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TUTORIAL

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SMART: A data reporting standard for mass spectrometry imaging

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Abstract

Mass spectrometry imaging (MSI) is an important analytical technique that simultaneously reports the spatial location and abundance of detected ions in biological, chemical, clinical, and pharmaceutical studies. As MSI grows in popularity, it has become evident that data reporting varies among different research groups and between techniques. The lack of consistency in data reporting inherently creates additional challenges in comparing intra- and inter-laboratory MSI data. In this tutorial, we propose a unified data reporting system, SMART, based on the common features shared between techniques. While there are limitations to any reporting system, SMART was decided upon after significant discussion to more easily understand and benchmark MSI data. SMART is not intended to be comprehensive but rather capture essential baseline information for a given MSI study; this could be within a study (e.g., effect of spot size on the measured ion signals) or between two studies (e.g., different MSI platform technologies applied to the same tissue type). This tutorial does not attempt to address the confidence with which annotations are made nor does it deny the importance of other parameters that are not included in the current SMART format. Ultimately, the goal of this tutorial is to discuss the necessity of establishing a uniform reporting system to communicate MSI data in publications and presentations in a simple format to readily interpret the parameters and baseline outcomes of the data.

KEYWORDS
data reporting standards, mass spectrometry imaging

1 | INTRODUCTION

Mass spectrometry imaging (MSI) allows for visualizing the spatial distribution and abundances of ions across an entire sample, which can provide insights into their biological roles and associated pathways. The MSI field has witnessed the growth of various techniques in the past years, including the continued development of laser-based ionization strategies. Among them, matrix-assisted laser desorption/ionization (MALDI),[1,2] laser ablation electrospray ionization (LAESI),[3,4] and infrared matrix-assisted laser desorption electrospray ionization

(IR-MALDESI)[5,6] employ an ultraviolet (UV) or infrared (IR) laser to ablate the sample for subsequent ionization. Non-laser-based methods collect imaging data in a similar fashion and include desorption electrospray ionization (DESI)[7] that sprays charged solvent onto a sample surface to desorb and charge the analytes, probe electrospray ionization (PESI)[8] that immerses the needle's tip into the sample to capture the components and then creates a miniaturized electrospray by applying a high voltage, and secondary ion mass spectrometry (SIMS) that applies an accelerated beam of primary ions directly to the sample for molecule ionization.[9] Despite their shared attributes, the critical parameters common to MSI techniques are defined ambiguously and reported differently across various platforms, creating unnecessary challenges when comparing methods. Additionally, studies often involve multiple samples and various sample preparation methods; thus, it is imperative to list the fundamental information relevant to all MSI experimental measurements, such as mass measurement accuracy (MMA) and duration of data acquisition, for easy understanding and comparison of experimental results within and between studies. Developing a standardized reporting protocol for MSI data can facilitate worldwide communication and avoid developing multiple confounding systems that could exacerbate the issue.[2]

Previous publications have suggested thorough data reporting guidelines for comparing MSI data in the clinical and pharmacological field and multicenter studies.[10,11] Although the parameters in prior guidelines should be reported in manuscripts,[10] this extent of detail may be difficult to include and interpret in a presentation format and may inhibit efficient scientific communication. A simplified reporting standard with essential MSI metrics may allow easier comprehension for both an experienced MS imaging scientist and a general audience member alike.

Herein, we propose the use of a standardized "key" for reporting MSI data: SMART. This acronym can be used as a marginal note next to the figures for brevity and ease of understanding. The recommendation for these unified criteria is to present essential information in a concise format. This also provides a simple and semi-quantitative way to compare different MSI approaches and sample preparative methods. However, we fully expect that these minimal reporting standards will evolve along with the MSI field.

2 | SMART TERMINOLOGY AND DEFINITIONS

Each parameter in the initial SMART data reporting metrics will be thoroughly discussed below regarding their definition and significance separately. Figure 1 presents an example of an ion image with the associated SMART aspects.

