

# Cross-modality Correlation of Multimodal Imaging Mass Spectrometry Data at Single-Pixel Resolution

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## Background

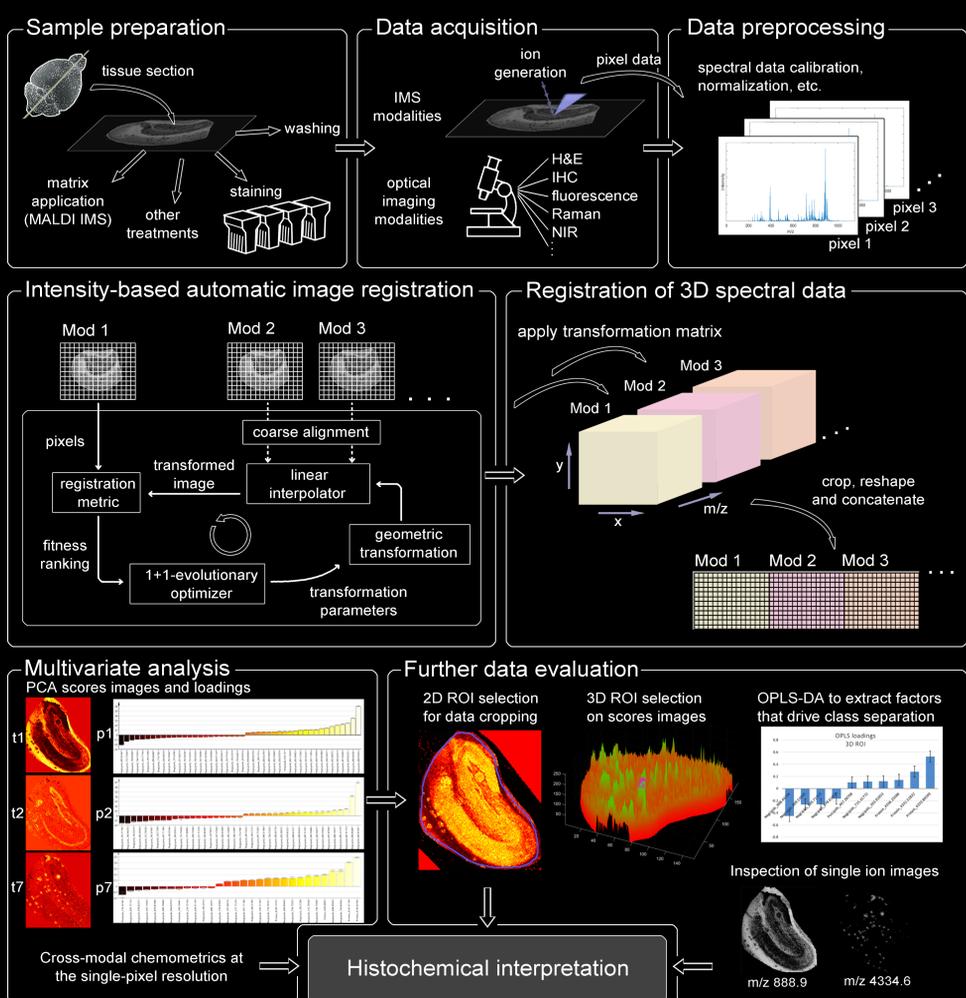
Imaging mass spectrometry (IMS) is a powerful technique for imaging spatial distributions of molecules on a sample surface. In particular, matrix-assisted laser desorption/ionization (MALDI) IMS is a popular approach for studying biochemical processes related to disease pathology, pharmacotherapy and drug metabolism. The understanding of chemically complex systems, such as biological tissues, benefits from the combination of multiple imaging modalities contributing with complementary information. Image data from various acquisitions are typically spatially offset and can be distorted. Therefore, to enable chemometrics analysis across the modalities, the image data need to be registered (aligned and distortions corrected). However, IMS data are commonly evaluated by qualitative comparative analysis where cross-modal correlations are subject to human interpretation. The aim of this study was to establish a workflow for effective processing and registration of IMS data to enable cross-modal chemometrics while minimizing human bias. We demonstrate the method in an application using MALDI IMS to investigate neuropathology associated distribution patterns of lipids and proteins in a transgenic mouse model of Alzheimer's disease (AD).

## Experimental

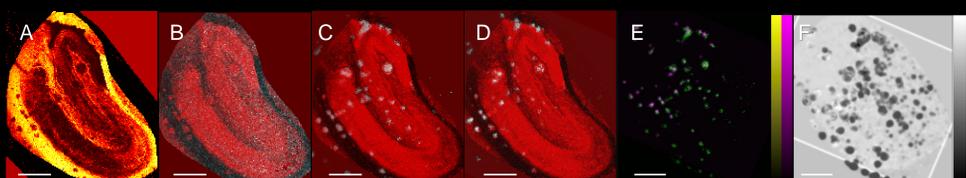
- AD mouse (tgSwe) brain tissue sections (hippocampal area) analyzed by MALDI IMS using an ultrafleXtreme instrument (Bruker) in three modalities: lipids in (1) negative and (2) positive ion mode, and (3) proteins.
- MATLAB routines for spectral data processing, registration and combination of IMS modalities, and data visualization
- Chemometrics analyses performed in SIMCA software (Sartorius Stedim Biotech)

