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Cell-Type-Specific Metabolic Profiling Achieved by Combining Desorption Electrospray Ionization Mass Spectrometry Imaging and Immunofluorescence Staining

Xin Yan,† Xiaoai Zhao,† Zhenpeng Zhou,‡ Andrew McKay, Anne Brunet,*, and Richard N. Zare*

ABSTRACT: Cell-type-specific metabolic profiling in tissue with heterogeneous composition has been of great interest across all mass spectrometry imaging (MSI) technologies. We report here a powerful new chemical imaging capability in desorption electrospray ionization (DESI) MSI, which enables cell-type-specific and in situ metabolic profiling in complex tissue samples. We accomplish this by combining DESI-MSI with immunofluorescence staining using specific cell-type markers. We take advantage of the variable frequency of each distinct cell-type in the lateral septal nucleus (LSN) region of mouse forebrain. This allows computational deconvolution of the cell-type-specific metabolic profile in neurons and astrocytes by convex optimization - a machine learning method. Based on our approach, we observed 107 metabolites that show different distributions and intensities between astrocytes and neurons. We subsequently identified 23 metabolites using high resolution mass spectrometry (MS) and tandem MS, which include small metabolites such as adenosine and N-acetylaspartate previously associated with astrocytes and neurons, respectively, as well as accumulation of several phospholipid species in neurons which have not been studied before. Overall, this method overcomes the relatively low spatial resolution of DESI-MSI and provides a new platform for in situ metabolic investigation at the cell-type level in complex tissue samples with heterogeneous cell-type composition.

The cellular metabolome can be defined as the set of metabolites present in cells, which capitalizes on the small molecules observed to construct a ‘fingerprint’ that can be uniquely assigned to individuals.1 Metabolome analysis can describe qualitatively and quantitatively the intermediates and products of cellular regulatory pathways.2,3 It allows us to understand cell phenotypes and can be seen as the ultimate response of a biologic system to genetic factors and/or environmental changes. Mass spectrometry (MS) plays an increasingly dominant role in the metabolomics field due to its inherent high sensitivity, specificity, and fast data acquisition.4-6 Cellular metabolomics has been reported in research for early diagnosis of disease and for understanding the molecular mechanism of disease progression, response, and resistance to therapeutics. It holds promise to inform the practice of precision medicine.7,8,5 For example, a number of investigations have been carried out to establish biomarkers for cancers,9,10 cardiovascular diseases,11,12 neurodegenerative diseases,13-15 diabetes,16,17 and obesity18. However, these studies all employed classic bulk “bind and grind” methods and conventional MS requiring cell homogenates either from tissue or cell culture for metabolic analysis; thus, information of spatial distribution is not preserved. In addition, contribution from different cell types, each of which could have very distinct metabolic profile, is lost. To fully understand the role of metabolites and cell function, it is crucial not only to identify but also to correlate cellular metabolites in the context of tissue anatomy and cell-type composition.

Mass spectrometry imaging (MSI), an emerging technology, enables simultaneous in situ detection of individual molecular species of metabolites while preserving tissue morphology.19,20 In contrast to common molecular biology techniques, including chemical staining, immuno-based imaging approaches, and in situ hybridization, MSI does not require any prior knowledge of the target species and is completely label-free. Ambient MSI techniques,21 such as desorption electrospray ionization (DESI) MSI, have become increasingly used for biological tissue imaging as they allow analysis to be performed in the open environment with minimal sample preparation and is perfectly suitable for the analysis of small molecules.19,22-25 Besides DESI, secondary ion mass spectrometry (SIMS),26-28 and matrix-assisted laser desorption ionization (MALDI),29-31 are two major alternative sample ionization methods of MSI. Other varieties are also available.20,32,33 DESI, SIMS, and MALDI imaging technologies are complementary, and the choice of ionization method depends on the user’s preferred requirements such as spatial resolution, m/z detection range, area to be imaged, the time required for sample preparation, data acquisition and data analysis.20

Importantly, MSI with cellular/subcellular resolution is an enormous challenge and remains a major driving force for making improvements in spatial resolution of MSI.34-39

