Answer for reviewers' comments

Respond for RC1:

Q1: It is awkward that the single-particle MS used in this study ("Bio-SPAMS") is a new instrument but is not fully described here, nor referenced. "Bio-SPAMS used in this study is not the same as Du et al.'s HP-SPAMS, and a different laser is employed." Can you add to that basic description? Presumably this instrument is designed to detect (large) biological particles?

A1: We thank the reviewer for the careful examination of the paper. To enhance the readability of the article, the author added a description in Section 2.3 regarding the optical design of Bio-SPAMS, highlighting the differences between the Bio-SPAMS used in this study and HP-SPAMS, particularly its biofluorescence detection capability. The details are as follows "Additionally, Bio-SPAMS introduces a laser-induced biofluorescence detection module, enabling the pre-screening of fluorescent particles. This allows for selective ionization of only the fluorescent particles, meeting the requirements for online monitoring of bioaerosols."

Due to Bio spams being a newly designed instrument, relevant articles are still being written

Q2: Fig 1. Add units to caption. Also, I think most small TSI OPC models use a fixed 2.8 lpm flow rate, ie, much higher than Bio-SPAMS – please confirm.

A2: Thank you for the valuable comments. The flow rate annotations in Fig. 2 have been revised, and the unit (L/min) has been added to the figure caption.

Q3: Fig 1/Section 2.1. Define "virtual impactor" here, as it is not currently listed in this section. It is necessary to clarify the authors' terminology because all injection systems employing the pressure-reduction orifice plus transverse pumpout design (common in many/all the other aerosol MS systems referenced in the paper) inherently include a virtual impactor, ie, the sample air is enhanced with larger particles compared to the pumpout air. I believe the authors mean that the addition of the "separation cone" is what defines a "virtual impactor" in this study, although technically the injection system already includes virtual impaction effect without the separation cone.

A3: Thank you for your feedback. To avoid confusion, the author revised "separation cone" to "virtual impactor" in the manuscript. Additionally, to enhance the readability of the article, the following statement was added: "In this study, the virtual impactor is defined as a device that enhances the concentration of larger particles in the sample air by employing a pressure-reduction orifice and transverse pumpout design. The addition of a separation cone further refines the virtual impaction effect, distinguishing it from the inherent virtual impaction observed in other aerosol MS systems referenced in the literature."

Q4: Also, list approx. pressures of all inlet components.

A4: Thank you to the reviewer for your meticulous review. In the revised manuscript, the author has added pressure annotations to Fig. 1.

Q5: Also, briefly describe the 2nd pumping stage downstream of the nozzle. Are one or both of the pumping lines fixed at a constant pressure, eg, using a commercial pressure controller?

A5: Thank you for your valuable feedback. In the revised manuscript, the author has added the pressure control method downstream of the nozzle, as described below: "Downstream of the nozzle, a second pumping stage is employed to further reduce the pressure and enhance particle acceleration into the vacuum chamber. This stage utilizes a molecular pump to achieve the necessary pressure drop, ensuring efficient particle transport and stable operation. Both pumping lines are maintained at a constant pressure using commercial pressure controllers, which provide precise control of the flow dynamics."

Q6: Line 120. A pressure reduction (critical) orifice is missing from this list. Also add labels in Fig 1 for separation cone and acceleration nozzle.

A6: Thank you for your valuable feedback. In the revised manuscript, the author has added descriptions of the critical hole in the key components section, modified the annotations in Fig. 1 to include labels for the acceleration nozzle, and replaced all references to the separation cone with the term “virtual impactor”.

Q7: Line 132. “Smooth” = tapered? Please clarify.

A7: To avoid confusion, the author has revised the description from “smooth nozzle” to “tapered nozzle.”

Q8: Section 2.2. Please state whether the model considers compressible flow (which is well known to occur downstream of each critical orifice and, importantly, through which all particles pass). State in the main text what symmetry was employed.

A8: Thank you to the reviewer for your meticulous review. To enhance the rigor of the article, the author has added the following description: “The numerical simulation employs an ideal gas as the material property, which means it considers the characteristics of compressible flow, particularly as the gas undergoes rapid expansion and acceleration downstream of the critical hole. In this region, the compressible Navier-Stokes equations are used to resolve pressure gradients, density variations, and inertia effects, all of which are essential for capturing transitions between supersonic and subsonic flow, as well as shock phenomena. To reduce computational complexity, axial symmetry is rigorously applied, assuming that flow properties and particle trajectories are symmetric around the central axis of the orifice.

Q9: Line 142. Do the authors mean that the pressures are chosen based on numerical simulations of this injection system from Zhang’s code? Please clarify.