## Results

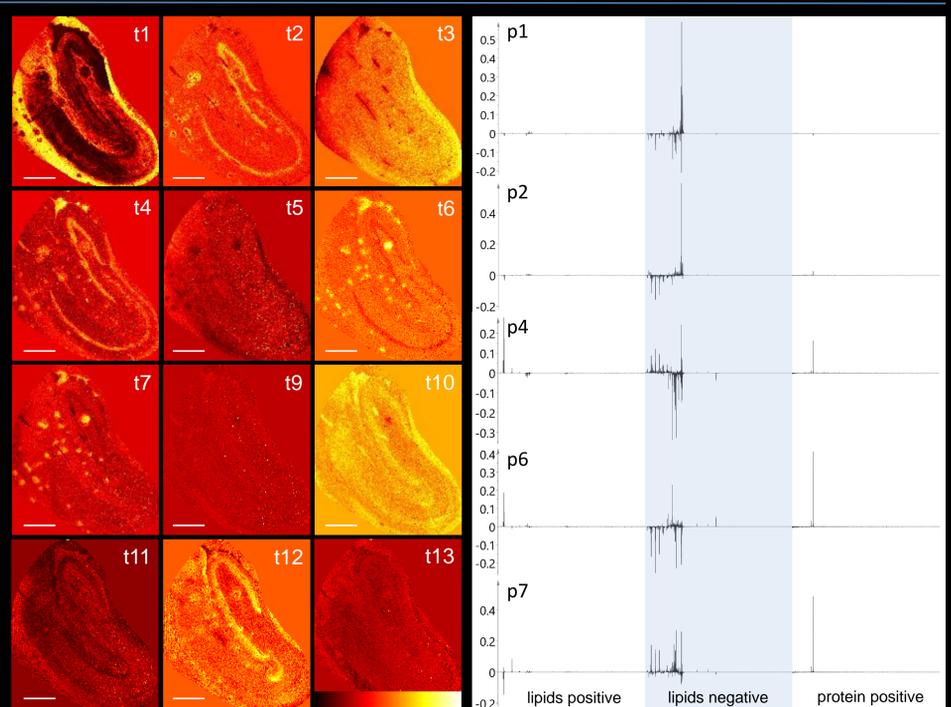
The presented methodology registers and combines multimodal IMS data enabling cross-modal multivariate analysis at the measured image resolution.



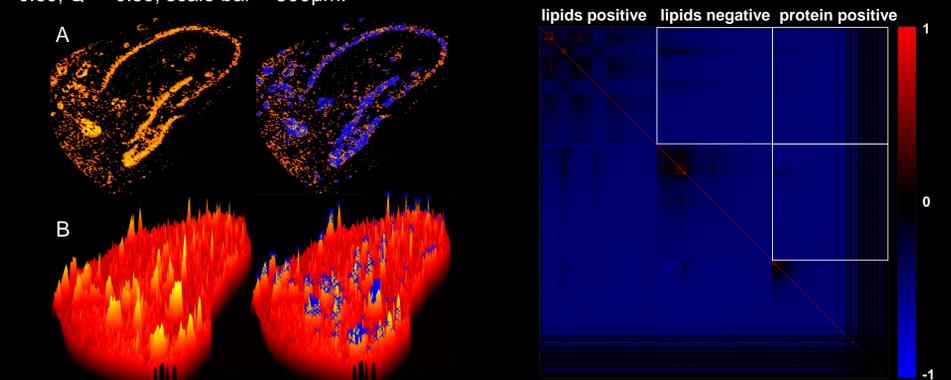
**Figure 1:** General workflow of the IMS-based strategy for cross-modal spatial chemometrics.



**Figure 2:** (A) PCA scores image t1 of uncropped data; red-cyan overlays of modalities (B) negative and positive (C) negative and protein from the same section, (D) negative and protein from the consecutive section; (E) green-magenta overlay of protein data from consecutive sections, (F) structural similarity score map of protein data from consecutive sections (SSIM = 0.6168); scale bar = 500µm.



**Figure 3:** PCA scores images and loadings for multimodal data mining. Scores t2 appear to delineate lipids localizing to plaques, while scores t7 appear to account for plaques peptide distributions.  $R^2 = 0.69$ ,  $Q^2 = 0.59$ , scale bar = 500µm.



**Figure 4:** 3D ROI selection for visualization and further data evaluation. Top scores of (A) t2 and (B) t7 are visualized and selected.

**Figure 5:** Correlation plot of the trimodal dataset. White squares mark plot areas comprising cross-modality correlations.

### Terms explained

**IMS:** Mass spectrometry application in which the sample surface is analyzed spot-by-spot to afford an image in which each pixel is represented by a mass spectrum.

**MALDI IMS:** IMS technique which uses laser (and a laser-absorbing matrix) to desorb analytes from the sample surface.

**Registration:** Alignment of different sets of image data by geometric transformation.

**AD:** Neurodegenerative disease of the brain that leads to cognitive decline and is characterized by neurotoxic peptide deposits

### Acknowledgments

The Swedish Research Council VR (#2014-6447, JH; #2012-1593, SS; #2013-2546, HZ), the European Research Council (#681712, HZ), Alzheimer Research UK (JH), Alzheimerfonden (JH, KB), Hjärfonden (KB), Jeansson Stiftelsen (JH), Ahlén Stiftelsen (JH), Stiftelsen Gamla Tjänarinnor (JH, KB, WM), Stohnes Stiftelse (JH, WM) and Torsten Söderberg Foundation (KB) are acknowledged for financial support. We thank the staff at Centre for Cellular Imaging (CCI), Core Facilities, The Sahlgrenska Academy, and University of Gothenburg for help with development of hyperspectral imaging paradigm and microscopy expertise.

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