Automated Workflow For Peptide-level Quantitation From DIA/ SWATH-MS Data

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Abstract:

Data Independent Acquisition (DIA) is a powerful method to acquire spectra from all ionized precursors of a sample. Considering the complexity of the highly multiplexed spectral data, sophisticated workflows have been developed to obtain peptides quantification directly from this data. Here we describe an open-source and easy-to-use workflow to obtain a quantitative matrix from multiple DIA runs. This workflow requires as prior information an “assay library”, which contains the MS coordinates of peptides. Our workflow consists of OpenSWATH, pyProphet and DIAlignR software. For the ease of installation and to isolate operating system related dependency, docker-based containerization is utilized in this workflow.

Key words OpenSWATH, pyProphet, DIAlignR, Data-Independent Acquisition, DIA, Retention time alignment, SWATH-MS

Introduction:

Liquid Chromatography coupled to tandem Mass-Spectrometer (LC-MS/MS) is widely used to analyze the proteome of biological samples. Currently, multiple methods using LC-MS/MS are available to practitioners which include targeted proteomics, shotgun proteomics and data-independent acquisition (DIA) [1]. Over the last decade, DIA has gained traction for high-throughput and reproducible analysis [1-3]. Compared to traditional shotgun proteomics, in DIA/SWATH-MS peptides are isolated using a larger m/z window and are co-fragmented, which results in rich multiplexed MS2 spectra from multiple precursors. Experimental guidelines to generate DIA data are explained in

Chapter 11 (by Hauck et al). Since DIA spectra are convoluted with fragment-ions from many precursors, innovative strategies have been developed to extract signal for each peptide of interest. These approaches are divided into two categories: library based (peptide-centric) and non-library based (spectrum-centric). In general, library-based methods tend to be more sensitive and produce accurate quantitative results, especially if the assay library is prepared from the same sample [4]. In this chapter, we will focus on the library-based workflow.

Efficient ways to obtain a high-quality library and guidelines are detailed in the article by Schubert et al [5], and in chapter 31 (by Eggers et at). Recent studies suggest that libraries can also be predicted using computational means [6, 7]. A library mostly consists of MS coordinates uniquely describing the peptide of interest. This includes elution time of peptides, their charge state, fragment-ions that are most representative (unique and detectable) and their relative intensities. Each precursor is called an “assay” and this assay library is the key for a successful DIA data analysis. In addition, the assay library must include decoy-peptides for statistical scoring [8, 9]. Note the DIA data contains spectra from all ionized precursors which makes it suitable for re-mining with a new library, however proper diligence is needed [10].

- **Automated data-analysis workflow:**

Compared to traditional mass-spectrometer data analysis in which spectra are matched against a database, DIA workflow focuses on identifying the correct chromatographic peak and its coelution profile; similar to targeted proteomics analysis [11]. Firstly, raw spectra files are converted from vendor-specific format to standard format such as mzML. For format-conversion, we are using MSConvert tool [12].

Feature extraction is performed by first aligning library retention time (RT) to spiked-in standard, iRT peptides, followed by fetching MS2 extracted-ion chromatograms (XICs) using library coordinates, as shown in Figure 1. OpenSWATH, supported by OpenMS, is an open-source software which performs this task and will be discussed in this chapter for picking features [11]. OpenSWATH picks multiple peaks from XICs of a precursor, therefore, a statistical method is needed to assign a probability

for a peak being the correct one, in addition chromatograms without any signal also need to be identified. pyProphet trains a semi-supervised model to calculate a discriminant score from which the p-value of each feature is estimated [8]. It also calculates the false discovery rate (FDR) or q-value (Figure 1) to correct for multiple testing [13].

OpenSWATH is run independently on each run, which can cause inconsistency in quantification due to altered peak-boundaries, selecting different peaks across runs, and also missing peaks resulting from error-control by pyProphet [14]. Alignment of MS2 chromatograms across multiple runs establishes consistency and correspondence between the features. For this purpose, a recently developed software DIAlignR is employed: DIAlignR provides highly accurate retention-time alignment across heterogenous SWATH runs [15]. DIAlignR uses q-values from pyProphet to pick reference run for each peptide and aligns its XICs to the chromatograms of other runs (Figure 2).