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Instrumentation advances allow SIMS and MALDI-MSI to achieve single-cell/subcellular spatial resolution, which enables cellular profiling of metabolites combining with fluorescence microscopy by antibody-labeling. In contrast, DESI-MSI so far has not achieved spatially single-cell resolution and therefore in situ cellular metabolic profiles could not be obtained. The lateral resolution of DESI-MSI can reach about 20 μm with optimal parameter settings, but the typical resolution is about 100 - 200 μm, which is determined by the effective surface area extracted by the spray solvent. Many efforts have been made to improve the resolution of DESI-MSI, including instrumental approaches such as the optimization of emitter capillary size, solvent composition, solvent flow rate, MS scan-rate and step-size, and image fusion methods such as the fusion of DESI-MSI with optical microscopy of hematoxylin and eosin (H&E) staining to produce the improved molecular distribution in tissues. To date, DESI-MSI cannot provide cell-type-specific information due to the inherent restriction of DESI probe. Metabolic information obtained from one pixel of DESI-MSI can neither distinguish between variations in different cell types and frequencies, nor identify the contributions of different cell types to the total measured metabolites. This prevents DESI-MSI from being used for complex tissues with a number of mixed cell types in each individual DESI-MSI scan. In order to acquire the above information, experiments such as analysis of different types of cells from cell culture or cell sorting from isolated subsets of tissue might provide the solution. However, cellular structures such as neuronal axon and metabolic states can be compromised simultaneously during tissue dissociation and sorting, which may introduce bias in understanding cell-type-specific metabolism under physiological conditions. Despite its relatively restricted spatial resolution compared to other technologies, one of the defining features of DESI-MSI is its histologically-compatible solvent system. The advantages that this capability brings are two-fold: 1) it allows cell-type identification on the very same tissue section following an MSI scan, providing an unambiguous correlation between MS profiling and its corresponding region on each given tissue; and 2) all staining and imaging can be done post-fix after MSI procedure, so that it does not introduce any possible external interference between tissue sample collection and assessment.

In this study, we develop a powerful new chemical imaging capability in DESI-MSI that enables metabolic profiling of cell types in tissues. This is accomplished by combining DESI-MSI and immunofluorescence staining with specific cell-type markers, and the metabolic features of cell types are recognized by machine learning. We take advantage of the heterogeneity of the frequency of two main cell types (astrocytes and neurons) in the lateral septal nucleus of the mouse forebrain, which allows us to apply convex optimization - a machine learning method to computationally deconvolve the contributing metabolic profiles from astrocytes and neurons distinctively. The method provides a unique tool to study in situ cell-type-specific metabolic profiles in complex tissues with heterogeneous cell-type composition.

### EXPERIMENTAL SECTION

**DESI-MSI.** A custom build DESI imaging stage was coupled to a hybrid LTQ-Orbitrap Mass Spectrometer (Thermo Fisher Scientific) for DESI-MSI. The mass spectra were acquired in the negative ion mode from m/z 50–1,000 using the Orbitrap as the mass analyzer with the resolving power set at 60,000. The spray voltage was set to ~3.5 kV, and the capillary voltage was set to ~65V. The tube lens voltage was set to ~120 V. Ion injection time was 100 ms, and one microscan was performed. The solvent system used is DMF : ACN = 1 : 1 (vol:vol) and provided at a flow rate of 0.8 μL/min. Assisted by a nebulizing gas (N2) at a pressure of 150 psi, molecules of interest from tissues are desorbed and ionized. DESI spray tip-to-surface distance was 2 mm; spray incident angle was 56°, and spray-to-inlet distance was 6.5 μm. Step size in the moving stage was set to 200 μm, and automatic gain control of mass spectrometer was switched off. These parameters were empirically found to yield the optimal MS signal from brain tissues. All experiments were carried out under identical experimental conditions to allow a comparison between measurements.

