



Minimizing ion competition boosts volatile metabolome coverage by secondary electrospray ionization orbitrap mass spectrometry

Jiayi Lan ^{a,1}, Jérôme Kaeslin ^{a,1}, Giorgia Greter ^b, Renato Zenobi ^{a,*}

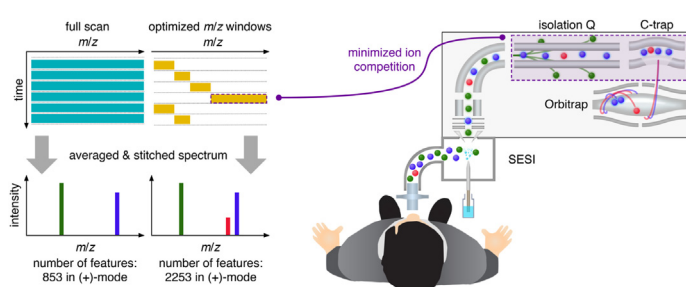
^a Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093, Zurich, Switzerland

^b Department of Health Sciences and Technology, ETH Zurich, CH-8093, Zurich, Switzerland

HIGHLIGHTS

- Ion competition limits the detection capabilities in SESI-Orbitrap Mass Spectrometry.
- Targeted data acquisition for SESI-Orbitrap studies alleviates this problem.
- A spectral stitching method detects more features, but with lower time resolution.
- Optimized m/z windows for feature detection with SESI-Orbitrap MS are found.
- Three times more features are detected using these optimized m/z windows.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 16 October 2020

Received in revised form

4 January 2021

Accepted 5 January 2021

Available online 16 January 2021

Keywords:

Secondary electrospray

Ion competition

Ion suppression

Volatile metabolites

Metabolomics

ABSTRACT

Secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) is an emerging technique for the detection of volatile metabolites. However, sensitivity and reproducibility of SESI-HRMS have limited its applications in untargeted metabolomics profiling. Ion suppression in the SESI source has been considered to be the main cause. Here, we show that besides ion suppression, ion competition in the C-trap of Orbitrap instruments is another important factor that influences sensitivity and reproducibility of SESI-MS. Instead of acquiring the full mass-to-charge ratio (m/z) range, acquisition of consecutive m/z windows to minimize the ion competition effect allows the detection of more features. m/z window ranges are optimized to fill the C-trap either with an equal number of features or an equal cumulative intensity per window. Considering a balance between maximizing scanning speed and minimizing ion competition, splitting the $m/z = 50\text{--}500$ range into 4 windows is selected for measuring human breath and bacterial culture samples on SESI-Orbitrap MS, corresponding to a duty cycle of 2.3 s at a resolution of 140,000. In a small cohort of human subjects, the proposed splitting into 4 windows allows three times more features to be detected compared to the classical full m/z range method.

© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail address: zenobi@org.chem.ethz.ch (R. Zenobi).

¹ Equal contribution.

1. Introduction

Volatile metabolites provide deep insight into metabolism. Various methods have been developed for the analysis of volatile metabolites and are commonly applied in clinical research, mainly using gas chromatography-mass spectrometry (GC-MS) [1], proton-transfer-reaction mass spectrometry (PTR-MS) [2] and selected-ion flow-tube mass spectrometry (SIFT-MS) [3]. Over the last decade, secondary electrospray ionization (SESI) has emerged and has been widely used to ionize metabolites in the gas phase. Given its modularity [4], SESI can be coupled with various commercial mass spectrometers. Particularly attractive is the coupling to high-resolution mass spectrometers (HRMS), e.g. an Orbitrap. Previous studies have shown that coupling to such a high-resolution instrument offers greater selectivity in feature detection and simplifies metabolite identification [5,6]. Besides the number of detected features and the resolving power, the reproducibility of the detected features is an important aspect and has been addressed in recent standardization studies [7,8].

Although efforts have been made to improve ionization efficiency [9] and feature detection stability [7] of SESI, reproducibility and sensitivity might still be impaired by ion suppression - an effect well known from classical electrospray methods in which a large number of analytes are introduced into the ion source and competition for charge carriers takes place [10]. Besides ion suppression, a large number of analytes are also problematic in other regards: (i) coalescence might result in fewer detected features and reduced mass accuracy [11], (ii) mass accuracy might deteriorate [12], and (iii) impaired sensitivity may lead to many undetected analytes. This reduced sensitivity is sometimes referred to as *ion competition* [13] or *post interface signal suppression* [14] on Orbitraps. This effect arises with matrix-heavy samples when high and low abundant ions compete for space in the capacity-limited C-trap [14]. Previous studies have shown that the ion competition effect can be minimized by applying a strategy called *spectral stitching*, in which smaller m/z windows are acquired and stitched together instead of acquiring the whole m/z range in one scan [15,16]. In the case of an Orbitrap, this avoids the overfilling of the C-trap with the most abundant ions when less abundant ions do not accumulate enough to be detected. However, since multiple scans are required to cover the whole m/z range, spectral stitching leads to a significantly longer duty cycle than a full MS scan. Instead of performing spectral stitching with evenly distributed m/z windows, it has recently been shown by Sarvin et al. in electrospray ionization based flow injection mass spectrometry, that optimized window positions and widths enhance sensitivity while keeping the duty cycle short [13]. In their study, the windows were optimized such that the number of features was equal in every window.

Here, we optimize m/z windows to mitigate ion competition on SESI-Orbitrap MS instruments for the real-time monitoring of gaseous analytes. We do this for human breath and bacterial culture headspace, where the monitored event are very time-limited (i.e., an exhalation, which lasts only a few seconds).