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2 Materials

An example dataset of *Streptococcus Pyogenes* is used to explain data-analysis workflow [11, 16]. The source data and intermediate result files can be downloaded from this link: http://www.peptideatlas.org/PASS/PASS01508. Although required applications (OpenMS, pyProphet) are publicly available, we suggest using docker based images as it makes installation of applications convenient. For this tutorial, at least 50 GB storage space is required to store spectra and intermediate chromatogram files. The following material is needed to get started:

1. Example dataset: The example dataset PASS01508 has all the intermediary files for this tutorial. Download this repository and name it as “PASS01508”. Docker scripts are available at the root level. “/lib” folder holds assay-library, alignment library, and SWATH acquisition scheme. “/data” directory has MSConvert output mzML files and raw files inside the “raw” folder. “/results” folder has OpenSwath output files in “OpenSwathOutput” folder. It also has “osw” and “mzml” directory with features and chromatogram files required for alignment by DIAlignR.

2. SWATH spectra files: A detailed protocol to acquire high-quality SWATH-MS data is provided in Chapter 11. In the example dataset, *S. pyogenes* (strain SF370) was grown in 0% and 10% human plasma in biological duplicates. Four technical replicates for each sample were analyzed on an Eksignet nanoLC (with two-hour linear gradient) coupled to an AB SCIEX TripleTOF 5600 in SWATH-MS mode. Thus, the dataset has a total of 16 runs. The raw files are available at /data/raw/ directory in PASS01508. The instrument produces two files per run, .wiff and .wiff.scan files, which should always be stored together. Besides the AB SciEx TripleTOF instrument, other types can be used to acquire spectra in data independent acquisition mode (*see* **Note 1**).

3. Assay library: In library-based workflow, the raw file is mined against an assay library which contains peptide precursors sequences, their retention times, their transitions m/z values and intensities. For statistical scoring of peptide identification, these libraries must contain suitable decoy transition groups [8, 9]. The assay library can be built from one or more shotgun run [5] or downloaded from SWATHAtlas database http://www.swathatlas.org. The SpyogenesAssayLibrary_decoy.pqp library used in this workflow is available in the /lib folder at PASS01508. The conversion to .pqp format is explained at the end of this chapter (*see* **Note 2**). To open library, either use online SQLite viewer (https://inloop.github.io/sqlite-viewer/) or install a local viewer such as the SQLite DB browser (https://sqlitebrowser.org/dl/).

4. Peptide assays for alignment: For targeted-extraction, the library retention time needs to be aligned to each DIA run using linear or nonlinear methods [17, 18]. Generally, spiked-in standard iRT assay library is used for this, however, it has also been shown that without the spike-in standards, library can be aligned using high-intensity endogenous peptides without sacrificing quantitative accuracy [18]. In the example dataset we have picked 20 endogenous peptides for linear alignment. This assay library is available in the /lib directory at PASS01508.

5. Docker software: Docker isolates dependencies between external software libraries and operating systems (Linux, Mac, Windows), providing reproducible computational results. Since docker images are self-contained, they are lightweight and can be easily ported.

   First, install the docker Engine on your machine. For linux system follow guidelines at https://docs.docker.com/install , for Windows 10 Home install Docker Toolbox from https://docs.docker.com/toolbox/toolbox_install_windows, for Windows 10 Pro or Mac install Docker Desktop from https://docs.docker.com/docker-for-windows.

   On a Linux setup, use the following commands to get OpenMS and pyProphet image:

```
sudo docker pull hroest/openms-executables-nightly:latest

sudo docker pull pyprophet/pyprophet:2.1.3
```

   For Windows 10 Home:

   Click on the start menu and type “Docker Quickstart Terminal”. In this terminal window enter previous commands without *sudo* (*see* **Note 3**)-

```
docker pull hroest/openms-executables-nightly:latest

docker pull pyprophet/pyprophet:2.1.3
```

6. Proteowizard: To convert raw files from vendor-specific format to open standardized mzML format, msconvert tool from Proteowizard software suite is used. This software is available at http://proteowizard.sourceforge.net/download.html. For software version and getting msconvert for a Linux system see **Note 4**.