A9: Thank you to the reviewer for your meticulous review. The selection of the physical model is indeed consistent with the design of Zhang et al., but the pressure values were chosen based on the design requirements of our instrument. To avoid misinterpretation, the author has moved this statement to a later section, where it is used to describe the related physical equations.

Q10: Line 162. A “three-way flow splitter”?

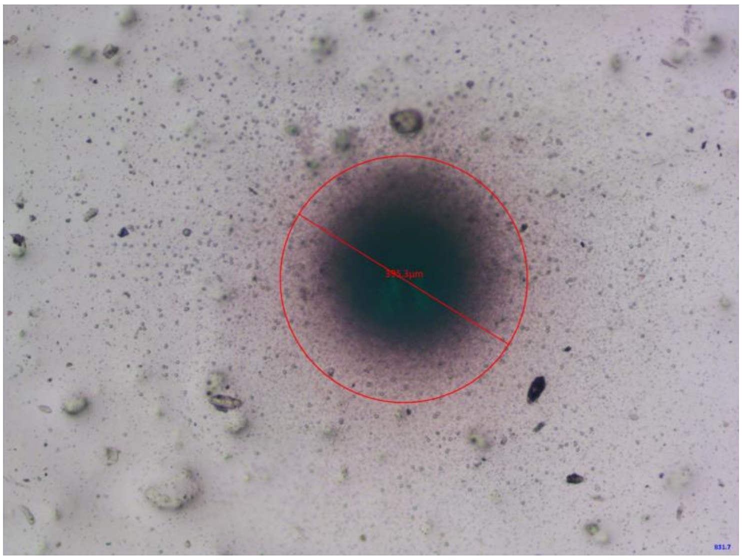
A10: We are grateful for the reviewer's recommendations. In the revised manuscript, the author has updated the relevant descriptions.

Q11: Line 178. APS = Aerodynamic Particle Sizer (TSI, Inc).

A11: We are grateful for the reviewer's recommendations. In the revised manuscript, the author has updated the relevant descriptions.

Q12: Line 179. For this new optical system, do the particle detection beams pass through focusing or shaping optics? State the approx. laser beam waists where the particles intersect the laser beams.

A12: In the novel optical system, the laser beam is shaped by optical components to form a rectangular spot of 600 μm × 30 μm. In this experiment, the particle beam width was measured at a position 110 mm downstream from the lens exit, which exceeds the distance between the lens and the beam profiling laser (67 mm), indicating that the actual particle beam width at the beam profiling laser position is narrower. Analysis of the microscope-captured distribution image of mixed particles (300 nm, 740 nm, 1 μm, 2 μm, 5 μm) at the beam profiling laser position (as shown in the figure below) reveals that the particle beam width at this location is smaller than the laser beam waist.



Q13: Line 185. Does the TE calculation also consider the flow difference?

A13: Thank you to the reviewer for your expert feedback. During the experimental pipeline connection, flow rate discrepancies between instruments were carefully considered, and a flow-balancing design was implemented to ensure consistent flow rates across all nodes.

Q14: Line 206. Again related to the "virtual impactor", please clarify what is actually being removed for this comparison (I think it is just the separation cone and not, eg, the entire upstream pumpout region).

A14: Thank you to the reviewer for your expert feedback. To prevent terminological ambiguity, the authors have revised the description of the "separation cone" to "virtual impactor" in the revised manuscript. Additionally, the statement regarding "removal of the virtual impactor and pre-focus structure" specifically indicates that within the flow path from the inlet to the buffer chamber, only the critical hole remain as core components, with all auxiliary structures being eliminated.

Q15: Fig 3. It surprising that the addition of a separation cone (aka "virtual impactor") has no theoretical effect on small particles (blue vs orange lines). One would expect that by adding the separation cone to enhance the inherent virtual impaction effect that is already there, the impactor's cutpoint diameter would shift to smaller sizes. But the blue line is already at 100% for the smallest particles shown. So although line 210 is technically correct ("Our team discovered that the virtual impactor used in this study is capable of transporting 100 nm particles downstream with an efficiency of over 90 %"), the same is apparently true for the injection system without the "virtual impactor". Consider clarifying.

A15: We deeply appreciate the reviewer's constructive criticism. First, the authors have standardized the terminology by revising the description of the "separation cone" to the standardized term "virtual impactor". Furthermore, in Section Q3, the functional role of this virtual impactor has been clarified: its primary purpose is inertial enrichment of large particles, whereas the 100 nm particle size threshold significantly exceeds the effective cutoff for size-selective classification by virtual impaction. In other words, the operational objective of the virtual impactor in this design is not to expel small particles via pumping mechanisms, but rather to selectively concentrate large particles to optimize aerosol transmission efficiency within the sampling system.