2. Methods

2.1. Real-time SESI-HRMS measurements of human breath and bacterial cultures

The SESI-HRMS system consisted of a commercial SESI source (Super SESI, Fossiliontech, Spain), a flow sensor (Exhalion, Fossiliontech) and an Orbitrap mass spectrometer (Q-Exactive Plus, Thermo Scientific, San Jose, CA). A 20 μm non-coated TaperTip emitter (New Objective, Woburn, MA) was used for electrospray using an aqueous solution of 0.1% formic acid. The sampling line

temperature of the SESI source was set to 130 °C and the ion chamber temperature was set to 90 °C. Breath was directly introduced into the sampling line and the ion chamber of the SESI source through a spirometry filter (MicroGard, Vyair Medical, USA; 3 cm ID) as previously described [7]. By controlling the exhaust mass flow cutoff of the SESI source at 0.65 L min⁻¹ and the nitrogen mass flow through the source at 0.35 L min⁻¹, 0.3 L min⁻¹ of breath was constantly sampled by the mass spectrometer while subjects were exhaling at a flow rate of 8 L min⁻¹, similar to the settings of a recent standardization study [7]. For preparing bacteria samples, *Escherichia coli* O9 HS was cultured to an optical density of 0.32, then filtered through a 0.22 μm filter to remove the bacteria. Hemin-supplemented (0.25 mg/ μL) brain heart infusion broth was used as medium for culturing bacteria. To sample the bacterial culture's headspace, 0.3 L min⁻¹ of humidified nitrogen was flushed through a culture flask containing either medium or culture supernatant, and introduced into the SESI source.

Data was acquired on the Orbitrap in profile mode. For both positive and negative mode acquisition, the following settings were used: an ion transfer tube temperature of 250 °C, a resolution of 140'000 at $m/z = 200$, a $m/z = 50$ –500 scan range, RF lens set at 55%, and a maximum injection time of 100 ms. The automatic gain control (AGC) target, which regulates the number of ions accumulated in the C-trap and later injects them into the Orbitrap analyzer, was set to $5 \cdot 10^6$. As an instrument blank, data was acquired without introducing human breath or bacterial culture headspace. In parallel to the MS data acquisition, the flow rate and total volume of the gas flows into the SESI source were measured by the Exhalation flow sensor.

Different MS acquisition methods with different scan ranges were used, including (a) full scan over a range from $m/z = 50$ –500; (b) targeted with the narrowest scan window possible from $m/z = 204.8$ –205.2; (c) twenty-two 20 Da windows with an overlap of 4 Da; (d) a variable number N of optimized windows covering the $m/z = 50$ –500 range split either according to the equal-feature number criterion (equal number of robust features per window - the same strategy as in Ref. [13]) or the equal-cumulative intensity criterion (equal cumulative intensity per window), with no overlap between windows. (Fig. 1) (i) shows how these ranges are allocated while a subject is exhaling.

2.2. Data processing

Raw data generated from the mass spectrometer were first converted into .mzXML format via MSConvert (ProteoWizard [17]). The converted files were then imported into Matlab (version R2019b, MathWorks, Natick, MA) and treated using a Matlab code developed in-house. Briefly, peaks were picked from an overall averaged spectrum of acquired spectra during the experiment. The acquired data was then centroided using the picked peaks, and a .csv file containing the intensities of the peaks per scan was generated for each of the acquired MS datasets.

Statistics and window suggestions were calculated using in-house developed R codes.¹ For each of the samples, the mean intensity value of the acquired scans was used for quantification. Overlapping regions between two windows were averaged. The peaks were grouped within 5 ppm ranges and the peak intensities were summed as the peak group intensity. The summed peaks were then used to identify robust features using the criteria described by Sarvin et al. [13]. Briefly, a feature was considered "robust" only if it

¹ The R codes and the raw data are made available in a curated data archive at ETH Zurich (<https://www.research-collection.ethz.ch>) under the DOI <https://doi.org/10.3929/ethz-b-000445560>

