Journal of Mass Spectrometry

WILEY

Journal of MASS SPECTROMETRY

PERSPECTIVE OPEN ACCESS

Spatial Multiomics Toward Understanding Neurological Systems

Elizabeth K. Neumann

Chemistry Department, UC Davis, Davis, California, USA

Correspondence: Elizabeth K. Neumann (ekneumann@ucdavis.edu)

Received: 15 December 2024 | **Revised:** 15 April 2025 | **Accepted:** 29 April 2025

Funding: The author thanks UC Davis and NIH NIA (1R21AG083965-01A1) for funding.

ABSTRACT

Dynamic biological processes in the brain involve complex interactions between various cell types, and these interactions span multiple biological scales. Each of these domains are crucial in maintaining brain health. Traditional methods, such as transcriptomics and protein labeling, provide valuable insights but fail to capture the full molecular landscape of neurological function. Multimodal imaging, combining multiple imaging techniques, offers a more comprehensive approach to studying biological systems by integrating different omics technologies. Spatial metabolomics involves using techniques like mass spectrometry imaging to enable detection of metabolites within their native tissue context and reveals functional roles that are crucial for understanding disease. Spatial transcriptomics and proteomics contribute information on gene expression and protein function but face challenges in resolution and integration with other omics approaches. Combining metabolomics, transcriptomics, and proteomics will enhance our understanding of cellular interactions, but challenges remain in optimizing sample preparation, maintaining molecular integrity, and integrating data across omics layers. Future advancements in spatial multiomics, incorporating epigenetics and extending to whole-body or nanoscale imaging, will significantly advance our understanding of neuroscience and complex diseases like Alzheimer's disease or autism spectrum disorder.

1 | Overview

Dynamic biological processes generally involve the active coordination of different, but functionally relevant, cell types. Memory, cognition, and learning are fundamental neurological processes that involve chemical communication between neurons and other cells, such as adjacent neurons, astrocytes, oligodendrocytes, and microglia [1–6]. These interactions span across size domains, ranging from organ systems to subcellular organelles and everything in between (Figure 1A). Neurons are involved in receiving, integrating, and communicating electrical signals that enable a variety of functions, including memory, and motor control [7–9], and are generally the most well studied. Within healthy systems, neuronal communication is reinforced and influenced by astrocytes, oligodendrocytes, microglia, and other adjacent cells [10–14]. These cells are often considered "passive," but I hypothesize that these cell types are the key to understanding neurological diseases. Each of these cell types of several subgroups vary as a function of brain region (e.g., hippocampus and cortex cerebellum) and developmental stage [15–17]. Clearly, this dynamic community of cells is spatially and molecularly complex, often involving multiple types of molecules, and any level of dysfunction within these communities often leads to disease or disorder. While important, this is a nontrivial pursuit that will proceed on for decades to come.

A great example of this complexity is Alzheimer's disease (AD), and the focus of many scientific pursuits. AD is a neurological disorder characterized by loss of memory, motor impairment, and fatality [18]. AD is pathologically characterized by the accumulation of Aβ plaques within multiple brain

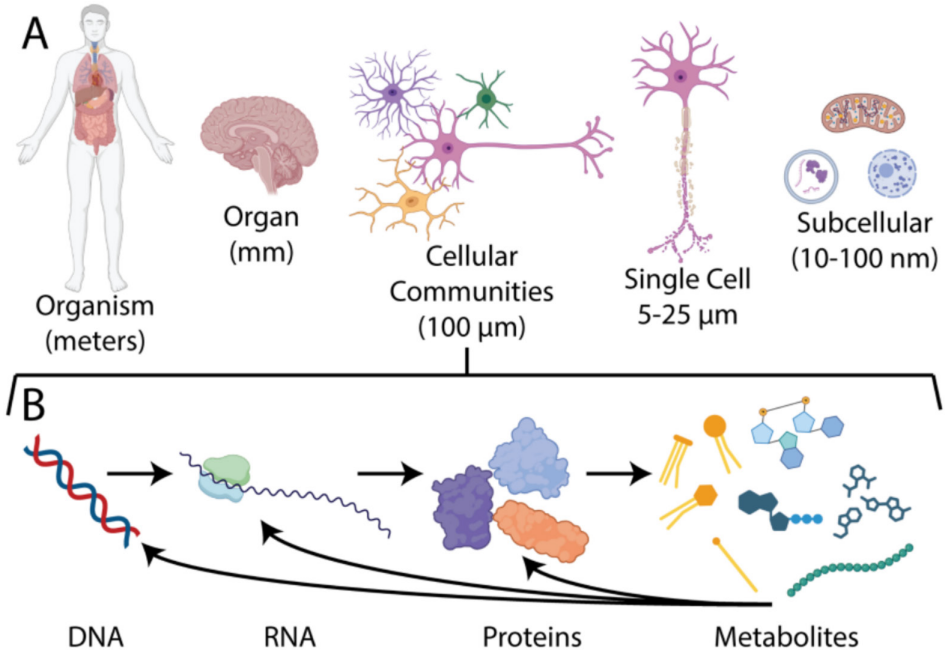

FIGURE 1 | Biological systems are highly complex systems on both physical (A) and molecular (B) scales. (A) Understanding neurological function and its transition toward disease requires a multipronged approach that spans from how the brain interacts with other organs, brain regions and subregions (e.g., cortex, hippocampus, cortex, and cerebellum), cellular interactions and communities, single cell responses, and to organelles. (B) At each of these domains, the molecular features also play unique, functional roles that are difficult to interpret by only studying one omics space. Indeed, metabolites are the broadest omics class, and they interact with all previous steps within the central dogma of biology.