Q16: Para starting line 217. Similarly, given the above interpretation of the enhanced virtual impaction effect, explain why the "virtual impactor has increased its ability to focus on large particles". If the VI cutpoint diameter was indeed reduced as I suspect, one would expect the large particle transmission to be relatively unchanged. The simulated D50 cutpoints for the two compared designs (with and without separation cone) are easy to estimate and may help illustrate.

A16: Thank you to the reviewer for your meticulous review. As detailed in the technical responses to Q15 and Q3, the virtual impactor in this sampling system not only achieves inertial enrichment efficacy for large particles through concentration enhancement, but also improves the flow-field distribution characteristics of large particles by optimizing aerosol dynamics, thereby effectively boosting the overall transmission efficiency. The newly added explanation states: "This improvement is primarily attributed to the virtual impactor, which refines the flow dynamics, reduces particle loss, and optimizes the impaction process, thereby enhancing the focusing efficiency for larger particles."

Q17: Lines 266-274. Most of this is repetitive with the previous paragraphs.

A17: We appreciate the reviewer's thorough evaluation. In the revised manuscript, the authors have implemented systematic optimizations. The newly states: "As shown in Fig. 4, the PFW-ALens-equipped injection system significantly reduces the radial width of the particle beam at various positions across different particle diameters, outperforming both the original design and Du et al.'s pre-focusing structure. Specifically, at the buffer chamber, the beam width is reduced by 70 % to 95 % compared to Du et al.'s design. Additionally, the radial distribution of particles in the buffer chamber exhibits an inverse correlation with particle size, a novel observation not seen in previous pre-focusing designs. This discovery offers a strong basis for optimizing the lens system's length and minimizing the number of lenses required."

Q18: Fig 5. The colors are inverted. Define the color bar and add units.

A18: Regarding the colorbar in Fig. 5, it actually represents the particle ID numbers, which are systematically distributed according to the particles' initial radial positions. Therefore, the numerical orientation of the colorbar is correct and consistent with the design logic. To enhance clarity, we have added an explicit definition of the colorbar in the figure.

Q19: Line 293. Enhancement compared to what?

A19: We greatly appreciate the reviewer's comments. The author has updated the relevant statements in the revised manuscript as follows: "Fig. 3(b) demonstrates a remarkable performance in the particle transmission range of the PFW-ALens."

Q20: Line 297-299. This is incorrect. The Du et al. 2023 system has a beam waist of 300 microns at the particle beam. Were the Du optical design used on the current system, it appears that a large fraction of the particles (having radial width ~0.5-0.6 mm, Fig 3b) would not intersect the laser beams. No details are given regarding the "improved" optical design employed here (line 179), though it is likely that new scattering laser beams are focused with converging lenses, as is done in other single-particle MS systems, such that the beam waists where particles pass through them may or may not be smaller than the simulated particle beam width. Although technically this consideration does not affect the transmission efficiencies reported here (but is highly relevant to experimental detection efficiencies in Fig 7), some context regarding the laser beam versus particle beam widths for the current system is necessary. Correct and reword as it pertains to the current system.

A20: We greatly appreciate the reviewer's comments. Although the optical system design in this study aligns with that of Du et al. (2021), we have implemented critical optimizations by incorporating focusing element and beam-shaping components to precisely modulate the output beam into a 600 μm × 30 μm rectangular spot. Regarding the observed variations in transmission efficiency in Figure 6, we have supplemented the revised manuscript with detailed analysis and experimental validation, further elucidating the optimization effects of beam shaping on aerosol transmission efficiency. Specifically, as follows "Although a beam width of 600 μm was employed in this study, the particle beam width for certain small particles still exceeds this threshold. According to Rayleigh scattering theory, the scattered light intensity is proportional to the sixth power of the particle diameter, leading to a dramatic decline in signal intensity as the particle size decreases. Due to the combined effects of these two factors, the detection sensitivity and transmission efficiency of small particles (e.g., 100 nm) are significantly compromised, as indicated by the low transmission efficiency of 100 nm particles in Fig. 6."

Q21: Fig 6. The red line is actually detection efficiency, which in addition to particle transmission also includes the efficiency of particle detection by light scattering. Also, add a caption and describe error bars.

A21: Thank you to the reviewer for your meticulous review. The error bars are plotted based on statistical data from five independent experiments, with the upper and lower bounds representing the maximum and minimum measured values, respectively, and the midpoint denoting the average value across these five experimental replicates. The revised text is presented as follows: "Error bars represent the range between the maximum and minimum values from five independent experimental replicates, with the midpoint denoting the average value."

