

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

A public repository for mass spectrometry imaging data

Andreas Römpp, Rui Wang, Juan Pablo Albar, Andrea Urbani, Henning Hermjakob,
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I. Information about the biological dataset used

Title:

Mass spectrometry imaging of phospholipids in mouse urinary bladder (imzML dataset)

Keywords:

Mass spectrometry imaging, tissue imaging, phospholipids, mouse urinary bladder, imzML

Project description:

The spatial distribution of phospholipids in a tissue section of mouse urinary bladder was analyzed by MALDI MS imaging at 10 micrometer pixel size with high mass resolution (using an LTQ Orbitrap mass spectrometer).

Sample preparation and experimental details:

All the experiments were performed with a AP-SMALDI imaging source. The laser was focused by a centrally bored objective lens to a diameter of 5 to 10 micrometer. Controller software and hardware for the scanning procedure were developed in-house. The imaging source was attached to a linear ion trap/Fourier transform orbital trapping MS (LTQ Orbitrap Discovery, Thermo Scientific GmbH, Bremen, Germany) with a mass resolving power of 30 000 at m/z 400 in positive-ion mode. This setup offers atmospheric pressure compatibility, MSn capability, and sub-ppm mass accuracy. A UV laser with a repetition rate of 60 Hz (LTB MNL-106, LTB, Berlin, Germany) was used for desorption/ionization. The mass range was m/z 100-1000 for measurements of phospholipids. Tissue sections (20 micrometer thickness) were coated with DHB (2,5-dihydroxybenzoic acid) matrix using a pneumatic sprayer. Assignments of lipids were confirmed by MS/MS analysis directly from tissue (isolation window dm/z 3). All images were generated with a bin width of dm/z 0.01. Details of sample preparation, data acquisition and data processing are described in: Römpf, A., S. Guenther, Y. Schober, O. Schulz, Z. Takats, W. Kummer and B. Spengler (2010). *Angewandte Chemie International Edition* 49(22): 3834-3838.

Data processing:

MS imaging data (Thermo RAW format) was converted to imzML (www.imzml.org) using the 'RAW to imzML' converter. For more information see: Schramm, T., A. Hester, I. Klinkert, J.-P. Both, R. M. A. Heeren, A. Brunelle, O. Laprevote, N. Desbenoit, M.-F. Robbe, M. Stoeckli, B. Spengler and A. Römpf (2012). *Journal of Proteomics* 75(16): 5106-5110. Selected ion images were generated using the software package MIRION developed at JLU Giessen. The imaging software imports raw data files as stored by the LTQ Orbitrap instrument software during image acquisition and couples this mass spectrometric information with additional scanning metadata, stored in separate data files by our ion source control program. This metadata includes the number of lines and columns of the image and the pixel size. The imaging software is able to create ion images from any of the detected mass-to-charge values with any selected mass window (bin width). A fast image browser of the MIRION software assists in selecting of images. In this work ion images of selected mass-to-charge values were created from the FT MS data set with a bin width of $dm/z = 0.01$. Up to three different ion images were overlayed in RGB images by the software to display different ion species in parallel. No other postprocessing steps such as interpolation or normalization to matrix signals were applied to the images.