**Immunofluorescence staining and imaging.** After each DESI scan, tissue sections were fixed with 4% paraformaldehyde (Electron Microscopy Science, Cat#15714) diluted in phosphate-buffered saline for 20 mins at -20°C. Antibodies against the astrocyte marker Glial Fibrillary Acidic Protein (GFAP) antibody (Abcam, ab53554) were used at 1:1000 dilution at 4°C overnight. Donkey anti-goat antibody conjugated with Alexa Fluor 647 (Thermo Fisher Scientific, Cat# A-21447) was used at 1:250 dilution at room temperature for 1.5 hours for a secondary staining. All sections were then mounted with 50 μl of ProLongTM Gold Antifade mounting media containing a fluorescent nucleic acid dye DAPI (4′,6-diamidino-2-phenylindole) (Thermo Fisher Scientific, P36931) before being imaged. Confocal microscopy was carried out using a Nikon Eclipse Ti confocal microscope equipped with a Zyla sCMOS camera (Andor) and NIS-Elements software (AR 4.30.02, 64-bit) using a 10x objective. No blinding was performed for taking pictures. The lateral septal nucleus region was defined using these anatomical landmarks shown on immunofluorescent images based on Allen Mouse Brain Atlas. Top: horizontal line immediately underneath the crossing of the bottom of corpus callosum with coronal section midline; bottom: 400 μm below top boundary; left and right: the left and right medial wall of the left and right lateral ventricle, respectively. A schematic figure showing the defined lateral septal nucleus region used in this study is represented in Figure 3a. The confocal images can be downloaded from MetaboLights at: http://www.ebi.ac.uk/metabolights/MTBLS1747.

**Image quantification.** Image quantification was performed using a custom Julia language script. Briefly, for each immunofluorescence-stained region, nuclei were detected using DAPI and a blob detection algorithm provided by Images.jl. Using the immediate region including and surrounding the detected nuclei, cells were classified as either GFAP-positive (astrocytes) or GFAP-negative (likely neurons). Although neuron, oligodendrocytes and microglia all exhibit negative GFAP...
staining, given the low level of mRNA expression detected from oligodendrocyte and microglia-specific marker genes in the lateral septal nucleus (Figure S2, and more in result section)\textsuperscript{54,55}, the vast majority of GFAP negative cells that were identified in our current study in the lateral septal nucleus region are likely neurons. To this end, we used Otsu’s method\textsuperscript{66} to determine a threshold for summed intensity within a given region. Once cells were labelled based on summed GFAP intensity with respect to the threshold, each stained region was given a value for the percentage of astrocytes and neurons based on the ratio of these GFAP-positive and GFAP-negative cells to total detected cells, respectively (Table S1).

**Machine learning approaches for mass spectra deconvolution.** The acquired raw data from Xcalibur were imported into BioMAP and MSIREader to obtain DESI-MS images and co-registered them with fluorescence images (see Supporting Information S3). Mass spectra from regions of interest as determined by immunofluorescence imaging were extracted and converted to text files. The raw mass spectra were first filtered by the frequency and relative intensity of the peaks. Peaks that only appear in less than two samples or peak intensities that were less than 0.1% of the maximum value were filtered out. The cell counts from the fluorescence image and the mass spectra were then combined to extract mass spectra for different cell types. Ordinary least square methods are not suitable for this problem as the solutions may contain negative peak intensity values. Instead, we formulated this problem as a convex optimization, which can then be solved by convex solvers. Briefly, let $X \in \mathbb{R}^{n \times d}$ be the set of mass spectra obtained, where $n$ is the number of samples, $d$ is the number of distinct peaks in the mass spectra, and $X_{ij}$ is the mass intensity of sample $i$ at peak $j$. $C \in \mathbb{R}^{n \times m}$ be the counting of the $m$ kinds of cells in the $n$ samples, and $M \in \mathbb{R}^{m \times d}$ be the mass spectra of the $m$ kinds of cells we are solving for. The convex optimization problem was then formulated as:

\[
\begin{align*}
\text{minimize} & \quad \|CM - X\|^2 \\
\text{subject to} & \quad M \geq 0
\end{align*}
\]