7. R and Rstudio: Alignment tool in this tutorial requires R (version > 3.5.0), therefore, install the latest version of R from https://cran.rstudio.com/. Rstudio integrates many tools to visualize data, documenting scripts to work efficiently with R. Rstudio can be downloaded from https://rstudio.com/products/rstudio/download/.

8. DIAlignR: This is an R-package which uses raw MS2 chromatograms for alignment of features across multiple runs. This software can be installed from Bioconductor in R. Use these commands in R environment (R version > 3.5.0).

```
if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")
BiocManager::install("DIAlignR")
```

---

3 Methods

Before proceeding to an automated analysis, it is crucial to understand DIA data, quality of chromatography and fragment-ion spectra. The Skyline software provides an open-source interactive solution for data visualization. We recommend using the Skyline workflow described previously by Röst et al (2017) to assess data quality and manually inspect few precursors and their transitions [14].

Step 1: File conversion to mzML

MSConvert, provided with ProteoWizard software suite, is used to convert vendor-specific raw spectra files into an open format such as mzML or mzXML. We will use mzML as it is a standardized format.

1. On a Windows setup, click on the *Start Menu* at the lower left corner (Windows icon). Type “MSConvert” in the *search* field and click on *MSConvert*.
2. Select *List of Files* then click on the *Browse* button and navigate to the folder where .wiff and .wiff.scan files are present. Select files and click the *Add* button. Choose an *Output Directory* using the second *Browse* button. In the *Options* field select following parameters- Output format: mzML; Binary encoding precision: 64-bit; Write Index: yes; Use zlib compression: yes; TPP compatibility: yes; Package in gzip: yes; Use numpress linear compression: yes. Other boxes can be left unchecked (Figure 3).

(Optional) To perform centroiding, in the *Filters* menu select *Peak Picking* from drop down; Algorithm: Vendor; MS levels : 1 - 2, click on *Add* and then click *Start*. OpenSWATH produces best results on profile data, however, to reduce disk-space and execution time, we chose to centroid in this workflow (*see* **Note 5**).

Step 2: OpenSWATH workflow:

OpenSwathWorkflow is the main command which efficiently integrates multiple tools from OpenSwath software. A basic command looks like this:

```
OpenSwathWorkflow -in filename.mzML.gz  \
                  -tr assayLibrary.pqp  \
                  -tr_irt iRTlib.TraML  \
                  -out_osw filename.osw \
                  -out_chrom filename.chrom.mzML
```

Where -in: spectra file; -tr: an assay library; -tr_irt: a library iRT peptides; -out_osw: output filename that shall contain picked peaks; -out_chrom: filename that shall contain MS2 chromatograms.

Firstly, move the MSConvert output files to the “data” directory which is located at the root of PASS01508. Make sure the assay library and the iRT library are present in the “lib” directory. For automated analysis-

On a **Linux system**, use the “oswDockerScript.sh” file to run this workflow:

1. Open “Terminal”. In the “Terminal” navigate to the root of PASS01508, where the “oswDockerScript.sh” script is present.
2. Enter the following command at the Terminal:

```
sudo docker run -v `pwd`:/data hroest/openms-executables-nightly /bin/bash
data/oswDockerScript.sh
```

On a **Windows system**, use the “oswDockerScriptWin.sh” file to run this workflow:

1. Click on the start menu and type “Docker Quickstart Terminal”. In the terminal navigate to the root of the data downloaded from PASS01508, where the “oswDockerScriptWin.sh” script is present.
2. Enter this command (*see* **Note 3**):

```
docker run -v `pwd`:/data hroest/openms-executables-nightly /bin/bash
data/oswDockerScriptWin.sh
```

This step yields two files (.osw and .chrom.mzML) for each run. The osw file is a collection of scored peak-groups in SQLite format. This contains potential peaks for each peptide and their scores. The chrom.mzML file contains all XICs of all transitions. For comparison, our results are stored at “results/OpenSwathOutput”.