II. Screenshots of all steps of submission process using the 'PX Submission Tool'



Fig. S1 Screenshot of the PX submission tool: Step 1

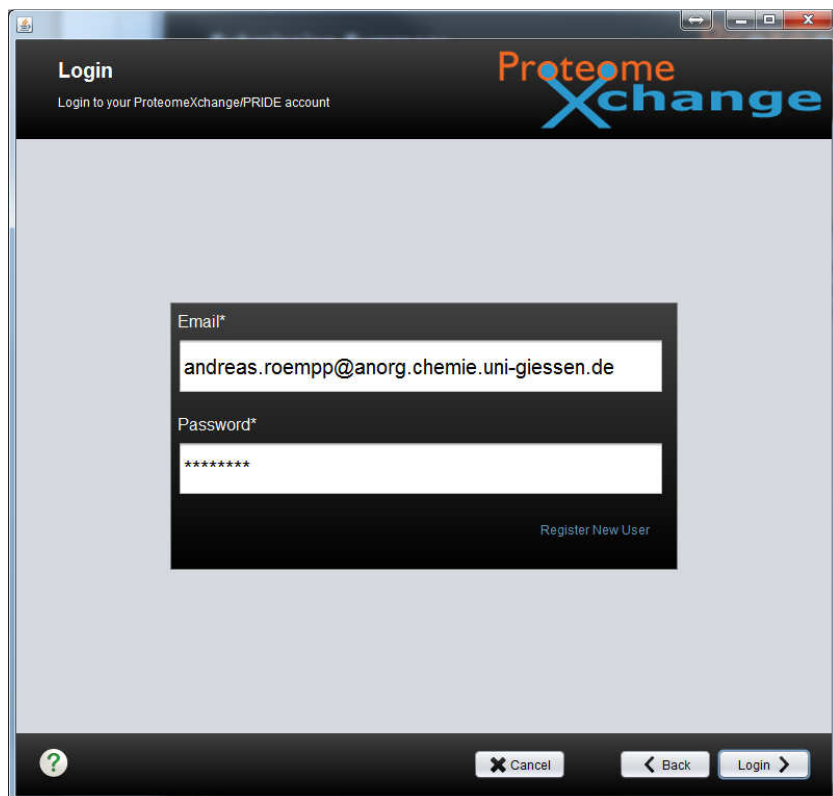


Fig. S2 Screenshot of the PX submission tool: Step 2

Dataset Details
Please provide some details about your dataset

Project title*
Mass spectrometry imaging of phospholipids in mouse urinary bladder (imzML dataset)

Keywords*
tissue imaging, phospholipids, mouse urinary bladder, imzML

Project description* (50 to 5000 characters)
The spatial distribution of phospholipids in a tissue section of mouse urinary bladder was analyzed by MALDI MS imaging at 10 micrometer pixel size with high mass resolution (using an LTQ Orbitrap mass spectrometer).

Sample processing protocol* (50 to 5000 characters)
All the experiments were performed with a AP-SMALDI imaging source. The laser was focused by a centrally bored objective lens to a diameter of 5 to 10 micrometer. Controller software and hardware for the scanning procedure were developed in-house. The imaging source was attached to a linear ion trap/Fourier transform orbital trapping MS (LTQ Orbitrap Discovery, Thermo Scientific GmbH, Bremen, Germany).

Data processing protocol* (50 to 5000 characters)
MS imaging data (Thermo RAW format) was converted to imzML (www.imzml.org) using the 'RAW to imzML' converter. For more information see: Schramm, T., A. Hester, I. Klinkert, J.-P. Both, R. M. A. Heeren, A. Brunelle, O. Laprevote, N. Desbenoit, M.-F. Robbe, M. S. Toeckli, B. Spengler and A. Rompp (2012). Journal of Proteomics 75(16): 5106-5110. Selected ion images were generated using the

Experiment type*
Choose experiment type here
Mass spectrometry imaging

Buttons: Cancel, Back, Next

Fig. S3 Screenshot of the PX submission tool: Step 3

Add Files
Add the files you want to submit

Buttons: Add Files, Which are the file types?

File Name	PATH / URL	File Type	Remove
HR2MSI mouse urinary bladder S096.ibd	V:\imzML\Beispiel-Dateien\Example set fr	RAW	✗
HR2MSI mouse urinary bladder S096.imzML	V:\imzML\Beispiel-Dateien\Example set fr	MS_IMAGE_DATA	✗
HR2MSI mouse urinary bladder S096 - optical image.tif	V:\imzML\Beispiel-Dateien\Example set fr	OPTICAL_IMAGE	✗
HR2MSI mouse urinary bladder S096 - results.bt	V:\imzML\Beispiel-Dateien\Example set fr	SEARCH	✗

