Workshop Report

Imaging MS: Present and Future of Multimodal Studies

Wednesday 8th of June, 5:45 - 7:00, Room 304, level 3

Presiding:

- Vilmos Kertesz
  Oak Ridge National Laboratory, Oak Ridge, TN, U.S.A.

- Raf Van de Plas
  Delft University of Technology, Delft, The Netherlands.
  Vanderbilt University, Nashville, TN, U.S.A.

Introduction

It is increasingly common for Imaging MS to make up part of multimodal imaging studies in which a set of different image types are brought to bear on the same or related samples. Analytical approaches that characterize a sample using different principles, measuring for example functional, chemical, as well as biological information within a single experiment, can often provide insights not available from a single modality alone.

While multimodal imaging that includes a mass spectral modality has greatly advanced, different research groups have gone about such studies with widely varying workflows and approaches. In this workshop, we have discussed how challenges could be tackled at the sample preparation, at the instrumental, as well as at the computational level. Furthermore, we wanted to see whether an overall direction for the field and a set of best practices could be distilled, complemented by a list of major challenges that still need to be addressed. The central topic of the workshop was therefore “Imaging by Mass Spectrometry as part of Multimodal Imaging Studies”.

Agenda

- Announcements & MSI award

- Discussion session on Imaging MS: Present and Future of Multimodal Studies
  - Experimental design
    - Intro by Jeff Spraggins
    - 15 min open discussion
  - Measurement & instrumentation
    - Intro by Vilmos Kertesz
    - 15 min open discussion
  - Data analysis
    - Intro by Raf Van de Plas
    - 15 min open discussion

- Election of next co-coordinator

Announcements & MSI award

At the start of the workshop three upcoming scientific events, relevant to the Imaging MS community, were highlighted:

- 12-15 Sep, 2016 – MSACL 2016 EU (Salzburg, Austria);
- 17-21 Oct, 2016 – Ourcon IV- Imaging Mass Spectrometry Conference (Ustron, Poland);
- 22-26 Jan, 2017 – MSACL 2017 US (Palm Springs, California);

After the announcements, Dr. Jonathan Stauber, president of ImaBiotech (Lille, France), presented the third ImaBiotech Mass Spectrometry Imaging Award for innovations in Imaging MS. The award went to Dr. John Cahill of the Oak Ridge National Laboratory in Tennessee for his contributions to the development of micro-sampling methods that allow for quantitative and submicron-resolution imaging.

Discussion session

The discussion session was split into three distinct 20-minute parts (Experimental Design, Measurement and Instrumentation, and Data Analysis). Each part examined multimodal studies with Imaging MS from a different viewpoint, and consisted of a 5-minute introduction followed by 15 minutes of open discussion.

Experimental design

Dr. Jeff Spraggins (Vanderbilt University, Nashville, TN, U.S.A.) gave a short introduction to multi-modal imaging studies from the experimental design perspective. From the literature, he highlighted a few example studies by several groups in the field that illustrate different types of multi-modal studies:

- Andreas Roempp and Bernard Spengler (Bhandari et al., *Analyst* (2015), **140**, 7696-7709)
- Jeff Spraggins and Richard Caprioli (Spraggins et al., *Proteomics* (2016), **16**, 1678-1689)
- Sabine Becker (Matusch et al., *Analytical Chemistry* (2012), **84**, 3170-3178)
- Theodore Alexandrov and Axel Walch (Oetjen et al., *Journal of Proteomics* (2013), **90**, 52-60)
- Liam McDonnell (Heijs et al., *Analytical Chemistry* (2015), **87**, 11978-11983)

Dr. Spraggins put forward for discussion three particular questions that, in his experience, tend to drive the decision process when it comes to designing multi-modal experiments with Imaging MS:

1. What is the biological question?
2. What imaging modalities are required to answer that question?
3. What are the challenges specifically tied to preparing samples for measurement by these different modalities?

In the open discussion, several community members recounted which modalities they had measured in combination with a form of Imaging MS, and what the underlying reason was for choosing those specific modalities. Examples included Raman imaging (e.g. for plant-related research), nano-SIMS (e.g. in microbial communities), and isotope-labeled modalities. In general, the particular combination of modalities seems to be a direct result of the biological question at hand, and the overall advice is to have your biological collaborators help suggest non-MS modalities on the basis of what are already known to be relevant image types in the specific application field. Once a set of relevant modalities has been established, however, the real challenge for the mass spectrometry scientist lies in developing wet-lab workflows that unify the required sample preparation steps for all these different modalities in the smallest number of tissue samples possible. The ideal situation would be that all modalities are collected from the same tissue sample, but in many cases the destructive nature of the different measurement processes (e.g. ablation) require that neighboring tissue sections be used. The question then becomes whether those distinct samples describe the same biological phenomenon. The discussion showed that whether neighboring tissue sections are acceptable depends strongly on the case study at hand, and more specifically on the size of the biological structures of interest (e.g. whether they are visible across multiple neighboring sections) and on the spatial resolution that is required to study them (e.g. morphological differences from section to section might be deal-breakers for very fine spatial resolution studies, but perfectly acceptable when a rougher spatial sampling is sufficient). In many multi-modal studies, the need to measure from neighboring tissue sections will come up at some point. The discussion showed that whether that is acceptable is a burden to be proven and demonstrated by the authors of the study, and it should be repeated for each new study and sample type.

