Automatic generic registration of Mass Spectrometry Imaging data to histology using nonlinear stochastic embedding

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Abstract

The combination of mass spectrometry imaging and histology has proven a powerful approach for obtaining molecular signatures from specific cells/tissues of interest, whether to identify biomolecular changes associated with specific histopathological entities or to determine the amount of a drug in specific organs/compartments. Currently there is no software that is able to explicitly register mass spectrometry imaging data spanning different ionization techniques or mass analyzers. Accordingly the full capabilities of mass spectrometry imaging are at present under exploited. Here we present a fully automated generic approach for registering mass spectrometry imaging data to histology, and demonstrate its capabilities for multiple mass analyzers, multiple ionization sources and multiple tissue types.

Keywords: Mass spectrometry imaging; image registration; tSNE; MALDI, SIMS

1. Introduction:

Mass spectrometry imaging (MSI) is a rapidly developing imaging modality that can provide the spatial distribution of hundreds of biomolecules directly from tissue[1]. It has already had a substantial impact in clinical and pharmacological research, uncovering biomolecular changes associated with disease[2] and providing low cost imaging of pharmaceuticals and their metabolites for drug formulation development[3]. The integration of the biomolecular information obtained by MSI with the anatomical structure provided by histology has proven essential for its clinical and pharmacological application[4], for example to identify biomolecular changes associated with specific histopathological entities[2] (e.g. tumors) or to determine the amount of a drug in specific organs/compartments[3].

The insignificant loss of histoanatomical structures after performing MSI experiments allows co-registration between MSI data and its histological image[4,5]. To date this is performed by most researchers either manually or, for datasets acquired on Bruker Daltonics instruments running the FlexImaging MSI data acquisition software, semi-automatically by using fiducial markers. Veselkov *et al.* recently reported using binary masks of the histological image and MSI data to perform the registration automatically[6]. In this approach the registration algorithm aligns the boundaries of the masks using a global transformation. While this approach is suited to the desorption electrospray ionization based MSI experiments reported in the paper the significant background in MSI datasets recorded using matrix assisted laser desorption/ionization[7,8] (MALDI) and secondary ion mass spectrometry[9] (SIMS) make defining the MSI binary mask more problematic.

Furthermore, MALDI and SIMS MSI datasets are frequently acquired from non-transparent mounting substrates (e.g. gold coated steel plate, or silicon wafer); in such cases the histological images are acquired from proximal tissue sections. Small histological differences between the tissue sections as well as local deformations resulting from their preparation (folds, tears) means that localized elastic transformations are necessary for their correct registration. A generic registration approach must therefore accurately trace the local differences in tissue structure and to make it robust to the background signals present in MALDI and SIMS measurements.

The main challenge is to automatically determine the spatial correspondences between the MSI data and the histological image. The multivariate techniques k-means clustering[10], principal component analysis (PCA)[11], probabilistic latent semantic analysis[12], and non-negative matrix factorization[13] have all been used to approximately demarcate, on the basis of the MSI signals, different histological regions. These are all linear dimensionality reduction algorithms that focus on representing dissimilar data points in a lower-dimensional space (e.g. the maximization of variance in PCA is determined by the most dissimilar data points in Euclidean space). One of the difficulties of using these methods is selecting the appropriate number of dimensions; a number of papers have shown that the images generated by these methods are dependent on the number of dimensions (components) selected for the analysis[10]. Another is that by focusing on keeping the most dissimilar data points far apart in the lower-dimensionality representation they can fail to preserve the local structure of the data[14]. In MSI this means that the analysis implicitly focuses on the largest differences in the dataset, and can merge regions whose molecular differences are minor in comparison[15]. While this merging may be alleviated by changing the number of dimensions used in the multivariate analysis, the dependence of the images on the number of dimensions (clusters) and the bias toward the largest Euclidian differences in the dataset make such techniques suboptimal for summarizing the spatial structures of MSI datasets.

Fonville *et al.* recently demonstrated that the non-linear technique t-distributed stochastic neighbor embedding (tSNE) outperforms linear dimensionality reduction techniques for summarizing MSI datasets[15]. tSNE is a non-linear dimensionality reduction technique developed by van der Maaten *et al.* that maps data points from high-dimensional space into a matrix of pairwise similarity in a lower-dimensional space[14]. The hallmark that characterizes tSNE is its ability to capture the local structures of high-dimensional data as well as preserving their global features. In MSI this means that relationships characterized by large differences in mass spectral profiles can be visualized concomitantly with those characterized by minor differences (that would be merged by linear techniques such as PCA).[15]

The tSNE representation of MSI data reveals clearly distinguishable anatomical regions that can be treated as landmarks for guiding the co-registration process with histology. Importantly the tSNE analysis

does not require any user input and so can be completely automated. Here we report tSNE-enabled automated alignment of MSI datasets with histology. The method is generic and we demonstrate its ability on datasets from different organs, different mass spectrometers, and different ionization methods.