Besides flags mentioned above, few other important flags to control the execution of *OpenSwathWorkflow* are explained in Table 1. These could be added into the docker script used above.

A web-portal http://www.openswath.org/en/latest/docs/openswath.html also goes into additional detail about OpenSWATH. Users are suggested to signup for mailing list General OpenMS discussion <open-ms-general@lists.sourceforge.net> for OpenSWATH related inquiries.

Step 3: False Discovery Rate (FDR) by pyProphet:

After running OpenSWATH, q-value for each feature across all runs is calculated using pyProphet. It requires the osw files and the corresponding assay library.

The following steps are performed by pyProphet:

1. Merges all osw files.
2. Trains a classifier on merged.osw. Classifier value can be either “LDA” (Linear Discriminant Analysis) or “XGBoost”. To see all flag use help command:

```
pyprophet score --help
```

3. Employs error (type I) rate control at peptide level. FDR-context can be “run-specific”, “experiment-wide” and “global”. To get explanation of all options use help:

```
pyprophet peptide --help
```

Basic commands for estimating q-value are given below:

```
pyprophet merge --template=assayLibrary.pqp --out=merged.osw *.osw

pyprophet score --in=merged.osw --classifier=XGBoost --level=ms2

pyprophet peptide --in=merged.osw --context=experiment-wide
```

For the ease of use we have included all the commands in a docker script. Before proceeding, make sure osw files from previous step are present in the “results” folder.

On a **Linux system**, open the “Terminal”. In the “Terminal” navigate to the root of PASS01508, where the “pyprophetDockerScript.sh” script is present, then, execute following command-

```
sudo docker run -v `pwd`:/data pyprophet/pyprophet:2.1.3 /bin/bash

data/pyprophetDockerScript.sh
```

On a **Windows system**, click on the start menu and type “Docker Quickstart Terminal”. In the terminal navigate to the root of PASS01508, where the “pyprophetDockerScriptWin.sh” script is present. Then, enter the following command-

```
sudo docker run -v `pwd`:/data pyprophet/pyprophet:2.1.3 /bin/bash

data/pyprophetDockerScriptWin.sh
```

pyProphet outputs a “merged.osw” file that has FDR value for each analyte, in addition, it also outputs pdf files which contains summary figures on calculated discriminant score (d-score), p-values and q-values. These must always be consulted so that common errors, such as having assay library without decoys, library with improper decoys and library from another organism can be identified. In a

successful analysis, pyProphet will have a clear separation between positives (true targets) and negatives (false targets and decoys), as shown in Figure 4. The decoy distribution is used to estimate the q-value. To directly get a quantitative matrix from this output, see **Note 6**.

Step 4: Multi-run alignments using DIAlignR:

DIAlignR has a set of functions for fetching XICs, selecting reference run for each analyte, performing chromatogram alignment and picking feature based on alignment. A single function *alignTargetedRuns* integrates all the functions and gives an intensity table. Prepare data by moving .chrom.mzml files to “results/mzml” folder and merged.osw to “results/osw” folder. To perform alignment:

1. Open Rstudio.
2. In “Console”, use *setwd* command to navigate to the “PASS01508/results”directory.

```
setwd("path/to/PASS01508/results")
```

3. To perform alignment and compute the intensity table, use the following command.

```
library(DIAlignR)
intensityTbl <- alignTargetedRuns(dataPath = ".", analyteInGroupLabel =
TRUE, samplingTime = 3.414)
write.table(intensityTbl, file = "strep_intensityTbl.csv", col.names = NA,
sep = ",")
```

alignTargetedRuns has many options to control the alignment algorithm and peak picking. A list of these options can be obtained using `?alignTargetedRuns` in RStudio. Several of these options are explained in Table 2.

