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Automatic 3D Non-linear Registration of Mass Spectrometry Imaging and Magnetic Resonance Imaging Data

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Abstract

Multimodal integration between mass spectrometry imaging (MSI) and radiology-established modalities such as magnetic resonance imaging (MRI) would allow the investigations of key questions in complex biological systems such as the central nervous system. Such integration would provide complementary multiscale data to bridge the gap between molecular and anatomical phenotypes, potentially revealing new insights into molecular mechanisms underlying anatomical pathologies presented on MRI. Automatic co-registration between 3D MSI/MRI is a computationally challenging process due to dimensional complexity, MSI data sparsity, lack of direct spatial-correspondences, and non-linear tissue deformation. Here, we present a new computational approach based on stochastic neighbor embedding to non-linearly align 3D MSI to MRI data, identify and reconstruct biologically-relevant molecular patterns in 3D, and fuse the MSI datacube to the MRI space. We demonstrate our method using multimodal high-spectral resolution MALDI 9.4 Tesla MSI and 7 Tesla *in vivo* MRI data, acquired from a patient-derived, xenograft mouse brain model of glioblastoma following administration of the EGFR inhibitor drug of Erlotinib. Results show the distribution of some identified molecular ions of the EGFR inhibitor

erlotinib, a phosphatidylcholine lipid, and cholesterol which were reconstructed in 3D and mapped to the MRI space. The registration quality was evaluated on two normal mouse brains using the Dice coefficient for the regions of brainstem, hippocampus, and cortex. The method is generic and can therefore be applied to hyperspectral images from different mass spectrometers and integrated with other established *in vivo* imaging modalities such as computed tomography (CT) and positron emission tomography (PET).

1. Introduction

Mass spectrometry imaging (MSI) is a technology that provides spatially-resolved distribution of a wide range of molecules directly from tissue samples in a label free manner¹. Based on the MSI sample preparation, images from different molecular classes can be acquired such as biomolecules (proteins, lipids, peptides, and metabolites)², as well as administered drugs. This makes MSI a promising discovery tool for application in many areas such as tumor heterogeneity³, drug discovery⁴, and neurological disorders^{5,6}. In a typical MSI acquisition a thin tissue section is raster scanned at a pre-defined spatial resolution grid in which each point (i.e. pixel) is subjected to desorption and ionization of tissue material releasing molecular ions. Measurement of these ions based on their physical property of mass-to-charge ratio (m/z) produces a mass spectrum at each analyzed pixel. Peaks from each mass spectrum reflect the relative abundance of different ions. Matrix-assisted laser desorption ionization Fourier transform ion cyclotron resonance (MALDI FT-ICR)⁷ and desorption electrospray ionization (DESI)⁸ are two ionization methods used for mass spectrometry imaging that have attracted interest in many pre-clinical studies^{9,10} and that hold potential for clinical applications^{3,11,12}.

Recent progress in imaging technology has enabled the acquisition of 3D MSI in a reasonable time frame¹³. Generally, 3D MSI data is obtained through 2D MSI acquisition of sequential tissue sections¹⁴. A 2D MSI experiment analyzes a tissue section and yields a hyperspectral image, i.e. a datacube of dimensions (x, y, k) such that x and y dimensions show spatial location of the spectral peaks and the k dimension represents m/z features. Repeating this process for several successive tissue sections results in multiple 2D MSI datacubes that can be co-registered and stacked together to form a 3D MSI dataset. Therefore, a 3D MSI dataset represents hyperspectral volume of dimensions (x, y, z, k) where x, y and z dimensions represent voxel spatial location and the k dimension represents m/z features.

A successful reconstruction of 3D hyperspectral volume requires those sequential 2D MSI data sets to be well aligned. The chemicals and manual tissue handling involved in MSI tissue preparation impose local deformations on the tissue section (i.e. non-linear distortions such as shrinkage). To date the reconstruction of 3D MSI data is generally restricted to a linear registration process that captures global deformations such as translation and rotation^{15–17}. Capturing global deformations can be considered an initial step to map the multiple 2D MSI datacubes into a common coordinate space. However, this step alone is insufficient to accurately construct 3D molecular maps. Ideally, the linear registration step

should be followed by a non-linear (i.e. known also as non-rigid/elastic) registration process to enable the capture of local deformations to achieve a more accurate reconstruction.

3D MSI data are collected from individual 2D sample sections that were originally dissected from a tissue volume. In the presence of local deformations those dissected tissue sections lose their inter-spatial structural integrity that might hinder reconstructing them back into their original 3D shape¹⁸. Mainly, the absence of geometrical constraints on the non-linear transformation model can lead to non-orthogonal structures being unrealistically warped into orthogonal structures on the reference template, a result known as “banana-into-cylinder” problem¹⁹. A blockface image or an *in vivo* imaging modality such as magnetic resonance imaging (MRI) provide a reference of the original tissue shape and thus can impose constraints on the transformation model to preserve the original geometrical volume entity while reconstructing the 3D molecular maps. The blockface approach is, however, more laborious and biologically and clinically less informative when integrated with MSI data compared to non-invasive *in vivo* imaging approaches such as MRI.

Multi-modal data integration between *in vivo* MRI and MSI has attracted interest and shown promise for addressing some complex biomedical questions¹⁷. This process would enable linking the anatomical structures provided by MRI with underlying molecular information provided by MSI. A framework providing seamless MSI/MRI integration could benefit surgical guidance^{20,21}, enable molecular biomarker identification^{3,22–24}, and the study of drug distribution²⁵.

Automatic registration of 3D MSI/MRI data is a challenging process; different dimensional complexities cause one-to-many mapping issues (hyperspectral vs. anatomical images) with differences in information contents limiting the establishment of spatial correspondences. This task has previously been addressed by using a further imaging modality to act as an intermediate reference to link 3D molecular maps to MRI^{16,17,26}. For example, Sinha *et al*¹⁷ used a blockface image while Oetjen *et al* used an optical image as an intermediate reference image¹⁶. In such approaches, the 3D MSI data was initially reconstructed by linear registration to an intermediate image and then mapped to the MRI space using a linear transformation matrix that was previously computed by aligning the intermediate image to MRI data.

In recent years, significant progress has been made in the automation of a non-linear registration process between 2D MSI and histology data. Abdelmoula *et al*²⁷ introduced the concept of using spatial organization of low dimensional spectral representation to overcome dimensional complexity, and thus enable direct non-linear alignment of 2D MSI/histology. In this approach, the dimensionality reduction method of t-distributed stochastic neighbor embedding (t-SNE)^{28,29} was used to non-linearly map the high dimensional mass spectra into a lower dimensional representation in 3D space. Those lower dimensional features were then spatially arranged to form a t-SNE image that was found to be structurally informative, revealing molecularly distinct regions. Those distinct regions could then be treated as landmarks to guide the registration and thus establish spatial correspondences with the other anatomical-based imaging modality. Verbeek *et al* has recently adopted the same concept, but based on principal component analysis (PCA), to align 2D MSI to MRI data²³.