2. Methods

Experimental datasets

The automatic alignment routine has been tested on datasets from four different mass spectrometers, representing four different types of MSI experiment, and spanning a wide range of spatial resolution. The algorithm was then validated on a sizeable animal cohort of 96 mouse brain coronal tissue sections. Table 1 provides a summary of the MSI datasets. Further experimental details about the MSI data acquisition can be found in the Supplementary Information.

Tissue	Sample type	Ion source	Mass analyzer	Pixel size	Molecular class measured	Histology
Thyroid cancer	FFPE	MALDI	TOF/TOF	150 μm	Proteolytic peptides	H&E
Mouse brain	Frozen	MALDI	TOF	100 μm	Proteins	Nissl
Mouse brain	Frozen	MALDI	Ion mobility-TOF	150 μm	Lipids	Nissl
Mouse brain	Frozen	SIMS	TOF	19.2 μm	metabolites	Nissl

Table 1. Overview of MSI datasets used in this study. Abbreviations used: FFPE, formalin-fixed and paraffin-embedded; H&E, hematoxylin and eosin. Note: The SIMS datasets were recorded with 0.3 μm pixel size but were rebinned to 19.2 μm for visualization of the entire area in a single 512 x 512 pixel image. All calculations were performed on this rebinned image.

Histology pre-processing

The stained histological images need first to be pre-processed to exclude the background noise, correct for potential image acquisition artifacts (e.g. inhomogeneous lighting and exposure, noise because of dust on the slides) and maximize contrast. We applied the histological pre-processing pipeline proposed by Abdelmoula *et al.*[16] in which the images were classified into two clusters using k-means (k=2) and then

followed by morphological operations (opening, closing, and region filling with a disk-shape structural element) to close any potential gaps in the clustered image. The resulting binary mask is then used to separate the tissue from the background.

MSI pre-processing

MALDI TOF-proteolytic peptides: Each pixel's mass spectrum was first processed using FlexAnalysis (version 3.4, Bruker Daltonics); mass spectral smoothing was performed with the Gauss algorithm (width 0.02 *m/z* and 2 cycles) and baseline subtraction with the top-hat algorithm. The MSI data was read into MATLAB R2013a (MathWorks, Natick MA, USA) where they underwent total-ion-count normalization[7]. Peak picking was performed on the global basepeak mass spectrum after smoothing, resampling, and baseline subtraction, and was performed using an adapted version of the data reduction code previously reported by McDonnell *et al.*[17] The basepeak spectrum displays the maximum intensity detected in the entire imaging dataset for every peak and is more effective for detecting peaks with localized distributions[17]. Peak areas were then extracted from every pixel's mass spectrum. This reduced and more computationally-manageable representation of a mass spectrum was then placed, based on its original coordinate information, as a pixel into a project-specific data cube[13] and was used for the subsequent registration with histology.

MALDI TOF-proteins: The dataset was processed identically to the MALDI TOF-proteolytic peptides dataset, except the mass spectral preprocessing parameters were adapted for intact proteins. Here, each pixel's mass spectrum was smoothed using the Savitsky-Golay algorithm with a width of 2.0 *m/z* and 5 cycles, and baseline subtracted with the top-hat algorithm (10% width).

MALDI Synapt: The data preprocessing was done employing our in-house developed ChemomeTricks toolbox for MATLAB (MathWorks, Natick MA, USA). In the first step the raw data was converted into a MATLAB format. Mass channels were binned into 0.1 Da wide mass bins. Peak picking was performed

on a global mean mass spectrum after smoothing. The peak picking algorithm has been described in detail elsewhere[18]. The created peak list consisted of 1707 mass channels each of which was defined by its center m/z and an m/z window (peak width at the baseline). The peak list was used to integrate each pixel's mass spectrum.

TOF-SIMS: The data preprocessing was done employing our in-house developed ChemomeTricks toolbox for MATLAB (MathWorks). Mass channels were binned into 0.05 Da wide mass bins. An average spectrum of all pixels was used for peak picking. Peak picking was performed on a global mean mass spectrum after smoothing as described in detail in Eijkel *et al.*[18] The created peak list consisted of 1400 selected mass channels. Pixels were spatially binned resulting in a 256x256 pixels dataset, resulting in a final spatial resolution of 19.2 µm. The peak list was used to integrate each pixel's mass spectrum. Subsequently a multiorder correction algorithm based on linear discriminant analysis (LDA) was applied to remove MS image distortions caused by the mosaic character of the data acquisition.[19] Finally the data was recalibrated on gold coating related peaks with well known m/z values.[20]

tSNE of MSI datasets:

Each processed MSI dataset was unfolded into a set of one-dimensional vectors, $X = [x_1, x_2, ..., x_k]$, in which each vector x_i represents the normalized mass spectral profile of the ith pixel. tSNE was then applied to find the low-dimensionality representation, in this case a 3D-representation $Y = (y1, y2, y3)$. The joint probabilities p_{ij} were first calculated to establish the pairwise similarities between data points x_i and x_j for all pairs in the high-dimensional space. Then, the joint probabilities q_{ij} were calculated for all pairs, y_i and y_j, in the low-dimensional space. The optimum low-dimensional representation (i.e. Y) that maximizes the similarities between p_{ij} and q_{ij} was found by minimizing the Kullback-Leibler divergence KL over all data points:

$$\sum_i KL(P_i||Q_i) = \sum_i \sum_j p_{ij} \log \frac{p_{ij}}{q_{ij}} \qquad (1)$$

Where P_i and Q_i represent the joint probabilities in the high- and low-dimensional spaces, respectively. The optimization problem was solved using the gradient descent method yielding an optimum 3D-representation of the original hyper-dimensional MSI dataset. For visualization, each of the three tSNE output dimensions was treated as a separate color channel and the results displayed as a 2D RGB image[15]. tSNE was performed using the default settings described by van der Maaten *et al.*[14] and the tSNE Matlab toolbox (http://homepage.tudelft.nl/19j49/t-SNE.html).

Image registration:

The high-resolution histological images and the MSI data were acquired from either the same tissue sections (MALDI data) or from adjacent sections (SIMS data). In the former case the histological images and MSI data differ only in their coordinate space and image resolution and thus can be registered using rotation, scaling and translation (rigid registration). For adjacent sections we also added an elastic deformation step to account for minor differences in brain regions size as well as artifacts introduced during sectioning and mounting of the tissue sections.

The registration algorithm transforms a moving image, $I_m(x, y)$, to be spatially aligned with a fixed image, $I_f(x, y)$. The moving image was the gray scale tSNE image, and the fixed image the pre-processed histological image. The rigid transform was used to model rotation, scaling and translation deformations through optimizing the standard registration problem given in Equation (2):

$$\hat{\mu} = \arg min_\mu C(I_f, I_m; T_\mu) \qquad (2)$$

μ is a vector which contains the transformation parameters that were optimized by minimizing the cost function C with respect to the transformation model T_μ using the adaptive stochastic gradient descent optimizer[21]. The statistical metric of the mutual information[22] was used as a cost function to assess the registration quality. Mutual information (MI) has demonstrated high efficiency in multimodal data registration, particularly when the intensity distributions of the images differ. MI measures the degree

of dependence in the intensity distributions between the moving and fixed images through measuring

their marginal and joint entropy, Equation (3). The best alignment is achieved through the

transformation metric in which the joint entropy $H(I_f, I_m)$ is minimal.

$$MI(I_f, I_m) = H(I_f) + H(I_m) - H(I_f, I_m) \qquad (3)$$

$H(I_f)$ and $H(I_m)$ represent the marginal entropy of the fixed and moving images, respectively.

For experiments that use the adjacent tissue section for histology an additional step was incorporated

in which the B-spline transform was used to correct any local deformations; mutual information was

again the cost function and the adaptive stochastic gradient descent optimizer used to achieve the best

similarity through optimizing the B-spline parameters. To capture deformations on different length-

scales the registration was applied using a multi-resolution scheme and implemented using elastix[23].

This elastic registration step is an adaptation of that previously reported for the registration of MSI

datasets to the Allen Brain Atlas,[16] in which experimental histological images were registered to the

reference histological images contained in the Allen Brain Atlas. In this paper we have adapted the

algorithm in order to directly map the MSI data onto the histological image of a proximal tissue

section.

3. Results and discussion:

To automatically co-register MSI with histology we have developed the pipeline shown in Figure 1. The

key elements of the pipeline are:

i) Mapping the MSI dataset to a 3D space using tSNE to determine the spatial correspondences

that are then used for the registration.

ii) Image registration algorithm – for MSI and histology of the same tissue section a rigid

transformation is used; for MSI and histology of adjacent sections elastic deformation is

permitted to account for small differences in the sizes of histological regions and for small

artifacts introduced during the sectioning/mounting procedure (e.g. folds, tears).

iii)   Statistical measure of MSI and histology fitness – mutual information[22] was used to

overcome the inherent independency of the intensity distributions of the tSNE and

histological images.

Figure 2 shows an example of an MSI dataset in which the mass spectral signatures are clearly associated

with the underlying histology. A thyroid cancer tissue section was first prepared for protein MALDI-MSI via

on-tissue tryptic digestion and then measured using an UltrafleXtreme MALDI-TOF/TOF. Following MSI

data acquisition and removal of excess MALDI matrix, the tissue was H&E-stained and a high-resolution

optical image recorded. Figure 2 shows the average mass spectrum, the original histological image and

example MS images. It can be seen that the MSI experiment detected a large number of proteolytic peptide

ions, many of which were localized to distinct histological regions of the thyroid cancer tissue section.