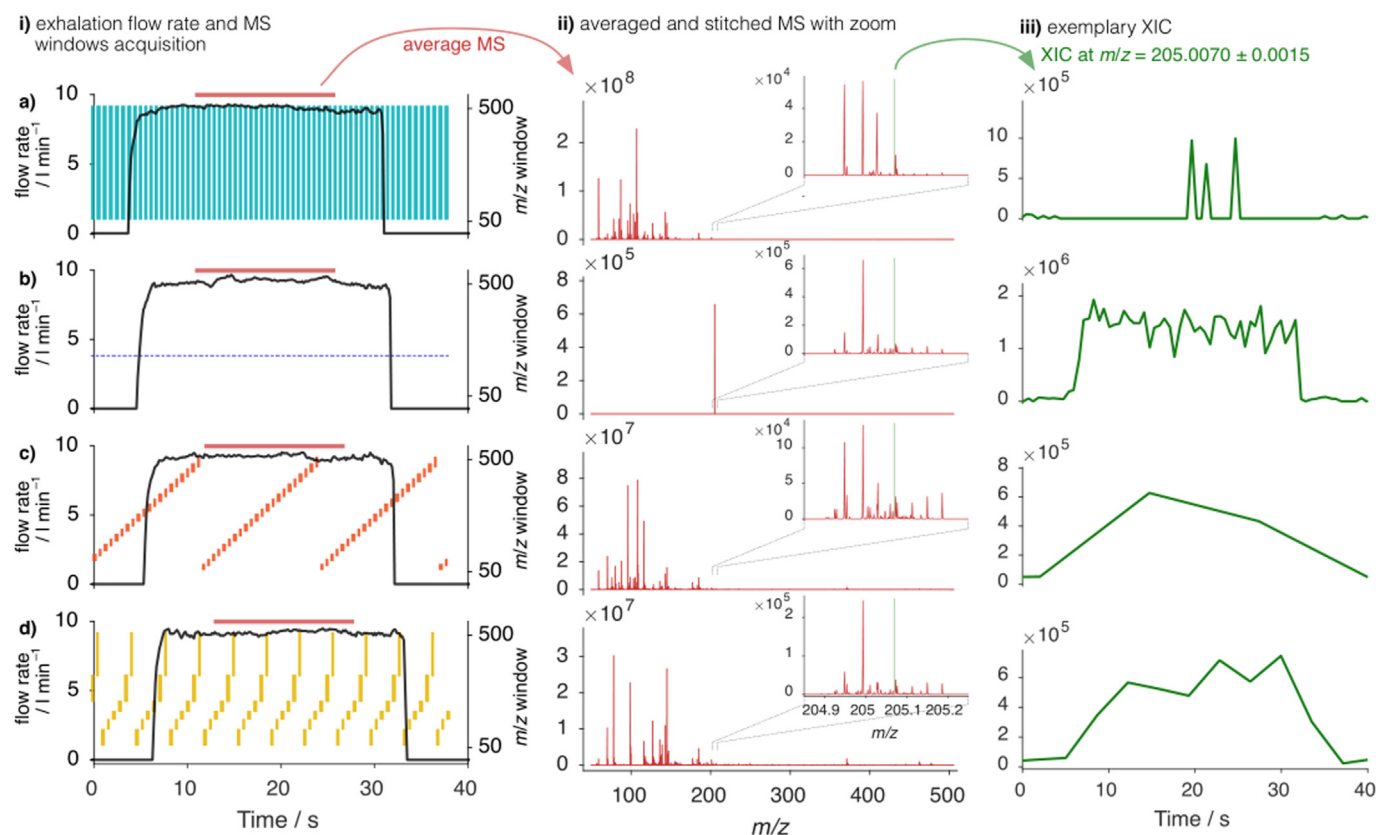


Fig. 1. Overview of different acquisition strategies; a) full scan from $m/z = 50$ – 500 ; b) targeted from $m/z = 204.8$ – 205.2 ; c) 22 sequential 20 Da windows; d) $N = 6$ intensity-optimized windows: i) shows one exhalation of a subject and plots the exhalation flow rate. The scan windows are shown in colors. ii) shows the average spectrum during the exhalation with a zoomed region. iii) shows the extracted ion chromatogram (XIC) of an exemplary selected feature. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

matched the following criteria: (i) observed in $\geq 80\%$ of the biological samples; (ii) the median intensity of the biological samples is above 1000 units; (iii) signal-to-noise ratio above 4; (iv) for breath samples, a relative standard deviation (RSD) across the biological sample injection lower than 30% and for bacteria culture, lower than 100%. The windows were then optimized to either contain an equal number of features per window (feature-optimized), or equal cumulative intensity per window (intensity-optimized).

3. Results and discussion

3.1. Targeted data acquisition method improves sensitivity of SESI-HRMS

In a classical SESI-MS study, full MS scans are acquired repeatedly to track a subject's exhalation (Fig. 1a) (i). From this data, an averaged spectrum can be calculated (Fig. 1a) (ii). Depicting a signal over time gives an idea as to whether the feature is elevated during exhalation or not – as in the case of (Fig. 1a) (iii) where the exemplary feature at $m/z = 205.007$ was inconsistently detected by the mass spectrometer. Such features below a certain exhalation-to-background threshold cannot be confidently quantified or attributed to the breath events. Consequently, they are typically filtered out during data analysis [18].

Based on the hypothesis that ion competition might limit sensitivity in SESI-MS, we investigated how the feature detection performance is influenced by the acquisition method and therefore by the extent that the C-trap is filled with analytes of interest. One

exhalation was acquired with a full scan method from $m/z = 50$ – 500 and with a targeted method from $m/z = 204.8$ – 205.2 (Fig. 1a) (i) and (b) (i). More peaks were observed in the region $m/z = 204.8$ – 205.2 with the targeted method (Fig. 1a) (ii) vs. (b) (ii). Additionally, the signals were clearly elevated during exhalation with the targeted method while the full scan method suffered from a poor exhalation-to-background ratio (exemplary time trace in Fig. 1a) (iii) vs. (b) (iii). Fig. S1 (iii) shows that this observation was persistent over multiple exhalations.

The full scan method, which fills the C-trap with all available ions, performs significantly worse than the targeted method, which only traps the ions of interest. This observation supports the hypothesis that ion competition limits sensitivity and robustness of the observed features in full scan SESI-Orbitrap MS. A similar behavior was observed for other targeted mass ranges ($m/z = 89.8$ – 90.2 , 253.9 – 254.3 , 397.9 – 398.3 , 412 – 412.4 , results not shown).

3.2. Defining the ion distribution of human breath and bacteria culture

Ion competition effects in untargeted studies can be minimized by spectral stitching [15,16]. We generated a spectral stitching method by using twenty-two 20 Da windows as shown in (Fig. 1) (i) (c). The stitched spectrum contained significantly more peaks compared to the full scan spectrum (Fig. 1) (ii) (a) vs. (ii) (c). Moreover, the exemplary $m/z = 205.007$ feature was reliably detected (Fig. 1) (iii) (c) and (Fig. S1) (iii) (c). Thus, ion competition is mitigated using twenty-two 20 Da windows, however the time

resolution is low due to long time to accomplish one duty cycle (≈ 14 s).

In a more systematic experiment, we compared the detected features by using twenty-two 20 Da windows over the mass range of $m/z = 50$ –500 both for human breath and bacteria culture headspace. An overview of the detected features comparing the two methods is given in Fig. 2. There, the robustly detected features in 22 scans are shown, i.e. comparing one $m/z = 50$ –500 full scan with twenty-two stitched 20 Da isolation windows. For breath and bacterial headspace, a 4- to 7-fold increase of robustly detected features is observed. Ion competition particularly suppresses analytes in the higher m/z region - presumably because the ion competition is mainly caused by high abundant low mass matrix

compounds. In fact, the ion competition-reduced feature distribution more closely resembles the distribution reported on a SESI-quadrupole time-of-flight (QTOF) instrument [19], where ion competition is not expected because of the ion-trap free nature of a QTOF instrument.