Q22: Line 316. The use of the "dual-peak signal in Bio-SPAMS" appears to contradict the statements in lines 183-184. Also, the explanation that follows is unclear. The authors appear to imply that the lower detection efficiency for 100 nm particles is simply due to a low signal-to-noise for the scattering detection…? If so, please state.

A22: We appreciate the reviewer's thorough evaluation. In the experimental design, a beam split mirror was employed to divide the incident beam into two parallel beams, with particle counting achieved by detecting the signal between the two beams, and the counting results recorded on the PMT1-1 detector. This design logic is free from contradictions. Regarding the detection efficiency, a detailed explanation has been provided in Q20, and the relevant supplementary content has been integrated into the main text, significantly enhancing the logical coherence and reading fluency of the manuscript.

Q23: Line 321. Delete 'over'.

A23: We are grateful for the reviewer's recommendations. In the revised manuscript, the author has updated the relevant descriptions.

Q24: Fig 7. Update the y-axis to dN/dlogD (with units) as described in line 330. Also, since the test dust is non-spherical and has high density, please clarify "diameter". The direct measurements from each instrument are aerodynamic diameter in the continuum regime for the APS and aerodynamic diameter in the vacuum (or near-vacuum?) regime for Bio-SPAMS. They are related but shifted a bit from one another (eg, see DeCarlo et al., 2004). No conversion is necessary for the figure.

A24: Thank you to the reviewer for the suggestion. The author has modified the coordinate axis in the revised manuscript and added relevant formulas and explanations for the particle size relationship between vacuum environment and continuous flow, as follows, "The APS measures the aerodynamic diameter (d_{ca}) in the continuum flow regime, while the Bio-SPAMS determines the vacuum aerodynamic diameter (d_{va}) under vacuum or near-vacuum conditions. Since the tested samples were high-density non-spherical particles (with particle density $\rho_p > \rho_0$, where $\rho_0 = 1 g/cm^3$ is the standard reference density), the theoretical ratio between the two aerodynamic diameters can be expressed as:

$$\frac{d_{va}}{d_{ca}} = \sqrt{\frac{\rho_p \chi_c}{\rho_0 (\chi_v)^2}}$$

where χ_c and χ_v (≥ 1) are dynamic shape factors correcting aerodynamic drag in the continuum (viscous-dominated) and free-molecular (collision-dominated) regimes, respectively. Since $\rho_p > \rho_0$ and $\chi_c/(\chi_v^2)$ >1 for such particles, d_{va} exceeds d_{ca}, resulting in a rightward shift of the Bio-SPAMS distribution curve relative to APS in Fig. 7, where X-axis represents aerodynamic diameter."

Q25: Line 350. The beam width does not actually decrease. I believe the authors mean to say that "the radial distribution of particles in the buffer chamber exhibits an inverse correlation with particle size" (line 274). Reword.

A25: We are grateful for the reviewer's recommendations. In the revised manuscript, the author has updated the relevant descriptions.

Respond for RC2:

Q1: On line 68, please say what you mean by large particles. Larger than 5 microns? Larger than 3 microns?

A1: Thank you to the reviewer for your helpful suggestion. The author has made modifications to the relevant description in the revised manuscript, as follows: "most reported lenses exhibit inefficient transport of particles larger than 5 μm."

Q2: On line 95, I would suggest starting a new paragraph after "critical hole." It's the start of a new topic discussing the pre-focus design.

A2: New paragraphs have been added to the revised manuscript as suggested.

Q3: Fig. 1 and the text are much clearer now that all parts are labeled in Fig. 1 and consistent terminology is used in the text. However, on line 121, I'm confused by the reference to "separation cone." Is that the same thing as the virtual impactor? If so, please use that term. If not, please identify it in Fig. 1.

A3: Thank you for your feedback. To avoid confusion, the author revised "separation cone" to "virtual impactor" in the manuscript.

Q4: On line 132, do you mean a tapered nozzle?

A4: We are grateful for the reviewer's recommendations. In the revised manuscript, the author has updated the relevant descriptions.

Q5: Lines 173-174 mention different flow rates to the OPC and Bio-SPAMS but the flow rate is indicated as 0.64 lpm for both in Fig. 2. Either update the figure or update the text.

A5: Thank you for the valuable comments. The flow rate annotations in Fig. 2 have been revised, and the unit (L/min) has been added to the figure caption.