Despite the high contrast of the MSI images it is far from straightforward to determine which of the distinct

MS images best follow the tissue section's histology.

In agreement with Fonville *et al.*[15] we found that a 3D representation of the MSI data using tSNE, and

visualized as an RGB image, reproducibly produces summary images that exhibit clear correspondences with

the tissue section's histology. Accordingly we surmised that the tSNE map could be used to automatically

guide the registration algorithm for finding the optimal transformation to spatially align MSI with histology.

The original histological image of the thyroid cancer tissue section was pre-processed to exclude the

background, normalize contrast and exclude potential image artifacts that might bias the registration

algorithm[16](Figure 3.a). The tSNE representation of the MSI data is shown in Figure 3.b; the color-coding

clearly highlights different histological regions. In this example the histology image and MSI data were from

the exact same tissue section. The tSNE image could thus be registered using a rigid registration (scale,

translation, rotation) and using the mutual information as the registration metric (as mutual information can

accommodate the different intensity distributions and color scales of the images). The high accuracy of the registration can be seen in Figure 3.c, in which the registered tSNE image (using the hot color map) is placed atop of the gray scale processed histology image.

To provide examples of the general applicability of the approach for different MSI platforms, different ionization methods and different application areas, three mouse brain tissue sections, which were sectioned differently (i.e. coronal and sagittal) and analyzed in different mass spectrometers are shown in Figure 4. The top row shows a high-spatial resolution SIMS-MSI analysis using a TOF-SIMS instrument, of the cerebellum region of a sagittal tissue section. The middle row shows a protein MALDI-MSI analysis, using a linear MALDI-TOF, of a coronal tissue section of a mouse brain. The bottom row shows a lipid MALDI-MSI analysis, using a MALDI ion-mobility-ToF instrument, of a sagittal tissue section of a mouse brain. In each case tSNE of the MSI data reveals clearly distinguishable anatomical features, for example: cerebellar cortex (Figure 4.b), corpus callosum (Figure 4.e) and cerebellum (Figure 4.h). The anatomical landmarks generated by the tSNE representations enable the MSI datasets to be registered to the histology images (Figures 4.c, 4.f, and 4.i). Overlaying the tSNE images on top of the histology images demonstrates the high-alignment accuracy. Additional examples of the registration of SIMS, MALDI-TOF and MALDI ion-mobility-TOF are included in Supplementary Figure S-1.

The SIMS-MSI and histology data shown in Figure 4 were of adjacent sections, and so there were minor differences between the histology image and the MSI data, due to the manual nature of mounting the thin tissue sections onto the target plate. In this instance an elastic registration step was necessary to account for the local deformations between the MSI data and the histology image (Supplementary Figure S-2).

To quantify the accuracy of the registration a set of control points were selected in the histological and MSI images. Supplementary Figure S-3 shows the control points selected for coronal mouse brain tissue sections as well as the results of the registration. After registration the errors ranged from under 10 µm for the SIMS dataset, to approximately 40 µm for the MALDI TOF analysis of mouse brain tissue sections, to 80 µm for

MALDI TOF analysis of tryptic peptides in thyroid cancer tissue. In each case the registration accuracy was sufficient that any errors were less than the size of a single MSI pixel (see table 1).

The tSNE-based automatic registration algorithm was then compared to the only commercial package currently available and *de facto* standard for registering histology and MSI data, namely FlexImaging from Bruker Daltonics. FlexImaging is Bruker's MSI data acquisition and data analysis software, and is only compatible with Bruker MALDI mass spectrometers. To record MSI data using FlexImaging the mass spectrometer's sample stage is first aligned to an optical image of the MALDI-matrix-coated tissue. This alignment is performed by manually selecting features in the matrix-coated-tissue-image and manually selecting the corresponding features in the mass spectrometer's sample visualization system. In this manner the mass spectrometer's coordinate system, and thus the MSI data, is aligned to the matrix-coated-tissue-image. After MSI data acquisition, the histology image is then registered to the MSI data through the matrix-coated-tissue-image, by selecting common features in the high-resolution histology image and the matrix-coated-tissue image.

