Biochimica et Biophysica Acta 1865 (2017) 967–977

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Connecting imaging mass spectrometry and magnetic resonance imaging-based anatomical atlases for automated anatomical interpretation and differential analysis☆

Nico Verbeeck [a], Jeffrey M. Spraggins [b,c,d], Monika J.M. Murphy [e], Hui-dong Wang [f], Ariel Y. Deutch [e,f,g], Richard M. Caprioli [b,c,d,h], Raf Van de Plas [a,b,c,*]

[a] Delft Center for Systems and Control (DCSC), Delft University of Technology, Mekelweg 2, 2628 CD Delft, the Netherlands
[b] Mass Spectrometry Research Center (MSRC), Vanderbilt University, 465 21st Ave. South, 9160 Medical Research Building III, Nashville, TN 37240, USA
[c] Department of Biochemistry, Vanderbilt University, 607 Light Hall, Nashville, TN 37205, USA
[d] Department of Chemistry, Vanderbilt University, 7330 Stevenson Center Station B 351822, Nashville, TN 37235, USA
[e] Program in Neuroscience, Vanderbilt University, U-1205 Medical Research Building III, 465 21st Ave. South, Nashville, TN 37232, USA
[f] Department of Psychiatry and Behavioral Sciences, Vanderbilt University Medical Center, 1211 Medical Center Dr. Nashville, TN 37232, USA
[g] Department of Pharmacology, Vanderbilt University, Nashville, 460B Preston Research Building, Nashville, TN 37232, USA
[h] Department of Medicine, Vanderbilt University Medical Center, 1161 21st Ave. South, D-3100 Medical Center North, Nashville, TN 37232, USA

ARTICLE INFO

Article history:
Received 9 August 2016
Accepted 13 February 2017
Available online 27 February 2017

Keywords:
Imaging mass spectrometry
Anatomical atlas
FTICR MS
MRI
Rat brain

ABSTRACT

Imaging mass spectrometry (IMS) is a molecular imaging technology that can measure thousands of biomolecules concurrently without prior tagging, making it particularly suitable for exploratory research. However, the data size and dimensionality often makes thorough extraction of relevant information impractical. To help guide and accelerate IMS data analysis, we recently developed a framework that integrates IMS measurements with anatomical atlases, opening up opportunities for anatomy-driven exploration of IMS data. One example is the automated anatomical interpretation of ion images, where empirically measured ion distributions are automatically decomposed into their underlying anatomical structures.

While offering significant potential, IMS-atlas integration has thus far been restricted to the Allen Mouse Brain Atlas (AMBA) and mouse brain samples. Here, we expand the applicability of this framework by extending towards new animal species and a new set of anatomical atlases retrieved from the Scalable Brain Atlas (SBA). Furthermore, as many SBA atlases are based on magnetic resonance imaging (MRI) data, a new registration pipeline was developed that enables direct non-rigid IMS-to-MRI registration. These developments are demonstrated on protein-focused FTICR IMS measurements from coronal brain sections of a Parkinson's disease (PD) rat model. The measurements are integrated with an MRI-based rat brain atlas from the SBA. The new rat-focused IMS-atlas integration is used to perform automated anatomical interpretation and to find differential ions between healthy and diseased tissue. IMS-atlas integration can serve as an important accelerator in IMS data exploration, and with these new developments it can now be applied to a wider variety of animal species and modalities. This article is part of a Special Issue entitled: MALDI Imaging, edited by Dr. Corinna Henkel and Prof. Peter Hoffmann.