DIAlignR also allows to plot aligned chromatograms. Use *getAlignObjs* and *plotAlignedAnalytes* functions to see aligned chromatograms.

```
runs <- c("hroest_K120809_Strep0%PlasmaBiolRepl2_R04_SW",
"hroest_K120809_Strep10%PlasmaBiolRepl2_R04_SW")
AlignObj <- getAlignObjs(analytes = "14299_QFNNTDIVLLEDFQK/3", runs = runs,
dataPath = ".", analyteInGroupLabel = TRUE)
plotAlignedAnalytes(AlignObj, annotatePeak = TRUE)
```

Any issues related to DIAlignR can be reported to https://github.com/shubham1637/DIAlignR/issues.

---

4 Notes

1. SWATH-MS was originally developed for a TOF based mass-analyzer on AB SciEx setup. However, SWATH-like data-independent acquisition (DIA) can be obtained from other types of instruments. One of the major considerations is the acquisition speed, which needs to allow sufficient sampling during elution of an analyte from the LC column. OpenSWATH can analyze data from multiple vendors, including data acquired on Waters Synapt TOF instruments, Thermo Fisher Q Exactive instruments and Bruker timsTOF Pro instrument. OpenSWATH, Skyline and other software have been used to analyze DIA data from Thermo Q Exactive instruments [19-22].

2. While OpenSWATH supports .TraML format, to reduce the downstream file size, SQLite based .pqp format is recommended. To convert library from TraML to pqp format, open “Terminal” (in Linux) or “Docker Quickstart Terminal” (in Windows), as explained in Methods. Navigate to the location where the lib.TraML is present, then run the following command:

```
sudo docker run -v `pwd`:/data hroest/openms-executables-nightly /bin/bash
```

```
-c "cd data; TargetedFileConverter -in lib.TraML -out lib.pqp"
```

3. To execute docker commands for OpenSwath and pyProphet on a Windows 10 Home setup, Following precautions must be taken:
   - Linux commands can be used to run docker containers without having leading *sudo*. It is only valid for Linux OS.
   - Make sure there are no spaces in the file path.
   - Make sure PASS01508 data is in C: drive (shared drive are not enabled for docker toolbox by default).

4. In this tutorial MSConvert is used from ProteoWizard release: 3.0.11252 (2017-8-22). For Linux systems, the docker image can be downloaded using the following command:

```
sudo docker pull chambm/pwiz-skyline-i-agree-to-the-vendor-licenses:latest
```

   To convert wiff files to mzML, use commands from “msconvertOnLinux.sh” file available at PASS01508.

5. OpenSWATH produces optimal results on profile data. For profile data, do not specify anything in *Filters* menu and remove any filter from lower-right box, if present (Figure 3). Centroiding reduces the number of peaks in the data and sometimes may remove low-intensity peaks, the XICs generated by OpenSWATH become less smooth and peak detection becomes less sensitive. If centroiding is performed, it is crucial to compare the results of OpenSWATH to those obtained on profile data on the same dataset to obtain an accurate estimation of how centroiding affects the identification rate [14].

6. Alignment is a necessary step for consistent analysis across multiple runs. Nonetheless, this is a time-consuming step and under some circumstances is not required. A (un-aligned) peptide data-matrix can be directly obtained from merged.osw file, using the following command:

```
sudo docker run -v `pwd`:/data pyprophet/pyprophet:2.1.3 /bin/bash -c "cd data; pyprophet export --in Strep.merged.osw --out Strep.csv"
```

---

5 References

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Figure 1: Peak-identification workflow for a peptide precursor with three transitions: red, blue and green is illustrated. First step is data conversion to standard mzML format, subsequently OpenSWATH is used for RT alignment of library to DIA run. OpenSWATH extracts MS2 chromatogram using library coordinates and identifies potential peaks. Successively, pyProphet identifies correct peak using statistical scoring and FDR calculation.

Figure 2: a) For retention-time alignment, DIAlignR picks a reference run for each precursor. b) The XICs of the reference run are aligned to the corresponding XICs of other runs using a non-linear approach. Thus, peak-boundaries from the reference run are mapped, which establishes consistency in quantification. Here, an alignment of XICs of run2 and run3 to a reference run1 is illustrated.