Buttons: Cancel, Back, Next

Fig. S4 Screenshot of the PX submission tool: Step 4

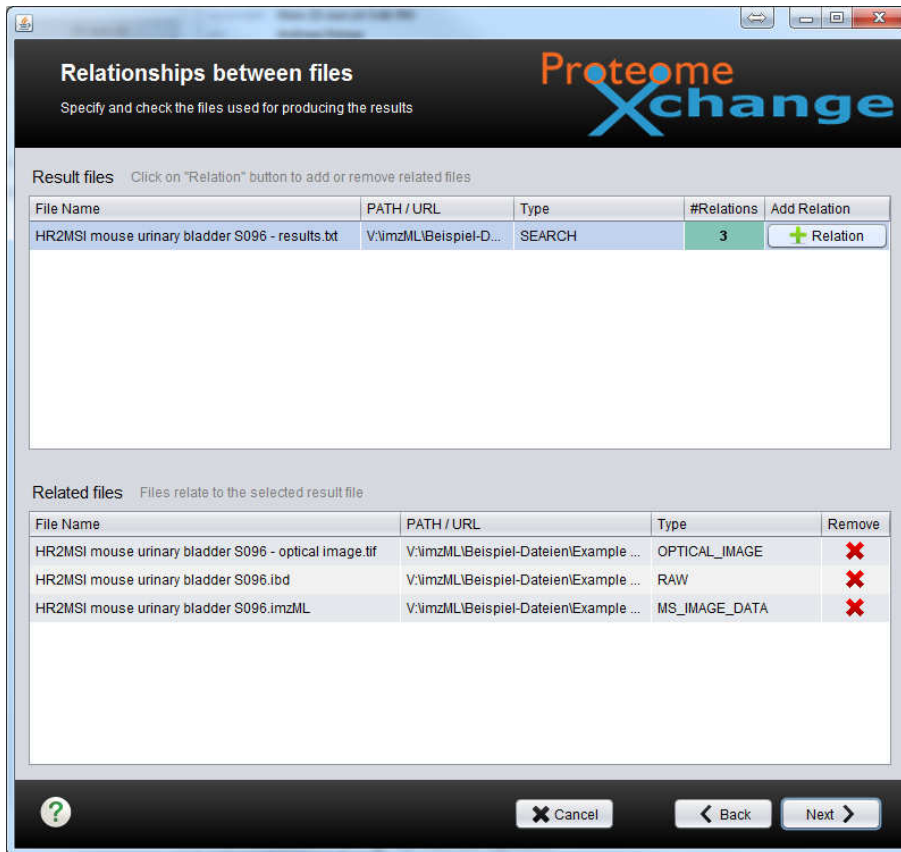


Fig. S5 Screenshot of the PX submission tool: Step 5

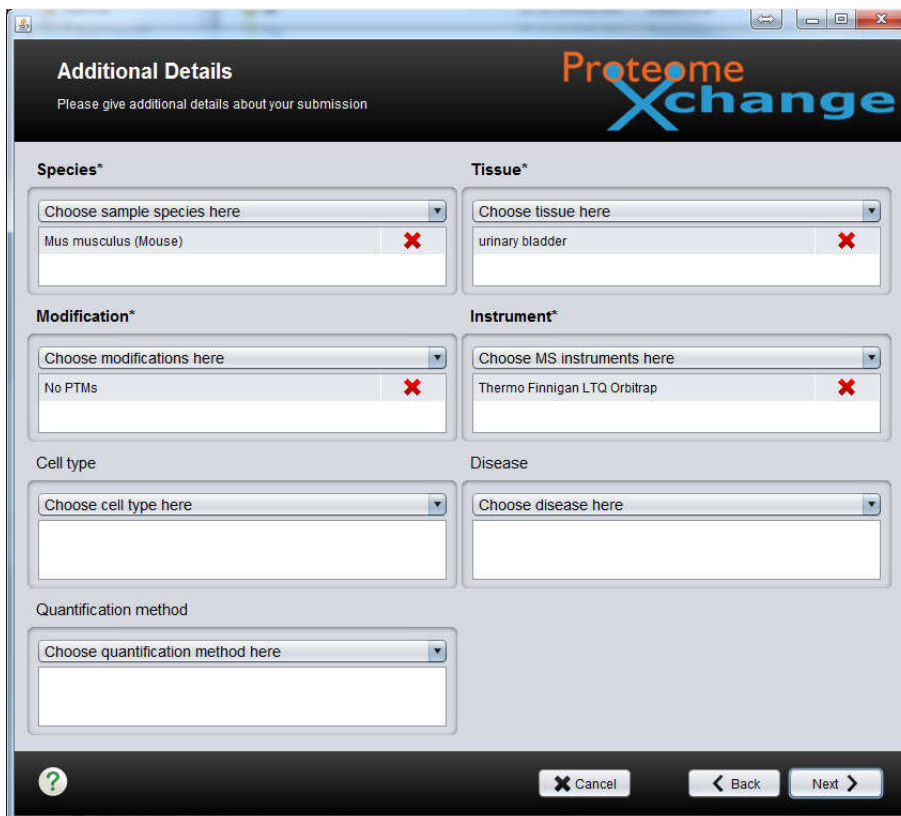


Fig. S6 Screenshot of the PX submission tool: Step 6

Lab Head
Please provide contact details of your lab head

ProteomeXchange

Name*
Bernhard Spengler

Email*
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Affiliation*
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NOTE: We are collecting this information for grouping submissions by lab and as a contact backup.