regions, and it is still heavily debated if these plaque features are disease causing or a symptom. Indeed, unanswered questions like these are fundamental but difficult to study, particularly by a single approach/perspective. For instance, the cellular microenvironment/neighborhood surrounding these plaques is cellularly and molecularly complex, heterogenous, and dynamic. Very similar observations can be drawn for many different neurological diseases, including Parkinson's disease [19], autism spectrum disorder [20], post-traumatic stress disorder [21], bipolar [22], and schizophrenia [23]. While understanding these different cellular processes has obvious implications, it is highly complicated and challenging. I believe that one of the only ways to understand neuroscience involves using multiple analytical techniques to assay multiple molecular classes. Traditional means of exploring the brain has often leaned on independent experiments involving transcriptomics-based analyses or antibody-labeling of protein markers [24, 25]. Because of this, brain cell types and subtypes are often defined by their protein or transcriptomic profiles. While overly reductive, it is still more related to function than cellular shape under a microscope (e.g., astrocytes are named for being star shaped). One additional means of stratifying cell types and subgroups would be to incorporate metabolites, which are the work horses of biological systems and interact with all other omics spaces. From an analytical perspective, there are few (if any) technologies that can directly measure more than a small subset of molecular features (e.g., mRNA, proteins, or lipids) within a complex matrix. One alternative strategy includes multimodal imaging, which can span across multiple omics spaces (Figure 1B) and will be the focus of this perspective.

2 | Multimodal Imaging

Multimodal imaging is the combination of two or more imaging technologies that access orthogonal information, providing a more comprehensive picture of the system than either individual approach alone [26–28]. In practice, however, multimodal imaging is rather complex; it consists of a delicate balance between the sampling and analytical requirements associated with each technique [27]. For example, most histological assessment is performed on formalin-fixed paraffin-embedded (FFPE) tissue as it best preserves tissue morphology, but mass spectrometry (MS)-based approaches prefer fresh-frozen tissues since any chemical additives can complicate resultant spectra, and the fixation process can remove molecules detectable by MS, including labile or chemically unstable molecules. What exactly is removed or crosslinked is still relatively unknown and likely depends on the sample and specific fixation protocol. Experiments that rigorously assess which molecules are removed and how will make these experiments more reproducible. As such, combining these two approaches requires strategically developing workflows that marry the sampling requirements without compromising data quality. While this takes extra planning, generally, the combination of two or more omic spaces improves classifications and better coverage of what constitutes biological systems [29–32].

Techniques that are relevant for neuroscience-based research and should be considered for spatial multiomics include next generation sequencing (genomics) [33, 34], spatial transcriptomics (gene expression/transcriptomics) [35, 36], microscopy (histology/proteomics/fluorescent molecules) [37–40], and MS imaging (proteomics/lipidomics/glycomics/metabolomics)

[41, 42]. Generally, most examples of multimodal imaging perform each approach on different samples to easily accommodate the different sampling requirements. While convenient, the data is not inherently registered nor integrated. These methods prevent single cell or organelle analysis, which are critical for neuroscience projects. As such, marrying these sampling requirements is often essential for performing multiple imaging experiments on the same tissue and will be discussed in detail within the sections below. I anticipate the community to continually push toward using the same sample. As this field develops, there will be additional issues associated with computationally combining and interpreting these datasets, including miss-matched spatial resolutions, drastically different dynamic ranges, and image registration. Largely, these computational approaches are spearheaded by academic and/or government institutions with commercial options severely lagging. From my experience, whenever new equipment is released, often, the commercial software that comes with it is ineffective. Often, it takes several years for the software to catch up with the hardware.

2.1 | Spatial Metabolomics

Of all the omics spaces, metabolomics is the least developed and would benefit the most from inclusion of orthogonal information. While excluded from the central dogma of biology, metabolites are some of the most important features of the brain. Metabolites are a class of biological molecules, such as lipids and signaling molecules, that participate in many critical physiological and disease processes [43–46]. Despite their importance, less is known about these molecules compared to their protein and gene counterparts. The chemical space that the term "metabolites" covers is huge both numerically and chemically. This often prevents holistic detection [47] as well as contributes to difficulties performing de novo identification and assessment of their exact function. From a stability standpoint, metabolites are often easily degraded or diffused [48–50]. Unlike DNA and RNA, metabolites cannot be amplified and often do not ionize as well as peptides/proteins. These differences have caused an incorrect assumption that genomics, transcriptomics, and proteomics are more important than metabolomics. I think metabolomics has lagged behind because of its difficulty.

There are dozens of analytical methods for detecting metabolites, but few are capable of spatial analysis at cellular resolutions. This is critical for neuroscience applications, where individual cells have unique functions. MS imaging is arguably one of the most widely used methods for spatially assaying metabolites, including matrix-assisted laser desorption/ionization (MALDI) [51–55], secondary ion MS (SIMS) [56, 57], and desorption electrospray ionization (DESI) [58, 59]. Of these, my lab primarily uses MALDI MSI, which is capable of measuring hundreds to thousands of molecules within a tissue without disturbing their spatial context [60, 61]. The tissue is coated in a UV-absorbing chemical matrix to enhance and facilitate desorption and ionization of endogenous metabolites. One of the benefits of this matrix is that it can be reactive toward specific metabolite classes [62–64], acts as a preservative [65, 66], and buffers the tissue from laser damage [67]. All of these features facilitate the addition of orthogonal imaging approaches. I believe that there are analogs that can be incorporated within SIMS and DESI workflows that would enable spatial multiomics. In particular, the preservation of different molecular classes is arguably the most important. SIMS experiments occur in a vacuum, while DESI occurs at ambient conditions, where alternative stabilization may need to be considered. SIMS is inherently the most destructive of the three, and any tissue damage will alter the results obtained by other spatial approaches, whereas DESI is the least destructive. Regardless, without orthogonal training, MSI is not the canonical method for cell classification and must be trained using other approaches, such as spatial transcriptomics and/or proteomics, for cell typing.