Figure 3: Converting DIA spectra data to mzML format and centroiding spectra using MSConvert GUI

Figure 4: Density-plot from calculated discriminant score (d-score). Generally, a bimodal distribution is expected for target peptides, whereas decoy peptides are expected to have unimodal distribution. Based on these distributions, pyProphet computes q-values which are used to select a set of results with a well-defined FDR.

Table 1: Few important flags in *OpenSwathWorkflow* command

Parameter	**Explanation**
--helphelp	displays all flags and their explanations.
Isolation window specific	
-swath_windows_file	A text file specifying the SWATH window out of which the fragment-ion traces will be extracted (*see* example file).
-sort_swath_maps	Sorts table specified in *swath_window_file*.
-min_upper_edge_dist	The overlap (in m/z) between neighboring SWATH windows used for data acquisition (Must not be used with *swath_window_file*). Usually set to 1.0 for regular SWATH-MS.
Speed and memory optimization	
-batchSize	The number of chromatograms that are loaded into memory and analyzed at once. The smaller this number, the less memory required.
-readOptions	Sets the memory strategy: by default, OpenSWATH loads all

	data into memory while “cache” will create a set of cached files on which OpenSWATH will work and not load any data into memory (more memory efficient). A good compromise between these two options is “cacheWorkingInMemory” which uses the cached files but loads the current Swath map into memory.
-tempDirectory	Temporary directory to store cached files. (Must be used if *readOptions* are using a caching strategy)
-threads	Defines number of threads to parallelize extraction of XICs and their scoring.
Extracted-ion-chromatogram (XIC)	
-RTNormalization:alignmentMethod	Selects method to align Library RT to sample RT space: ‘linear’, ‘interpolated’, ‘lowess’ and ‘b_spline’. Recommended value with iRT peptides is ‘linear’ and with endogenous peptides select ‘lowess’.
-rt_extraction_window	Size of the RT window in seconds to extract XICs from that peaks should be picked and scored. The RT window is centered around the aligned library RT. Default value is 600 seconds, which means extracting +/- 300 seconds around the expected RT. This value should be changed depending on the quality of library RT alignment.
-extra_rt_extraction_window	Adds additional time to *rt_extraction_window* only for XIC extraction. Peaks will not be picked and scored from this extra XIC. It is useful for visual inspection of chromatograms and for