While the twenty-two windows method significantly enhances the number of observed features, it also prolongs the duty cycle. To account for the longer duty cycle of the twenty-two windows method, the two strategies can be benchmarked by comparing them, with respect to an identical acquisition time (22 scans, roughly 14 s). This comparison takes into account that the $m/z = 50$ –500 full scan method has a better counting statistic, i.e. a potential feature is scanned 22 times more in the same period of

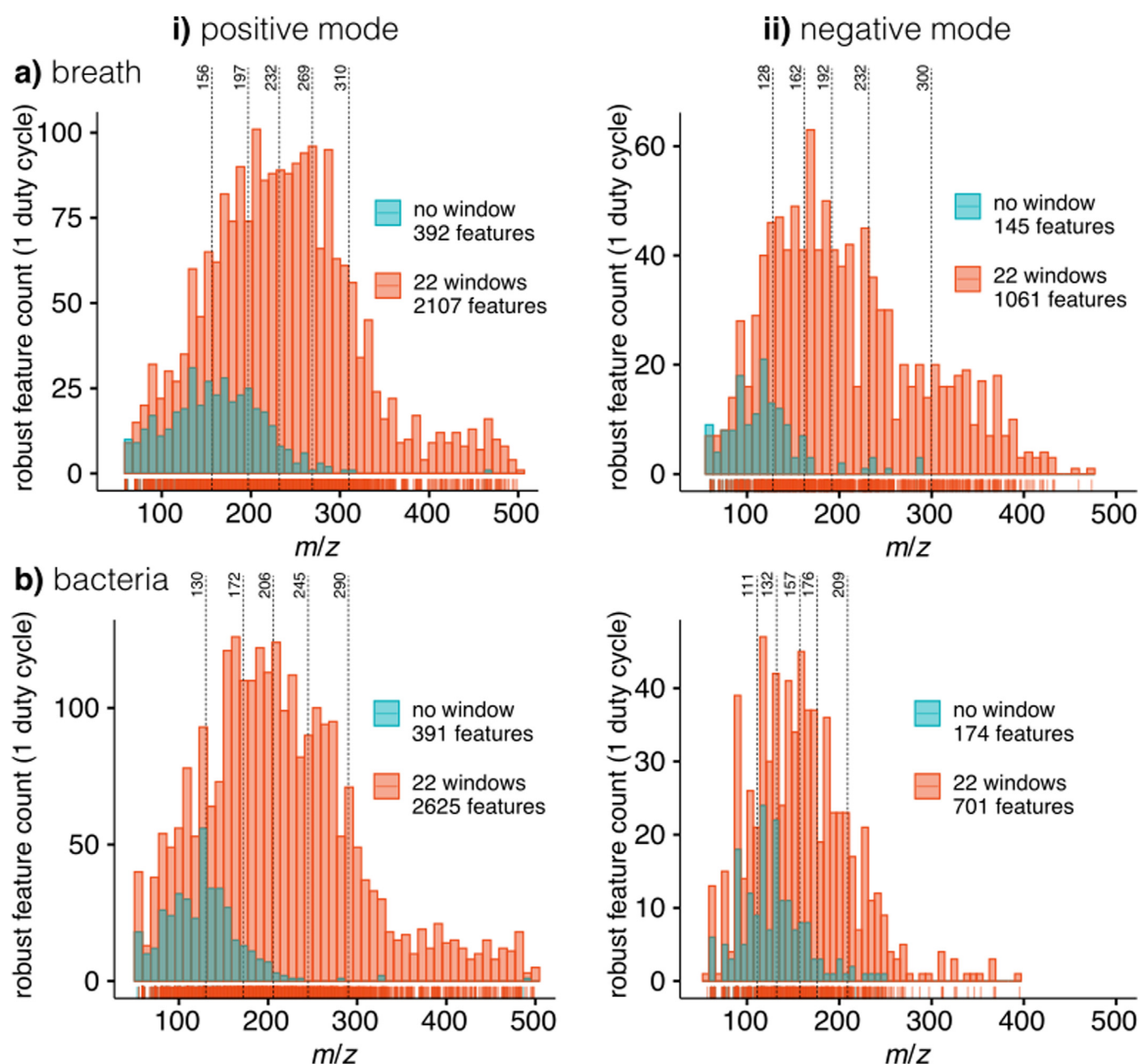


Fig. 2. Feature distribution for different acquisition methods with one duty cycle (one $m/z = 50$ –500 scan vs. twenty-two stitched 20 Da windows): shows the feature distribution in breath (a) and bacterium culture headspace (b) observed with no m/z windows (i.e. full scan $m/z = 50$ –500) in blue. Using twenty-two 20 Da windows, more features are detected (in red) due to minimized ion competition. The feature distribution is shown for positive i) and negative ii) polarity. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

time. Nonetheless, Fig. S2 shows that the twenty-two windows strategy outperforms the no windows strategy by a factor of two in terms of detected robust features for positive mode in breath and bacteria. The same holds true for the negative mode in breath, but not in bacteria where both methods perform similarly well. Overall, the performance of the two methods within an identical acquisition time is comparable in the low mass region. However, the twenty-two windows method clearly performs better in the mid-to high mass region.

3.3. Optimized windows for human breath and bacteria headspace acquisition

Assuming that the twenty-two windows feature distribution in Fig. 2 represents the ion-competition free distribution, the optimized windows' center and widths can be determined by allocating an equal number of robust features into one optimized window as outlined before in Ref. [13]. The borders of these "feature optimized" windows are marked with a dashed line in Fig. 2 for $N = 6$

windows. Additionally, we hypothesized that ion competition is more efficiently controlled by allocating the windows not to contain an equal number of robust features but instead to contain an equal cumulative intensity. This is because ion competition is not driven by the robust features themselves but rather by highly abundant ions - independent from whether they arise from the sample or from the instrument. Consequently, we also calculated "intensity-optimized" windows to containing an equal amount of cumulative intensity per window during exhalation or headspace acquisition as shown in Fig. S3.