? Cancel < Back Next >

Fig. S7 Screenshot of the PX submission tool: Step 7

Additional dataset details
Please provide additional details about your dataset

ProteomeXchange

Parent project (optional)
If your project is part of a larger project, please select the parent project from the table below. If you would like to propose a new parent project, please contact us at: pride-support@ebi.ac.uk

Parent Project
<input type="checkbox"/> Human Proteome Project
<input type="checkbox"/> Biology/Disease-Driven Human Proteome Project (B/D-HPP)
<input type="checkbox"/> Chromosome-centric Human Proteome Project (C-HPP)
<input type="checkbox"/> PRIME-XS Project
<input type="checkbox"/> CPTAC Consortium
<input type="checkbox"/> Bioinformatics Infrastructure for Life Sciences (BILS) network (Sweden)

PubMed ID(s) (optional)
20397170

Reanalysis ProteomeXchange accession(s) (optional)
Only applicable if your results are based on the reprocessing of one or several previously submitted PX dataset(s)

Links to other 'Omics' datasets (optional)
Only applicable if proteomics results can be linked to other biological data submitted to other resources (e.g. ArrayExpress, GEO)

? Cancel < Back Next >

Fig. S8 Screenshot of the PX submission tool: Step 8

← □ ×

Dataset Details

Please provide some details about your dataset

Project title* 💡

Keywords*

Project description* (50 to 5000 characters)

The spatial distribution of phospholipids in a tissue section of mouse urinary bladder was analyzed by MALDI MS imaging at 10 micrometer pixel size with high mass resolution (using an LTQ Orbitrap mass spectrometer).

Sample processing protocol* (50 to 5000 characters)

All the experiments were performed with a AP-SMALDI imaging source. The laser was focused by a centrally bored objective lens to a diameter of 5 to 10 micrometer. Controller software and hardware for the scanning procedure were developed in-house. The imaging source was attached to a linear ion trap/Fourier transform orbital trapping MS (LTQ Orbitrap Discovery, Thermo Scientific GmbH, ...)

Data processing protocol* (50 to 5000 characters)

MS imaging data (Thermo RAW format) was converted to imzML (www.imzml.org) using the 'RAW to imzML' converter. For more information see: Schramm, T., A. Hester, I. Klinkert, J.-P. Both, R. M. A. Heeren, A. Brunelle, O. Laprevote, N. Desbenoit, M.-F. Robbe, M. S toeckli, B. Spengler and A. Rompp (2012). Journal of Proteomics 75(16): 5106-5110. Selected ion images were generated using the

Experiment type*

Choose experiment type here

Mass spectrometry imaging ✖

?