2.1 | S: Step size; spot size; scan total

Step size is the distance that the sampling stage moves between adjacent pixels in the *x* and *y* directions. Alternatively, spot size is defined as the effective diameter of the ablation or desorption area which results from using a given MSI approach. Some groups report the smallest achievable spot size as their spatial resolution without considering the stage movement, whereas other groups define their spatial resolution as the step size since spatial resolution can be improved by adapting a step size smaller than the spot size (i.e., oversampling[14]). To our knowledge, spot size would likely be inconsistent on different tissue types and/or different matrices,[15] and step size can be tailored to the scope of studies.[16] Therefore, we recommend clarifying and reporting both spot size and step size with ion images for pulsed imaging studies. In the case of continuous MSI, spot size can be disregarded, and only pixel/step size be inferred and reported. Should a top-hat or other type of beam shape be used in a study, we suggest using parenthesis with this metric to specify this information.

The "S" term can be further described as the number of total scans collected in a region of interest (ROI) and is noted here as "scan

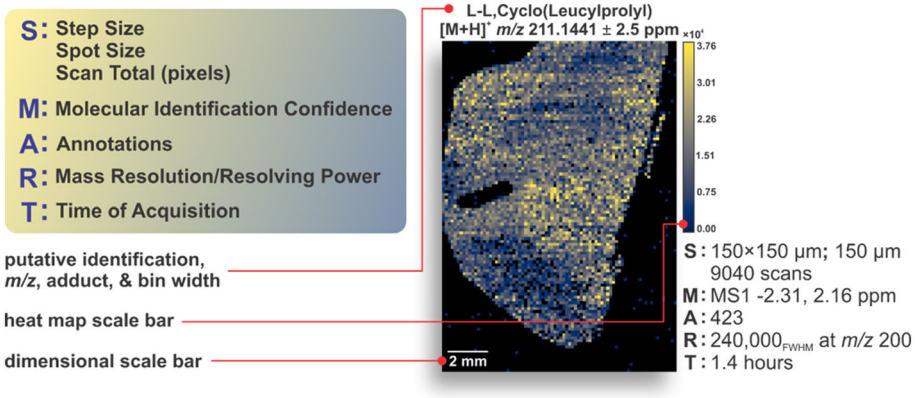

FIGURE 1 An overview of the meaning of each letter in SMART. The ion image example on the right was generated from MSiReader[12,13] using the *cividis* colormap with the relevant experimental parameters. The putative identification, the detected adduct, the respective *m/z* with the bin width, heatmap scale bar, and dimensional scale bar were shown accordingly. No normalization techniques (e.g., global or local TIC) were applied to this ion image. This experimental example was a part of amyotrophic lateral sclerosis patient's brain analyzed by IR-MALDESI MSI. The data are available publicly here: https://metaspace2020.eu/project/SMART_Tutorial_2022.

total." ROI is the sample area that researchers are interested in investigating for a particular purpose and is conventionally defined as the number of scans or pixels sampled. For the purposes of this definition, a scan will be counted with the assumption that there was only one scan collected per *XYZ* location. Specifying the number of scans within an ROI demonstrates the scale of the study and aids statistical comparison of two or more sampled ROIs containing different numbers of scans.

2.2 | M: Molecular identification confidence

"M" generally refers to molecular identification (ID) confidence,[17] which can be representative of a few potential parameters, such as the following: as the range of experimental MMA when molecule identification is based on MS1 data, as MS/MS when MS2 experiments are done for structure validation, as reference standard when appropriate, or as the experimental drift time or collision cross section (CCS) value or ΔCCS when the ion mobility technique adds a separate dimension to further improve the identification confidence. Other analytical techniques for molecular identification, including but not limited to NMR and optical spectroscopy, should also be stated in "M."