2 to 16 optimized windows were calculated based on the "feature-optimized" and the "intensity-optimized" criteria. The corresponding window limits can be found in Table S1 for breath and S2 for bacterial headspace, respectively. It should be mentioned that these suggested windows are dependent on the matrix and the general background level of ions arising from the instrument and environment. However, we assume that the matrix of human breath has an inter-subject similarity. Moreover, the window splitting is expected to mitigate ion competition even if the exact

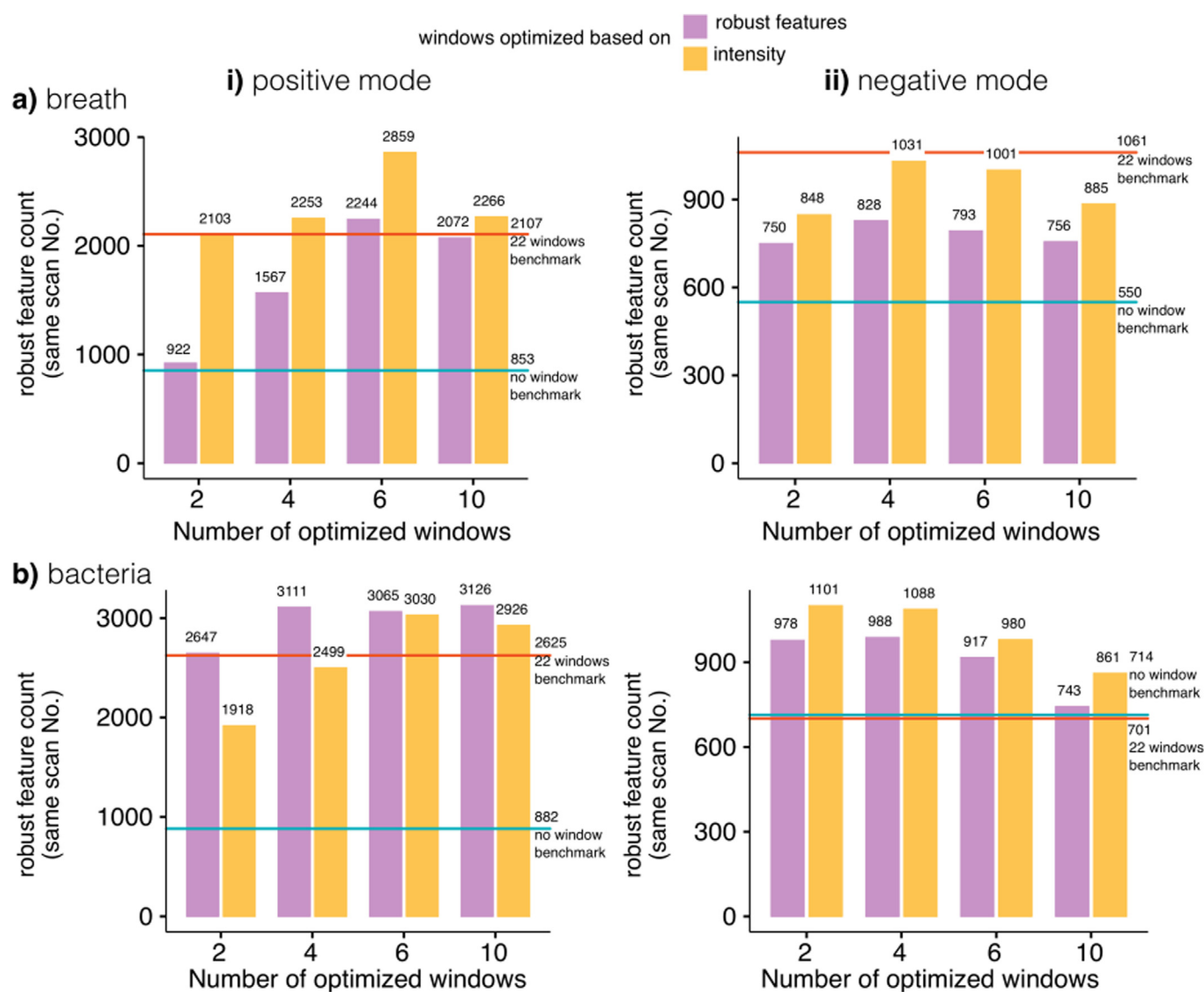


Fig. 3. Numbers of robust features detected using $N = 2, 4, 6, 10$ windows in (a) breath and in (b) bacterium culture headspace in 20 scans (≈ 11 s of scanning time). Both feature-optimized (purple) and intensity-optimized (orange) windows were tested. Moreover, benchmarks from the no windows $m/z = 50$ –500 full scans and twenty-two 20 Da windows are represented as a line. Both polarities are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

window breaking point might not be exactly ideal. Thus, the reported window limits could directly be adapted by other groups. An example for 6 intensity-optimized windows is shown in (Fig. 1d and S1d). The exemplary feature $m/z = 205.007$ is robustly detected. Therefore, the advantages of window optimization are that it allows the detection of suppressed features (Fig. 1a) vs. (d), is untargeted (Fig. 1b) vs., (d) and has an acceptable time resolution ((Fig. 1c) vs. (d), time for one duty cycle reduced from ≈ 14 s for 22 windows to ≈ 3 s for 6 windows).