Where $\| \cdot \|_2$ stands for $l_2$ norm, and $M \geq 0$ represents $M$ is elementwise greater than or equal to 0. The convex optimization problem was then solved by the open source package of cvxpy.\textsuperscript{37,58} Note that this method is limited by the intrinsic properties of linear systems. For example, when all samples have the same ratio of one cell-type to another, the matrix $C$ becomes singular, and no unique solution exists. Therefore, the successful application of this procedure depends on the existence of a heterogeneous distribution of the cell types, which is the common situation. Representative pixel metabolic profiles (Figure S1) show the variety of cellular metabolites in each pixel. Other aspects that are required to apply this method are 1) the frequency at which the cells in the selected pixels are intermingled differs from pixel to pixel, allowing robust deconvolution; 2) the different cell types have very different metabolic characteristics (e.g., neurons and astrocytes); and 3) the number of cell types is relatively low (e.g., two types in this study) so as not to over-complicate the solution. The method itself has no limit to the number of cell types as long as enough pixels are provided to generate a unique solution. The deconvolved spectra were visualized by mapping to a two-dimensional space using partial least square – discriminant analysis (PLS-DA), and the differences between metabolic distributions were tested by the Wilcoxon rank-sum test. We obtained 10 samples and extracted 10 pixels from each sample. The deconvolution was performed using the 10 pixels for each sample separately. Therefore, 10 deconvoluted spectra for each cell type were obtained, with each of them coming from a different sample. The 20 deconvoluted spectra were used in the Wilcoxon rank-sum test. The analysis was done using the package of scikit-learn.\textsuperscript{39} All code used for imaging analysis and cell-type-specific deconvolution by machine learning are available on Github repository for this paper.

https://github.com/lightingghost/cell_type_mass_spec_imaging

**RESULTS AND DISCUSSION**

**Cell-type-specific metabolite measurement using dual-modality imaging methods combined with machine learning.** We performed differential metabolic analysis for two cell types, neurons and astrocytes, in mouse brain tissue in situ by dual-modality imaging combined with machine learning (Figure 1), which was illustrated by the following four steps: (1) Spatial distribution of metabolites of lateral septal nucleus in adult mouse forebrain was measured by DESI-MSI coupled to a mass spectrometer (Thermo LTQ Orbitrap XL) having both high mass resolution and tandem MS for structural analysis. A typical spatial resolution of DESI-MSI, 200 μm was used; therefore, the total number of cells included in one scan pixel ranged from 48 -156 cells. (2) After DESI-MSI experiments, cells were labelled by cell-type-specific antibodies. Neurons and astrocytes were identified, and the frequency of each cell-type was quantified. (3) MSI images were then overlaid with fluorescence images to obtain pixel information of metabolic distribution, cell numbers, and cell types. Mass spectrum extracted from one MSI scan pixel reflects the composition of all the cells in that pixel. Different MSI pixels include mixed cell types and different cell numbers, which leads to differences in the mass spectra of MSI pixels. (4) Cell-type-specific metabolic profiles were then deconvolved by solving a convex optimization problem, a machine learning method. This method relies on the variation in the frequency of different cell types in each given MSI pixel, which is an inherent nature for almost all complex tissue samples with mixed cell types. In our study, we focused on the lateral septal nucleus region in the forebrain which is dominated by neurons and astrocytes. Details of our results in each step are elaborated in the following sections.

**Extraction of pixel mass spectra from DESI-MSI data.** DESI-MS imaging was carried out on coronal sections of ten mouse brain samples. A histologically compatible solvent system, composed of N, N-dimethylformamide (DMF): acetonitrile (1:1, vol:vol) was used in DESI-MSI experiments.\textsuperscript{53} This method allowed immunofluorescence staining to be performed on the very same tissue section previously analyzed by DESI-MSI, which provides an
unambiguous correlation between metabolic signatures and cell-type identity. Reproducibility was investigated by analyzing the same mouse brain tissue section on different days. Very small variations were found for MS peaks in the pixels (RSD = 8% when the cutoff value of the noise intensity was set at 0.005). The representative mass spectra of a pixel obtained from DESI-MSI of the same tissue section on two different days are shown in Figure S5. The main metabolites observed in the examination of mouse brain tissues in the mass range of $m/z$ 100 to 1,000 (Figure 2a) were majorly amino acids, nucleosides, saturated and unsaturated fatty acids (FA), glycerophosphoinositols (PI), glycerophosphoserines (PS), glycerophosphethanolamines (PE), phosphatidic acid (PA) and sulfatides (ST). Representative images of metabolites including $N$-acetylaspartate ($m/z$ 174.0), adenosine ($m/z$ 302.1), arachidonic acid ($m/z$ 303.2), PS (40:6) ($m/z$ 834.5), PI (38:4) ($m/z$ 885.5), and ST (24:1) ($m/z$ 888.6) were shown in Figure 2b, and they present very different distributions in the brain sections. Metabolic profiling on neurons and astrocytes has always been of great interest in the field. MSI offers the possibility to provide new insights whereas...
traditional MS technology relies heavily on mechanical dissociation of cells which is challenging due to intricate cellular structures, such as axon in neurons. In this study, we focus on lateral septal nucleus areas in the forebrain because this region is demarcated with distinct anatomical landmarks on coronal section (Figure 3a and S2a).

