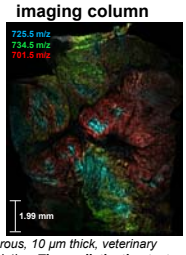
AutoDESI imaging column	LA-REIMS imaging column	Comparative observations
		• AutoDESI smoother due to sprayer spot size • LA-REIMS has spot-by-spot detail due to laser spot size • Overall higher intensities with AutoDESI (10*e^5), magnitude lower for LA-REIMS • Setup specific intensive peaks

Figure 6. *Fresh frozen, tumorous, 10 µm thick, veterinary section sampled on 50 µm resolution: **Tissue distinction test***

		• Sampling of deparaffinized FFPE section not possible with AutoDESI • Sampling of deparaffinized FFPE section possible with LA-REIMS • Very little phospholipids present with LA-REIMS • Very low overall intensity for LA-REIMS (10*e^3)

Figure 7. *Deparaffinized FFPE, tumorous, 10 µm thick, human section sampled on 50 µm resolution: **deFFPE application test***

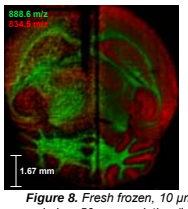
		• AutoDESI has light removal of tissue but still enough remaining for resampling • Complete ablation of tissue for LA-REIMS, no resampling possible

Figure 8. *Fresh frozen, 10 µm thick, mouse brain section sampled on 50 µm resolution (left side sampled twice): **Tissue destruction test***

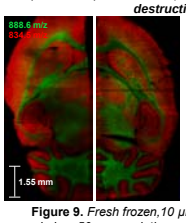
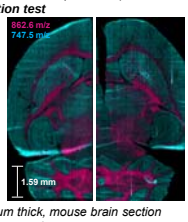
		• Slightly better resolution difference noticeable for AutoDESI on similar signal intensities • LA-REIMS (focus spot size of ~48 µm): Oversampling occurs for 25 µm resolution • Very low intensity on 25 µm for LA-REIMS

Figure 9. *Fresh frozen,10 µm thick, mouse brain section sampled on 50 µm resolution on the left and 25 µm resolution on the right side: **Sampling resolution test***

CONCLUSION

- LA-REIMS Imaging data can be compared with LA-REIMS CO_2 data, but further investigation is needed to ensure more precise comparability
- LA-REIMS spectra feature more abundant PEs with ammonia loss (e.g. 699.5 m/z) and AutoDESI spectra featuring more abundant PIs (e.g. 885.5 m/z)
- AutoDESI is better for samples where a high signal strength and resampling is needed
- LA-REIMS Imaging allows visualization of deparaffinized FFPE samples

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.