1. Introduction

Imaging Mass Spectrometry (IMS) is a powerful imaging technology that allows thousands of molecules to be detected throughout a tissue sample using a single experiment [1]. IMS enables direct comparison of proteomic, peptidomic, lipidomic, or metabolomic content between various tissue areas without the need for prior labeling of a target molecule [2,3]. These attributes have led to increased application of IMS in exploratory biomedical studies of a wide variety of diseases such as cancer [4–6], Parkinson's disease [7,8], Alzheimer's disease [7], macular degeneration [9], and diabetes [10,11]. Over the last decade, sample preparation and instrumental advances have greatly enhanced the depth of data that can be obtained by IMS [12], as well as the speed

Abbreviations: AMBA, Allen Mouse Brain Atlas; MRI, Magnetic resonance imaging; PD, Parkinson's disease; SBA, Scalable Brain Atlas; t-SNE, t-distributed stochastic neighbor embedding; FTICR, Fourier transform ion cyclotron resonance; PCA, Principal component analysis.

☆ This article is part of a Special Issue entitled: MALDI Imaging, edited by Dr. Corinna Henkel and Prof. Peter Hoffmann.

* Corresponding author at: Delft Center for Systems and Control (DCSC), Delft University of Technology, Mekelweg 2, 2628 CD, Delft, the Netherlands.
E-mail addresses: n.verbeeck@tudelft.nl (N. Verbeeck), jeff.spraggins@vanderbilt.edu (J.M. Spraggins), monika.murphy@vanderbilt.edu (M.J.M. Murphy), huidong.wang@vanderbilt.edu (H. Wang), ariel.deutch@vanderbilt.edu (A.Y. Deutch), richard.m.caprioli@vanderbilt.edu (R.M. Caprioli), raf.vandeplas@tudelft.nl (R. Van de Plas).

http://dx.doi.org/10.1016/j.bbapap.2017.02.016
1570-9639/

with which high-spatial-resolution experiments can be conducted [13]. These improvements, however, have also led to a large increase in the amount of data that is obtained by IMS experiments, with data sizes of individual experiments approaching 1 TB. In addition to being impractical and time consuming, manual interpretation often introduces human bias and drift into the exploration, which ultimately leads to inexhaustive extraction of biologically relevant information. As a result, an increasing number of publications study multivariate data analysis techniques for extracting in an automated way useful information from IMS measurements. Examples of such techniques include principal component analysis (PCA) [14–16], probabilistic latent semantic analysis (pLSA) [17], CUR matrix decomposition [18], t-distributed stochastic neighbor embedding (t-SNE) [19,20], and various clustering techniques [21,22], which all provide means of representing IMS data more concisely. While these techniques have been valuable in advancing the ways in which IMS data are explored, they tend to operate in an IMS-centric fashion and often do not take into account other (non-IMS) types of information that are known about the sample in question. One way of using such additional types of data in IMS analysis is by data-driven multi-modal fusion [23], where direct mining of relationships between IMS and its accompanying histology are used to predictively gain insights beyond the original IMS measurements. Another important source of external information, which is often used by researchers in the biological interpretation of IMS data, is anatomical knowledge on the tissue under examination. While such anatomical information is widely available in the form of anatomical atlases, its use is often limited by the fact that the (spatial and content-wise) link between ion images of interest and the anatomical atlas information usually needs to be established by manual interpretation. Such manual matching of molecular patterns to anatomical patterns is not only cumbersome and time consuming, but it is also prone to human bias and drift. In order to move the usage of anatomical information towards a more objective platform, we and other research groups [24,25] have recently proposed using spatial registration to establish a computational link between IMS data, obtained from mouse brain sections, and the Allen Mouse Brain Atlas (AMBA) [26].

Such a computer-traversable link between both data sources opens the door towards more advanced anatomy-based methods for IMS analysis. Examples of such approaches were developed in our previous work [24], where we examined a number of different IMS analysis methods that leverage information from a registered anatomical atlas to guide a user towards relevant findings within a single IMS experiment. The simplest methods were correlation-based queries, in which the user specifies an anatomical structure of interest and the query returns ions relevant to that structure in terms of high correlation. Vice versa, when supplied with an ion image of interest, the query returns the anatomical structures that have the highest correlation with that ion image. However, in the publication we also demonstrated that correlation-based queries fall short in cases where there is "multi-membership", i.e. the ion of interest is present in multiple anatomical structures concurrently. Since multi-membership is a common occurrence in biological tissues, we introduced a novel method capable of handling this property, termed 'automated anatomical interpretation'. The method makes it possible to have the spatial distributions in an ion image automatically interpreted as a combination of the anatomical structures provided by the atlas, similar to how a human investigator would deliver an interpretation. Since this method can automatically deliver anatomical annotations for hundreds of ion images in a time span much smaller than that needed for manual examination, it can serve as a powerful tool in the exploration of IMS data, particularly for the histological non-expert.