Identification of informative 3D molecular patterns, which may hold biological value, and cross-correlating them with MRI data is a natural step following a successful co-registration process. The large size of 3D MSI data, and more specifically ultra-high mass resolution data provided by MALDI Fourier transform ion cyclotron resonance mass spectrometry imaging (MALDI FT-ICR MSI)¹, which may contain up to millions of mass spectra each of which has thousands of m/z features, poses computational challenges for spectral feature learning and identification of molecular patterns¹⁵. Recently, Abdelmoula *et al* presented an efficient computational method based on hierarchical stochastic neighbor embedding (HSNE)³⁰ to analyze 3D MSI data and identify multi-scale molecular patterns with manageable computational complexities³¹. HSNE is a scalable extension of t-SNE used for unsupervised non-linear dimensionality reduction of large and complex datasets³², and it is applied here to identify molecular patterns from the 3D MALDI MSI data and fuse it to the MRI.

We introduce an automatic non-linear image registration method to directly integrate 3D MSI datasets with *in vivo* MRI without using an intermediate imaging modality. Our computational pipeline is also able to identify and reconstruct biologically-relevant molecular patterns in 3D, and fuse the MSI datacube to the MRI space. We demonstrate a biological application of our method using multimodal high-spectral resolution MALDI 9.4 Tesla MSI and 7 Tesla *in vivo* MRI data, acquired from a patient-derived xenograft (PDX) mouse brain model of glioblastoma (GBM39) following administration of the EGFR inhibitor drug of Erlotinib. The registration quality was evaluated on two normal mouse brains using the Dice coefficient. While it is applied in this paper to MSI and MRI, the proposed automatic alignment method is independent of the imaging technology and can be applied to integrate different *in vivo* imaging modalities (X-ray, computed tomography, PET-CT.) with hyperspectral biomolecular imaging modalities equipped with different mass spectrometers and different ionization sources.

2. Materials and Methods:

Intracranial xenograft model of GBM39 was established in 6–8 week old female Athymic Nude mouse (Harlan Sprague Dawley Athymic Nude-Foxn1nu mouse) ordered from Envigo (Indianapolis, IN) as previously described³³. The mouse received a single dose of 100 mg/kg erlotinib via oral gavage in a 0.5% methocellulose solution, and the brain was scanned by MRI. Two hours post drug administration the treated mouse was euthanized by cervical dislocation following CO₂ inhalation. The brain was then harvested, flash-frozen in consecutive three second dips in liquid nitrogen, and stored at –80⁰ C for a week until imaging using a MALDI 9.4 Tesla FT-ICR mass spectrometer. Two healthy and untreated Black6 mice (Harlan Sprague Dawley C57BL/6 mice) were processed in the same manner for MRI and scanned using MALDI- TOF/TOF mass spectrometer. The protocol was reviewed and approved by the Brigham and Women's Hospital Center for Comparative Medicine Institutional Animal Care and Use Committee.

2.1. Magnetic resonance imaging:

MR images were acquired with a Bruker BioSpec® 70/20 USR 7T MRI scanner, using an 86 mm transmit volume coil and a 2 cm surface receiving coil. Standardized pulse sequences of T1 pre-Gadolinium, T1 post-Gadolinium and T2-RARE (Rapid Imaging with Refocused Echoes) were applied. The MR slice thickness was 600 μm and the in-plane spatial resolution was 60 μm . MR images were first pre-processed through skull stripping to exclude non-brain tissues such as bone and muscle. Skull stripping was performed semi-automatically using morphological operations and region growing³⁴. The spatial correspondence between the MR image and its associated MSI datacube was visually determined by comparing anatomical landmarks (hippocampus, ventricles, and tumor location/shape) in both the MR image and the hematoxylin and eosin (H&E) stained tissue image that was successive to each MSI tissue section.

2.2. MALDI mass spectrometry imaging:

Mass spectrometry imaging of the GBM39 samples was performed using a Matrix Assisted Laser Desorption Ionization (MALDI) ionization source coupled to a 9.4 Tesla Solarix XR FT-ICR mass spectrometer (Bruker Daltonics, Billerica, MA) for high spectral mass resolution. And mass spectrometry imaging of the healthy mice brains was performed using a Rapiflex (Bruker Daltonics, Billerica, MA), Matrix Assisted Laser Desorption Ionization (MALDI)-TOF/TOF mass spectrometer. Detailed information on MALDI MSI acquisition process is given in the Supplementary Materials and Methods. Eventually, the spectra were pre-processed using the software SCiLS 2019a (Bruker Daltonics, Bremen, Germany) for peak picking and exported to the standardized format of imzML for further analysis^{35,36}.

2.3. t-SNE maps of 3D MSI data:

The 3D MSI dataset can be mathematically formatted as a high dimensional array $M_{x \times y \times k \times z}$ where x and y represent the in-plane spatial dimensions of the hyperspectral data with k dimensions of m/z features measured at the z^{th} tissue section. For simplicity, this hyper-dimensional matrix M can be re-arranged in a vectorized format $X_z^{(n_z)} \in \mathbb{R}^k$; where X is a set k -dimensional vectors each of which holds n_z high dimensional data points (i.e. spectra) for the z^{th} tissue section. For example, the 2D MSI datacube from the first tissue section (i.e. $z=1$) is unfolded into $X_1^{(n_1)} = \{x_1, x_2, \dots, x_{n_1}\}$ where x_i represents a total-ion-count (TIC) normalized spectrum³⁷. Mathematically, we aim at mapping the high dimensional data X into a lower dimensional representation Y as such, $X_z^{(n_z)} \in \mathbb{R}^k \rightarrow Y_z^{(n_z)} \in \mathbb{R}^3$. The dimensionality reduction method of t-SNE was used to non-linearly map the hyperspectral datapoints into a 3D space (embedding space)²⁹.

