Cross-continental, multisite round robin REIMS study for the evaluation of REIMS fundamentals and technology

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INTRODUCTION

- Rapid Evaporative Ionization Mass Spectrometry (REIMS) is an emerging technology based on the mass spectrometric analysis of aerosol generated during the thermal ablation of biological samples
- The technology is capable of the quasi real-time, in situ characterization of a wide variety of samples including tissues, microorganisms and food items
- Our long term goal is to introduce the REIMS technology into surgical environment around the world at routine level for real-time, in vivo margin assessment in cancer surgery
- In order to be successful, we need to understand the fundamentals of the method and the variation of signal acquired at different sites
- We report here the results of the first cross-site REIMS study, including repeatability, reproducibility and robustness

AIMS

- **To evaluate the repeatability and reproducibility**
- **Instrument-to-instrument comparison within and cross-site**
- **Testing of robustness of the technology by using multiple instruments, multiple users at multiple locations and multiple time slots**
- **To gain more understanding of REIMS mechanism and identify key experimental parameters**

METHODS and DATA ANALYSIS

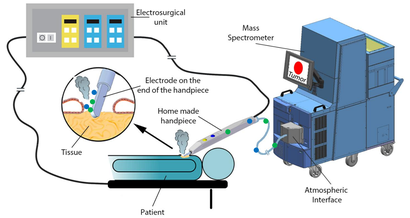

Fig. 1. iKnife REIMS setup used throughout this study.

- A total of 6 Xevo G2-XS instruments were used at 4 sites
- All parameters were set and instrument status was checked according to the checklist shown on Figure 2.

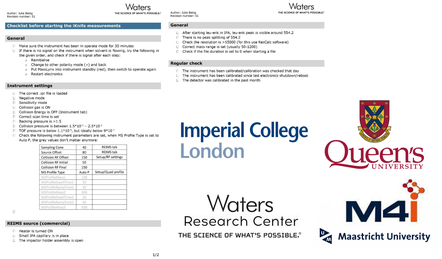

Fig. 2. Checklist for parameter and instrument settings. The 4 participating institutions are: M4I Maastricht University, Imperial College London, Queens University, Kingston, ON and Waters Research Center, Budapest with 1-1-1-3 instruments.

- 0.05 ng/µl Leucine Enkephalin in isopropanol
- 150 µl/min flow rate
- Measurements on 3 different days, 3 times per day
- Repeated twice in a year
- NIST reference meat homogenate sample, two batches of pork liver samples shipped around and local samples were also used

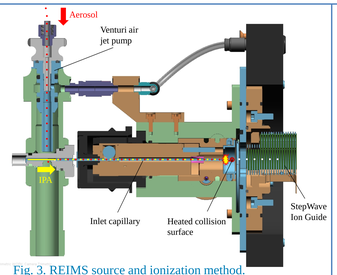

Fig. 3. REIMS source and ionization method.

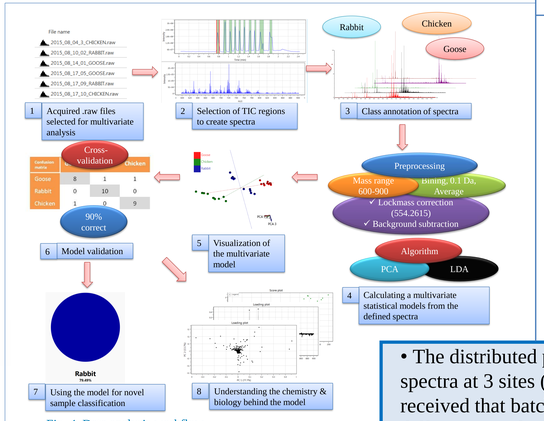

Fig. 4. Data analysis workflow.

RESULTS

Multisite Comparison of Instruments

Using Leucin-enkephalin, NIST reference meat homomgenate and pork liver shipped from the UK

- There were two separate batches of pork liver samples shipped from the UK
- The second batch never arrived to Canada, thus Queens used the first batch for all experiments
- NIST reference was purchased by all institutions separately
- The instrument parameters where changed between the two batches of experiments

Fig. 5. Spectrum of Leu-enkephalin external lockmass compound and the calculated resolution of the instruments at 4 sites.

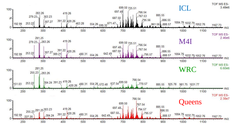

Fig. 6. Spectra of the first batch of pork liver acquired at all four sites.

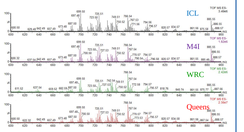

Fig. 7. Spectra of the first batch of pork liver (phospholipid range).

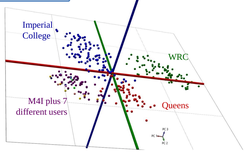

Fig. 8. 3D PCA plot of the first batch of pork liver samples sampled at all four sites

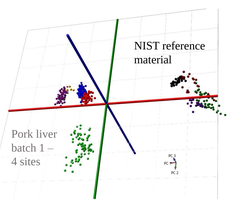

Fig. 9. 3D PCA plot of the first batch of pork liver sample data and NIST reference samples acquired at all four sites.