MMA, also termed "mass measurement error," denotes the difference between the experimentally determined mass and the theoretical mass of a given ion.[18] The MMA is calculated using Equation (1):

$$MMA = \frac{(m_i - m_a)}{m_a} \times 10^6 \, (ppm) \tag{1}$$

where m_i is the experimental measured m/z of an ion and m_a is the theoretical m/z of the measured ion and is usually reported in parts-per-million (ppm). The MMA range based on MS1 data contains the range of all MMA values of the given ion in each pixel on the ion image. It gives an overview of the MS data quality and also reflects the instrument condition when combined with the mass resolving power for "R". For example, if a $240\,000_{FWHM}$ resolving power is applied but MMA values are above 10 ppm, this may imply that the instrument is not operating under the optimal conditions. When annotations are assigned by matching the measured m/z with a theoretical m/z of a molecule ion in the database, the MMA range serves as a basis for setting the searching threshold, as the threshold should contain the MMA range and be stringent enough to reduce false positive identifications.

An alternative or supplemental option for the "M" metric can be to specify that MS/MS, otherwise called MS2 or tandem MS, is performed on the m/z of interest. Tandem MS is a technique to record spectra of fragment (also referred to as product) ions resulting from the dissociation of selected precursor ions[19] with the intent to elucidate probable structural features of the precursor ions. As not a single value can specifically represent MS/MS, it is recommended to indicate "MS/MS" for the letter "M" in SMART and include annotated MS/MS spectra alongside the publication. We offer an example in Figure S1, where two ion images of lipids derivatized with di-cationic ions in mouse liver from a previous study[20] are shown with their respective SMART metrics. "MS/MS" is added to the "M" parameter and is accompanied by annotated MS^2 spectra, where all fragments are reported with their mass-to-charge ratio, chemical formula, and adduct detected.

Should ion mobility measurements be collected to improve the confidence of identifications, we suggest that the experimental drift time or CCS value or ΔCCS value be reported for this parameter. In ion mobility-mass spectrometry experiments, CCS measurements reflect an ion's rotationally averaged cross sectional area based on its interaction with the neutral gas when traveling through a drift tube.[21–23] One way that CCS values can be calculated uses the Mason-Schamp Equation (2):

$$CCS = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_b T)^{1/2}} \left[\frac{1}{m_i} + \frac{1}{m_b}\right]^{1/2} \frac{t_A E}{L} \frac{760}{P} \frac{T}{273.15} \frac{1}{N}. \tag{2}$$

This physiochemical property of an analyte provides a complementary descriptor for more confident identifications and is usually coupled with the m/z ratio of the ion and/or the following MS/MS fragmentation. ΔCCS is the difference between the experimental and theoretical CCS values, which can be obtained from databases or from running a reference standard under the identical experiment operation.

2.3 | A: Annotations

This metric refers to the number of analytes, or ions-of-interest, detected, and tentatively annotated in each sample with confirmed isotope spectral accuracy. When other orthogonal analytical methods are applied to identify and confirm molecular identifications, the way of counting IDs for "A" depends on the researchers' choice but should be consistent within a publication to avoid any confusion. Should the researcher elect to report the number of annotations detected by either an MSI technique or by an orthogonal method, the basis of these annotations should be reported in the figure caption or manuscript (i.e., the number of annotations reported in the SMART metric results from analytes detected by orthogonal methods). Further, any methods of filtering or post-processing annotations should be mentioned in the publication. As opposed to the number of annotations in untargeted/discovery studies, "targeted" may be used for "A" in targeted analyses. Additionally, imaging data should be made publicly available in repositories such as METASPACE[24] and PRIDE[25] with the requested referencing format or statement.

2.4 | R: Mass resolution/resolving power

Mass resolving power and mass resolution are often used interchangeably in the field of mass spectrometry. In accordance with IUPAC,[19] mass resolution in a mass spectrum is expressed as Equation (3):

$$Mass\ Resolution = \frac{m}{\Delta m}, \tag{3}$$

where m is the measured m/z of the ion of interest and Δm is often the peak width measured at a specific fraction of the maximum peak height. The full width at half (50%) maximum (FWHM) definition is commonly used, though the mass resolution may be defined at other percentages as well. The definition of mass resolving power is the measure of the ability of a mass spectrometer to provide a specified value of mass resolution,[19] indicating its capability to resolve adjacent peaks at a specified m/z. For example, the instrument setting of Thermo Scientific Orbitrap Exploris 240 Mass Spectrometer includes resolving power of $240\,000_{FWHM}$, $120\,000_{FWHM}$, and $60\,000_{FWHM}$ at m/z 200, which can resolve peaks at different levels.[26] The Agilent 6546 LC/Q-TOF is able to reach over 60 000 mass resolution for high masses (m/z 1521).