2.2 | Spatial Transcriptomics and Cell States

Advances in next generation sequencing technologies have changed our understanding of biological systems. Transcriptomics provides extremely rich information on the physiological state of cells and tissues by measuring mRNA that encode for specific proteins [68–71]. Moreover, many cells and their subtypes are classified by their genetic profiles, and they often participate in unique functions in many organ systems [72–74]. Single-cell spatial transcriptomics technologies can quantify the transcripts of thousands of genes with a resolution close to 200 nm, although most technologies having a spatial resolution closer to hundreds of microns [75, 76]. While there are many other types of spatial transcriptomics technologies, they almost all rely on fluorescence-based instrumentation [35], are limited in imaging areas, and/or are expensive. These limitations have prevented their regular use in the clinic and diagnostics. Even within basic research, they are inappropriate for assaying large patient cohorts or even large pieces of tissue.

In the context of multiomics, there is some disconnect between gene expression profiles and abundance of proteins and metabolites [77]. I believe this discrepancy reduces the reproducibility and rigor of spatial transcriptomic experiments and has led to delayed biological understanding. There are many potential causes for this discrepancy, ranging from technical variables to downstream biochemical regulators, but understanding how gene expression affects secondary metabolites is critical for understanding healthy function and disfunction, especially in the brain, where these connections often span cellular communities. To date, few studies have successfully combined these two approaches [78–80], particularly in a spatial context for large tissue areas. One reason for this is the mismatched sample preparation methods for transcriptomics and metabolomics. Single-stranded mRNA is easily degraded [81] at ambient conditions and from RNases. As such, RNase inhibitors are added. While not known, I anticipate that adding these inhibitors may cause diffusion of small metabolite and that some types of MSI can cause mRNA degradation. Additionally, traditional spatial transcriptomics experiments require fixation [82], which crosslinks and/or removes metabolites [52]. The combination of spatial transcriptomics with spatial proteomics is straight forward as these two approaches follow similar protocols, unlikely standard spatial metabolomic protocols. While straightforward, I think there is a significant overlap between the types of information spatial proteomics and spatial transcriptomics can provide.

While the combination of transcriptomics and MSI is difficult, this is a promising direction for both communities. For one, MSI can analyze large pieces of tissue and could be used to guide where spatial transcriptomics should be performed. Their combination comes down to mass spectrometrists learning what factors contribute to their degradation, such as not using RNase free water, contamination/degradation that occurs at atmosphere, and original tissue integrity. Generally, RNA integrity numbers (RIN) are critical for assessing if sample preparation methods affect mRNA integrity, but these are often not measured on tissue undergoing MSI experimentation. While difficult, I think the most compelling results that can come from the combination of transcriptomics and metabolomics is understanding the mechanisms behind their discrepancy, as it may come from cellular interactions that are unknown. Additionally, the combination may lead to the development of more effective gene therapies for psychiatric diseases.

2.3 | Spatial Proteomics and Relation to Cell Type

Within the central dogma of biology, mRNA is translated into protein features and these proteins often interface with metabolites. Unlike the other omics spaces, proteins are responsible for structural integrity, chemical catalysis, and transport within biological systems and are intricating involved in function with neuroscience [83, 84]. Because of a protein's connection to function, proteins are often used to define different types of cells [85, 86]. Traditional means of spatial detection proteins involve the use of antibodies that are attached to a fluorophore or another measurable label [87, 88]. By targeting these antigens, cells can be visualized by a variety of common microscopy approaches for large tissue areas. Traditional immunofluorescence experiments are limited in plexity to ~4–7 markers because of the spectral overlap between fluorescent channels [89]. Most organs have dozens of cell types that can be further divided into subtypes, requiring highly multiplexed labeling approaches for appropriately studying a tissue.

There are numerous examples of combining spatial metabolomics or transcriptomics with spatial proteomics [67, 90–93], and this has been exceptionally fruitful in understanding metabolism as well as posttranslational regulations. Often, these experiments are performed on fixed tissue and/or embedded tissue, which requires antigen retrieval. Antigen retrieval has detrimental effects on metabolomics [94] because proteins are inherently more stable [95] and can withstand the chemical washes and pressure associated with antigen retrieval. A side effect of their stability is that this has readily enabled the combination of spatial proteomics with spatial metabolomics. This must be taken into consideration when determining the order of operations within multiomic experiments. Indeed, targeting proteins first in an imaging sequence will most likely result in degradation or removal of other chemical species, including mRNA and metabolites. Additionally, validating the specificity of an antibody is also important if MSI is performed first as the effects of UV on the epitope of a protein is often unknown. While minimally published and studied [67], MALDI matrix choice is also a critical feature that influences protein integrity and epitope stability. I encourage those combining MSI with spatial proteomics to include both the clone and lot associated with each antibody.

