Imaging MS: Developing Quantitative Imaging Workshop
Workshop Report
Tuesday 22nd May, 5:45 - 7:00, Room 118-120

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Introduction

Mass spectrometry imaging is growing strongly owing to its importance in biological and materials sciences and technologies. Last year, a successful workshop was held titled 'Towards Quantitative Imaging' with very lively discussions. This found that one of the key obstacles to quantitative imaging was the need to get rid of ion suppression effects, perhaps through blotting, better internal standards, or new ionisation techniques. The aim of this workshop was to explore the present research available and being developed to deal with this key issue. The current gaps and opportunities that will further the aim of reducing ion suppression effects as well as data processing for quantitative mass spectrometry imaging were debated. The meeting followed the format introduced last year with discussion leaders distributed in the audience to stimulate debate.

This year at ASMS we have had 157 presentations (cf 120 in 2011) in mass spectrometry imaging:

Oral (3 sessions)	**Poster (8 sessions)** Number of posters given in parentheses
Biological applications	Pharmaceutical applications (17)
Instrumentation and ionization sources	Quantitative analysis (6)
Pharmaceutical applications	Small molecules (30)
	Instrumentation (20)
	Method development (25)
	Disease markers (23)
	Large molecules (8)
	Software (10)

To ensure continuity of the workshop an election of three candidates was conducted to enable the interest group to make a recommendation to the ASMS Board of Directors for the workshop chair 2015-2016 (co-chair 2013-2014). The three candidates (Liam McDonnell, Andreas Römpp, Amina S. Woods) gave a short introduction of themselves and their vision for the workshop. Liam McDonnell was selected.

Discussion (for free discussion comments are not attributed)

1. Ionisation and ion-suppression

Quantification still seems to be the Holy Grail for most, advanced, imaging mass spectrometry groups. Funnily, less experienced people have no trouble, they just take it for granted that there are 3 orders of magnitude of dynamic range, and one can calibrate the method, while often there is no dynamic range at all, at least not for the whole sample. The conclusion was, that one has to take sample inhomogeneity into account and validate quantification for each tissue type using an exhaustive and quantitative method (e.g. LSSP-LC-MS) in combination with actual desorption ionization method.

There is a need for a standard sample which allows comparison of techniques and instruments. Most of the work being published does not give any quantitative information and few labs investigate in this field. Also, published data is often not validated and or no validate method is used. In view of the diverse sample preparation techniques and the tissue/substance-specific suppression it is often not possible to distinguish a real distribution pattern from an artefact.

The MSI field is growing strongly and as a result there are many new people in the field. At present there is limited information available to help them, for example practical “tricks” and methods. As a community, it is important that we are more open regarding sharing both experience and software tools. This is a serious hurdle for the technique regarding finding its way to real-life applications. Possible ways to help include documenting methods in teaching books, websites, protocols and tutorials.

It is important to be clear about the terminology. For example, “ionization bias” it is essential to accurately characterize the phenomenon by separating the different processes (which are often included in the *catch-all* term of “ionization biases”) – this may include differential sampling of the analytes during sample preparation, ionization bias, differences in ablation characteristics (MALDI), different chemical backgrounds. Only with an accurate description of the issue can the process be properly addressed. As a community we need to define agreed terms.

The use of normalization by the total ion counts (TIC) is insufficient for correcting for ionization biases for quantification. Post ionization could be very effective method to reduce ionization bias for ambient ionization sources – e.g. LA-ESI, APCI, APPI.

What is quantitation? Each MS image contains (relative) quantitative information, i.e. the intensity distribution of an analyte ion. If we talk about ‘quantitation’ in imaging, we usually refer to absolute quantitation. Possibilities to avoid/reduce suppression effects:

- Decouple desorption and ionization, for example 2 laser techniques
- Use isotopically labeled standards for targeted experiments, i.e. only one analyte of interest
- Use ICP-MS as a reference method, if possible

Generally, “be careful” a) how you perform your experiment (control the whole workflow) and b) how you present your quantitative results (report uncertainty).

The use of labelled standards for normalisation was recommended by one group with deposition on and off the tissue and bearing in mind that ion suppression will vary depending on the type of tissue. This issue was highlighted by another group; the ionisation suppression is tissue specific. It was recommended ensuring the matrix matched as well as possible. There is, unfortunately, no universal method. Washing the sample was one way to reduce these effects. However, another group was against washing since this may remove important analytes. Chemical treatment with ammonium acetate to remove electrolytes could be better. The point was moot with, probably, most favouring washing. It was noted that ionisation suppression is an issue for all MSI methods. It is important to validate measurements with other techniques such as LC-MS.

2. Software and methods for data interpretation and quantification

Our group had a vision of building an open-source MS imaging software. The idea will be discussed by the COST group (MSI: New tools for healthcare research) and might be feasible in the near future.

Regarding software, practically everybody is unhappy with the current situation. We should make efforts to have a high-end, open source software which can process arbitrary imaging data. Something similar to the Mozilla project, maybe we could get EU FP7 funding for that.

One group considered the different software tools they use and the effectiveness:

- Working well: Biomap – functions well and is free
- Not being used: imzML: currently a lack of data format convertors and data analysis tools has meant no-one of the group is using imzML
- Being used: matlab (6 people), R (3 people), C++ (1 person) – though this reflected the heavy bioinformatics presence in our group
- Data availability: imaging MS data needs to be made available – as the raw data is too large, a reduced data format (image data of all detected peaks) would be sufficient provided tools are available that confirm the reduced data is an accurate representation of the original data. In all cases the raw data needs to be made available if requested.

