Version 2

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Data Processing and Image Registration V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bt8jnrun

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ABSTRACT

Scope:
Annotate lipid species detected by MALDI IMS analysis, and register MALDI IMS images to different types of microscopy images.

Expected Outcome:
A list of lipid identifications with low mass errors (<5 ppm) that correlate to ion images produced by MALDI IMS analysis, and the ability to layer different images from different assays.

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Data Processing

1 Convert Bruker (.d) raw file into a custom binary data format in profile mode at native spectral resolution.

2 Perform mass alignment and calibration of the dataset.

Spectral misalignment is estimated for each pixel and corrected using PCHIP interpolation or linear shift. Alignment is carried out using a Python msalign package (https://github.com/lukasz-migas/msalign). All spectra are first aligned based on automatically selected peaks and then recalibrated using at least three of the following well-characterized lipids:

1. Positive mode: m/z 703.5748, 734.5694, 760.5851, 810.6001.
2. Negative mode: m/z 673.4814, 744.5549, 834.5291, 885.5499.

3 Create a mean mass spectrum by taking the mean of each mass bin in the dataset.

4 Calculate ppm error associated with common lipids, such as [PC(32:0)+H]+ and [PC(34:1)+H]+. If mass error is above 3 ppm, re-calibrate the entire dataset with additional well-characterized ions.

5 Compute total ion current (TIC)-based normalization factors for each pixel after alignment and calibration has been performed.

6 If LC-MS/MS has been performed, annotate each m/z value against the LC-MS/MS list, otherwise move to 6.1

6.1 Use LIPIDMAPS database to annotate each m/z value with the following search criteria: https://www.lipidmaps.org/resources/tools/bulk_structure_searches.php?database=COMP_DB

1a. Positive Mode Adducts: [M+H]+, [M+H-H2O]+, [M+Na]+, [M+K]+
1b. Negative Mode Adducts: [M+H]-, [M+Cl]-, [M+HCOO]-, [M+OAc]-
2. Specify Mass Tolerance: 0.005 m/z
3. Specify Chains: Even Chains Only
4. Sort: Delta

7 Calculate the ppm error associated with each assignment and remove assignments with errors larger than 5 ppm, although most have errors lower than 3 ppm.

8 Extract peak intensity from the calibrated dataset using one of the available options:

1. Extract ion images based on an untargeted feature list that has not been narrowed down by the previous step.
2. Extract ion images based on the identified feature list generated using the previous step.

Note: In either case, narrow 3-5 m/z bin extraction windows are created for each selected peak and the ion images are generated. The width of the extraction window is important because if it is too wide, several poorly resolved ions can be captured within one ion image.

9 Export the extracted peak intensities to a columnar CSV file by concatenating the x and y-coordinates from the registration step with the preselected ions in the form: x, y, m/z 1, m/z 2 … m/z N.

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10 Read-in the table into Python and export the peak intensities to an imzML file using the Python pyimzML library (https://github.com/lukasz-migas/pyimzML).

Image Alignment and Registration

11 Generate a MALDI imaging mass spectrometry pixel map using regToolboxMSRC (https://github.com/nhpatterson/regtoolboxmsrc).

12 Using FIJI ImageJ, select corresponding laser ablation marks and IMS pixels in the post-acquisition autofluorescence image map and IMS pixel map, respectively.

13 Use a "Landmark Correspondences" FIJI plugin to find an affine transformation between the two images, resampling the post-AF image to the IMS pixel map by selecting post-AF image as source image, and IMS pixel map as the template image within the plugin.

14 Save the transformed and registered post-AF image.

15 Using wsireg (https://github.com/nhpatterson/wsireg) to align other microscopy modalities, such as CODEX multiplexed IF, preIMS AF, PAS or any other associated microscopy image, to the newly saved post-AF image. wsireg will generate pyramidal OME-TIFF images for all images after registration.

16 When complete, all images will be sampled in the same coordinates as the IMS pixel map and IMS can be overlaid onto microscopy by scaling the IMS data to the same resolution as the microscopy data.

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