Measurement & instrumentation

For an instrumental perspective on multi-modal imaging, Dr. Vilmos Kertesz (Oak Ridge National Laboratory, Oak Ridge, TN, U.S.A.) delivered a short introduction based on his experiences with building instruments that combine different modalities into one device. He introduced two instruments, the first of which combines optical (and fluorescent) images with Imaging MS, and the second combined Atomic Force Microscopy with Imaging MS. Dr. Kertesz specifically highlighted some of the advantages in acquiring different measurements using the same device. One of the most prominent advantages is that a singular instrument acquires all its measurement types within the same spatial reference plane, and as a result there is little to no need for post-acquisition registration of different image types (and the uncertainty that can potentially introduce). For these types of instruments, the different modalities are implicitly registered as they roll out of the instrument.

The open discussion elaborated on the differences between tackling multi-modal studies with a singular instrument versus using separate instruments. Some of the advantages of a singular instrument that were discussed included ease-of-use, the avoidance of an (often less than optimal) image registration step that aligns the different image types, and also the fact that this allows you to avoid that fiducials need to be introduced into the samples. On the other hand, some of the disadvantages of a singular instrument that were voiced included the human resource and economical cost involved with modifying instruments, and that in certain implementations the physical combination of two measurement principles might result in less than optimal capability for either modality. Dr. Marshall (GlaxoSmithKline, U.K.) brought up the matter of flexibility, mentioning that in some cases combining into a single instrument could potentially take away flexibility in terms of what you can do with your instruments. He mentioned that in the pharmaceutical industry, as the target in question changes, the parameters of particular modalities change and maybe even the type of modalities you want to acquire. Dr. Van Berkel (Oak Ridge National Laboratory, U.S.A.) denoted that many of the combined instruments are becoming more plug-and-play in a similar fashion to how ion sources are relatively plug-and-play these days. Increasingly, the combined instruments can be built up as combinations of pluggable components, and a singular instrument does not necessarily need to be hard-wired for a single-modality combination. Summarizing, the argument for a singular multi-modality-capable instrument seems to rest primarily on a cost/benefit analysis for the particular case at hand.

Data analysis

The computational perspective on multi-modal imaging was introduced by Dr. Raf Van de Plas (Delft University of Technology, Delft, The Netherlands). He remarked that computational research and analysis in a multi-modal imaging setting is increasingly capable of solving problems that are difficult to address physically at the sample preparation or instrumental level. He also mentioned that currently there are a lot of multi-modal studies producing different types of imaging, but a lot of that potential goes underutilized at the moment and there is relatively little true integration of information across modalities being performed. Dr. Van de Plas highlighted three common data approaches that can be found in the multi-modal studies literature:

1. Modalities are not explicitly linked
   The different types of image data are not explicitly linked to each other in any way. The findings in the data are interpreted by a human, without transforming the raw image data spatially, nor content-wise. - *These approaches consist largely of making cross-modal connections in the head of the researcher, not in the computer (e.g. simply placing two images side-by-side in a figure).*
2. Modalities are linked spatially
   The different types of image data are spatially transformed to the same spatial coordinate system. - *These data analysis approaches include image registration and overlays.*
3. Modalities are linked content-wise
   The different types of image data are spatially transformed to the same spatial coordinate system, and subsequently the content of both types of measurements is computationally related to each other. - *These data analysis approaches include image fusion and cross-modality data mining.*

Further topics addressed in the intro included at what point a data analysis approach changes from an image registration-based method to an image fusion-based method, with examples both in interpolation and in de-noising. Dr. Van de Plas furthermore addressed two different sub-categories within the fusion approaches that have been applied to Imaging MS:

i. User-specified fusion
   The fusion model that determines how observations from different modalities are combined, is specified by the user beforehand. The disadvantage of this approach is that it requires the user to have a complete understanding of the image types and their sensors beforehand. This makes the approach less suitable for exploratory Imaging MS studies.
ii. Data-driven fusion
   The fusion model that determines how observations from different modalities are combined, is learned empirically from the measurements using machine learning. This approach does not require prior knowledge on the sensors, making it more suitable for exploratory Imaging MS applications. These approaches can determine empirically for which ions in the data set useful cross-modal predictions can be made.

Since cross-modality data mining and fusion approaches often take a next step after registration has been performed, it stands to reason that image registration is one of the most pressing computational problems at this time in multi-modal imaging studies with Imaging MS.

The open discussion session was primarily focused on the registration problem and what sort of tissue deformations an ideal registration approach should be able to accommodate for. Some of the more pressing deformations that were mentioned included sample preparation-induced damage to the tissue (and missing pieces), with non-rigid deformations as a close second. It was also remarked that similar to the neighboring tissue sample validity issue mentioned earlier, the needs for the registration approaches are largely dependent on the particular spatial resolution being pursued. As we all push for higher and higher spatial resolution, registration will become an increasingly important issue to address.

One issue that came up is an open question to the vendors. Currently, there are forms of registration present in many vendor software packages. However, there is little known about what type of registration is being used and what its parameters are. As a result, it is very hard to record, approximate, or analyze the registration transformation performed by vendor software. **Similar to the efforts being undertaken to export raw Imaging MS data from vendor-specific data formats, there is the request to the vendors to provide some form of exporting their registration transform, together with sufficient information to understand the coordinate system that was used.**

Election of next co-coordinator

The final point of the workshop was the election of a new co-chair for the coming two years. An open call for candidacies was performed. Dr. Reid Groseclose (GlaxoSmithKline, NC, U.S.A.), who could not be present but had submitted his candidacy prior to the workshop, was elected to be the next co-chair of the interest group, replacing Vilmos Kertesz.

Estimated attendance

Participation was estimated to be around 300-350 attendees.

Acknowledgements

We thank the ASMS for the opportunity to hold the workshop as part of the conference as well as for all their help and support in doing so. The presenters are gratefully acknowledged for stimulating the discussion and getting everyone involved and excited about the discussions. We furthermore express our gratitude to everyone in the audience for their participation and lively discussion.