We reanalyzed human breath and bacterial headspace with $N = 2, 4, 6, 10$ feature- and intensity-optimized windows. The robustly detected features for these window settings are benchmarked with our previously detected robust features of the $m/z = 50\text{--}500$ full scan and the twenty-two 20 Da windows strategy in Fig. 3 using an equal acquisition time. Multiple conclusions can be drawn from this comparison: first, the window splitting always outperforms the $m/z = 50\text{--}500$ full scan method. Second, 4 or 6 windows seem to be a good compromise between minimized ion competition and satisfying counting statistics. Third, the intensity-optimized windows have a tendency of outperforming the feature optimized windows, except in the bacterial headspace where the opposite behavior is observed. This supports our hypothesis that windows should preferentially be optimized to contain equal

cumulative intensity per window since the sum of charges ultimately drives ion competition.

$N = 4$ intensity-optimized windows seem to be the best compromise between reduced ion competition, counting statistics and time resolution. This corresponds to ≈ 2.3 s to scan through the $m/z = 50\text{--}500$ range once at a resolution of 140'000 on the Orbitrap Q-Exactive Plus.

3.4. Validating the proposed optimized windows in a larger cohort

The human breath windows optimization process from the previous sections is based on only one subject. However, the applicability of these optimized windows may be dependent on inter-subject variability of the breath matrix. We hypothesize that the breath matrix is comparable among different subjects. To support this assumption, we assessed our suggested optimized windows with a cohort of seven people, mimicking the setting of a small scale clinical study. In both positive and negative mode, the median number of robust features detected in individual subjects significantly increased by using 4 intensity-optimized windows, compared to the classical no window approach (Fig. 4a). Moreover, consistency of detected features among subject is also critical for data completeness and statistics. Defining a feature to be consistent

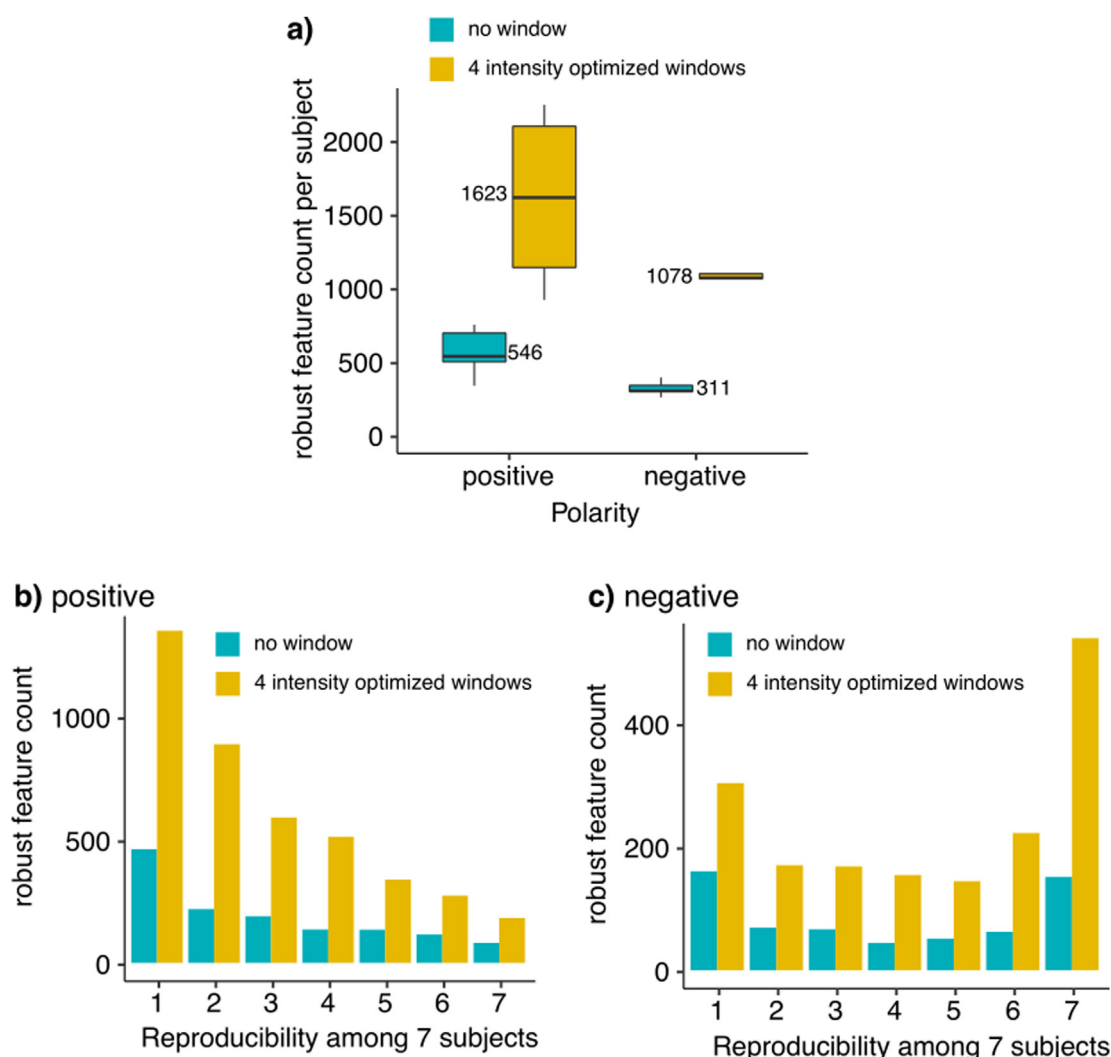


Fig. 4. a) Robust features detected per subject. Reproducibility of the detected features among seven subjects in b) positive and c) negative mode.

if detected in at least 80% of the subjects (≥ 6 subjects), 455 consistent features were detected using 4 intensity-optimized windows in positive mode and 760 in negative mode. In both cases, the consistent features are significantly increased compared to 196 (positive mode) and 212 (negative mode) obtained using the full scan approach (Fig. 4b and c).