Ultimately, I think the direction that is most compelling is the combination of spatial metabolomics with highly multiplexed spatial proteomics, which has not been robustly explored. Indeed, the combination of proteomics with metabolomics enables cell-specific contextualization of small molecules. This usually requires 20 or so protein targets and their respective antibodies, making this a costly endeavor. Another exciting direction is direct detection of proteins by MSI for connection with metabolites. This provides insight into any post-translational modifications that may occur. While important, these post translational modifications are probably the reason why spatial proteomics by MSI is difficult. Each ion is difficult to identify, and few instruments can resolve these ions without the addition of multiple charges. This, ultimately, makes DESI MSI the standout choice for these experiments. Initial steps toward this will require greater consideration for sample integrity during long imaging runs as well as how to handle highly abundant proteins, such as albumin, that prevent the detection of other proteins related to cell typing, such as glial fibrillary acid protein that is used to identify astrocytes.

2.4 | Future Directions

The future of multimodal imaging is bright. Most exciting is the concept of understanding what discrete metabolites are functionally doing within complicated cellular systems. Largely, function is only related to bulk molecular classes as opposed to discrete molecules. While I discussed some of the exciting directions of spatial metabolomics with both spatial transcriptomics and spatial proteomics, I think the combination of all three omics spaces on one tissue will be game changing for our understanding of neurological diseases as well as other diseases. This would allow us to trace a biochemical pathway from the genome all the way to secondary metabolism and everything in between. Additionally, I think spatial multiomic measurements that incorporate aspects of epigenetics are the next major advancement in the field. In brief, epigenetics is the study of DNA regulation, where DNA or histones are chemically modified to change which genes are transcribed into mRNA. Spatial epigenetics is an up and coming field [96] and likely explains some discrepancies between genomics and proteomics or metabolomics. A gene can have drastically changed expression based on epigenetics and likely occurs on the single-cell level in neurological systems. Beyond epigenetics, the integration of other molecules, including glycans and carbohydrates, will also be critical, since these molecules are key when thinking about immune responses.

Finally, most multiomics measurements are performed on only a single organ (or even just a single section from a portion of an organ) in an imaging context or are single-cell profiling approaches. These experiments ignore the connection between organ systems as well as organelles. Indeed, organs are connected; the brain bidirectionally interfaces with every other organ in an organism. The central and peripheral nervous system spans the entire body, and brain health is maintained by organs, such as the heart, kidney, and liver. Thus, viewing the brain in isolation is a very narrow view of neuroscience. With that, there are significant methodological and computational challenges associated with datasets that are exceptionally large, such as whole body or 3D in nature, making them natural next

directions for spatial multiomics. Methods have to be incredibly reproducible, as any artifact or missing tissue will be apparent in the final 3D stack. These datasets are also incredibly large (>Tb for the MSI alone) and require alignment and 3D segmentation, of which no current commercial software will do. Currently, spatial proteomics and spatial metabolomics are the only fields capable of imaging such large volumes of tissue. Spatial transcriptomics has to almost be redesigned to enable this type of work.

On the other end of the spectrum is targeting features that are in the range of nanometers in diameter, such as mitochondria and extracellular vesicles (EVs), that are functional in nature and can lead to different diseases. While important, these small sizes make them incredibly difficult to study. From a MS standpoint, there are often only tens to hundreds of copies of every discrete molecule in EVs. As a natural first step, MALDI or DESI MSI can learn from the SIMS community and connect to ion-based microscopies like scanning electron microscopy and tunneling electron microscopy. There are certainly computational difficulties with this; the spatial resolution is an order of magnitudes different between the approaches. From a methodological standpoint, this could be aided by incorporating fiduciaries that are active in both modalities that will help with image alignment.

Finally, and arguably most importantly, I think that spatial multiomics will cause these different, often siloed communities, to connect. Through this, we will be able to learn and obtain insight that will otherwise remain siloed. For instance, as MALDI and DESI MSI reach smaller and smaller spatial resolutions, our stages will need the accuracy and precision to accommodate those spatial resolutions. Microscopes and spectroscopic stages regularly achieve high precision within those spatial scales. Microscopists have already developed computational approaches for 3D stacking and rendering as well as segmentation across these stacks that can probably be adapted for MSI. Spatial transcriptomics and proteomics also have methods for calculating and assessing cellular neighborhoods, which can also be commandeered. Regardless of these points, neuroscience and other biological fields will benefit by the combination and development of spatial multiomic methods.

Acknowledgments

The author thanks UC Davis and NIH NIA (1R21AG083965-01A1) for funding.

Conflicts of Interest

The author declares no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

1. J. J. Wade, L. J. McDaid, J. Harkin, V. Crunelli, and J. A. S. Kelso, "Bidirectional Coupling Between Astrocytes and Neurons Mediates Learning and Dynamic Coordination in the Brain: A Multiple Modeling Approach," *PLoS ONE* 6, no. 12 (2011): e29445.

2. M. E. Gibbs, D. Hutchinson, and L. Hertz, "Astrocytic Involvement in Learning and Memory Consolidation," *Neuroscience & Biobehavioral Reviews* 32, no. 5 (2008): 927–944.

3. R. Moraga-Amaro, J. M. Jerez-Baraona, F. Simon, and J. Stehberg, "Role of Astrocytes in Memory and Psychiatric Disorders," *Journal of Physiology-Paris* 108, no. 4 (2014): 240–251.

4. P. W. Frankland and S. A. Josselyn, "Starring Role for Astrocytes in Memory," *Nature Neuroscience* 23, no. 10 (2020): 1181–1182.

5. A. Pereira and F. A. Furlan, "Astrocytes and Human Cognition: Modeling Information Integration and Modulation of Neuronal Activity," *Progress in Neurobiology* 92, no. 3 (2010): 405–420.

