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

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Potential competing interests: No potential competing interests to declare.

General comments:

This manuscript presents simulated results of projection-type mass spectrometry imaging using a modelled commercially-available system and reports on the utility of using PEDA for improved mass resolution in this simulated system. The paper makes use of SIMION for the simulations. Similar simulations have been demonstrated using SIMION to some success, and the reinvigoration of projection-type imaging MSI requires such novel simulation work. Although I appreciate the article's topic and results, I find some general areas that can be improved in this article's current form:

- The introduction and discussion of the topic of MSI are lacking in nuance and are, in some cases, incorrect.
- Although citations do not always need to be recent, there have been some advancements in both the MSI field as well as projection-based MSI in the last 5-8 years. Citing these advancements may be helpful for readers interested in the field's impact and potential for growth. MSI advancements that may be of value to include are the improvements in TOF-based MSI instruments' mass resolution (both generally as well as for multi-reflection TOF), improvements in spatial resolution (Some groups have pushed some forms of Nano-SIMS like imaging beyond 50 nm pixels, and MALDI has been demonstrated at sub-1 micrometer pixel sizes). Projection-mode MSI improvements that could be mentioned are improvements in detectors, field-of-view size, and throughput.
- It is not clear why some of the simulated variables were given such high significance compared to other, possibly more experimentally relevant variables, such as the precise documentation and control of ion velocity but not kinetic energy, and why so many different masses were simulated but the einzel lens voltage was not adjusted. A holistic explanation of the reasoning behind the choices of which variables to simulate would be appreciated.

Many of the specific comments below discuss these points.

Specific comments:

1. Page 1, Abstract: ", then projecting them onto an ion detector of timeof-flight mass spectrometry." This is incomplete, as magnetic sector-based projection mode mass spectrometry was the initial and a popular form of projection mode MSI. (https://doi.org/10.1002/jms.4800)

2. Page 1, Abstract: "Compared to conventional MSI, projection-type MSI can deliver overwhelmingly higher spatial

resolution.” This is ambiguous. If “spatial resolution” means “pixel count,” then I partially agree, as projection-type MSI can be faster than conventional MSI; although pixel count is image/experiment specific. If “spatial resolution” means “ability to see spatial details,” then this is incorrect, as NanoSIMS is capable of ~50 nm pixel sizes, whereas the highest resolution projection/stigmatic/mass microscopy technique that I am aware of has demonstrated only imaging resolutions down to ~110 nm (https://doi.org/10.1063/1.337240). Please clarify.

3. Page 1, I suggest citing a recent review article or two to help readers find pharmaceutical and semiconductor applications of MSI.

4. Page 1, I suggest clarifying at the first mention what is meant by “focused-beam.” E.g., adding something like “focused ion or laser beam.”

5. Page 2, I’d suggest changing the “10 µm” to “5 µm,” as the Bruker microGRID is a commercial technology that can image at 5 µm. Mention of 1 µm MALDI could also be helpful, as transmission-mode MALDI can achieve smaller than 1 µm spot sizes for some time now (https://doi.org/10.1038/s41592-019-0536-2).

6. Page 2, “In other words, projection-type MSI can be realized only with time-of-flight mass spectrometry (TOFMS)”, see specific comment #1. This is incorrect as magnetic sector-based projection MSI is also possible and indeed was the first form of MSI.

7. Page 3, “Although the concept of projection-type MSI was proposed a long time ago [12]” Reference 12 is not incorrect but omits a large body of work prior to 1992 (from 1962 onwards). I suggest adding earlier references or clarifying that by “projection-type MSI” it refers only to TOF-based MSI. By stating “Although the concept of TOF-based, projection-type. . .”

8. Page 4, “no commercially available equipment has emerged or become widespread to date.” It might be worthwhile noting the TRIFT style TOF-based stigmatic/projection-style mass spectrometers that were commercially available but are not currently available with projection-style modes of imaging.

9. Page 4, “Relying solely on TOFMS would be a major disadvantage in terms of analytical specificity” This sentence is not exactly clear in context. Possibly adding some qualifiers or restating it could improve clarity. I also think it could be removed without affecting the meaning of the surrounding text.