The t-SNE algorithm aims at optimizing the cost function, given in equation (1), using stochastic gradient descent to minimize the of the Kullback-Leibler (KL) divergence³⁸ between two joint probability distributions P and Q of high and low dimensional data points,

respectively. The joint probability p_{ij} was computed to model the pairwise similarities between high dimensional data points x_i and x_j using a Gaussian distribution given in equation (2), whereas q_{ij} was computed to model the pairwise similarities of their matched points y_i and y_j in the low dimensional space using a Student's t-distribution given in equation (3).

$$KL(P, Q) = \sum_i \sum_j p_{ij} \log \frac{p_{ij}}{q_{ij}}, \quad (1)$$

$$p_{ij} = \frac{\exp - \left(\frac{\|x_i - x_j\|^2}{2\sigma^2} \right)}{\sum_{r \neq l} \exp - \left(\frac{\|x_r - x_l\|^2}{2\sigma^2} \right)} \quad (2)$$

$$q_{ij} = \frac{(1 + \|y_i - y_j\|^2)^{-1}}{\sum_{r \neq l} (1 + \|y_r - y_l\|^2)^{-1}} \quad (3)$$

Of note, we have parametrized the high dimensional data vector $X_z^{(n)}$ based on tissue location, z , due to the t-SNE computational considerations, and thus a low dimensional embedding is separately computed at different z locations for each 2D MSI datacube. The hallmark of the t-SNE is its ability to preserve the local distances (i.e. molecular structures) of the high dimensional data in a low dimensional representation. This means similar data points that are located near each other in the high dimensional space will be projected close to each other in a low dimensional representation, whereas dissimilar high dimensional data points will be projected far apart. In addition, t-SNE proposes a solution for the common dimensionality reduction issue known as the “crowding problem” which hinders faithful representation of high dimensional data points in the embedding space²⁸. Eventually, the separation distances between distinct data points in the t-SNE scatter space translate into different intensity levels when they are spatially-mapped to form a t-SNE image and thus show molecularly distinct regions²⁷. The t-SNE image was colored using the perceptually linear color system L*a*b* as such each of the t-SNE features was fed into a separate color channel³⁹.

2.3. 3D MSI-MRI non-linear image registration:

Image registration is typically performed between two images, namely: fixed image I_f and moving image I_m . The moving image, in this case a t-SNE image, is warped to be spatially aligned with the fixed image, the MR image. The proposed registration scheme is presented in Figure 1. We have based our approach on the parametric image registration framework proposed by the insight toolkit (ITK)⁴⁰ and implemented using the publicly available toolbox elastix⁴¹. Computationally, image registration is formulated as an optimization problem, given in equation (4), which determines the transformation parameters μ that minimize the cost function $\mathcal{C}$ with respect to the transformation model T .

$$\hat{\mu}_z = \arg \min_{\mu_z} \mathcal{C}(I_f^z, I_m^z; T_{\mu_z}) \quad (4)$$

Parameters of equation (4) were all parametrized based on the tissue location z because we have performed a series of 2D image registration in slice-to-slice fashion between the t-SNE and MR images, as shown in Figure 1. MR slice correspondences to our experimental MSI tissue sections were visually identified based on knowledge of sampling locations and acquisition settings. The registration process was initialized using affine transformation to compute the global deformation parameters (translations, rotation, scaling, and shearing), and followed by increasing the deformations degree-of-freedom to model the local deformations using the cubic BSpline transform. The cost function of mutual information was used as a similarity measure to assess the multi-modal registration quality⁴². The non-linear registration was implemented in a multi-resolution manner, in which 4 levels of Gaussian smoothing pyramids were used. Such a multi-resolution scheme is used to speed up the optimization convergence⁴³. Eventually, the computed transformation parameters were applied to each m/z image in the corresponding MSI datacube to spatially align it with the associated MR image.

It should be noted that capturing local deformations depends on the control point spacing of the BSpline transformation model⁴⁴. One control point spacing grid is usually insufficient to capture local deformations across multi-scale structures. For example, very large distances would focus on modeling deformations of big structures at the expense of smaller structures, whereas very small distances might lead to texture distortions. Therefore, the proposed multi-resolution scheme was equipped with different BSpline control point spacing as such it was empirically set to 80 pixels at the coarsest level and gradually decreased by a factor of 2 across the different resolution levels.

2.4. 3D MSI data segmentation using HSNE:

We applied hierarchical stochastic neighbor embedding (HSNE)³⁰ to concurrently analyze the large data array, $X_{n \times k}$, that encompasses the entire 3D MSI dataset, where n is the number of spectra and k is number of m/z features. HSNE enables visualizing multi-scale 2D non-linear embeddings of the high dimensional data. The visualization concept of the HSNE is based on the concept “overview first, details on demand” in which dominant

structures are first identified at the coarser embedding level, and then a more detailed embedding can be reconstructed for a selected structure from the previous embedding level.

The hierarchical nature of HSNE means that the original high dimensional data points are represented, across different scales, by parental points called landmarks. Briefly, the set of landmarks at scale s is denoted by L^s and it is a subset of landmarks at the previous finer scale L^{s-1} ; L^1 refers to a set of landmarks at the finest scale which represent the original input data points. A typical application of the HSNE encounters two main phases, namely: construction and exploration. In the construction phase the low dimensional embedding is computed based on the pairwise similarities of those automatically selected high dimensional landmarks. Landmarks that are similar in high dimensional space will be projected close to each other, whereas dissimilar ones will be projected further away. In the exploration phase, densely distributed landmarks in the embedding space were manually selected to get L_c^s , which is a set of landmarks that form a cluster c at level s . The selected HSNE cluster, L_c^s , assigns a probability for each of the high dimensional datapoints based on their likelihood of being represented within that cluster. A spatial mapping of those datapoints, which were held within an HSNE cluster, would reveal a region of interest of a 3D molecular structure. The Pearson correlation coefficient was calculated between an HSNE cluster and MSI datacube to find co-localized m/z ion features within that HSNE cluster. For more information about HSNE we refer the reader to Pezzotti *et al*³⁰.

3. Results:

3.1. Spatially-mapped t-SNE for MSI-MRI integration

We have proposed an automatic method for direct multi-modal non-linear alignment of 3D MSI and MRI data. The proposed concept, as illustrated in Figure 1, is based on simplifying the dimensional complexity of the MSI data to extract structural features common to the two modalities and thus can establish spatial correspondence; enabling one-to-one transformation mapping using a parametric image registration algorithm. The dimensionality reduction method of t-SNE was used to compute a non-linear mapping of the high dimensional mass spectra into a lower dimensional representation, in 3D space. Spatial organization of those reduced features resulted in the formation of a t-SNE image that revealed structural information of spectrally distinct regions. Each hyperspectral image (datacube) of the 2D MSI dataset is represented by a single, t-SNE image, which was used as a moving image in the image registration process. The 3D MSI/MRI alignment problem was approximated and implemented using a series of sequential 2D alignment in slice-to-slice fashion between the t-SNE and MR images, as illustrated in Figure 1. Image registration computes a transformation matrix to non-linearly warp the moving image which becomes spatially aligned with the corresponding MR slice with an overall running time of about 70 seconds per each 2D MSI/MRI dataset. Eventually, that computed transformation matrix was used to map each m/z image in the MSI datacube to its corresponding MR slice within less than 4 seconds. Supplementary Figures S1. and S2 show original t-SNE images of 3D MALDI MSI data from two normal mice brains. Each t-SNE image was warped and fused with its corresponding MR image after the linear and non-linear registration. The

alignment quality has significantly been improved after modeling the non-linear deformations using the cubic BSpline transformation.