Figure 5.a shows the preprocessed high resolution optical image of a coronal tissue section of a mouse brain, Figure 5.b the spatial distribution of a selected mass (m/z=1241). FlexImaging was then used to align the histology image and the MSI data (Figure 5.c), and the tSNE-based automatic registration algorithm applied to the same data (Figure 5.d). Visual inspection of the automatic and semi-automatic co-registration results shows a close consensus in the MS distribution with respect to the tissue's anatomy. To validate the automatic registration algorithm, its results were compared with those from FlexImaging's semi-automatic registration for datasets from 60 coronal mouse brain tissue sections spanning three different molecular classes (20 metabolite MSI datasets, 20 peptide MSI datasets and 20 protein MSI datasets). Supplementary Figures S4a-S4l visualize the results of the tSNE-based automatic registration algorithm. The Pearson correlation between the automatically registered results, and those from the FlexImaging's semi-automatic method, were then calculated. A histogram of the resulting correlation coefficients, Figure 5e, demonstrates excellent agreement between the two methods, with a mean correlation coefficient of 0.97 and a standard

deviation of 0.01. Supplementary Figure S-4m shows the histogram of the DICE coefficients, another image

overlap metric that again confirms the high quality of the automatic registration algorithm.

We have developed an automatic generic technique to co-register MSI-datasets to their histological images;

we have demonstrated its applicability to MSI datasets measured on different mass spectrometers, using

different ionization mechanisms, different tissue samples and validated the results using a large series of

mouse brain tissue sections. The tSNE representation plays a vital role in the registration by summarizing the

spatio-molecular organization of the tissue, which has clear correspondences with the tissue section's

histology. While even a single tSNE dimension was sufficient to reveal the spatio-molecular organization,

the 3D tSNE map was significantly smoother and so was used here (Supplementary Figure S-5).

The computational and memory requirements of the original tSNE algorithm[14], as used by Fonville *et al.*[15],

scale with the square of the number of data points. An MSI dataset of just 200 x 100 pixels, and 500 detected

peaks, contains 10M data points. Accordingly tSNE analyses could run very slowly. A new implementation,

termed the Barnes-Hut implementation,[24] scales as NlogN for computation and N for memory, and thus

enables tSNE of MSI datasets to be run much more practically. Freely available code, for many different

platforms, is available from the tSNE website.[25]

All the experiments referred to here were recorded using MALDI or SIMS, ionization methods that generate

a substantial background signal and so not well suited to previously reported methods based on the rigid

registration of binary images[6]. Supplementary Figure S-6 shows a comparison of the registration results for

MALDI MSI of a coronal mouse brain tissue section using the binary image registration method with those

obtained using tSNE. It is immediately apparent that there is a translation error in the registration performed

using binarized images (due to the background in MALDI MSI datasets). Furthermore, high spatial

resolution analyses such as those presented in Figures 4a-c often focus on specific regions of tissue rather

than analyze the entire section because of the measurement time / memory demands of the experiment. These

MSI datasets do not contain the tissue border regions necessary for the binary image registration method[6].

Finally binary images do not contain the internal structures needed for elastic registration algorithms to align

MSI and histological data from adjacent tissue sections (Figures 4a-c and Supplementary Figures S-1 and S-2).

This automatic histology-MSI registration pipeline will enable joint histology-MSI experiments to be performed irrespective of the ionization method or mass analyzer used to acquire the MSI data. Accordingly virtual microdissection can be used to extract region-specific mass spectra from disease entities, e.g. tumors, to enable biomarker discovery experiments utilizing the full repertoires of MSI approaches. Furthermore by combining the automatic histology-MSI registration pipeline with that previously reported by Abdelmoula *et al.*[16] MSI datasets of mouse brain tissue sections can be automatically aligned to the Allen Brain Atlas[26]. The Allen Brain Atlas alignment routine requires the MSI-dataset and its associated histology to be already registered to each other. Previously this was performed using fiducial markers in Bruker Daltonics' FlexImaging software. However, this limited the approach to MALDI MSI data recorded using instruments from Bruker Daltonics. The generic and automated histology-MSI co-registration pipeline reported here means that all MSI data may be analyzed in the context of the reference atlas and gene expression data contained in the Allen Brain Atlas.

tSNE can also be used as a distinct classification tool[27]. In a process termed Automatic Classification of Cellular Expression by Nonlinear Stochastic Embedding (ACCENSE) Shekhar *et al.* utilized tSNE and a density-based partitioning of the tSNE space to demarcate T-cells into groups on the basis of the expression levels of 35 proteins, measured using mass cytometry[27]. The application of a similar density-based partitioning to the results of a tSNE analysis of MSI data would enable the identification of clusters without the need to pre-define their number (as is necessary in NMF, PLSA, k-means clustering). It is expected that the combination of automatic MSI-histology alignment reported here and a classifier (whether based on tSNE or other classification algorithm) will enable the automated identification of specific regions/organs of interest and thereby the automated extraction of their mass spectral profile. Such capabilities would greatly facilitate the biomedical application of MSI, whether clinical biomarker discovery experiments or quantifying the level of a drug in different animal organs.