6. M. Santello, N. Toni, and A. Volterra, "Astrocyte Function From Information Processing to Cognition and Cognitive Impairment," *Nature Neuroscience* 22, no. 2 (2019): 154–166.

7. I. A. Meinertzhagen, S.-y. Takemura, I. A. Meinertzhagen, et al., "From Form to Function: The Ways to Know a Neuron," *Journal of Neurogenetics* 23, no. 1–2 (2009): 68–77.

8. R. Yuste, "From the Neuron Doctrine to Neural Networks," *Nature Reviews Neuroscience* 16, no. 8 (2015): 487–497.

9. J. Ma and J. Tang, "A Review for Dynamics in Neuron and Neuronal Network," *Nonlinear Dynamics* 89 (2017): 1569–1578.

10. R. Siracusa, R. Fusco, and S. Cuzzocrea, "Astrocytes: Role and Functions in Brain Pathologies," *Frontiers in Pharmacology* 10 (2019): 1114, https://doi.org/10.3389/fphar.2019.01114.

11. Y. Kim, J. Park, and Y. K. Choi, "The Role of Astrocytes in the Central Nervous System Focused on BK Channel and Heme Oxygenase Metabolites: A Review," *Antioxidants* 8, no. 5 (2019): 121.

12. K. D. Barron, "The Microglial Cell. A Historical Review," *Journal of the Neurological Sciences* 134 (1995): 57–68.

13. E. Solito and M. Sastre, "Microglia Function in Alzheimer's Disease," *Frontiers in Pharmacology* 3 (2012): 14.

14. A. I. Rojo, G. McBean, M. Cindric, et al., "Redox Control of Microglial Function: Molecular Mechanisms and Functional Significance," *Antioxidants & Redox Signaling* 21, no. 12 (2014): 1766–1801.

15. J. R. Ecker, D. H. Geschwind, A. R. Kriegstein, et al., "The BRAIN Initiative Cell Census Consortium: Lessons Learned Toward Generating a Comprehensive Brain Cell Atlas," *Neuron* 96, no. 3 (2017): 542–557.

16. J. B. Sylvester, C. A. Rich, Y.-H. E. Loh, M. J. van Staaden, G. J. Fraser, and J. T. Streelman, "Brain Diversity Evolves via Differences in Patterning," *Proceedings of the National Academy of Sciences* 107, no. 21 (2010): 9718–9723.

17. B. Raj, J. A. Farrell, J. Liu, et al., "Emergence of Neuronal Diversity During Vertebrate Brain Development," *Neuron* 108, no. 6 (2020): 1058–1074.e6.

18. P. dos Santos, C. Leide, P. F. Ozela, et al., "Alzheimer's Disease: A Review From the Pathophysiology to Diagnosis, New Perspectives for Pharmacological Treatment," *Current Medicinal Chemistry* 25, no. 26 (2018): 3141–3159.

19. N. Panicker, P. Ge, V. L. Dawson, and T. M. Dawson, "The Cell Biology of Parkinson's Disease," *Journal of Cell Biology* 220, no. 4 (2021): e202012095.

20. D. Pinto, E. Delaby, D. Merico, et al., "Convergence of Genes and Cellular Pathways Dysregulated in Autism Spectrum Disorders," *American Journal of Human Genetics* 94, no. 5 (2014): 677–694.

21. K. J. Ressler, S. Berretta, V. Y. Bolshakov, et al., "Post-Traumatic Stress Disorder: Clinical and Translational Neuroscience From Cells to Circuits," *Nature Reviews Neurology* 18, no. 5 (2022): 273–288.

22. G. Rajkowska, "Cell Pathology in Bipolar Disorder," *Bipolar Disorders* 4, no. 2 (2002): 105–116.

23. H.-G. Bernstein, J. Steiner, P. C. Guest, H. Dobrowolny, and B. Bogerts, "Glial Cells as Key Players in Schizophrenia Pathology: Recent Insights and Concepts of Therapy," *Schizophrenia Research* 161, no. 1 (2015): 4–18.

24. A. Khodadadian, S. Darzi, S. Haghi-Daredeh, et al., "Genomics and Transcriptomics: The Powerful Technologies in Precision Medicine," *International Journal of General Medicine* 13 (2020): 627–640.

25. M. G. Kohale, A. V. Dhobale, N. J. Bankar, O. Noman, K. Hatgaonkar, and V. Mishra, "Immunohistochemistry in Pathology: A Review," *Journal of Cellular Biotechnology* 9, no. 2 (2023): 131–138, https://doi.org/10.3233/JCB-230110.

26. L. Martí-Bonmatí, R. Sopena, P. Bartumeus, and P. Sopena, "Multimodality Imaging Techniques," *Contrast Media & Molecular Imaging* 5, no. 4 (2010): 180–189.

27. E. K. Neumann, K. V. Djambazova, R. M. Caprioli, and J. M. Spraggins, "Multimodal Imaging Mass Spectrometry: Next Generation Molecular Mapping in Biology and Medicine," *Journal of the American Society for Mass Spectrometry* 31, no. 12 (2020): 2401–2415.

28. X. Jiang, J. Ma, G. Xiao, Z. Shao, and X. Guo, "A Review of Multimodal Image Matching: Methods and Applications," *Information Fusion* 73 (2021): 22–71.

29. S. Sangeetha, S. K. Mathivanan, P. Karthikeyan, et al., "An Enhanced Multimodal Fusion Deep Learning Neural Network for Lung Cancer Classification," *Systems and Soft Computing* 6 (2024): 200068.