Similar issues were identified in another group. Many people use home-built software tools to analyze/process MS imaging data. One way to make this more efficient is a common data format. One such possibility is imzML. It can be used

1. To choose a processing software that fits best to a certain application
2. To display data from different instruments in the same software in order to compare them with identical settings

Several tools for imzML (converters, displaying/processing software) are already available. A number of additional programs are currently being developed. Additional information on imzML can be found on www.imzml.org.

There are clearly many ways in which data analysis tools could be improved; one recommendation was to be able to draw specific regions of interest in an image and then reconstruct the spectrum. The data files are very large which makes these, seemingly, simple tasks difficult.

Discussion - Conclusions

Each group consisted of around 15 to 30 people. Overall the discussion worked well and the majority of people (>95 %) felt that the workshop was successful. The essential points from the discussion are:

- Ionisation suppression is tissue type dependant and the tissue is heterogeneous. It is necessary to validate / calibrate the MSI technique with traditional methods such as LC-MS.
- There is a need for a standards sample for comparison of techniques and instruments and confirmation of performance.
- More tutorial / educational material is needed for MSI.
- The terminology needs to be accurately defined.
- There is a need for open-source MSI imaging software.
- A common data format, imzML, is available.
- The functionality of the software needs to be improved. It is currently limited owing to the large data sizes.

Based on the discussion and from the survey responses the following oral and poster sessions are recommended for the 61st ASMS (2013) sessions:

Oral	**Poster**
Biological applications	Pharmaceutical applications
Fundamentals, instrumentation and ionization sources	Sample preparation
Pharmaceutical applications	Small molecules
	Instrumentation
	Method development
	Disease markers
	Large molecules [perhaps combine with one of the others – only 8 posters this year]
	Data analysis, interpretation and software
	Materials science applications

Acknowledgements

We thank ASMS for the opportunity to hold the workshop as part of the conference and all their help and support in doing so. The Discussion Leaders are gratefully acknowledged for stimulating the discussion and getting everyone involved and excited about the discussion. Tara Salter is also gratefully acknowledged for help preparing the workshop and taking notes. The Discussion Leaders are (in first name alphabetical order):

Amina S. Woods, NIDA IRP, USA
Andreas Römpp, Justus Liebig University, Germany
Garry Corthals, University of Turku, Finland
Jeremy L. Norris, Vanderbilt University, USA
Josephine Bunch, Birmingham University, UK
Katherine Kellersberger, Bruker Daltonics, Inc.
Liam McDonnell, Leiden University Medical Center, The Netherlands
Malcolm R Clench, Sheffield Hallam University, UK
Markus Stoeckli, Novartis, Switzerland
Michelle Reyzer, Vanderbilt University, USA
Sarah Trimpin, Wayne State University, USA.
Walter Korfmacher, Independent Consultant, USA
Zoltan Takats, Imperial College, UK

Workshop Survey

Results of the workshop survey are shown below (64 returns)

Workshop

Do you think this workshop has fulfilled the outline aims and objectives?

Not Sure 0	Poor 3	OK 31	Good 32

Have you found the format of the workshop successful?

Not Sure 0	Poor 8	OK 26	Good 30

Has the workshop been useful?

Not Sure 2	Poor 2	OK 32	Good 29

Do you have any suggestions or requests for next year's workshop?

- *Workshop provides cohesion – need to find ways to help group get to know each other.*
- *Mix groups up more so that they are not self-assembled from colleagues from same laboratory / company.*
- *More focused discussion on quantification with sub-groups covering small molecules and proteins separately.*
- *Quantification and sample preparation uses different methods for exogenous and endogenous molecules – split groups to cover topics separately (see above).*
- *Many important issues have been identified – need to identify someone to take the "to do list" forward.*
- *Protein imaging would be a good topic.*
- *It would be good to have short updates from vendors.*
- *Plan an ASMS presence at the World Molecular Imaging Conference*
- *Continue Quantitation topic but choose narrower topic for more focussed discussion.*
- *Multivariate analysis*
- *Identification strategies, including data pre-processing and multivariate statistics.*
- *Metabolomics / lipidomics*
- *If continue with quantitation then provide a short update / review of progress in field at the beginning.*
- *Shorter response time, fewer group leaders*
- *Pick 4 discussion leaders to summarize each point or divide evenly-was too redundant*
- *Doesn't seem much progress from last year*
- *Software for image generation/processing*
- *Sample prep issues*
- *Separate into groups based on common themes (e.g. ionization, anlyate). Too many different approachs/applications to have a useful conversation*
- *Opn sharing of data with different software: what are the barriers and how to break, features from 3rd party packages?*
- *Rounded groups*
- *More pracatical issues for quantitation*

Conference

Has the number and topics of Imaging MS oral sessions been adequate?

Yes 60	No 8

Has the number and topics of Imaging MS poster sessions been adequate?

Yes 56 No 6

Have the oral sessions been useful?

Mostly people answered yes (~90%). The following comments were mentioned several times: Too applications focussed.

Suggestions for oral and poster session topics next year?(titles in bold were suggested more than once)

Oral	Poster
MSI in Material Sciences	**MSI Sample Preparation**
Quantitation (methods and solutions)	Plant imaging
Data handling / processing	Computational analysis
Computational analysis	Software development
Forensic imaging	Imaging MS with other imaging methods
Phyto? applications	MS/MS in imaging
Inorganic	
Same topics as 2012	
Sample prep	
Proteomic imaging	
High spatial resolution imaging	
Metabolomics	
Cancer research	
Multimodal imaging	
In-vivo imaging	
A focus on software tools	