To assess the quantification stability of the intensity-optimized 4 windows method, an intensity ratio was calculated between 4 feature pairs for each of the replicates. The coefficient of variation within a subject was used as an indication of the quantification stability, assuming the ratio should be stable for one subject over all the replicates (Fig. 5). When features are robustly detected in both methods, variations are expected to be comparable, independent of whether the pair of features was measured in the same scan window (positive: pyridine/isoprene) or in different scan windows (positive: phenylalanine/isoprene, negative: valeric acid/ $m/z = -89.023$). For these features, the intensity ratio's coefficient of variation was comparable between the two methods, indicating that they perform similarly in terms of quantification. For one of the feature pairs in different windows in negative mode (2-hydroxyisobutyric acid/ $m/z = -89.023$), the coefficient of variation was lower using 4 intensity-optimized windows. This is due to the fact that an analyte signal is underestimated if ion competition takes place [20]. This can be seen in Fig. S6, where the signal variation of 2-hydroxyisobutyric acid is much higher when using the no window approach comparing to the intensity-optimized 4 windows method.

Sensitivity was also assessed by plotting the distribution of signal-to-noise ratios of features detected in both the full scan method and the 4 intensity-optimized window method (Fig. S5). Only features that have a noise intensity above zero have been

considered. Both methods show similar distributions, suggesting the sensitivity of the two methods are at the same level as we expected, since ion suppression hampers both the detection and quantification of very low abundant features while high abundant features are not affected. Linear detection range of the two SESI-MS methods was not compared directly, due to complexity of preparing standards mimicking breath (humid VOC/aerosol mixture). We believe the post-ionization behavior would be identical to what has been described for electrospray ionization (ESI) [15], that the linear dynamic range was extended by preventing an overfilling of the C-trap. In summary, the quantification capabilities of the detected features seems to be comparable or even better using the intensity-optimized 4 windows method compared to $m/z = 50$ –500 full scan method.

4. Conclusions

We show that ion competition indeed limits the sensitivity and robustness of SESI-Orbitrap MS for analyzing volatiles. For targeted applications, our findings show that a narrow m/z window is superior to an untargeted full MS acquisition. For untargeted studies, using spectral stitching with 20 Da windows for acquisition enhances the sensitivity and robustness but limits the sampling frequency due to the prolonged duty cycle. By optimizing the m/z window's positions and widths, a compromise between sensitivity and duty cycle can be found. We demonstrate this approach by using 4 optimized m/z windows for the analysis of volatiles in breath and bacteria culture. A significant increase in sensitivity and robustness is observed while the duty cycle is still acceptable. Moreover, different sets of optimized windows for the analysis of breath or bacteria culture volatiles are proposed based on the

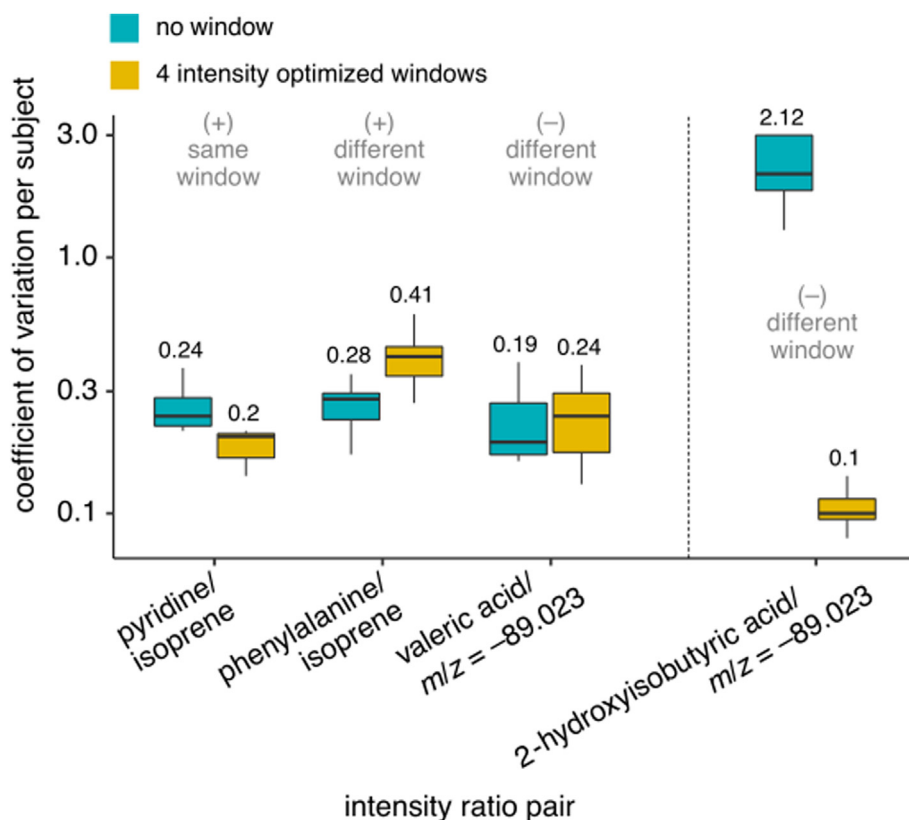


Fig. 5. Coefficient of variation of the intensity ratio per subject between 4 features pairs. The labels state the polarities of the features and whether they are located in the same or a different window.

feature number distribution or the cumulative intensity distribution.

CRediT authorship contribution statement

Jiayi Lan: Data curation, Formal analysis, Writing - original draft, recorded the data, performed the data analysis, prepared a draft of the figures and the manuscript text. **Jérôme Kaeslin:** Data curation, Formal analysis, Writing - original draft, recorded the data, performed the data analysis, prepared a draft of the figures and the manuscript text. **Giorgia Greter:** Data curation, Formal analysis, Writing - original draft, recorded the data, performed the data analysis. **Renato Zenobi:** Funding acquisition, Writing - original draft, obtained financial support for the research, All authors wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is part of the Zurich Exhalomics flagship project under the umbrella of "University Medicine Zurich/Hochschulmedizin Zürich". Jérôme Kaeslin acknowledges a fellowship from the Scholarship Fund of the Swiss Chemical Industry (SSCI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aca.2021.338209>.