Additionally, it contains robust and heterogeneous distribution of GFAP-positive (likely astrocytes) and GFAP-negative (likely neurons) cells (Figure 1, S2b-S2c and 3b). As a proof of principle effort to start delineating the cell-type-specific metabolic profiling in neurons and astrocytes, this provides an ideal setup for accurate data acquisition and downstream analysis. Functionally, the lateral septal nucleus also occupies one of the most strategically important positions in the forebrain, it is known to receive reciprocal connection from the olfactory bulb, hippocampus, hypothalamus, and midbrain and play roles in critical physiological function in animals such as regulating social behavior, reward, and anxiety.\(^{60,61}\) So far, cell-type-specific metabolic study on neurons and astrocytes in septal areas is largely unexplored, which hinders our understanding of their functional roles. We extracted mass spectrometric information of metabolites from septal nucleus areas and displayed it in forms of pixel mass spectra (Figure 2c). Ten pixels from each individual mouse were extracted from the overlay images of DESI-MSI and fluorescence images in lateral septal nucleus of the mouse forebrain. Due to different numbers of cells and cell types in each pixel, the metabolic profile varies from pixel to pixel. In total, 100 pixels from lateral septal nucleus were subjected to analysis.

**Figure 2.** (a) Representative mass spectrum of mouse brain scanning using DESI-MSI; (b) Ion images of metabolites, N-acetylaspartate, adenosine, arachidonic acid, PS (40:6), PI (38:4) and ST (24:1), showing different spatial distributions in mouse brain. Mass intensity was reduced by a factor of two for N-acetylaspartate and arachidonic acid and increased by a factor of ten for adenosine to visualize using the same scale; (c) Representative mass spectra of pixels exacted from DESI-MSI, showing metabolic variation due to different cell number and cell types in each pixel. Each representative mass spectrum originates from different pixels.
Immunofluorescence imaging of neurons and astrocytes. We took advantage of the fact that cellular structure and protein remain intact following DESI-MSI scan. This enables us to use the very same tissue section that has been subjected to DESI-MSI for cell-type identification and quantification using immunofluorescence staining. Brain sections were stained with antibody against astrocyte specific marker Glial Fibrillary Acidic Protein (GFAP). We also applied a commonly used fluorescent nucleic acid dye DAPI (4′,6-diamidino-2-phenylindole) to identify the nucleus of each cell. Following confocal fluorescence microscopy, we quantified the total number of intact cells using blob detection (see methods section for more details) on areas that are positively stained with DAPI in each pixel. Using the immediate region including and surrounding the detected nuclei, cells were then classified as either GFAP-positive (likely astrocytes) or GFAP-negative (likely neurons) using Otsu’s method.66

While astrocyte marker GFAP can also label neural stem cells in neurogenic regions of mouse brain, such as the subventricular zone and the hippocampal dentate gyrus, to our knowledge, cells that are positively labelled by GFAP in the lateral septal nucleus region should almost exclusively be astrocytes.62 Neuron, oligodendrocyte and microglia are the other three differentiated cell types in adult brain besides astrocyte, all of which exhibit negative GFAP staining. Given the low level of mRNA expression detected by in situ hybridization54, 55 of Myelin basic protein (Mbp) (an oligodendrocyte marker) (Figure S2d) or Allograft Inflammatory Factor a (Aif1, also known as Iba1) (a microglia marker) (Figure S2e) in the lateral septal nucleus,63 the vast majority of GFAP negative cells that were identified in our current study in the lateral septal nucleus region are likely neurons. However even with very low levels of oligodendrocyte present, we cannot entirely exclude the possibility that myelin sheaths, which are generated by oligodendrocytes and wrap around neuronal axons, are being captured in the lateral septal nucleus region. Once cells were characterized as either GFAP-positive astrocytes or GFAP-negative neurons, the frequency of each of these two cell types were quantified in all 100 pixels for downstream computational analysis and metabolic profile deconvolution.