4. Concluding remarks

MSI experiments can now be performed using a diverse array of ionization methods and mass analyzers that offer complementary capabilities. The development of the imzML data standard[28] and open source data analysis tools[29,30] now enable the MSI data from different platforms to be more readily compared and combined, the latter for greater biomolecular depth of coverage. The automated generic MSI-histology registration tool reported here represents an important development in the efforts to increase the impact, accessibility and inter comparison of MSI data, because it delivers one of the principal strengths of MSI for biomedical analysis (the ability to acquire cell/region specific mass spectra from tissues with complex histologies) for any combination of mass analyzer and ionization method.

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Figure 1. The proposed pipeline to automatically align MSI data to its histological image. The method is generic as it can be applied to different tissues, MSI datasets recorded using different types of mass spectrometer, and equipped with different ion sources.
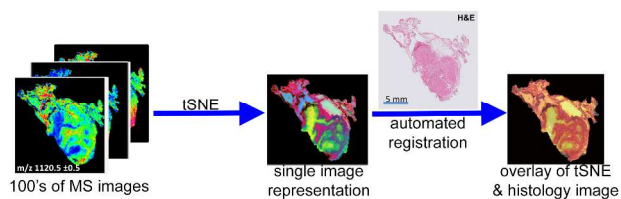
Figure 2. MALDI MSI analysis of a human oncocytic follicular thyroid cancer tissue using on tissue tryptic digestion and measured using a MALDI-TOF/TOF. The MSI data contains hundreds of proteolytic peptide ions, many of which exhibit highly structured distributions (top row). A comparison with the histological image (tissue section H&E stained after the MSI experiment) reveals that many ions are associated with specific histological features.

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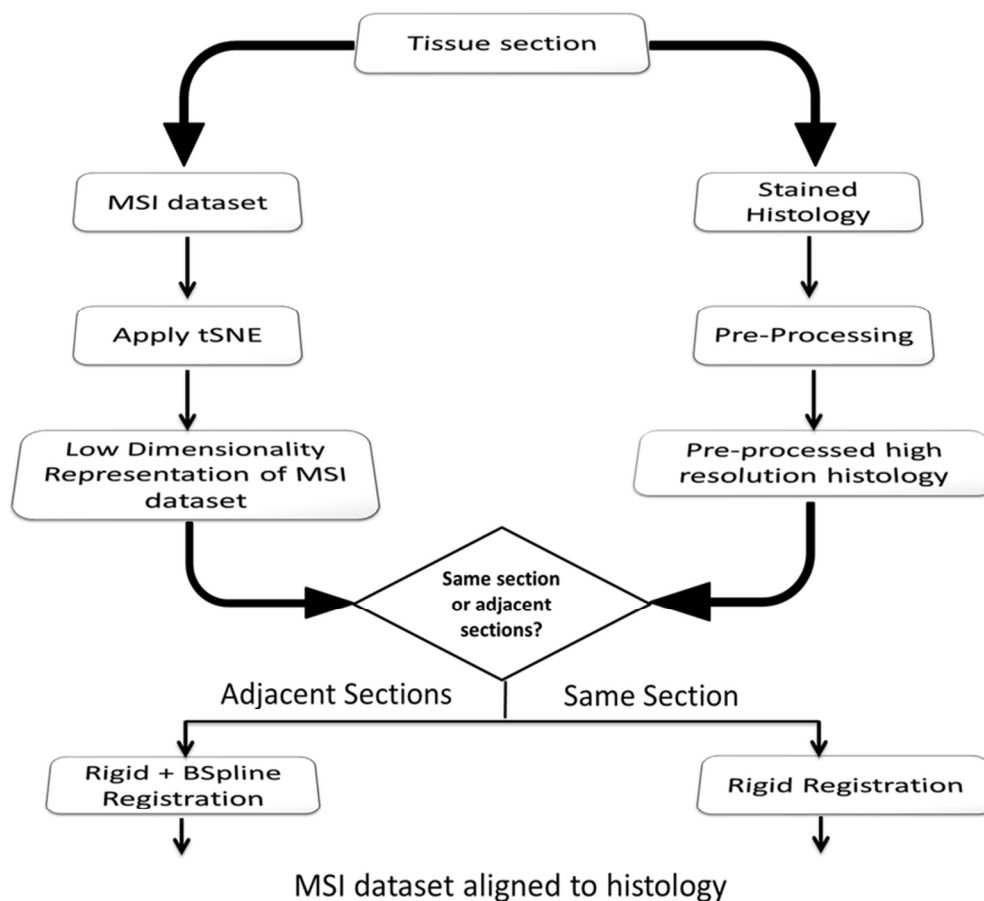41
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Figure 3. Co-registration of MALDI MSI data and histological image of thyroid cancer tissue: (a) Pre-

processed histological image; (b) The low dimensionality representation of the high-dimensional MALDI

MSI data using tSNE (and which is used as the moving image in the registration process); (c) Fusion

result – overlay of the processed histological image and registered tSNE results.