Fig. S9 Screenshot of the PX submission tool: Step 9

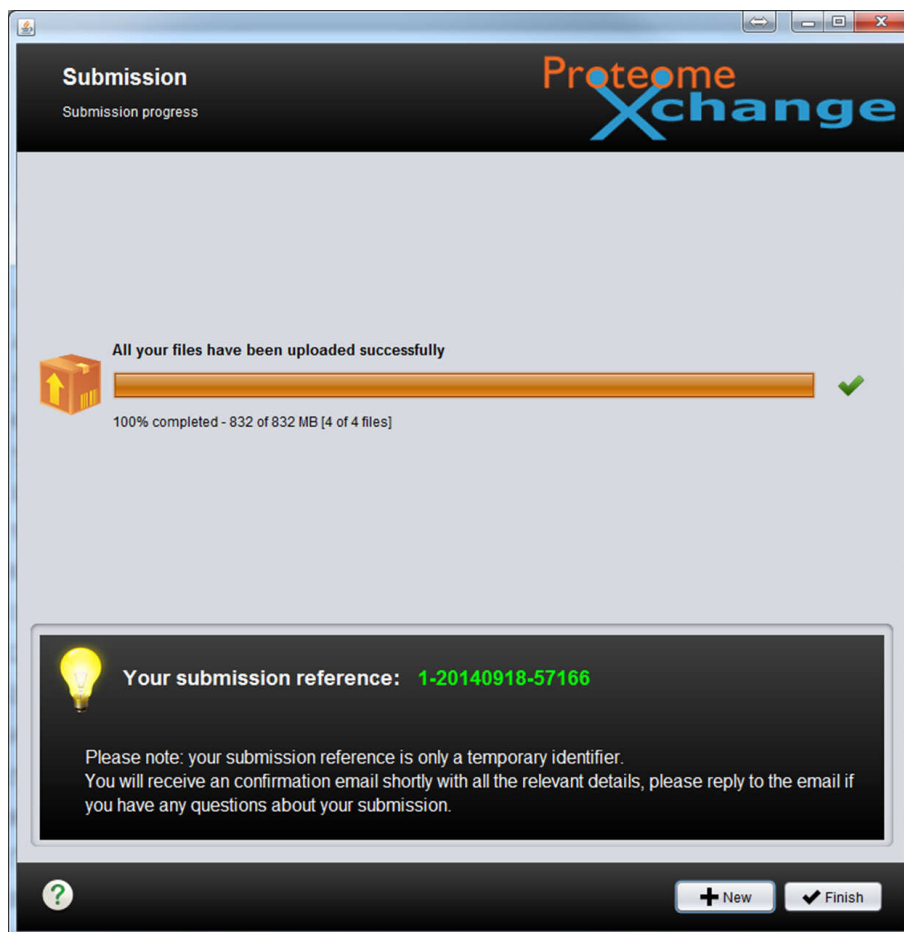


Fig. S10 Screenshot of the PX submission tool: Step 10

III. 'SEARCH' file included in the exemplary dataset

HR2MSI mouse urinary bladder S096 – results.txt (in csv format)

High resolution mass spectrometry imaging of mouse urinary bladder (project title);;;

As published in Roempp A, Guenther S, Schober Y, Schulz O, Takats Z, Kummer W and Spengler B (2010) Angewandte Chemie International Edition 49 (22):3834-3838. (Figure 1, Figure S2);;;

;;;

Parameters used to generate MS images in publication;;;;

Pixel size: 10 æm; ;;;

Bin width ;;;

Normalization: none; ;;;

Interpolation: none; ;;;

;;;

;m/z;Compound;Bin width;

Figure 1A, RGB overlay; ;;;

Red;743.5482;unknown;unknown;

Green;798.541;PC(34:1);[M+K]+;

Blue;741.5307;SM(34:1);[M+K]+;

Figure 1B, RGB overlay; ;;;

Red;616.1767;Heme;M+;

Green;812.5566;PE(38:1);[M+K]+;

Blue;798.541;PC(34:1);[M+K]+;

Figure S2 (supporting information), grey scale images; ;;;

;743.5482;Unknown;;

;741.5307;SM(34:1);[M+K]+;

;798.541;PC(34:1);[M+K]+;

;616.1767;Heme;M+;

;772.5253;PC(32:0);[M+K]+;

IV. Other screenshots

The screenshot shows a web browser window displaying the PRIDE Archive website. The browser's address bar shows the URL www.ebi.ac.uk/pride/archive/projects/PXD001283. A cookie notice is visible at the top, stating that the website uses cookies for functionality and that they have already been set. The PRIDE Archive logo and navigation menu are present, including links for Home, Submit data, Browse data, Help, About PRIDE Archive, Logout, Profile, and Feedback. The main content area displays the project details for PXD001283, including assigned tags (Biological Dataset and Biomedical Dataset), a summary, and a detailed description. The description mentions the use of MALDI MS imaging at 10 micrometer pixel size with high mass resolution. A table on the right side of the page provides key metadata for the dataset.

Species	Tissue
Mus musculus (Mouse)	urinary bladder
Instrument	Software
LTQ Orbitrap	Not available
Modification	Quantification
No PTMs are included in the dataset	Not available
Experiment Type	
Mass spectrometry imaging	