The tissue geometrical reference provided by MRI was used to impose constraints on the non-linear transformation model, allowing smooth and non-orthogonal (i.e. curved) transitions between the slices of the 3D ion image for accurate reconstruction (Figure 1.d-f). The non-linearly deformed tissues were stacked together to form a volumetric shape that looks consistent with the original tissue shape as depicted by MRI. Figure 1.e shows clearly natural curvature transitions between slices that were otherwise highly difficult to preserve without those deformation constraints.

3.2. Integrated molecular and anatomical phenotypes in a normal brain

Multi-modal integration of 3D MSI and MRI data has enabled fusion of multi-scale data at the molecular and organ levels, respectively. Figure 2 shows the distribution of two ion features at m/z 864.5 $\pm$ 0.1 and m/z 840.5 $\pm$ 0.1 that were non-linearly deformed and overlaid atop of T2-RARE MR volumetric image within the region of interest in the normal brain of the first mouse. The spatial distributions of these two ions reveal co-localization with distinct MR anatomical regions. Specifically, the distribution of m/z 864.5 is highly present in the corpus callosum and brainstem, whereas m/z 840.5 is elevated in the hippocampus. The 3D reconstruction of the aligned ion image at m/z 864.5 is presented as a rendered volume using ImageJ⁴⁵ and the 3D ion image at m/z 840.5 was rendered and fused with the MR volumetric image, see Figure 2. The volume rendering of these two m/z images show natural curvatures that resemble the original tissue shape. Supplementary Figure S3 shows two ion features at m/z 840.5 $\pm$ 0.1 and m/z 868.5 $\pm$ 0.1 from a second normal brain that were reconstructed in 3D and fused with the associated T2-RARE MR image. This MSI-MRI fusion also confirms accurate co-localization of these two ion signatures with the MR anatomical regions of striatum, corpus callosum, and cortex.

3.3. Drug distribution volume fused with MRI in a PDX glioblastoma model

The 3D reconstruction of the distribution of the EGFR inhibitor erlotinib in the patient-derived xenograft mouse brain model of glioblastoma (GBM39), at m/z 394.1757 $\pm$ 0.001, is presented in Figure 3.a. An integration between this ion distribution and the T2-RARE MR volumetric image is given in Figure 3.b as an overlay of rendered volumes that was visualized using ImageJ⁴⁵ (see Supplementary Movie 1). This integration reveals elevated intensity of the drug in the tumor region compared to the normal brain regions, as shown in Figure. 3.c. A major advantage of such 3D MSI/MRI integration is to visualize and assess the drug distribution throughout the entire tissue volume.

3.4. Identification of 3D molecular patterns and integration with MRI

The hierarchical stochastic neighbor embedding (HSNE) is a scalable version of t-SNE and it enables analysis of large dataset such as 3D MSI data^{30,31}. The aligned 3D MALDI MSI datacube of the normal mouse brain was analyzed using the HSNE algorithm which identified molecular patterns as shown in Figure 4. The HSNE automatically constructed 3 embedding levels based on the 3D MSI data distribution in the high dimensional space. The coarsest embedding level L^3 , see Figure 4, reveals three dominant structures based on their

molecular similarities; forming densely distributed landmarks for different HSNE clusters as given in the embedding scatter plot. A set of landmarks that form an HSNE cluster was manually delineated and their spatial distribution was constructed to form an HSNE spatial cluster. The HSNE spatial cluster assigns a probability value for each voxel (i.e. spectrum) to estimate the likelihood of being represented by that selected HSNE cluster. The HSNE spatial clusters at the overview level of L^3 reveals structures for sparse background, noise background, and foreground (tissue). A more detailed embedding of the foreground structure at L^3 was computed and constructed at the subsequent finer level L^2 in which it depicts molecular patterns that form the following structures: 1) lateral ventricles, 2) corpus callosum and brainstem, 3) third ventricle, 4) cortex, 5) hippocampus, and 6) tissue edges and holes. These anatomical-like molecular structures were integrated to constitute a 3D composite image that were rendered (Figure 4.b) and fused with the T2-RARE MR image (Figure 4.d). These molecular patterns reconcile the MR anatomical structures (Figure 4.c), and their overlay on the MR image visually confirms high accuracy of the non-linear registration at distinct anatomical regions (Figure 4.d).

3.5. 3D molecular patterns in PDX model integrated with MRI

The aligned 3D MALDI MSI datacube of the GBM39 brain model was analyzed using HSNE and the identified molecular structures are shown in Figure 5. The HSNE algorithm automatically constructed 3 embedding levels based on the 3D MSI data distribution in the high dimensional space. The HSNE spatial clusters at the overview level of L^3 reveals structures for background and foreground (normal and tumor). A more detailed embedding of the foreground structure at L^3 was computed and constructed at L^2 in which it depicts finer spatial structures for both tumor and normal regions and identifies noise-related structures as well (noise and tissue outer edge). The 3D reconstruction of the segmented tumor and normal regions are shown in Figure 5.b as red and green clusters, respectively. These two clusters were overlaid on the MRI volumetric data; leading to an integrated visualization of the tissue anatomy and the molecular-based segmented structures, Figure 5.c and 5.d. Simplifying the dimensional complexities of the 3D MSI data using HSNE will allow building models of molecular distributions as they relate to MRI parameters.

3.6. Tumor specific molecule mapped to MRI:

Co-localized m/z ion features within each of the HSNE clusters were identified by calculating the Pearson's correlation between the selected HSNE cluster and the spectral information of the GBM39 model. The highly co-localized m/z features are expected to have higher correlation values. The distributions of the spectral correlation within each of the HSNE clusters of normal and tumor are shown in the Supplementary Figure S4. It was found that m/z 369.3512 ± 0.001 and m/z 744.4946 ± 0.001 were highly specific to the normal and tumor HSNE clusters with correlation values of 0.9427 and 0.9387 respectively. The 3D reconstructions of these two ions and their integration with T2-RARE MR volumetric image are shown in Supplementary Figures S5 and S6. Identities of these two ions were proposed by searching accurate mass on the Human Metabolome Database (HMDB)⁴⁶. Ions with m/z 744.4946 were tentatively assigned as the $[M+K]^+$ adduct of phosphatidylcholine: PC(30:0), associated with a ppm of 0.81. Ions with m/z 369.3512 were tentatively assigned as the

[M-H₂O+H]⁺ species of cholesterol, with ppm = 2.7. The drug signal at m/z 394.1757 $\pm$ 0.001 was found highly correlated with the HSNE tumor cluster with Pearson correlation coefficient of 0.8098.