Figure 4. Co-registration of MSI datasets and their histological images; datasets from different mice and different mass spectrometers (SIMS, MALDI-TOF, and MALDI-Synapt): tSNE representations of the MSI datasets (2nd column) show clear spatial correspondences with their associated histological images (1st column), enabling registration to be performed successfully (3rd column, for improved clarity the histological image and tSNE representation are shown in grey scale and hot color scale, respectively).

Figure 5. Comparison between semi-automatic and automatic co-registration of mouse brain datasets: (a) Pre-processed histology. (b) Original spatial distribution of a selected mass (m/z = 1241 Da). (c) Fusion result combining the histological image and the MS image – co-registration performed semi-automatically and is based on manually selected fiducial markers. (d) Fusion result combining the histological image and the MS image – co-registration performed automatically using tSNE. (e) Histogram of correlation coefficients between 60 MSI datasets of coronal mouse brain tissue sections automatically registered using the tSNE-based pipeline and semi-automatically registered using FlexImaging. Supplementary Figure S-4 shows the results of the automatic registration for all 60 tissue sections.

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Figure 1. The proposed pipeline to automatically align MSI data to its histological image. The method is generic as it can be applied to different tissues, MSI datasets recorded using different types of mass spectrometer, and equipped with different ion sources.
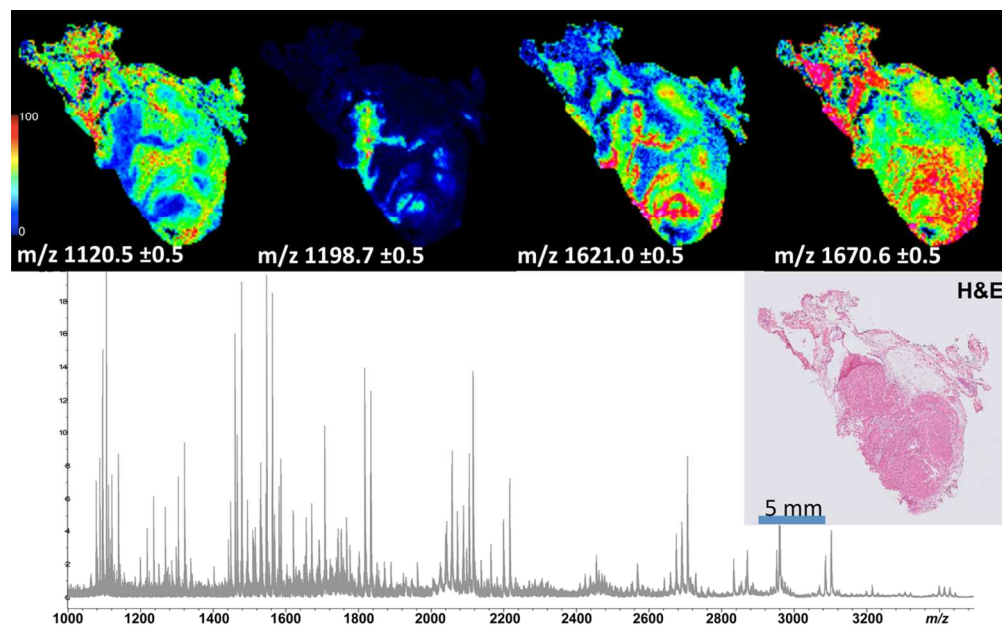75x67mm (300 x 300 DPI)