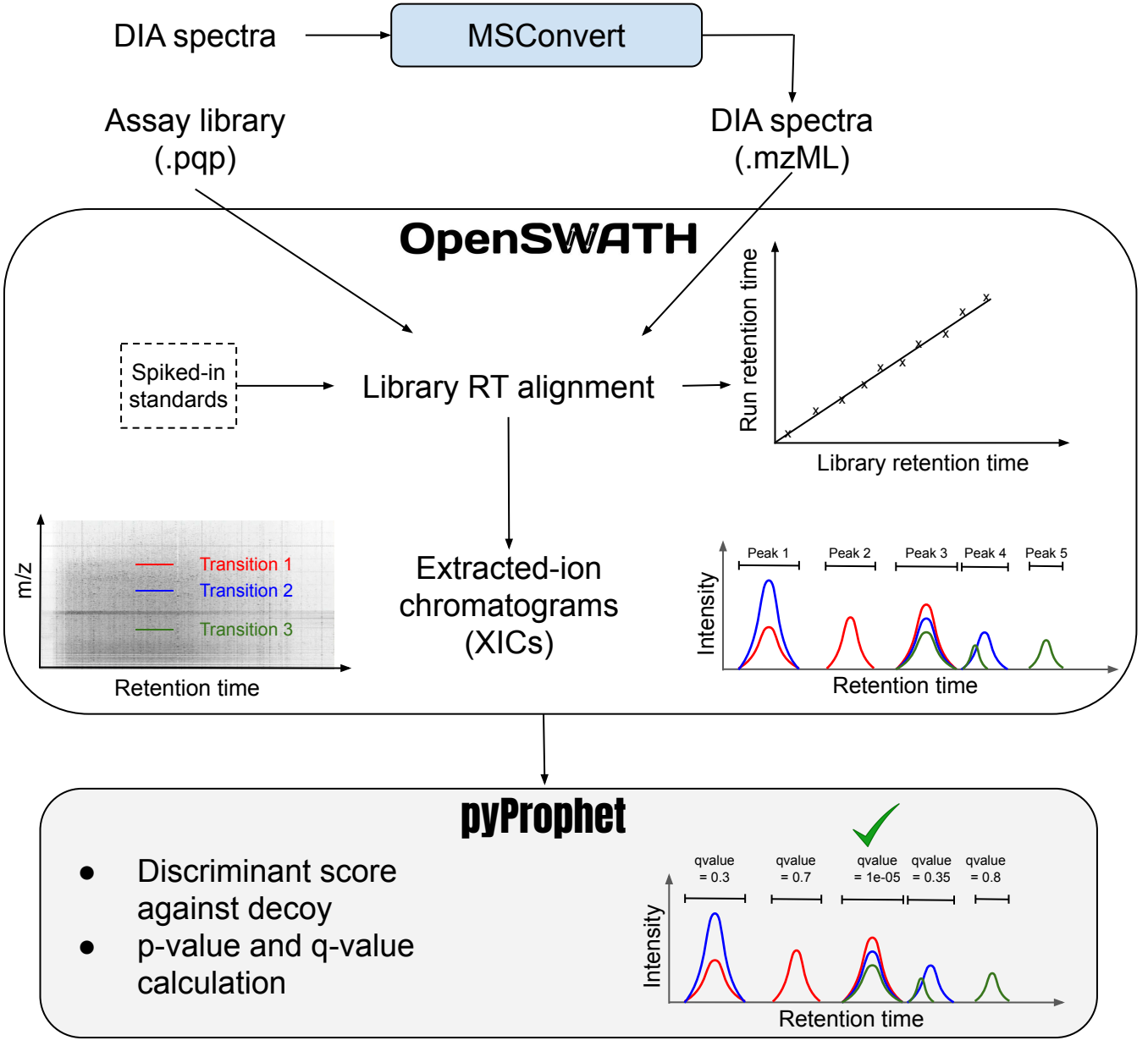
	alignment tools such as DIAlignR.
-mz_extraction_window	Size of m/z extraction window (in Thomson) centered around library m/z. This value needs to be adjusted according to the instrument resolution. It's unit can be changed with *mz_extraction_window_unit*.
-mz_extraction_window_unit	Defines the unit of *mz_extraction_window* and can be set to ppm.
Peak smoothing	
-Scoring:TransitionGroupPicker:PeakPickerMRM:peak_width	Peak-width in seconds required to define a non-spurious peak (adjust to your chromatography).
-Scoring:TransitionGroupPicker:PeakPickerMRM:sgolay_frame_length	Number of points required for convolution by Savitzky-Golay smoothing. Increasing this parameter leads to stronger smoothing.
-Scoring:TransitionGroupPicker:PeakPickerMRM:sgolay_polynomial_order	Degree of polynomial fitted by Savitzky-Golay smoothing. Decreasing this parameter leads to stronger smoothing.
-Scoring:TransitionGroupPicker:PeakPickerMRM:use_gauss	If set as 'true', Gaussian filter is used for smoothing.
-Scoring:TransitionGroupPicker:P	Gaussian width in seconds, estimated peak size in seconds.