3.7. MSI data integrated with multiparametric MRI:

The proposed registration method enables the integration of MSI data in an MRI volume independent of the MR sequence. In this work, an *in vivo* MRI was obtained using different pulse sequences (such as T1 pre-contrast, T1 post-contrast, and T2-RARE). Generally, the resulting *in vivo* MR images created using different sequences are either in the same orientation or suffer from global deformations that could possibly arise during handling of the scanned subject (rotation/translations). Those globally deformed MR images could easily be corrected using rigid transform, and thus multiple MR images from various pulse sequences become spatially aligned with each other. Eventually, the previously aligned MSI/MRI data can now be propagated to different sequences of MR images. Supplementary Figures S7 and S8 show the ion distribution of m/z 369.3512 $\pm$ 0.001, m/z 394.1757 $\pm$ 0.001, and m/z 744.4946 $\pm$ 0.001 overlaid atop of the T1 pre-contrast and T1 post-contrast 3D MR image, respectively.

4. Discussion:

We opted to approximate the 3D MSI/MRI alignment problem using a series of sequential 2D alignment processes over existing algorithms for 3D image registration^{41,47} to overcome the sparsity of our 3D moving image. While each MRI plane is 600 μ m, the thickness of each MSI tissue section is only 10 μ m. The reconstruction of a 3D volume from a 10 μ m image slice every 600 μ m significantly suffers from a lack of reference, but the initial 2D alignment to each MRI plane provided the 3D reference to more accurately reconstruct the 3D MSI volume. This is in agreement with previous work that found it more efficient to perform slice-to-slice non-linear registration for sparse volumetric histology reconstruction¹⁸. This approach initially requires establishment of slice correspondences with the MRI volume, which we performed visually based on prior knowledge of sampling locations and acquisition settings. Nevertheless, the slice correspondences could be established automatically through optimizing a pairwise similarity measure between each slice in the moving image and the MRI slices¹⁸. For example, this similarity measure could be based on comparable geometrical features⁴⁸ or using a statistical measure such as mutual information⁴⁹, but first after capturing global distortions.

The proposed alignment approach assumes a mostly orthogonal tissue sectioning was performed using the microtome. From a computational perspective, the literature has addressed alignment of oblique sections, also known as “out-of-plane”, through slice-to-volume registration^{50,51}. Such approaches though, are typically restricted to radiology-based imaging modalities, and not imaging microscopy, in which the slice represents a projective image (e.g. an X-ray image) that typically has spatial correspondences with the volumetric image. Such approaches have not been adopted for direct histology-slice-to-MRI volume registration because the histological slice is not a projective image. The alignment of an oblique histological image to an MR image is a computationally challenging problem and it

would need extra information to provide reference such as that can be provided by an intermediate ex vivo modality to acquire a blockface image⁵². Moreover, from a biological perspective the approach could induce artifacts by presenting in-plane biochemical information originating from different 3D anatomical locations. This should be avoided to decrease the likelihood of misleading interpretations especially in applications that need to compare biomolecular signatures at different anatomical entities within the same tissue⁵.

Validation of registration results, particularly in the medical and biomedical imaging area, is an active research topic especially in non-linear registration¹⁸ that was described as important two decades ago by Maintz and Viergever⁴⁷. Recently, it has even been classified as more urgent by Viergever and co-workers who have presented retrospective assessment on image registration developments over the last two decades⁵³. Validation techniques vary from qualitative assessment^{48,54,55} to quantitative assessment^{56–58}. In this work we have adopted both quantitative and qualitative assessment approaches and our results were visually examined by experts in MSI. The current quantitative assessment approaches would generally rely on measuring the Euclidean distances between common landmarks or measuring the overlap between segmented structures in the two imaging modalities. We think such current quantitative approaches are not necessarily precise to describe the registration quality between spatially-resolved omics data (e.g., MSI data) and anatomical images (e.g., MRI), mainly because of the observer variability in segmenting common structures in complementary modalities that can even be more challenging to identify especially in presence of certain abnormalities such as the presence of tumor. For example, Supplementary Figure S9. shows the Dice similarity coefficient between the two segmented structures from the GBM39 mouse brain model, namely: the T1 post-contrast MRI tumor region and the HSNE spatially-mapped cluster of the molecularly-based segmented tumor. The Dice coefficient is a similarity metric that measures the overlap between two segmented images; a Dice coefficient of one means a complete overlapping whereas zero means there is no overlap⁵⁹. The mean Dice coefficient was 0.7218 ± 0.1992 but might not necessarily be representative of the registration quality. The T1 post-contrast image segments the tumor based on gadolinium contrast enhancement that highlights the tumor's aberrant vasculature, while the HSNE segments the tumor based on molecular characteristics of the tissue. In this application, HSNE images provide complementary information that cannot be fully reconciled to evaluate registration accuracy, suggesting differences in tumor presentation on contrast enhanced MRI and tumor distribution at the molecular level into tissue. The registration accuracy of the method itself was then evaluated using healthy and untreated mouse brains where we have identified molecular patterns that more closely reconcile with the MRI anatomy. Figure 6 shows the distribution of Dice coefficient in three main anatomical regions of one healthy animal with overall values of 0.8935 ± 0.0079 , 0.7630 ± 0.0619 , and 0.8452 ± 0.0237 in the regions of brainstem, hippocampus, and cortex, respectively. The Dice coefficient of a second healthy animal was 0.8996 ± 0.0243 , 0.7815 ± 0.0443 , and 0.8501 ± 0.0296 in the regions of brainstem, hippocampus, and cortex, respectively. The overlapping of these three anatomical structures in both modalities is shown in Supplementary Figures S10 and S11.

It is worth noting that the far-right column of Supplementary Figure S9 shows the lowest Dice coefficient in which the hyper-intensity region revealed in the T1 post-contrast MR image is much larger than that depicted by the tumor-colocalized molecule. The corresponding high-resolution H&E stained histological images are shown in Supplementary Figure S12, in which both the tumor location and morphology are faithfully represented. The MSI data were automatically aligned to the histological images by the t-SNE based non-linear alignment using the registration parameters previously described by Abdelmoula *et al.*²⁷. In concordance with histology, the distribution of the tumor-based molecular ion at m/z 744.4946 was mapped to the histology-based tumor region, as shown Supplementary Figure S12.c. The bottom-right image in Supplementary Figure S12.c. shows the tumor region is located near the cortical layer, whereas its counterpart T1 post-contrast MR image in Supplementary Figure S12.a. reveals hyper-intensity region within the thalamus region. We have observed such discrepancy in a different study where the contrast agent diffused outside of the viable tumor region as evidenced by MSI of both molecules⁶⁰. Herein, the concordance of the HSNE identified tumor biomarker with the histological delineation of the tumor by H&E further support the MSI-MRI registration accuracy by validating the HSNE-based biomolecular delineation of the tumor despite the apparent misalignment, which warrants further biological investigation.

