Qeios

Peer Review

Review of: "A Computer Simulation Study on Ion Optics Aiming at the Realization of Projection-Type Mass Spectrometry Imaging"

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Y. Naito proposed the rationalization of ion trajectories using SIMION for the future development of projection mode in mass spectrometry imaging, an emerging technique and one of the very last instrumental developments of MALDI TOF instruments.

This is an interesting initiative and merits being published.

Nevertheless, I think that several points need to be addressed before publishing.

N.B: I rated 3 stars for the current draft, and it will likely gain at least 1 more star for the improved next version of the draft.

In my opinion, the current version of the article is lacking in pedagogy.

For example, in the introduction, the author assumes that any reader is aware of the projection mode.

To fix it, I think the author should briefly introduce the mass spectrometry imaging technique, what the projection mode does, and rework Figure 1 accordingly, stipulating that the squared area represents the spatial distribution (at micrometric scale) of the ions in a single pixel (laser shot), then a magnification step of the spatial distribution by making diverging the ion beam, measuring the time of flight, and finally using a multichannel detector plate that can retrieve the (X,Y) spatial distribution of the ions from the single pixel of the target plate.

Also, Figure 1 showed larger m/z peak widths when the m/z ratio increased. In theory, the mass resolving power of the time of flight is almost unaffected by the increasing m/z value, contrary to the

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FTMS instruments such as Orbitrap and FT-ICR instruments. I think that you should mention or explain that it was due to, e.g., energy dispersion, lack of kinetic energy focalization in the absence of pulse delay extraction, and the use of the post extraction differential acceleration (PEDA), or any other realistic explanation.

Figures 3 and 4 deserve to be annotated, and a correspondence of the different sections of Figure 4 should be highlighted in regard to Figure 3.

I understand the challenge and the computational cost of modeling more ion paths. Nevertheless, I think it could be useful to simulate how a full mass spectrum of, e.g., some lipids (let's say couples of PC, PE, and isobar ions of PC/PE, PS, SM, MG-DG-TG, and add some -C2H4- and -C2H2- units, typical for lipid congeners) would look after being submitted to PEDA. I would recommend the use of the toolbox from, e.g., the lipidmaps website for this purpose (https://lipidmaps.org/).

Lipids are the most investigated compounds by MALDI mass spectrometry imaging because of their high abundance in cell membranes and the vast literature about sample prep, matrix deposition, and instrument set-up.

When I checked your Figure 15, I estimated the peak widths of m/z 340 and 460 to be slightly higher than 2 ns. According to Figure 3 from J. Am. Soc. Mass Spectrom. 2024, 35, 5, 992−998 (https://doi.org/10.1021/jasms.4c00018), one can deduce that the delta time of flight of a modified first gen Ultraflex would be around 2 to 6 ns.

I think it would be interesting to evaluate if the peak annotation of lipid m/z peaks (in terms of mass accuracy and mass resolving power) is still guaranteed in the context of projection mode MSI experiments, at least according to this modeling study of projection mode MSI.

I am mentioning it in view of your Figures 11 to 14, which are the predictions of the m/z peak shapes from the TOF mass analyzer in the mass region of actually m/z 100 to 1600, which is typically the mass range used for mass spectrometry imaging of lipids.

Figures 7 to 10: I am not sure that a vertical layout of the 3D plots is the best. A panel or mosaic should be more appropriate (to be tested).

The current draft is also lacking concluding remarks. In my opinion, several critical points need to be briefly discussed:

1/ The projection mode mass spectrometry imaging assumes the retention of the spatial distribution of the ions on the desorbed pixel, i.e., the spatial localization of ions is unaffected during the

desorption and ionization steps.

For secondary ion mass spectrometry (SIMS) and its related method nanoSIMS, this should be more likely true. Depending on the type of substrates, I think this postulate is reasonably true during surface-assisted laser desorption ionization experiments (SALDI) if one can prove that there is no ion cloud expansion during the desorption steps of SALDI.

Nevertheless, for matrix-assisted laser desorption ionization experiments (MALDI), I do not think that this assumption is still true, due to the ions cloud expansion also known as "the MALDI plume." To date, it is not clear yet if this plume expansion is homogeneous and if the plume expansion is isotropic (at least in the meaning of isotropic expansion in the directions of the electric field and its orthogonal axis used for ions extraction).

Additionally, depending on the "hardness" of the MALDI matrix, a significant amount of ion-neutral collisions would occur. Moreover, the dissociation of matrix clusters could release the ions when colliding at a different apparent spatial localization than where it was coming from.

As a consequence, one can imagine that there could be a significant scrambling concerning the retention of the spatial distribution of the ions in the MALDI pixel image.

In this regard, I think that the author should critically discuss in the conclusion the field of validity of the very first postulate concerning the retention of the spatial distribution within the pixel where the ions are submitted to desorption.

In fact, I think that such a modified instrument and the modeling of ions trajectories is a great opportunity to go experimentally further and deeper into the mechanisms involved during the desorption and ionization steps of ions inside the MALDI plume, and a good opportunity to determine if SALDI also produces some "plumes" and ion cloud expansion or not, i.e., revisiting again the fundamentals of MALDI and SALDI.

2/ What would/could be the influence of laser energy distribution when projection mode MSI is used? How can we anticipate the laser energy dispersion to (negatively) affect the final ion distribution on the detector?

Also, what would be the effect of the initial velocity of ions after the desorption and ionization? (For example, see Asakawa and coworkers, IJMS (2013) pages 29-33, Influence of initial velocity of analytes on in-source decay products in MALDI mass spectrometry using salicylic acid derivative matrices, https://doi.org/10.1016/j.ijms.2012.12.008)

I would imagine that it would negatively/positively affect the magnification factor of the instrument?

The same question could be raised for the in-source decay fragments that will be obtained and have different velocities and different spatial distributions in the MALDI plume?

3/ Gaining about 50 times magnification of the spatial distribution of the ions would make sense in the context of the use of, e.g., the MicroGrid (Bruker). Indeed, MicroGrid theoretically offers the capability of 5µm lateral resolution of MALDI MS images. Gaining this factor of 50 would theoretically allow reaching a final equivalent lateral resolution of the image of 0.1µm (50µm/50), allowing the discrimination of the co-localization of ions at the sub-compartments level of a cell… A major breakthrough for MALDI MSI if it could be done.

I do not require that all of these points be answered, but at least critically assessed and to make the community aware of the future challenges of projection mode MSI.

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Declarations

Potential competing interests: No potential competing interests to declare.