30. R. Choudhary, P. Thatoi, and S. S. Rout, 2024.

31. C. Tang, M. Wei, J. Sun, S. Wang, Y. Zhang, and Alzheimer's Disease Neuroimaging Initiative, "CsAGP: Detecting Alzheimer's Disease From Multimodal Images via Dual-Transformer With Cross-Attention and Graph Pooling," *Journal of King Saud University, Computer and Information Sciences* 35, no. 7 (2023): 101618.

32. C. Cui, H. Yang, Y. Wang, et al., "Deep Multimodal Fusion of Image and Non-Image Data in Disease Diagnosis and Prognosis: A Review," *Progress in Biomedical Engineering* 5, no. 2 (2023): 022001.

33. T. Hu, N. Chitnis, D. Monos, and A. Dinh, "Next-Generation Sequencing Technologies: An Overview," *Human Immunology* 82, no. 11 (2021): 801–811.

34. B. E. Slatko, A. F. Gardner, and F. M. Ausubel, "Overview of Next-Generation Sequencing Technologies," *Current Protocols in Molecular Biology* 122, no. 1 (2018): e59.

35. L. Moses and L. Pachter, "Museum of Spatial Transcriptomics," *Nature Methods* 19, no. 5 (2022): 534–546.

36. C. G. Williams, H. J. Lee, T. Asatsuma, R. Vento-Tormo, and A. Haque, "An Introduction to Spatial Transcriptomics for Biomedical Research," *Genome Medicine* 14, no. 1 (2022): 68.

37. J. W. Lichtman and J.-A. Conchello, "Fluorescence Microscopy," *Nature Methods* 2, no. 12 (2005): 910–919.

38. P. W. Harms, T. L. Frankel, M. Moutafi, et al., "Multiplex Immunohistochemistry and Immunofluorescence: A Practical Update for Pathologists," *Modern Pathology* 36, no. 7 (2023): 100197.

39. R. H. Garman, "Histology of the Central Nervous System," *Toxicologic Pathology* 39, no. 1 (2011): 22–35.

40. A. Javaeed, S. Qamar, S. Ali, M. A. T. Mustafa, A. Nusrat, and S. K. Ghauri, "Histological Stains in the Past, Present, and Future," *Cureus* 13, no. 10 (2021): e18486.

41. E. R. A. van Hove, D. F. Smith, and R. M. Heeren, "A Concise Review of Mass Spectrometry Imaging," *Journal of Chromatography A* 1217, no. 25 (2010): 3946–3954.

42. A. Römpp and B. Spengler, "Mass Spectrometry Imaging With High Resolution in Mass and Space," *Histochemistry and Cell Biology* 139 (2013): 759–783.

43. N. Ishida, "Circadian Clock, Cancer and Lipid Metabolism," *Neuroscience Research* 57, no. 4 (2007): 483–490.

44. J. A. Allen, R. A. Halverson-Tamboli, and M. M. Rasenick, "Lipid Raft Microdomains and Neurotransmitter Signalling," *Nature Reviews Neuroscience* 8, no. 2 (2007): 128–140.

45. J. M. Dietschy and S. D. Turley, "Thematic Review Series: Brain Lipids. Cholesterol Metabolism in the Central Nervous System During Early Development and in the Mature Animal," *Journal of Lipid Research* 45, no. 8 (2004): 1375–1397.

46. R. Aizen, K. Tao, S. Rencus-Lazar, and E. Gazit, "Functional Metabolite Assemblies—A Review," *Journal of Nanoparticle Research* 20, no. 5 (2018): 125.

47. F. S. Mirnaghi and A. A. Caudy, "Challenges of Analyzing Different Classes of Metabolites by A Single Analytical Method," *Bioanalysis* 6, no. 24 (2014): 3393–3416.

48. A. Gil, D. Siegel, H. Permentier, D.-J. Reijngoud, F. Dekker, and R. Bischoff, "Stability of Energy Metabolites—An Often Overlooked Issue in Metabolomics Studies: A Review," *Electrophoresis* 36, no. 18 (2015): 2156–2169.

49. G. A. Reed, "Stability of Drugs, Drug Candidates, and Metabolites in Blood and Plasma," *Current Protocols in Pharmacology* 75, no. 1 (2016): 7.6.1–7.6.12.

50. J. K. Lukowski, A. Pamreddy, D. Velickovic, et al., "Storage Conditions of Human Kidney Tissue Sections Affect Spatial Lipidomics Analysis Reproducibility," *Journal of the American Society for Mass Spectrometry* 31, no. 12 (2020): 2538–2546.

51. E. K. Neumann, J. F. Ellis, A. E. Triplett, S. S. Rubakhin, and J. V. Sweedler, "Lipid Analysis of 30000 Individual Rodent Cerebellar Cells Using High-Resolution Mass Spectrometry," *Analytical Chemistry* 91, no. 12 (2019): 7871–7878.

52. E. K. Neumann, T. J. Comi, S. S. Rubakhin, and J. V. Sweedler, "Lipid Heterogeneity Between Astrocytes and Neurons Revealed by Single-Cell MALDI-MS Combined With Immunocytochemical Classification," *Angewandte Chemie International Edition* 58, no. 18 (2019): 5910–5914.

53. E. K. Neumann, T. J. Comi, N. Spegazzini, et al., "Multimodal Chemical Analysis of the Brain by High Mass Resolution Mass Spectrometry and Infrared Spectroscopic Imaging," *Analytical Chemistry* 90, no. 19 (2018): 11572–11580.