Multi-modal integration between MSI and MRI data is a natural and possibly foundational step in paving the way to harvest benefits of interesting complementary information for building more robust models of tumor growth and/or response to treatment. An MRI voxel would then be associated with a high dimensional feature vector of molecular information, and a highly complex non-linear relationship is expected to govern this association. This would impose mathematical challenges to model MSI/MRI data relationships. Identifying meaningful molecular patterns prior to the mathematical modeling alleviates the complexity of dealing with original large 3D MSI dataset.

5. Concluding Remarks:

We have proposed an automatic method to non-linearly align hyperspectral molecular images of MSI with MRI. The method is easily generalizable to hyperspectral images from different mass spectrometers and it can be integrated with other established imaging modalities in radiology such as computed tomography (CT) and positron emission tomography (PET). Integration of such multi-modal imaging data would bridge the gaps between anatomical and biomolecular phenotypes for better understanding of various biological problems and paving the way for establishing predictive models between those modalities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References:

- (1). McDonnell LA; Heeren RM Imaging Mass Spectrometry. *Mass Spectrom Rev* 2007, 26 (4), 606–643. [PubMed: 17471576]
- (2). Caprioli RM; Farmer TB; Gile J Molecular Imaging of Biological Samples: Localization of Peptides and Proteins Using MALDI-TOF MS. *Anal Chem* 1997, 69 (23), 4751–4760. [PubMed: 9406525]
- (3). Abdelmoula WM; Balluff B; Englert S; Dijkstra J; Reinders MJT; Walch A; McDonnell LA; Lelieveldt BPF Data-Driven Identification of Prognostic Tumor Subpopulations Using Spatially Mapped t-SNE of Mass Spectrometry Imaging Data. *Proc. Natl. Acad. Sci.* 2016, 113 (43), 12244–12249. [PubMed: 27791011]
- (4). Rubakhin SS; Jurchen JC; Monroe EB; Sweedler JV Imaging Mass Spectrometry: Fundamentals and Applications to Drug Discovery. *Drug Dsccovery Today* 2005.
- (5). Carreira RJ; Shyti R; Balluff B; Abdelmoula WM; van Heiningen SH; van Zeijl RJ; Dijkstra J; Ferrari MD; Tolner EA; McDonnell LA; et al. Large-Scale Mass Spectrometry Imaging Investigation of Consequences of Cortical Spreading Depression in a Transgenic Mouse Model of Migraine. *J Am Soc Mass Spectrom* 2015.
- (6). Stoeckli M; Knochenmuss R; McCombie G; Mueller D; Rohner T; Staab D; Wiederhold KH MALDI MS Imaging of Amyloid. *Methods in Enzymology*. 2006, pp 94–106. [PubMed: 17046654]
- (7). Karas M; Hillenkamp F Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10,000 Daltons. *Anal. Chem.* 1988, 60 (20), 2299–2301. [PubMed: 3239801]
- (8). Takáts Z; Wiseman JM; Gologan B; Cooks RG Mass Spectrometry Sampling under Ambient Conditions with Desorption Electrospray Ionization. *Science* (80-.). 2004, 306 (5695), 471–473.
- (9). Patterson NH; Doonan RJ; Daskalopoulou SS; Dufresne M; Lenglet S; Montecucco F; Thomas A; Chaurand P Three-Dimensional Imaging MS of Lipids in Atherosclerotic Plaques: Open-Source Methods for Reconstruction and Analysis. *Proteomics* 2016, 16 (11–12), 1642–1651. [PubMed: 26958804]
- (10). Hanrieder J; Phan NTN; Kurczyk ME; Ewing AG Imaging Mass Spectrometry in Neuroscience. *ACS Chemical Neuroscience*. 2013, pp 666–679. [PubMed: 23530951]
- (11). Calligaris D; Norton I; Feldman DR; Ide JL; Dunn IF; Eberlin LS; Graham Cooks R; Jolesz FA; Golby AJ; Santagata S; et al. Mass Spectrometry Imaging as a Tool for Surgical Decision-Making. *J. Mass Spectrom.* 2013, 48 (11), 1178–1187. [PubMed: 24259206]
- (12). Eberlin LS; Norton I; Orringer D; Dunn IF; Liu X; Ide JL; Jarmusch AK; Ligon KL; Jolesz FA; Golby AJ; et al. Ambient Mass Spectrometry for the Intraoperative Molecular Diagnosis of Human Brain Tumors. *Proc. Natl. Acad. Sci. U. S. A.* 2013, 110 (5), 1611–1616. [PubMed: 23300285]
- (13). Spraggins JM; Rizzo DG; Moore JL; Noto MJ; Skaar EP; Caprioli RM Next-Generation Technologies for Spatial Proteomics: Integrating Ultra-High Speed MALDI-TOF and High Mass Resolution MALDI FTICR Imaging Mass Spectrometry for Protein Analysis. *Proteomics* 2016.
- (14). Seeley EH; Caprioli RM 3D Imaging by Mass Spectrometry: A New Frontier. *Anal Chem* 2012, 84 (5), 2105–2110. [PubMed: 22276611]
- (15). Trede D; Schiffler S; Becker M; Wirtz S; Steinhorst K; Strehlow J; Aichler M; Kobarg JH; Oetjen J; Dyatlov A; et al. Exploring Three-Dimensional Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry Data: Three-Dimensional Spatial Segmentation of Mouse Kidney. *Anal. Chem.* 2012, 84 (14), 6079–6087. [PubMed: 22720760]
- (16). Oetjen J; Aichler M; Trede D; Strehlow J; Berger J; Heldmann S; Becker M; Gottschalk M; Kobarg JH; Wirtz S; et al. MRI-Compatible Pipeline for Three-Dimensional MALDI Imaging Mass Spectrometry Using PAXgene Fixation. *J Proteomics* 2013, 90, 52–60. [PubMed: 23558029]