Because a high mass resolution/resolving power mass spectrometry is necessary to distinguish isobaric species more confidently, mass resolution/resolving power at which the experiment is conducted is a critical parameter to report. Therefore, we highly recommend reporting the operating mass resolution/resolving power that is applied in the MSI experiment; the researcher can also report the mass resolution of a peak in the experimental mass spectra when the instrument setting of resolution/resolving power is not accessible.

2.5 | T: Time of acquisition

The time of acquisition is the duration of time required to collect the respective information within the specified ROI (i.e., mass spectra in each scan/pixel). This metric is impacted by the ion source, mass resolution, stage characteristics, and the focus of the study. Generally, a larger-sized sample, a higher spatial resolution, and/or a higher mass resolution extend the duration of a study.[28] Further, a 3D imaging experiment inherently demands more time than 2D imaging as it requires sampling multiple layers or sections. Knowing how the time of acquisition is affected by different parameters helps readers select the proper method within their time constraints.

2.6 | Other considerations for reporting imaging data

Although the SMART acronym encapsulates the most notable figures of merit for imaging data, it is not all-encompassing. There are several other aspects of data reporting that should remain consistent throughout the field that were not included within the current scope of SMART. In the following discussion, we would like to mention these aspects that are crucial to avoid misinterpretation of data.

All ion images should be presented with the respective m/z value(s) and the mass spectral bin width or bin size, for example, "m/z = 758.5694 ± 0.002 Da" or "m/z = 211.1441 ± 2.5 ppm." The mass precision of m/z values, that is, the number of decimal places of the m/z values, is up to the mass resolving power/resolution of the instrument allows. The bin width is the integration window on the m/z scale for displaying ion signals.[1] Abundances of any peak(s) in the integration range will be accounted for the resulting ion abundance of the given m/z. The bin width also has an impact on the experimental MS peak resolution. Therefore, it is important to choose and show an appropriate bin width at the m/z values for MSI data. More, the putative identity of the analyte and its associated adduct ($[M + H]^+$, $[M + Na]^+$, etc.) should also be noted either alongside the image or in the text (examples shown in Figures 1, S1, and S2).

While the number of pixels is stated in the "S" term as previously discussed, the dimensional scale bars should also be added on the ion image. In addition to dimensional scale bars, each ion image should be coupled with heatmap scale bars in a linear, color vision deficiency (CVD)-compliant color scheme. Although rainbow color maps (e.g., *jet*) are esthetically pleasing and commonly used in MSI publications, these should be substituted for scientifically derived color maps (e.g., *cividis*, *magma*, *inferno*, and *viridis*) to avoid data misinterpretation.[29–31] Additionally, we would suggest stating any accompanying normalization strategy and data post-processing information with the ion image (e.g., Normalized to TIC) if applicable. Other data processing methods to report may include but are not limited to the minimum or maximum abundance threshold cutoff and hot spot removal percentage. Including these measures in conjunction with SMART metrics will help to accurately communicate the size of the sample and correctly display the ion abundances of the analyte(s) presented in an ion image.

3 | CONCLUSION

A standardized reporting system, SMART, was defined to improve the consistency of MSI data reporting among research groups. The purpose of SMART is to share fundamental experimental information in scientific publications and presentations. We fully expect that SMART is a starting point and that this concept will evolve over time as the MSI field evolves. We anticipate that more researchers in the MSI field will join the discussion and contribute to the future community data reporting system.