References

- [1] C. Wang, R. Dong, X. Wang, A. Lian, C. Chi, C. Ke, L. Guo, S. Liu, W. Zhao, G. Xu, et al., Exhaled volatile organic compounds as lung cancer biomarkers during one-lung ventilation, *Sci. Rep.* 4 (2014) 7312.
- [2] P. Trefz, M. Schmidt, P. Oertel, J. Obermeier, B. Brock, S. Kamysek, J. Dunkl, R. Zimmermann, J.K. Schubert, W. Miekisch, Continuous real time breath gas monitoring in the clinical environment by proton-transfer-reaction-time-of-flight-mass spectrometry, *Anal. Chem.* 85 (21) (2013) 10321–10329.
- [3] D. Smith, P. vSpanvel, B. Enderby, W. Lenney, C. Turner, S.J. Davies, Isoprene levels in the exhaled breath of 200 healthy pupils within the age range 7–18 years studied using sift-ms, *J. Breath Res.* 4 (1) (2009), 017101.
- [4] J. Lan, F. Blanco Parte, G. Vidal-de Miguel, R. Zenobi, Secondary electrospray ionization, in: *Breathborne Biomarkers and the Human Volatilome*, Elsevier, 2020, pp. 185–199.
- [5] M.T. Gaugg, D.G. Gomez, C. Barrios-Collado, G. Vidal-de Miguel, M. Kohler, R. Zenobi, P.M.-L. Sinues, Expanding metabolite coverage of real-time breath analysis by coupling a universal secondary electrospray ionization source and high resolution mass spectrometry—a pilot study on tobacco smokers, *J. Breath Res.* 10 (1) (2016), 016010.
- [6] B. Rochat, From targeted quantification to untargeted metabolomics: why lc-high-resolution-ms will become a key instrument in clinical labs, *Trac. Trends Anal. Chem.* 84 (2016) 151–164.
- [7] K.D. Singh, G. Tancev, F. Decrue, J. Usemann, R. Appenzeller, P. Barreiro, G. Jaumà, M.M. Santiago, G.V. de Miguel, U. Frey, et al., Standardization procedures for real-time breath analysis by secondary electrospray ionization high-resolution mass spectrometry, *Anal. Bioanal. Chem.* (2019) 1–16.
- [8] J. Lan, A. Gisler, T. Bruderer, P. Sinues, R. Zenobi, Monitoring peppermint washout in the breath metabolome by secondary electrospray ionization-high resolution mass spectrometry, *J. Breath Res.* 15 (2) (2021), 026003.
- [9] G. Vidal-de Miguel, M. Macia, P. Pinacho, J. Blanco, Low-sample flow secondary electrospray ionization: improving vapor ionization efficiency, *Anal. Chem.* 84 (20) (2012) 8475–8479.
- [10] T.M. Annesley, Ion suppression in mass spectrometry, *Clin. Chem.* 49 (7) (2003) 1041–1044.
- [11] A. Kaufmann, S. Walker, Coalescence and self-bunching observed in commercial high-resolution mass spectrometry instrumentation, *Rapid Commun. Mass Spectrom.* 32 (6) (2018) 503–515.
- [12] A. Makarov, E. Denisov, A. Kholomeev, W. Balschun, O. Lange, K. Strupat, S. Horning, Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer, *Anal. Chem.* 78 (7) (2006) 2113–2120.
- [13] B. Sarvin, S. Lagziel, N. Sarvin, D. Mukha, P. Kumar, E. Aizenshtein, T. Shlomi, Fast and sensitive flow-injection mass spectrometry metabolomics by analyzing sample-specific ion distributions, *Nat. Commun.* 11 (1) (2020) 1–11.
- [14] A. Kaufmann, M. Widmer, K. Maden, Post-interface signal suppression, a phenomenon observed in a single-stage orbitrap mass spectrometer coupled to an electrospray interfaced liquid chromatograph, *Rapid Commun. Mass Spectrom.* 24 (14) (2010) 2162–2170.
- [15] A. Kaufmann, S. Walker, Extension of the q orbitrap intrascan dynamic range by using a dedicated customized scan, *Rapid Commun. Mass Spectrom.* 30 (8) (2016) 1087–1095.
- [16] A.D. Southam, R.J. Weber, J. Engel, M.R. Jones, M.R. Viant, A complete workflow for high-resolution spectral-stitching nano-electrospray direct-infusion mass-spectrometry-based metabolomics and lipidomics, *Nat. Protoc.* 12 (2) (2017) 310–328.
- [17] R. Adusumilli, P. Mallick, Data conversion with proteowizard msconvert, in: *Proteomics*, Springer, 2017, pp. 339–368.
- [18] M.T. Gaugg, Y. Nussbaumer-Ochsner, L. Bregy, A. Engler, N. Stebler, T. Gaisl, T. Bruderer, N. Nowak, P. Sinues, R. Zenobi, et al., Real-time breath analysis reveals specific metabolic signatures of copd exacerbations, *Chest* 156 (2) (2019) 269–276.
- [19] T. Bruderer, M. Gaugg, L. Cappellin, F. Lopez-Hilfiker, M. Hutterli, N. Perkins, R. Zenobi, A. Moeller, Detection of volatile organic compounds with secondary electrospray ionization and proton transfer reaction high-resolution mass spectrometry: a feature comparison, *J. Am. Soc. Mass Spectrom.* 31 (8) (2020) 1632–1640.
- [20] A. Kaufmann, S. Walker, Accuracy of relative isotopic abundance and mass measurements in a single-stage orbitrap mass spectrometer, *Rapid Commun. Mass Spectrom.* 26 (9) (2012) 1081–1090.