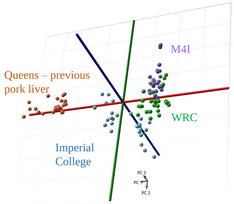

Fig. 10. 3D PCA plot of the second batch of pork liver sample data analysed at three sites, Queens used the first batch.

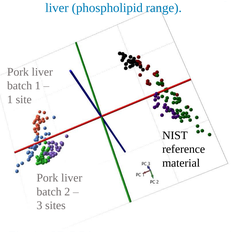

Fig. 11. 3D PCA plot of the second batch of pork liver sample data (Queens used the first batch) and NIST reference samples analysed at all four sites.

- There was no significant difference in the acquired signal, when the same sample was measured by multiple untrained users
- There was a clear difference in the fatty acid/phospholipid ratio between the same liver sampled at multiple sites in the first batch
- After selecting and adjusting all instrumental parameters, in the second batch, there still was a variation in the signal, however it was not significant, data could be reproduced at all sites

Fragmentation of molecules in REIMS

Using the fragmentation of Leu-enk as an indicator

- As leucin-enkephalin is present in all scans as an external lockmass compound, we monitored the fragments of leu-enk in all experiments in order to observe if there is any fragmentation occuring on the impact on the heated collision surface of the REIMS interface

Leucin Enkephalin fragments	Relative intensity (ESI negative)
130.0868	15
179.0821	10
219.077	10
236.1035	70
293.125	15
334.1767	10
379.1766	10
391.1988	10

Table. 1. Leucin-Enkephalin fragment ions and their relative intensities @30 eV CID energy.

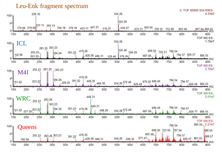

Fig. 12. Pork liver spectra from all four sites and leu-enk fragment spectrum.

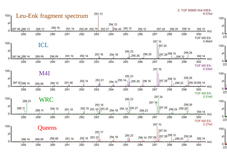

Fig. 13. Pork liver spectra from all four sites and leu-enk fragment spectrum focusing on m/z 293.13.

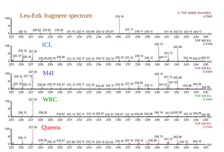

Fig. 14. Pork liver spectra from all four sites and leu-enk fragment spectrum focusing on m/z 236.10.

- No fragmentation or dimer formation of the injected lockmass compound Leucin-enkephalin was observed
- Fragmentation of the phospholipids could occur at the sampling point, however the sampling circumstances were fixed for all sites

Comparison of locally supplied samples and instruments

Calf liver, chicken liver, chicken breast, turkey breast

- Food grade meat was purchased at the local supermarket. Models were built at each site and used to classify samples from the other sites
- A total number of 487 sampling events, 2435 scans were selected (calf liver = 126 (630), chicken breast = 105 (525), chicken liver = 147 (735), turkey breast = 109 (545)) analysed on 6 instruments from 4 sites

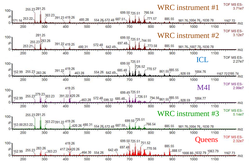

Fig. 15. Full spectra of calf liver analysed at 4 sites with a total of 6 instruments.

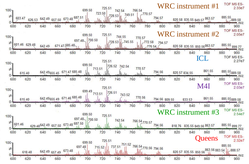

Fig. 16. Phospholipid spectra of calf liver analysed at 4 sites using a total of 6 instruments.

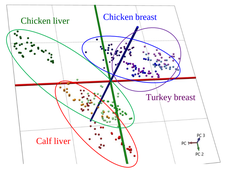

Fig. 17. 3D PCA plot of calf and chicken liver, turkey and chicken breast purchased at local suppliers. The different shades of color represent each site.

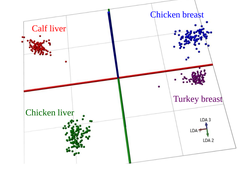

Fig. 18. 3D pseudo LDA plot of calf and chicken liver, turkey and chicken breast purchased at local suppliers. This model was used for classification.

Model training	Correct Class. Rate	ICL	WRC main instrument	M4I	WRC instrument #2	WRC instrument #3	Queens
ICL	**75.67%**	-	64.7	100	50.0	50.0	75.0
WRC	**100%**	100	-	100	-	-	100
M4I	**88.34%**	99.1	73.3	-	50.0	92.9	100
Queens	**69.87%**	83.1	59.0	77.2	50.0	50.0	-
WRC instrument #1	**97.12%**	95.6	-	100	100	100	94.6

Table. 2. Correct classification rate building the model on the data acquired by one site and classifying all data acquired on the other sites.

- The 4 different samples from local suppliers were not identical. Running cross-validations between sites resulted in 64-100% correct classification rate
- Interestingly WRC model performed at 100%, however the WRC spectra were mostly misclassified by other models. As at WRC 3 instruments were used, it is suggested that the variance covered by the classifier was greater compared to the other models

CONCLUSIONS

- The distributed pork liver (second batch) had very similar spectra at 3 sites (ICL, M4I and WRC), while Queens never received that batch and used a previous one, which gave different signal suggesting that the differences are not instrument related
- **Our findings demonstrate, that the reproducibility, repeatability and robustness of the instruments are adequate throughout all four sites**
- We observed no significant fragmentation of species, however the ratio of fatty acid and phospholipid signals was different throughout the sites, suggesting an interference from the pre-analytical processing at each site

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