Metabolic profiling of neurons and astrocytes. By overlaying MSI scans with immunofluorescence images, metabolic profiles from each matching MSI pixels in lateral septal nucleus were extracted. Given the 200 µm diameter of the spray nozzle, variations of metabolites in mass spectrum obtained from each pixel could be due to the differences in cellular metabolic states or cell-type
composition in different pixels of the tissue. With the cell-type frequency quantification of neurons and astrocytes from each pixel, we were then able to apply convex optimization in all samples, which provided us the cell-type-specific metabolic profiles of astrocyte (Figure 4a) and neuron (Figure 4b) from all samples. Metabolites ranging from m/z 100 to 1000 show distinct patterns in astrocytes and neurons.

To visualize the variation and separation of the deconvolved metabolic profiles from neurons and astrocytes, we performed partial least squares-discriminant analysis (PLS-DA) using all 4450 detected metabolic features from mass spectra. Computationally deconvolved metabolic profile of neurons and astrocytes from all samples were clustered into two populations based on their cell-type identification (Figure 5), suggesting very distinct and reproducible metabolic signatures between these two cell types. The Wilcoxon rank-sum test reveals 107 metabolites show statistically distinct abundance between two cell types (Figure 6a), with 31 metabolites displaying enriched intensity in astrocytes and 76 showing accumulation in neurons (Figure 6b, Figure S3). Molecular compound characterization was then performed using high resolution MS and tandem MS, and 23 distinct metabolites (Table S1) were identified. Interestingly, adenosine is highly enriched in astrocytes and neurons, showing cells were clustered into two populations which are known to have very sparse organelles than neurons.71,72

We also made novel observations: for example, we observed a list of phospholipids that are significantly more abundant in neurons than astrocytes, such as lipids in classes of PE, PA, PG, PI, PS and sulfatide (Table S1). Besides previous report suggesting an overall higher level of lipid turnover in neurons compared to astrocytes, how individual phospholipid could be differentially regulated between these two cell types are largely unexplored.69 PE, PG, PI, PS and sulfatide are major membrane components that can become building blocks of plasma membrane as well as a variety of organelle membranes. Interestingly, phospholipid composition has been implicated in regulating important neuronal functions such as synaptic vesicle fusion and receptor accessibility,70 which could potentially contribute to the relative abundance of these phospholipids in neurons. Moreover, given that the astrocytes in septal nuclei are mostly fibrous astrocytes, which are known to have very sparse organelles than neurons.71,72

Table S1). NAA is a metabolite specific to the nervous system which is synthesized in neurons.68 Importantly, previous studies indicated that NAA shows high concentration in medial and lateral septal nucleus, which we were able to corroborate in metabolic profile of neurons from the same region. Thus, our identification of metabolites such as adenosine and NAA, which were previously associated with astrocytes and neurons, respectively, provides validation for our experimental and in silico pipeline.

![Figure 4. Cell-type-specific metabolic profile in (a) astrocytes; (b) neurons obtained by convex optimization of cell-type frequency quantification of neurons and astrocytes from 100 pixels in lateral septal nucleus region of ten different mouse brain tissues.](image)

![Figure 5. Result of Wilcoxon’s rank-sum test revealing 107 metabolites that show statistically distinct abundance between astrocytes and neurons in the lateral septal nucleus region of mouse brain tissue, with 31 metabolites displaying enriched intensity in astrocytes and 76 showing accumulation in neurons.](image)

![Figure 6. Result of Wilcoxon’s rank-sum test revealing 107 metabolites that show statistically distinct abundance between astrocytes and neurons in the lateral septal nucleus region of mouse brain tissue, with 31 metabolites displaying enriched intensity in astrocytes and 76 showing accumulation in neurons.](image)
CONCLUSIONS