10. Page 8, I am confused by the numbers regarding the ions and request some clarification. If I understand correctly, there were 4 groups of ions (100 Da, 400 Da, 900 Da, and 1600 Da) but then it is said that, “to investigate the influence due to small differences in mass within each mass region, six different masses were added in the range of ±30 Da to ±120 Da for each mass region.” I do not understand this, as 100 Da couldn’t have -120 Da added to it (it would be negative). Were the additional masses chosen randomly/pseudorandomly, or were they distributed evenly? It then says that there were 28 masses, with which I have difficulty aligning the math (4 groups x 6 is 24, so were 6 ions added to each group, making it 4 x 7?). Honestly, this is all quite confusing. To add to my confusion, Table 1 only shows 20 velocities (not 28). I would suggest removing the confusing description, adding all 28 mass values as well as 28 velocities (or 20 velocities if 28 masses were flown at 20 velocities each) to Table 1 so that they are easy to see, and just saying “we chose

the mass values shown in Table 1 for testing."

11. Page 8, Table 1, please add another column with the sample/extraction voltage. Or mention the sample/extraction voltage in the table if it is constant.

12. Page 8, Table 1, how is Velocity "Defined as kinetic energy for each mass"? This table note confuses me, as KE depends on both mass and velocity squared. I might just remove the table note or clarify what is meant, as I assumed I understood "Velocity" without the table note but am confused by it, as it seems unnecessary to define what velocity is.

13. Page 8, "The magnitudes of the initial ion velocities were set to 20 levels in the range of 439–1965 m s−1 equally for all masses." This is very confusing; why were velocities specified at all? Shouldn't they either be generated by initial voltage settings or be assumed based on a distribution if the initial ejection is what is being modeled? I suggest clarifying - possibly clarification won't be needed if clarification to the general discussion of KE/V is provided in response to another comment.

14. Page 8, " In SIMION, initial conditions of ion motion are set by kinetic energy rather than velocity." I am unsure if this is true of SIMION 7, but this is not true of SIMION 8, as the ions can be set in one of four velocity formats (Velocity vector, Direction + KE, Direction + Speed, and Direction + Momentum). Also, this adds to my confusion, as the default ability to set ions by kinetic energy appears to me to be more experimentally relevant than to set ions' initial velocities. So it appears that slightly more work was performed to enable slightly less experimentally-relevant results – briefly explaining this discrepancy would improve the paper's clarity.

15. Page 8, "Therefore, the start value and increment of the initial kinetic energies within a definition group of ions were adjusted according to mass to fulfill the same initial velocity range for all groups. For example, the start value and increment were 0.07 eV for 70 Da, and for 1720 Da they were 1.72 eV." Please provide a bit more explanation as to why keeping velocities identical per group was done rather than kinetic energies (as in a "real" TOF, velocities are not able to be controlled on a per-mass basis, only total imparted kinetic energy).

16. Page 11 and Figure 6 C-E, it is stated, "Figures 6C–6E show enlarged views of the vicinity of the potential array instance corresponding to the ion detector." Please provide a scale bar on Figures 6C-E, as these images are lacking recognizable detail that allows a reader to understand the scale of the images. Also, please provide the amount of focus (in units of length) rather than relative observations (e.g., how much more focused is 6D than 6C/E?). Additionally, please specify what is considered successful point convergence (e.g., 1 nm radius or some other metric) and why successful point convergence is a hallmark of successful projection/image formation. Adding a citation on this last point that provides more information/theory would be helpful.

17. Page 11, "It is considered that the electrostatic lens formed by the target plate and the extract electrode has the ion optical aberration of a substantial magnitude." Please comment on what the specific cause of this ion optical aberration may be. Please discuss whether using a smaller simulation voxel size would help reduce this ion optical aberration or if additional optics would be needed for corrections. Is the grounded Einzel lens able to correct for this, or does it contribute meaningfully one way or another?

18. Figures 7-10 are all somewhat similar, and the specific details of each individual plot do not appear to be as important as the changes between plots. Consider combining them into a single full-page figure (possibly with smaller individual plots) where each current figure is a “column” so that the combined single figure is composed of 4 columns and 7 rows. This will make visual comparisons slightly easier for readers.

19. Figure 11, as Figure 11 is essentially a straight line, consider removing this figure and just stating that there is a very high correlation (R2 0.9999) and the equation of the line in text.

20. Page 22, I do not feel that the graphics in Figure 15 or the paragraph discussing SIMION’s simulation quality factor are of interest or relevance for this study. These can be omitted and replaced with a single sentence summarizing that no significant quality change was observed for higher SIMION simulation quality settings.