54. I. Kaya, H. Zetterberg, K. Blennow, and J. Hanrieder, "Shedding Light on the Molecular Pathology of Amyloid Plaques in Transgenic Alzheimer's Disease Mice Using Multimodal MALDI Imaging Mass Spectrometry," *ACS Chemical Neuroscience* 9, no. 7 (2018): 1802–1817.

55. J. Hanrieder, H. Zetterberg, and K. Blennow, "MALDI Imaging Mass Spectrometry: Neurochemical Imaging of Proteins and Peptides," in *Neuroproteomics*, ed. K. W. Li (New York, NY: Springer New York, 2019): 179–197.

56. F. Jia, X. Zhao, and Y. Zhao, "Advancements in ToF-SIMS Imaging for Life Sciences," *Frontiers in Chemistry* 11 (2023): 1237408.

57. S. Mas, R. Perez, R. Martinez-Pinna, J. Egido, and F. Vivanco, "Cluster TOF-SIMS Imaging: A New Light Forin Situmetabolomics?," *Proteomics* 8, no. 18 (2008): 3735–3745.

58. C. S. Clendinen, M. E. Monge, and F. M. Fernández, "Ambient Mass Spectrometry in Metabolomics," *Analyst* 142, no. 17 (2017): 3101–3117.

59. M. Iqfath, S. N. Wali, S. Amer, E. Hernly, and J. Laskin, "Nanospray Desorption Electrospray Ionization Mass Spectrometry Imaging (Nano-DESI MSI): A Tutorial Review," *ACS Measurement Science Au* 4, no. 5 (2024): 475–487.

60. L. D. Manzanares-Meza, C. I. Gutiérrez-Román, and O. Medina-Contreras, "MALDI Imaging: Beyond Classic Diagnosis," *Boletín Médico del Hospital Infantil de México* 74, no. 3 (2017): 212–218.

61. K. Schwamborn, M. Kriegsmann, and W. Weichert, "MALDI Imaging Mass Spectrometry—From Bench to Bedside," *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1865, no. 7 (2017): 776–783.

62. R. S. Borisov, M. D. Matveeva, and V. G. Zaikin, "Reactive Matrices for Analytical Matrix-Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry," *Critical Reviews in Analytical Chemistry* 53, no. 5 (2023): 1027–1043.

63. Z. Yang, Z. Chang, K. Deng, et al., "Reactive Matrices for MALDI-MS of Cholesterol," *Analytical Chemistry* 95, no. 46 (2023): 16786–16790.

64. X. Lin, C. Xiao, L. Ling, L. Guo, and X. Guo, "A Dual-Mode Reactive Matrix for Sensitive and Quantitative Analysis of Carbohydrates by MALDI-TOF MS," *Talanta* 235 (2021): 122792.

65. D. Calderón Guzmán, F. Trujillo Jiménez, E. Hernández García, and H. Juárez Olguín, "Assessment of Antioxidant Effect of 2,5-Dihydroxybenzoic Acid and Vitamin A in Brains of Rats With Induced Hyperoxia," *Neurochemical Research* 32, no. 6 (2007): 1036–1040.

66. J. G. Kiselar and K. M. Downard, "Preservation and Detection of Specific Antibody—Peptide Complexes by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry," *Journal of the American Society for Mass Spectrometry* 11, no. 8 (2000): 746–750.

67. C. C. Shafer and E. K. Neumann, "Optimized Combination of MALDI MSI and Immunofluorescence for Neuroimaging of Lipids Within Cellular Microenvironments," *Frontiers in Chemistry* 12 (2024): 1334209.

68. A. Kulkarni, A. G. Anderson, D. P. Merullo, and G. Konopka, "Beyond Bulk: A Review of Single Cell Transcriptomics Methodologies and Applications," *Current Opinion in Biotechnology* 58 (2019): 129–136.

69. A. Noori, A. M. Mezlini, B. T. Hyman, A. Serrano-Pozo, and S. Das, "Systematic Review and Meta-Analysis of Human Transcriptomics Reveals Neuroinflammation, Deficient Energy Metabolism, and Proteostasis Failure Across Neurodegeneration," *Neurobiology of Disease* 149 (2021): 105225.

70. Y. Zhou, P.-E. Lutz, E. C. Ibrahim, et al., "Suicide and Suicide Behaviors: A review of Transcriptomics and Multiomics Studies in Psychiatric Disorders," *Journal of Neuroscience Research* 98, no. 4 (2020): 601–615.

71. J. Liu, J. Chen, N. Perrone-Bizzozero, and V. D. Calhoun, "A Perspective of the Cross-Tissue Interplay of Genetics, Epigenetics, and Transcriptomics, and Their Relation to Brain Based Phenotypes in Schizophrenia," *Frontiers in Genetics* 9 (2018): 343.

72. L. Larsson, J. Frisén, and J. Lundeberg, "Spatially Resolved Transcriptomics Adds a New Dimension to Genomics," *Nature Methods* 18, no. 1 (2021): 15–18.

73. S. Darmanis, S. A. Sloan, Y. Zhang, et al., "A Survey of Human Brain Transcriptome Diversity at the Single Cell Level," *Proceedings of the National Academy of Sciences* 112, no. 23 (2015): 7285–7290.

74. Q. Weng, J. Wang, J. Wang, et al., "Single-Cell Transcriptomics Uncovers Glial Progenitor Diversity and Cell Fate Determinants During Development and Gliomagenesis," *Cell Stem Cell* 24, no. 5 (2019): 707–723.e8.