Fig. S11 Screenshot of the exemplary dataset in the PRIDE website

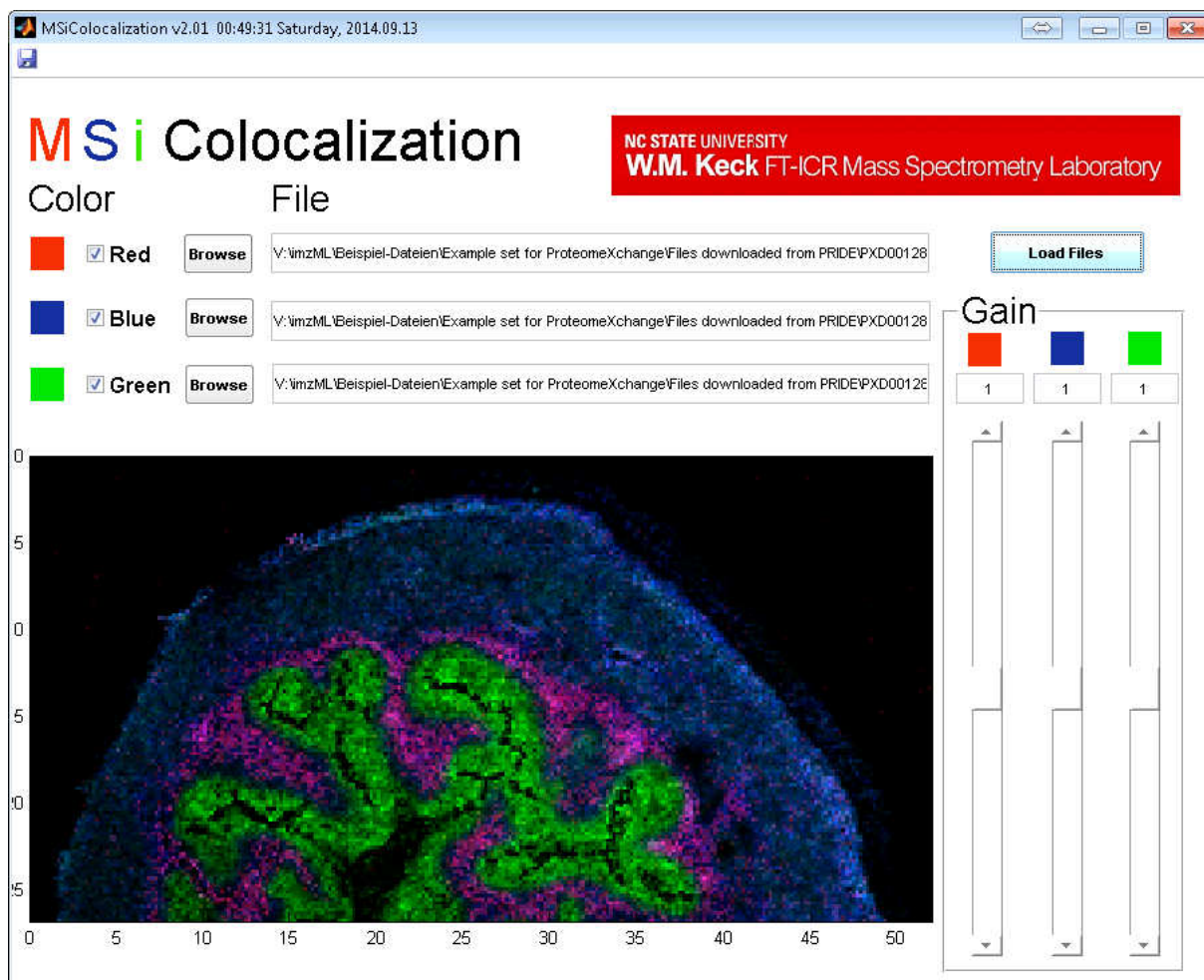


Fig. S12 Screenshot of MS imaging data displayed in MSiReader (data shown in Figure 2C)



Fig. S13 Screenshot of MS imaging data displayed in MSiReader (data shown in Figure 2D)

V. Handling and modification after submission

How to access data privately in PRIDE

Private data set files can be accessed by journal editors/reviewers in two ways: *via* the PRIDE Archive web or *via* PRIDE Inspector.

(i) The PRIDE Archive web site (<http://www.ebi.ac.uk/pride/archive>). Reviewers need to log-in using the reviewer account (including username and password) provided by PRIDE. The details should be communicated by the authors, ideally in the main text.

(ii) In the PRIDE Inspector tool (which can be downloaded at <http://code.google.com/p/pride-toolsuite/wiki/PRIDEInspector>), select ‘Review Project’ and enter the account details (username and password). For MS imaging data, it is just possible to download all the files to a given directory since they are always ‘partial’ submissions).

How to modify an already submitted data set

A given data set can be modified while it remains private. This can be done through the ‘Resubmission’ option using the PX submission tool (available in Step 1). The whole data set needs to be submitted again.

How to make a data set public or add the corresponding published reference

By default, a dataset will be made publicly available after the related manuscript has been accepted, or when PRIDE staff is notified to do so by the original submitter. There are two ways to do it: (i) contacting the PRIDE team by e-mail, or (ii) using the PRIDE Archive website (<http://www.ebi.ac.uk/pride/archive>). To use the web option, the user will need to be logged in and click on the ‘Publish’ button located next to each unpublished dataset.

The corresponding reference associated with a given dataset can also be provided in both ways. It is encouraged that the final version of the reference is always provided. This could potentially be available quite some time after the actual acceptance of the manuscript.