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REFERENCES

1. Römpp A, Spengler B. Mass spectrometry imaging with high resolution in mass and space. *Histochem Cell Biol*. 2013;139(6):759-783. doi:10.1007/s00418-013-1097-6
2. Buchberger AR, DeLaney K, Johnson J, Li L. Mass spectrometry imaging: a review of emerging advancements and future insights. *Anal Chem*. 2018;90(1):240-265. doi:10.1021/acs.analchem.7b04733
3. Nemes P, Vertes A. Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry. *Anal Chem*. 2007;79(21):8098-8106. doi:10.1021/ac071181r
4. Nemes P, Barton AA, Li Y, Vertes A. Ambient molecular imaging and depth profiling of live tissue by infrared laser ablation electrospray ionization mass spectrometry. *Anal Chem*. 2008;80(12):4575-4582. doi:10.1021/ac8004082
5. Bokhart MT, Muddiman DC. Infrared matrix-assisted laser desorption electrospray ionization mass spectrometry imaging analysis of biospecimens. *Analyst*. 2016;141(18):5236-5245. doi:10.1039/C6AN01189F
6. Bagley MC, Garrard KP, Muddiman DC. The development and application of matrix assisted laser desorption electrospray ionization: the teenage years. *Mass Spec Rev*. 2021;42(1):mas.21696. doi:10.1002/mas.21696
7. Yin R, Burnum-Johnson KE, Sun X, Dey SK, Laskin J. High spatial resolution imaging of biological tissues using nanospray desorption electrospray ionization mass spectrometry. *Nat Protoc*. 2019;14(12):3445-3470. doi:10.1038/s41596-019-0237-4
8. Chen LC, Yoshimura K, Yu Z, et al. Ambient imaging mass spectrometry by electrospray ionization using solid needle as sampling probe. *J Mass Spectrom*. 2009;44(10):1469-1477. doi:10.1002/jms.1632
9. Tuck M, Blanc L, Touti R, et al. Multimodal imaging based on vibrational spectroscopies and mass spectrometry imaging applied to biological tissue: a multiscale and multiomics review. *Anal Chem*. 2021;93(1):445-477. doi:10.1021/acs.analchem.0c04595
10. McDonnell LA, Römpp A, Balluff B, et al. Discussion point: reporting guidelines for mass spectrometry imaging. *Anal Bioanal Chem*. 2015;407(8):2035-2045. doi:10.1007/s00216-014-8322-6
11. Römpp A, Both JP, Brunelle A, et al. Mass spectrometry imaging of biological tissue: an approach for multicenter studies. *Anal Bioanal Chem*. 2015;407(8):2329-2335. doi:10.1007/s00216-014-8410-7
12. Robichaud G, Garrard KP, Barry JA, Muddiman DC. MSiReader: an open-source Interface to view and analyze high resolving power MS imaging files on Matlab platform. *J am Soc Mass Spectrom*. 2013;24(5):718-721. doi:10.1007/s13361-013-0607-z
13. Bokhart M, Nazari M, Garrard KP, Muddiman DC. MSiReader v1.0: evolving open-source mass spectrometry imaging software for targeted and untargeted analyses. *J am Soc Mass Spectrom*. 2018;29(1):8-16. doi:10.1007/s13361-017-1809-6
14. Jurchen JC, Rubakhin SS, Sweedler JV. MALDI-MS imaging of features smaller than the size of the laser beam. *J am Soc Mass Spectrom*. 2005;16(10):1654-1659. doi:10.1016/j.jasms.2005.06.006
15. Kibbe RR, Mellinger AL, Muddiman DC. Novel matrix strategies for improved ionization and spatial resolution using IR-MALDESI mass spectrometry imaging. *J Mass Spectrom*. 2022;57(8):e4875. doi:10.1002/jms.4875
16. Müller MA, Kompauer M, Strupat K, Heiles S, Spengler B. Implementation of a high-repetition-rate laser in an AP-SMALDI MSI system for enhanced measurement performance. *J am Soc Mass Spectrom*. 2021;32(2):465-472. doi:10.1021/jasms.0c00368
17. Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA. Untargeted metabolomics strategies—challenges and emerging directions. *J am Soc Mass Spectrom*. 2016;27(12):1897-1905. doi:10.1007/s13361-016-1469-y
18. Brenton AG, Godfrey AR. Accurate mass measurement: Terminology and treatment of data. *J am Soc Mass Spectrom*. 2010;21(11):1821-1835. doi:10.1016/j.jasms.2010.06.006
19. Murray KK, Boyd RK, Eberlin MN, Langley GJ, Li L, Naito Y. Definitions of terms relating to mass spectrometry (IUPAC recommendations 2013). *Pure Appl Chem*. 2013;85(7):1515-1609. doi:10.1351/PAC-REC-06-04-06
20. Xi Y, Muddiman DC. Enhancing Metabolomic coverage in positive ionization mode using Dicationic reagents by infrared matrix-assisted laser desorption electrospray ionization. *Metabolites*. 2021;11(12):810. doi:10.3390/metabo11120810
21. Guntner AS, Thalhamer B, Klampfl C, Buchberger W. Collision cross sections obtained with ion mobility mass spectrometry as new descriptor to predict blood-brain barrier permeation by drugs. *Sci Rep*. 2019;9(1):19182. doi:10.1038/s41598-019-55856-7
22. Paglia G, Angel P, Williams JP, et al. Ion mobility-derived collision cross section as an additional measure for lipid fingerprinting and identification. *Anal Chem*. 2015;87(2):1137-1144. doi:10.1021/ac503715v
23. Stow SM, Causon TJ, Zheng X, et al. An Interlaboratory evaluation of drift tube ion mobility–mass spectrometry collision cross section measurements. *Anal Chem*. 2017;89(17):9048-9055. doi:10.1021/acs.analchem.7b01729
24. Palmer A, Phapale P, Chernyavsky I, et al. FDR-controlled metabolite annotation for high-resolution imaging mass spectrometry. *Nat Methods*. 2017;14(1):57-60. doi:10.1038/nmeth.4072
25. Perez-Riverol Y, Bai J, Bandla C, et al. The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Res*. 2022;50(D1):543-552. doi:10.1093/nar/gkab1038
26. Cooper J, Roberts D, Cojocariu C. *Mass resolving power of 240,000: For confident compound identification; TN10730-EN 0720S*; Thermo Fisher Scientific Inc.: Runcorn, UK; 2020. Accessed October 14, 2022. http://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-10730-gc-ms-power-confident-compound-identification-tn10730-en.pdf
27. Yannell KE, Klein C, Zamboni N. *Agilent 6546 LC/Q-TOF: Gaining Higher Confidence and Throughput in Metabolite Analysis; 5994-0724EN*; Agilent Technologies, Inc.: Santa Clara, CA, USA; 2019. Accessed October 14, 2022. https://www.agilent.com/cs/library/technicaloverviews/public/technicaloverview-6546-q-tof-metabolomics-5994-0724en-agilent.pdf
28. Prentice BM, Caprioli RM. The need for speed in matrix-assisted laser desorption/ionization imaging mass spectrometry. *Postdoc J*. 2016;4(3):3-13. doi:10.14304/SURYA.JPR.V4N3.1
29. Knizner KT, Kibbe RR, Garrard KP, Nuñez JR, Anderton CR, Muddiman DC. On the importance of color in mass spectrometry imaging. *J Mass Spectrom*. 2022;57(12):e4898. doi:10.1002/jms.4898
30. Crameri F, Shephard GE, Heron PJ. The misuse of colour in science communication. *Nat Commun*. 2020;11(1):5444. doi:10.1038/s41467-020-19160-7
31. Nuñez JR, Anderton CR, Renslow RS. Optimizing colormaps with consideration for color vision deficiency to enable accurate interpretation of scientific data. *PLoS 1*. 2018;13(7):e0199239. doi:10.1371/journal.pone.0199239

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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