Figure 2. MALDI MSI analysis of a human oncocytic follicular thyroid cancer tissue using on tissue tryptic digestion and measured using a MALDI-TOF/TOF. The MSI data contains hundreds of proteolytic peptide ions, many of which exhibit highly structured distributions (top row). A comparison with the histological image (tissue section H&E stained after the MSI experiment) reveals that many ions are associated with specific histological features.
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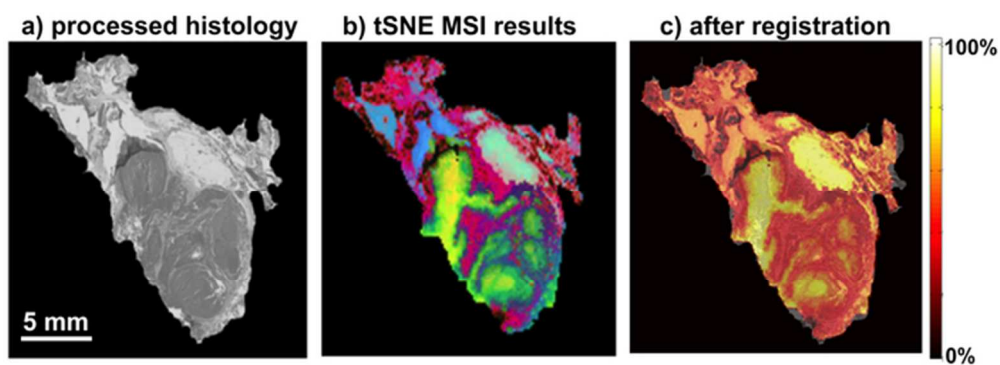43
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Figure 3. Co-registration of MALDI MSI data and histological image of thyroid cancer tissue: (a) Pre-processed histological image; (b) The low dimensionality representation of the high-dimensional MALDI MSI data using tSNE (and which is used as the moving image in the registration process); (c) Fusion result – overlay of the processed histological image and registered tSNE results.
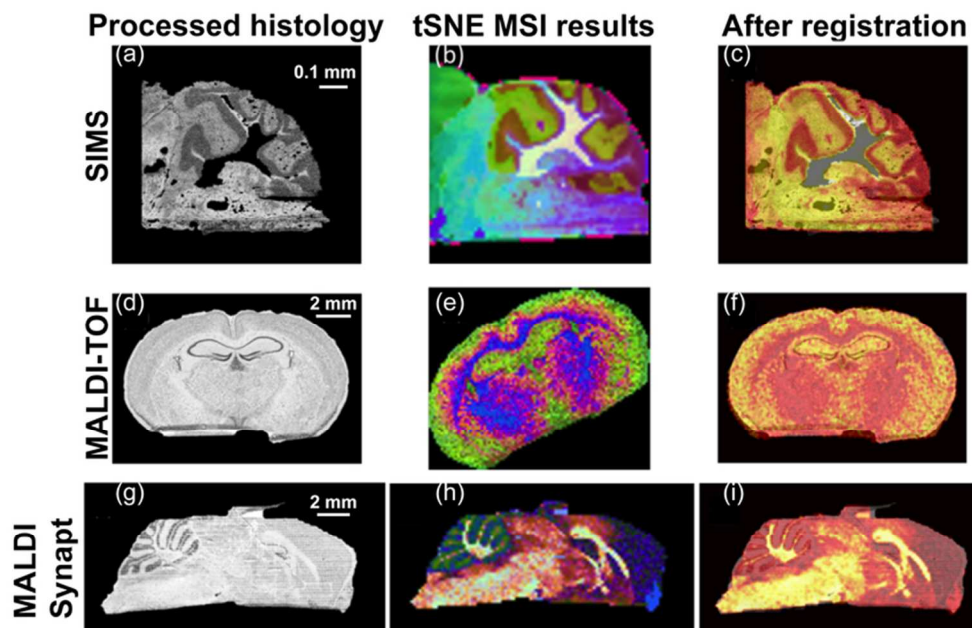
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Figure 4. Co-registration of MSI datasets and their histological images; datasets from different mice and different mass spectrometers (SIMS, MALDI-TOF, and MALDI-Synapt): tSNE representations of the MSI datasets (2nd column) show clear spatial correspondences with their associated histological images (1st column), enabling registration to be performed successfully (3rd column, for improved clarity the histological image and tSNE representation are shown in grey scale and hot color scale, respectively).
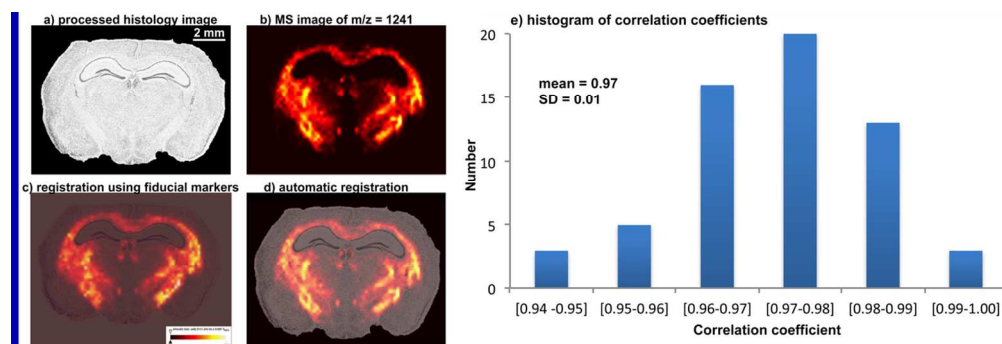73x46mm (300 x 300 DPI)

Figure 5. Comparison between semi-automatic and automatic co-registration of mouse brain datasets: (a) Pre-processed histology. (b) Original spatial distribution of a selected mass (m/z = 1241 Da). (c) Fusion result combining the histological image and the MS image – co-registration performed semi-automatically and is based on manually selected fiducial markers. (d) Fusion result combining the histological image and the MS image – co-registration performed automatically using tSNE. (e) Histogram of correlation coefficients between 60 MSI datasets of coronal mouse brain tissue sections automatically registered using the tSNE-based pipeline and semi-automatically registered using FlexImaging. Supplementary Figure S-4 shows the results of the automatic registration for all 60 tissue sections.

60x20mm (600 x 600 DPI)