Systems biology

**mzRAPP: a tool for reliability assessment of data pre-processing in non-targeted metabolomics**

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Abstract

**Summary:** Reliability assessment of automated pre-processing of liquid chromatography-high resolution mass spectrometry data presents a significant challenge. Here, we present a tool named mzRAPP, which generates and validates a benchmark from user-supplied information and later utilizes it for reliability assessment of data pre-processing. As a result, mzRAPP produces several performance metrics for different steps of the pre-processing workflow, supporting five of the most commonly used pre-processing tools.

**Availability and implementation:** mzRAPP is implemented in R and can be downloaded from GitHub under GNU GPL v.3.0 licence. Extensive documentation, background and examples are available at (https://github.com/YasinEl/mzRAPP).

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**Supplementary information:** Supplementary data are available at Bioinformatics online.

1 Introduction

To date, several different software solutions for data pre-processing have been proposed, and new ones are published continuously. However, reproducibility, comparability and reliability of results generated via such algorithms remain challenging and difficult to assess or compare. Difficulties in the evaluation are primarily due to complex parameter options used for pre-processing within various tools and the complexity of the analysis, which can potentially affect and vary the outcome (Li et al., 2018). In metabolomics and particularly for non-targeted metabolomics, there are no ‘gold standard’ experimental datasets or a trusted set of methods to evaluate parameter choices and tools [e.g. Sanger sequencing for genomics (Sanger et al., 1977)]. Moreover, differences in instrumentation, acquisition strategies or sample complexity might not draw a general conclusion from a potential gold standard dataset. While there are software packages for automatized non-targeted data pre-processing (NPP) parameter optimization and adaption [e.g. IPO (Libiseller et al., 2015), Autotuner (McLean et al., 2020)], they are not meant to assess the general quality of NPP results (More details in Supplementary Material S1). Hence there is a need for a strategy to assess the reliability of non-targeted data pre-processing (NPP) for LC-HRMS data on a routine case by case basis. This led us to develop the R package mzRAPP (Reliability Assessment for NPP), which enabled the extraction of well-defined NPP performance metrics from existing experimental datasets, by generating a benchmark (BM) subset to evaluate NPP-results (Fig. 1). For benchmarking, mzRAPP relies on user-provided input, specific to the experimental chromatographic setup [approximated retention time (RT) boundaries for a subset of compounds with known molecular formulas] for the generation of a BM. This BM is then used as a reference point for deriving performance metrics for NPP conducted on the same raw data. The reliability of NPP performance metrics is ensured by considering a wide range of orthogonal information. Broad applicability in the interdisciplinary field of metabolomics is enabled via graphical user interface (GUI) and support of more than five high cited NPP tools.

2 Implementation and methods

The mzRAPP-package has been developed with the R environment (version 4.0.3) and implemented as a shiny app for ease of use. It can be navigated in a two-step procedure, resulting in ten different NPP performance metrics. The first step is a module responsible for confirming the consistency of user-supplied information (RT boundaries for a subset of compounds with known molecular formulas) for the generation of a BM. This BM is then used as a reference point for deriving performance metrics for NPP conducted on the same raw data. The reliability of NPP performance metrics is ensured by considering a wide range of orthogonal information. Broad applicability in the interdisciplinary field of metabolomics is enabled via graphical user interface (GUI) and support of more than five high cited NPP tools.
NPP-performance metrics for the evaluation of chromatographic peak picking and alignment as well as the aligned features, respectively. The output metrics include measurements to judge the proportion of missed peaks, quality of reported peak abundances, nature of missed peaks and alignment error counts. Interactive plots providing an overview and context to all metrics can be inspected interactively within the shiny app. As a result, several different NPP algorithms generated BM is then utilized to assess the performance of NPP peak picking, peak alignment and feature processing across NPP methods. Subsequently, different performance metrics can be derived as a result. The generated BM can then be used for NPP reliability assessment as described below. More details are provided in Supplementary Material S2.

2.2 Key functions

2.2.1 BM generation

BM generation was facilitated via a cascade of three functions performing IT pattern prediction (get_mz_table), extraction of chromatogram information (get_ROIs) and ultimately peak extraction, consistency checks and filtering (find_bench_peaks). Each function returns a data.frame object serving as argument for the subsequent proceeding functions. In doing so mzRAPP relies on functionalities from enviPat, XCMS (xcmsRaw and findmzROI) and MSnbase (readMSData and chromatogram) architectures. The generated BM can then be used for NPP reliability assessment as described below. More details are provided in Supplementary Material S2.

2.2.2 Assessing NPP reliability

Since the BM can be edited by the user its data structure is checked via check_benchmark_input before being used for NPP assessment. The mzRAPP-package supports output formats from the five most common NPP tools, such as XCMS, MS-DIAL (Tsugawa et al., 2015) and MZmine 2 (Pliska et al., 2010). The check_nonTargeted_input function checks the presence of all components necessary for subsequent matching procedures and generates a generic format output for downstream processing. Next mzRAPPs compare_peaks function performs a series of non-equivalent joins between the BM and supplied NPP output tables. The result is returned as a list object which serves as input for different downstream analysis and plotting functions [e.g. derive_performance_metrics (details are provided in Supplementary Material S2) and plot_sunburst_peakQuality]. Elements of the list are themed around conducted joins or classifications, details of which can be viewed using function-help. Output metrics include measurements to judge the proportion of missed peaks, quality of peak abundances, nature of missed peaks and the alignment error counts. Empirical confidence intervals (alpha = 0.95) for all metrics are estimated by bootstrapping (as employed in the boot package) for the supplied molecules.

2.2.3 Graphical user interface

A comprehensive GUI was developed to help metabolomics scientists with different computational skill sets access mzRAPP software solutions for NPP evaluation. The callmzRAPP function allows users to generate BMs and assess NPP without requiring any command-line functions.

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