A second important advantage of linking IMS data to an anatomical atlas, is that it facilitates automated comparisons between multiple IMS experiments. Since spatial locations are generally not matched across experiments, i.e. the *x*th pixel in experiment A does not necessarily correspond to the *x*th pixel in dataset B, direct comparisons between IMS experiments are not straightforward. Mapping the individual data sets to a common anatomical atlas, however, can provide a reference plane in which spatial locations from different IMS experiments can be directly compared. This provides the opportunity to explicitly link these locations to the anatomical structures in which they reside. Carreira et al. [27], for example, have used the AMBA reference plane in a cohort study on cortical spreading depression (CSD) to investigate biomolecular changes at the anatomical level. Škrášková et al. [28] have made use of this setup to accurately pinpoint the anatomical locations of lipids in SIMS data obtained from sagittal sections of mouse brain.

In this work, we extend the IMS-atlas integration beyond the mouse-focused studies that introduced it, and we expand the applicability of the automated anatomical interpretation framework [24] by means of the following developments:

(1) *New anatomical atlases, expanding the interpretation framework to non-mouse species.* Previous work on IMS-atlas integration has focused on mouse brain, using the AMBA as the source for curated information. To expand atlas-guided analysis to a broader set of animal species, we make the link to a new set of anatomical atlases available through the Scalable Brain Atlas (SBA) project [29] (http://scalablebrainatlas.incf.org). SBA is a web-based platform that provides access to a large collection of brain atlas templates for (currently) six different species, namely mouse, rat, marmoset, human, macaque, and opossum.
(2) *Direct non-rigid spatial registration of IMS to Magnetic Resonance Imaging (MRI).* The AMBA was defined on a basis of Nissl-stained microscopy images, allowing for straightforward registration to the histological images available in most state-of-the-art IMS experiments. Many atlases available through the SBA, however, are defined on the basis of MRI measurements rather than microscopy. To be able to establish a spatial link between IMS data and the SBA atlases, we developed a non-rigid registration pipeline that directly maps IMS data to an MRI reference image, without the need to go through histology. This is a non-trivial task as going from the intact organ (as observed by MRI) to tissue sections introduces significant morphological distortions (e.g. due to tissue shrinkage and cutting artifacts).
(3) *Application of the IMS-atlas integration for finding biomolecular differences between IMS data sets, and providing automated anatomical interpretation for these differences.* While automated anatomical interpretation holds a lot of potential for exploring a single IMS experiment, here we demonstrate that an IMS-atlas framework can also be used to discover differentially expressed ions between IMS experiments. Additionally, these differential findings can be immediately and automatically tied to the relevant anatomical structures.

These innovations are demonstrated in a case study where protein-focused Fourier transform ion cyclotron resonance (FTICR) IMS data from coronal brain sections of a Parkinson's Disease (PD) rat model is integrated with the Waxholm Space Sprague Dawley rat atlas of Papp et al. [30–32], available through the SBA.

2. Methods

This section describes the methodology used to integrate IMS data with an MRI-based rat brain atlas. We first introduce the two data sources used in this work: (a) the protein-focused FTICR IMS data obtained from a coronal rat brain section as part of a case study on Parkinson's disease, and (b) the MRI-based anatomical rat atlas available through the SBA. Subsequently, details are provided on the registration pipeline we developed to perform direct non-rigid registration between IMS and MRI data. This is followed by a more detailed look into the methods that use the IMS-atlas integration to analyze the IMS data.