Q6: Lines 212-216. Is the 110 mm downstream of the detection laser? In the paragraph describing the Bio-SPAMS (lines 176 to 188), it would be helpful to have the distance from the lens exit to the first measuring laser, to the second measuring laser and to the detection laser. That would give the 110 mm a better context.

A6: The length refers to the distance of 110 mm from the lens. To improve readability, the revised manuscript now includes the distances from the lens exit to PMT1 (42 mm) and PMT2 (67 mm). The modified text reads: "The reason for choosing the 110 mm position is that it is downstream of all lasers, including the acceleration nozzle to PMT1 (42 mm) and PMT2 (67 mm), allowing for a clearer evaluation of the particle transport and focus effect."

Q7: Lines 208 to 221, please indicate which figure the various lines are in. I would not use the word "original" on line220 because it is not referring to the line labeled original in the figure.

A7: To avoid confusion, the author has revised the corresponding text. The modified statement reads: "As shown in Fig. 3, by comparing the particle transmission efficiency curves before and after adding a virtual impactor, it can be found that after the addition of the virtual impactor (orange diamond symbol line), the focusing ability of the injection system for particles larger than 1 μm has increased to varying degrees, and the transmission efficiency of 7 μm particles has increased from the initial 5 % to 30 %."

Q8: Figure 4. Can you label the panels as a,b,c,d as you have for the other figures? I would use the same y-axis scale for all panels. It is very hard to compare when the scales are all different. For example, if all are scaled the same, then the statement on lines274-277 would be easy to evaluate. I would include the particle size in each panel.

A8: Thank you to the reviewer for your constructive comments. To avoid confusion, the author has revised the labels of the subfigures in Fig. 4. Additionally, the position of the title in Fig. 4 has been adjusted accordingly to ensure clarity and prevent overlap with the labels in the figure.

Q9: Figure 5. I would put the particle size in each panel.

A9: Thank you to the reviewer for your helpful suggestions. To further enhance readability, the author has added particle size descriptions to the subfigures in Fig. 5, ensuring a clearer presentation of the relevant information.

Q10: Figure 6. I would put the numbers in a table rather than in the figure. The numbers clutter up the figure.

A10: To avoid confusion, the author has removed the numerical labels in Fig. 6 and added Table 1 to summarize the transmission efficiencies. Additionally, the following explanation has been included: "The trend in transmission efficiency for 10 μm particles is consistent with the simulation results, registering only 25 % efficiency. The specific transmission efficiencies for different particle sizes are summarized in Table 1."

Table 1. Comparison of Transmission Efficiency Across Different Particle Sizes

Particle diameter (nm)	Transmission efficiency (%)	Particle diameter (nm)	Transmission efficiency (%)
100	35	4000	95
200	100	5000	91.65
300	107	6000	88.3
500	104	7000	72.1
800	101	8000	65.7
1000	108.5	9000	64.9
2000	101	10000	25
3000	107.5		

Q11: Lines 326-330. You refer to the colors of the lines before you have referred to the figure. Please reorder these sentences.

A11: We appreciate the reviewer's careful reading and constructive suggestions. The author has added a description indicating that the lines are derived from Fig. 7. Additionally, the related content on particle size distribution has been revised for greater clarity and conciseness.

Q12: Line 360. Where is the data available? Give the URL.

A12: The data used in the report are directly accessible, and no additional URLs are provided.

Q13: There are still a few minor issues with English language usage, such as missing words (e.g., missing "and" before "a five-stage lens" in line 16 and missing "the" before "Aerodynamic Particle Sizer" in line 28 and many other instances.) There are minor issues with citations, such as incorrect capitalization of "Mcmurry" in line 67, and inclusion of author name in the parentheses after citing the name in the text. It should just be the year in the parentheses, e.g., line 74 and many other instances.

A13: We are thankful to the reviewer for the valuable input. The revisions have been made in accordance with the reviewers' comments, such as including only the year after the authors' names in the references. For example:

"We typically assess the particle transmission capacity of injection systems by considering the range where the transmission efficiency exceeds 50 %, such as the 25-250 nm of Liu et al. (1995), the 100-900 nm and the 340-4000 nm of Schreiner et al. (1998; 1999), the 60-600 nm of Zhang et al. (2004), and the 125-600 nm of Zelenyuk et al. (2015). For example, Lee et al.(2013) designed a seven-stage lens for particle detection in the range of 30 nm to 10 µm, but this study does not consider the impact of critical hole on the transmission loss of large particles and it is not applied in practice. Cahill et al. (2014) designed a high-pressure lens, and used a very long buffer chamber combined with a seven-stage lens to transport 4-10 µm particles."

Other modifications have been highlighted in red in the manuscript.