- (17). Sinha TK; Khatib-Shahidi S; Yankeelov TE; Mapara K; Ehtesham M; Cornett DS; Dawant BM; Caprioli RM; Gore JC Integrating Spatially Resolved Three-Dimensional MALDI IMS with in Vivo Magnetic Resonance Imaging. *Nat Methods* 2008, 5 (1), 57–59. [PubMed: 18084298]
- (18). Pichat J; Iglesias JE; Yousry T; Ourselin S; Modat M A Survey of Methods for 3D Histology Reconstruction. *Med. Image Anal.* 2018.
- (19). Malandain G; Bardinet É; Nelissen K; Vanduffel W Fusion of Autoradiographs with an MR Volume Using 2-D and 3-D Linear Transformations. *Neuroimage* 2004.
- (20). Pirro V; Alfaro CM; Jarmusch AK; Hattab EM; Cohen-Gadol AA; Cooks RG Intraoperative Assessment of Tumor Margins during Glioma Resection by Desorption Electrospray Ionization-Mass Spectrometry. *Proc. Natl. Acad. Sci.* 2017.
- (21). Santagata S; Eberlin LS; Norton I; Calligaris D; Feldman DR; Ide JL; Liu X; Wiley JS; Vestal ML; Ramkissoon SH; et al. Intraoperative Mass Spectrometry Mapping of an Onco-Metabolite to Guide Brain Tumor Surgery. *Proc. Natl. Acad. Sci.* 2014, 111 (30), 11121–11126. [PubMed: 24982150]
- (22). Seeley EH; Caprioli RM Molecular Imaging of Proteins in Tissues by Mass Spectrometry. *Proc. Natl. Acad. Sci.* 2008.
- (23). Verbeek N; Spraggins JM; Murphy MJM; Wang H. dong Deutch AY Caprioli RM Van de Plas R Connecting Imaging Mass Spectrometry and Magnetic Resonance Imaging-Based Anatomical Atlases for Automated Anatomical Interpretation and Differential Analysis. *Biochim. Biophys. Acta - Proteins Proteomics* 2017.
- (24). Jones EA; Deininger SO; Hogendoorn PC; Deelder AM; McDonnell LA Imaging Mass Spectrometry Statistical Analysis. *J Proteomics* 2012, 75 (16), 4962–4989. [PubMed: 22743164]
- (25). Randall EC; Emdal KB; Laramy JK; Kim M; Roos A; Calligaris D; Regan MS; Gupta SK; Mladek AC; Carlson BL; et al. Integrated Mapping of Pharmacokinetics and Pharmacodynamics in a Patient-Derived Xenograft Model of Glioblastoma. *Nat. Commun.* 2018, 9 (1).
- (26). Thiele H; Heldmann S; Trede D; Strehlow J; Wirtz S; Dreher W; Berger J; Oetjen J; Kobarg JH; Fischer B; et al. 2D and 3D MALDI-Imaging: Conceptual Strategies for Visualization and Data Mining. *Biochimica et Biophysica Acta - Proteins and Proteomics.* 2014, pp 117–137.
- (27). Abdelmoula WM; Skraskova K; Balluff B; Carreira RJ; Tolner EA; Lelieveldt BP; van der Maaten L; Morreau H; van den Maagdenberg AM; Heeren RM; et al. Automatic Generic Registration of Mass Spectrometry Imaging Data to Histology Using Nonlinear Stochastic Embedding. *Anal Chem* 2014, 86 (18), 9204–9211. [PubMed: 25133861]
- (28). van der Maaten L; Hinton G Visualizing Data Using T-SNE. *J. Mach. Learn. Res.* 2008, 9, 2579–2605.
- (29). van der Maaten L Accelerating T-SNE Using Tree-Based Algorithms. *J. Mach. Learn. Res.* 2014, 15, 3221–3245.
- (30). Pezzotti N; Höllt T; Lelieveldt B; Eisemann E; Vilanova A Hierarchical Stochastic Neighbor Embedding. *Comput. Graph. Forum* 2016, 35 (3), 21–30.
- (31). Abdelmoula WM; Pezzotti N; Höllt T; Dijkstra J; Vilanova A; McDonnell LA; Lelieveldt BPF Interactive Visual Exploration of 3D Mass Spectrometry Imaging Data Using Hierarchical Stochastic Neighbor Embedding Reveals Spatiomolecular Structures at Full Data Resolution. *J. Proteome Res.* 2018.
- (32). van Unen V; Höllt T; Pezzotti N; Li N; Reinders MJT; Eisemann E; Koning F; Vilanova A; Lelieveldt BP F. Visual Analysis of Mass Cytometry Data by Hierarchical Stochastic Neighbour Embedding Reveals Rare Cell Types. *Nat. Commun.* 2017, 8 (1), 1740. [PubMed: 29170529]
- (33). Carlson BL; Pokorny JL; Schroeder MA; Sarkaria JN Establishment, Maintenance and in Vitro and in Vivo Applications of Primary Human Glioblastoma Multiforme (GBM) Xenograft Models for Translational Biology Studies and Drug Discovery; 2011.
- (34). Park JG; Lee C Skull Stripping Based on Region Growing for Magnetic Resonance Brain Images. *Neuroimage* 2009.
- (35). Römpp A; Schramm T; Hester A; Klinkert I; Both J; Heeren RMA; Stöckli M; Spengler B ImzML: Imaging Mass Spectrometry Markup Language: A Common Data Format for Mass Spectrometry Imaging. *Methods Mol. Biol.* 2011, 696, 205–224. [PubMed: 21063949]