75. Q.u. Ain, N. Frei, A.M. Khoshiwal, et al., bioRxiv 2023, 2023.11.02.565001.

76. N. Rao, S. Clark, and O. Habern, "Bridging Genomics and Tissue Pathology," *Genetic Engineering & Biotechnology News* 40, no. 2 (2020): 50–51.

77. A. R. Fernie and M. Stitt, "On the Discordance of Metabolomics With Proteomics and Transcriptomics: Coping With Increasing Complexity in Logic, Chemistry, and Network Interactions Scientific Correspondence," *Plant Physiology* 158, no. 3 (2012): 1139–1145.

78. Z. Wang, H. Shi, S. Yu, et al., "Comprehensive Transcriptomics, Proteomics, and Metabolomics Analyses of the Mechanisms Regulating Tiller Production in Low-Tillering Wheat," *Theoretical and Applied Genetics* 132, no. 8 (2019): 2181–2193.

79. I. Angelidis, L. M. Simon, I. E. Fernandez, et al., "An Atlas of the Aging Lung Mapped by Single Cell Transcriptomics and Deep Tissue Proteomics," *Nature Communications* 10, no. 1 (2019): 1–17.

80. L. Kreutzer, P. Weber, T. Heider, et al., "Simultaneous Metabolite MALDI-MSI, Whole Exome and Transcriptome Analysis From Formalin-Fixed Paraffin-Embedded Tissue Sections," *Laboratory Investigation* 102, no. 12 (2022): 1400–1405.

81. S. H. Boo and Y. K. Kim, "The Emerging Role of RNA Modifications in the Regulation of mRNA Stability," *Experimental & Molecular Medicine* 52, no. 3 (2020): 400–408.

82. X. Ma, P. Chen, J. Wei, et al., "Protocol for Xenium Spatial Transcriptomics Studies Using Fixed Frozen Mouse Brain Sections," *STAR Protocols* 5, no. 4 (2024): 103420.

83. J. Choudhary and S. G. N. Grant, "Proteomics in Postgenomic Neuroscience: The End of the Beginning," *Nature Neuroscience* 7, no. 5 (2004): 440–445.

84. A. Drabik, A. Bierczynska-Krzysik, A. Bodzon-Kulakowska, P. Suder, J. Kotlinska, and J. Silberring, "Proteomics in Neurosciences," *Mass Spectrometry Reviews* 26, no. 3 (2007): 432–450.

85. R. E. Banks, M. J. Dunn, D. F. Hochstrasser, et al., "Proteomics: New Perspectives, New Biomedical Opportunities," *Lancet* 356, no. 9243 (2000): 1749–1756.

86. K. Sharma, S. Schmitt, C. G. Bergner, et al., "Cell Type– and Brain Region–Resolved Mouse Brain Proteome," *Nature Neuroscience* 18, no. 12 (2015): 1819–1831.

87. I. Paul, C. White, I. Turcinovic, and A. Emili, "Imaging the Future: The Emerging Era of Single-Cell Spatial Proteomics," *FEBS Journal* 288, no. 24 (2021): 6990–7001.

88. E. Lundberg and G. H. H. Borner, "Spatial Proteomics: A Powerful Discovery Tool for Cell Biology," *Nature Reviews Molecular Cell Biology* 20, no. 5 (2019): 285–302.

89. W. Schubert, B. Bonnekoh, A. J. Pommer, et al., "Analyzing Proteome Topology and Function by Automated Multidimensional Fluorescence Microscopy," *Nature Biotechnology* 24, no. 10 (2006): 1270–1278.

90. J. Dunne, J. Griner, M. Romeo, et al., "Evaluation of Antibody-Based Single Cell Type Imaging Techniques Coupled to Multiplexed Imaging of N-Glycans and Collagen Peptides by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging," *Analytical and Bioanalytical Chemistry* 415, no. 28 (2023): 7011–7024.

91. M. Machálková, B. Pavlatovská, J. Michálek, et al., "Drug Penetration Analysis in 3D Cell Cultures Using Fiducial-Based Semiautomatic Coregistration of MALDI MSI and Immunofluorescence Images," *Analytical Chemistry* 91, no. 21 (2019): 13475–13484.

92. N. Wang, X. Li, R. Wang, and Z. Ding, "Spatial Transcriptomics and Proteomics Technologies for Deconvoluting the Tumor Microenvironment," *Biotechnology Journal* 16, no. 9 (2021): 2100041.

93. S. Gooding, C.-Y. Wang, W. Baker, et al., "Development of a Spatial Multiomics Platform to Integrate Genomic, Transcriptomic and Proteomic Features for Translational Research in Multiple Myeloma," *Blood* 144 (2024): 6848.

94. R. T. Steven, M. Niehaus, A. J. Taylor, et al., "Atmospheric-Pressure Infrared Laser-Ablation Plasma-Postionization Mass Spectrometry Imaging of Formalin-Fixed Paraffin-Embedded (FFPE) and Fresh-Frozen Tissue Sections With No Sample Preparation," *Analytical Chemistry* 94, no. 28 (2022): 9970–9974.

95. W. Shao, T. Guo, N. C. Toussaint, et al., "Comparative Analysis of mRNA and Protein Degradation in Prostate Tissues Indicates High Stability of Proteins," *Nature Communications* 10, no. 1 (2019): 2524.

96. D. Zhang, Y. Deng, P. Kukanja, et al., "Spatial Epigenome–Transcriptome Co-Profiling of Mammalian Tissues," *Nature* 616, no. 7955 (2023): 113–122.