Complex tissue samples with mixed cell populations present a challenge in determining the metabolic profiles of different cell types. We present a method that integrates the rich chemical information found within mass spectra recorded by DESI-MSI, with the well-defined cellular classifications obtained by immunohistochemical staining from thousands of cells. Downstream machine learning enables computational deconvolution. Based on this method, we report cell-type-specific metabolic profiles from neurons and astrocytes in the lateral septal nucleus of adult mouse forebrain. Featured metabolites such as adenosine and N-acetylserinapectate are in line with our current understanding of their functions in astrocytes and neurons, respectively. Excitingly, our method also reveals accumulation of phospholipid species from a few different classes in neurons that could provide new opportunities for future functional metabolic studies between these two important cell types - neurons and astrocytes - in the central nervous system. Our method can infer the spectra of distinct cell types in a mixture when we know the spectrum of the mixture and the percentage of each cell type. Conversely, this method could also be used to infer the percentage of each component when we know the spectrum of the mixture and the spectra of different components, and thereby could reveal cellular heterogeneity in a tissue. For example, many studies have shown that cancer cells have distinct metabolic profiles from normal cells. We envision this method could be applied to obtain the metabolic profiles of cancer cells vs. normal cells, especially for tumors that have cancer cells sparsely distributed throughout normal cells. Thus, this method should allow several applications in cellular metabolomics in samples with heterogeneous cell types, provided that there are enough differences between the cell types.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website.

Supporting Information Figures S1-S3 and Table S1: Metabolic profiles of pixels; cell-type-specific marker gene visualization; identified metabolites; and Wilcoxon’s rank-sum test result (PDF)

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REFERENCES


DESIR-MSI

Metabolic Profiles of Pixels in LSN

Overlay of Two Images

Cell Types and Frequencies in Pixels in LSN

Computational Deconvolution to Obtain Cell-Type-Specific Metabolic Profile

Cell Type
- Astrocyte
- Neuron

Test Statistics

m/z
Immunofluorescent staining

Astrocytes (GFAP+)

Neurons (GFAP−)

GFAP DAPI

DESI-MSI

Test Statistics

Cell Type

m/z

Relative Abundance

Computational Deconvolution to Obtain Cell Type-Specific Metabolic Profile
Figure 2. (a) Representative mass spectrum of mouse brain scanning using DESI-MSI; (b) Ion images of metabolites, N-acetylaspartate, adenosine, arachidonic acid, PS (40:6), PI (38:4) and ST (24:1), showing different spatial distributions in mouse brain. Mass intensity was reduced by a factor of two for N-acetylaspartate and arachidonic acid and increased by a factor of ten for adenosine to visualize using the same scale; (c) Representative mass spectra of pixels exacted from DESI-MSI, showing metabolic variation due to different cell number and cell types in each pixel. Each representative mass spectrum originates from different pixels.
Figure 3. (a) Schematic showing the region of interest in this study. A rectangular area (red dashed rectangle) within the lateral septal nucleus region (black dashed symmetrical regions) in coronal section (Bregma 0.445 mm) of mouse brain tissue, image obtained from Allen Developing Mouse Brain Atlas;54,55 (b) fluorescence images of mouse brain tissue after cell labelling using GFAP antibody and DAPI; (c) fluorescence images of one MSI pixel with a dimension of 200 μm by 200 μm showing cell numbers and types using GFAP labelling in green and DAPI labelling in blue; (d) image after DAPI staining; (e) blob detection showing total number of cells in one MSI pixel; (f) fluorescence image with grey circle showing the number of astrocytes in one MSI pixel; (g) fluorescence image with grey circle showing the number of neurons in one MSI pixel.
Figure 4. Cell-type-specific metabolic profile in (a) astrocytes; (b) neurons obtained by convex optimization of cell-type frequency quantification of neurons and astrocytes from 100 pixels in lateral septal nucleus region of ten different mouse brain tissues.

910x988mm (119 x 119 DPI)
Figure 5. Visualization of partial least squares-discriminant analysis using 4450 detected metabolite features in astrocytes and neurons, showing cells were clustered into two populations which suggests very distinct and reproducible metabolic signatures between these two cell types. Each dot represents one type of cells from an individual mouse brain. Mass spectra of ten pixels were obtained for each individual mouse brain. The circle around each cluster represents data ellipse drawn at 95% level. The outlier may come from the heterogeneity of different samples.
Figure 6. Result of Wilcoxon’s rank-sum test revealing 107 metabolites that show statistically distinct abundance between astrocytes and neurons in the lateral septal nucleus region of mouse brain tissue, with 31 metabolites displaying enriched intensity in astrocytes and 76 showing accumulation in neurons (The m/z values of 107 featured metabolites and the annotations of identified metabolites are shown in Figure S3).
860x369mm (119 x 119 DPI)