- (36). Race AM; Styles IB; Bunch J Inclusive Sharing of Mass Spectrometry Imaging Data Requires a Converter for All. *J. Proteomics* 2012, 75 (16), 5111–5112. [PubMed: 22641155]
- (37). Deininger SO; Cornett DS; Paape R; Becker M; Pineau C; Rauser S; Walch A; Wolski E Normalization in MALDI-TOF Imaging Datasets of Proteins: Practical Considerations. *Anal Bioanal Chem* 2011, 401 (1), 167–181. [PubMed: 21479971]
- (38). Kullback S; Leibler RA On Information and Sufficiency. *Ann. Math. Stat.* 1951, 22 (1), 79–86.
- (39). Hunter RS Accuracy, Precision, and Stability of New Photoelectric Color-Difference Meter. *J. Opt. Soc. Am.* 1948, 38 (12), 1094.
- (40). Ibanez L; Schroeder W; Ng L; Cates J The ITK Software Guide. *ITK Softw. Guid.* 2005, Second (5), 804.
- (41). Klein S; Staring M; Murphy K; Viergever MA; Pluim JP Elastix: A Toolbox for Intensity-Based Medical Image Registration. *IEEE Trans Med Imaging* 2010, 29 (1), 196–205. [PubMed: 19923044]
- (42). Viola P; Wells III WM Alignment by Maximization of Mutual Information. *Int. J. Comput. Vis.* 1997, 24 (2), 137–154.
- (43). Unser M; Aldroubi A; Gerfen CR A Multiresolution Image Registration Procedure Using Spline Pyramids. *Proc. SPIE-Mathematical Imaging Wavelets Appl. Signal Image Process.* 1993, 2034 (Nov), 160–170.
- (44). Unser M Splines: A Perfect Fit for Signal and Image Processing. *Signal Process. Mag. IEEE* 1999, 16 (6), 22–38.
- (45). Abràmoff MD; Magalhães PJ; Ram SJ Image Processing with ImageJ. *Biophotonics International*. 2004.
- (46). Wishart DS; Tzur D; Knox C; Eisner R; Guo AC; Young N; Cheng D; Jewell K; Arndt D; Sawhney S; et al. HMDB: The Human Metabolome Database. *Nucleic Acids Res.* 2007.
- (47). Maintz JB; Viergever MA A Survey of Medical Image Registration. *Med. Image Anal.* 1998, 2 (1), 1–36. [PubMed: 10638851]
- (48). Abdelmoula WM; Carreira RJ; Shyti R; Balluff B; van Zeijl RJ; Tolner EA; Lelieveldt BF; van den Maagdenberg AM; McDonnell LA; Dijkstra J Automatic Registration of Mass Spectrometry Imaging Data Sets to the Allen Brain Atlas. *Anal Chem* 2014, 86 (8), 3947–3954. [PubMed: 24661141]
- (49). Xiao G; Bloch BN; Chappelow J; Genega EM; Rofsky NM; Lenkinski RE; Tomaszewski J; Feldman MD; Rosen M; Madabhushi A Determining Histology-MRI Slice Correspondences for Defining MRI-Based Disease Signatures of Prostate Cancer. *Comput. Med. Imaging Graph.* 2011.
- (50). Ferrante E; Paragios N Slice-to-Volume Medical Image Registration: A Survey. *Med. Image Anal.* 2017.
- (51). Markelj P; Tomaževič D; Likar B; Pernuš F A Review of 3D/2D Registration Methods for Image-Guided Interventions. *Med. Image Anal.* 2012.
- (52). Park H; Piert MR; Khan A; Shah R; Hussain H; Siddiqui J; Chenevert TL; Meyer CR Registration Methodology for Histological Sections and In Vivo Imaging of Human Prostate. *Acad. Radiol.* 2008.
- (53). Viergever MA; Maintz JBA; Klein S; Murphy K; Staring M; Pluim JPW A Survey of Medical Image Registration – under Review. *Med. Image Anal.* 2016.
- (54). Gaffling S; Daum V; Steidl S; Maier A; Köstler H; Hornegger J A Gauss-Seidel Iteration Scheme for Reference-Free 3-D Histological Image Reconstruction. *IEEE Trans. Med. Imaging* 2015.
- (55). Wirtz S; Fischer B; Modersitzki J; Schmitt O Super-Fast Elastic Registration of Histologic Images of a Whole Rat Brain for Three-Dimensional Reconstruction. *Med. Imaging 2004 Image Process. Pts 1–3* 2004.
- (56). Muenzing SEA; van Ginneken B; Murphy K; Pluim JPW Supervised Quality Assessment of Medical Image Registration: Application to Intra-Patient CT Lung Registration. *Med. Image Anal.* 2012.
- (57). Ward AD; Crukley C; McKenzie CA; Montreuil J; Gibson E; Romagnoli C; Gomez JA; Moussa M; Chin J; Bauman G; et al. Prostate: Registration of Digital Histopathologic Images to in Vivo MR Images Acquired by Using Endorectal Receive Coil. *Radiology* 2012.

- (58). Heijs B; Abdelmoula WM; Lou S; Briaire-de Bruijn IH; Dijkstra J; Bovée JVMG; McDonnell LA Histology-Guided High-Resolution Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging. *Anal. Chem.* 2015, 87 (24), 11978–11983. [PubMed: 26595321]
- (59). Dice LR Measures of the Amount of Ecologic Association between Species. *Ecology* 1945, 26 (3), 297–302.
- (60). Kim M; Ma DJ; Calligaris D; Zhang S; Feathers RW; Vaubel RA; Meaux I; Mladek AC; Parrish KE; Jin F; et al. Efficacy of the MDM2 Inhibitor SAR405838 in Glioblastoma Is Limited by Poor Distribution across the Blood-Brain Barrier. *Mol. Cancer Ther.* 2018.

Figure 1.

Proposed framework for automatic integration of 3D MSI and MRI: a) 3D MSI dataset consists of a set of sequential 2D MSI datacubes each of which holds hundreds of m/z ion images. b) t-SNE images simplify the dimensional complexity of each 2D MSI datacube in a single image representation. The color of the t-SNE images is arbitrarily chosen to visualize spectrally differentiated regions that created edge structures to guide the multi-modal registration. The 3D MSI-MRI image registration was implemented in a slice-to-slice fashion between the t-SNE and MR images as illustrated in b and c. Panels (d-f) shows simple visualization of inter-slice deformations of the MSI tissue sections before and after non-linear registration with their corresponding MR slices. MRI provides a volumetric reference to preserve the original tissue shape while non-linearly warping the 3D MSI data. The binary representation is for illustrative purposes, but the registration process took image contents in considerations.

Figure 2.

Multi-modal integration of MSI and MRI data of normal mouse brain: 3D distribution of two ion features at m/z 864.5 $\pm$ 0.1 and m/z 840.5 $\pm$ 0.1 that were non-linearly deformed and overlaid atop of T2-RARE MR volumetric image. The upper row shows the distribution of m/z 864.5 is highly expressed and co-localized with the MRI anatomical regions of Corpus Callosum and Brainstem. Contrary, the bottom row shows high expression of m/z 840.5 in the Cortex and Hippocampus. The volume rendering of these two ions, in the right column, reveals natural curvatures that resemble the original tissue shape.

Figure 3. Multi-modal integration of MSI and MRI data of PDX glioblastoma mouse brain model GBM39: (a) non-linear 3D reconstruction of ion feature at m/z 394.1757 (erlotinib) that are highly expressed and localized in the tumor region. This ion feature was volume rendered and overlaid atop of T2-RARE MR volumetric image (b). Coronal cross sections show the original anatomical structures of T2-RARE MR image within the region of interest before (c) and after (d) fusing with drug molecule.

(a)

(b)

(c)

(d)

Figure 4.

Hierarchical stochastic neighbor embedding (HSNE) analysis of 3D MSI data from normal brain reveals multi-scale spectral patterns that were fused to the MR volumetric image to visualize the registration quality. (a) The overview embedding separates tissue foreground from various background noise, and the detailed embedding only on the tissue structure reveals distinct molecular patterns that agree with MR anatomical structures (c). The HSNE molecular patterns were volume rendered (b) and fused atop of T2-RARE MR image (d) and the zoomed-in images visualize high registration quality.

Figure 5.

Hierarchical stochastic neighbor embedding (HSNE) identifies spectral patterns associated with tumor and normal tissue types in the GBM39 mouse brain model. The overview embedding shows the major identified structures, whereas a more detailed embedding at level L^2 depicts finer segmented structures of both tumor and normal tissues. Spectral-based segmentation of tumor (red) and normal (green) structures using HSNE were volumetric rendered (b) and overlaid atop of T2-RARE MRI (c) and (d).

Figure 6.
Evaluation of the registration quality using the Dice metric in three main anatomical regions for two healthy and untreated mouse brains.