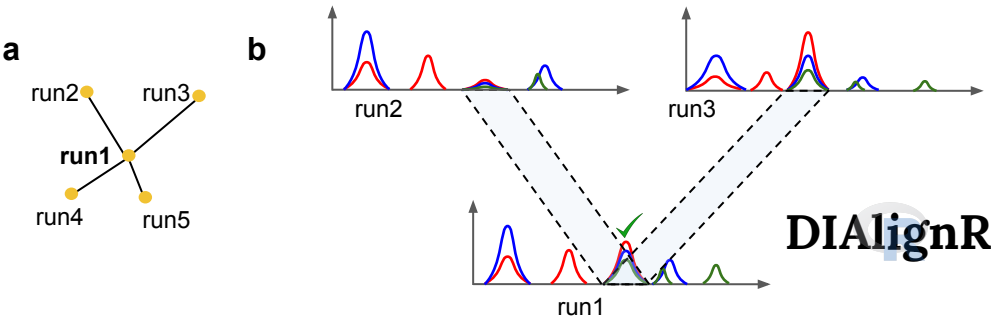
eakPickerMRM:gauss_width	
Debug mode	
-debug	Set as 1 to print all debug output.
Quantification and Scoring	
-TransitionGroupPicker:background_subtraction	Set as 'exact' to remove background from peak signal. This option may increase the accuracy of the quantification.
-use_ms1_traces	Extract the precursor ion trace(s) and use them for scoring

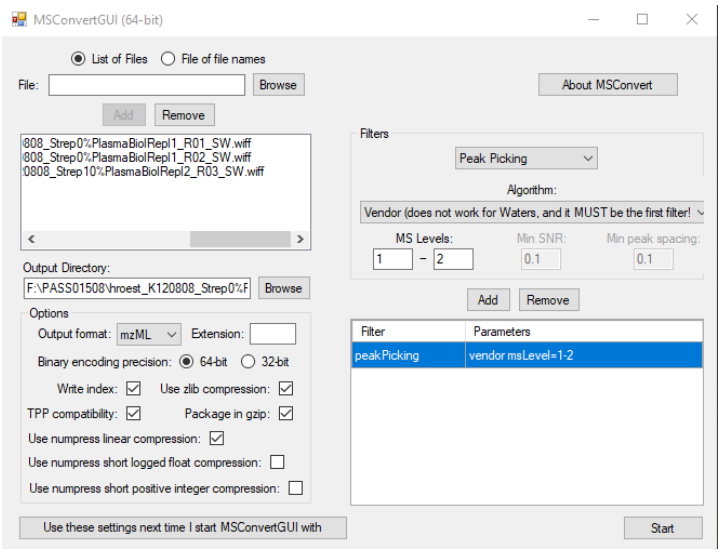
Table 2: Few options in *alignTargetedRuns* to control alignment of chromatograms

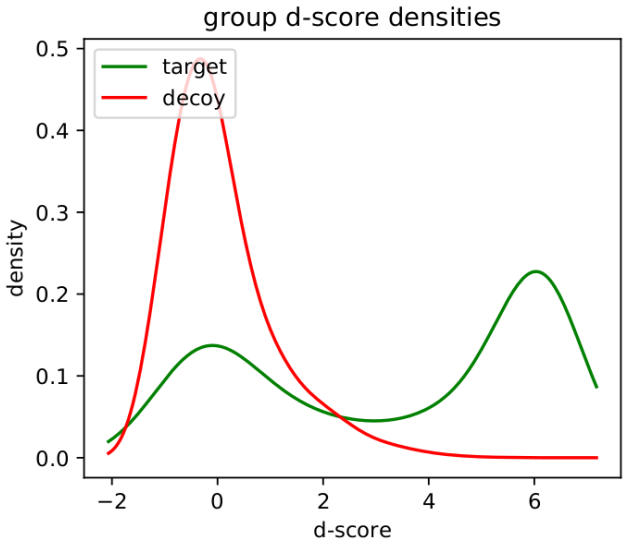
Options	Explanation
analytes	Specify analytes ("PEPTIDE_Charge") to be aligned. If unspecified, all analytes found in osw files are aligned.
runs	Names of runs to be aligned. If unspecified all runs mentioned in RUN table of osw files are aligned.
alignType	Defines the alignment strategy (default: 'hybrid'). There are three options- global: only aligns features from osw, this does not use XICs. local: only uses XICs for alignment. hybrid: uses XICs for alignment that is constrained using features from osw.
simMeasure	Defines the type of metric should be used to construct pointwise similarity between two XIC-groups. XICs of a transition-group have fragment-ion

	intensities for each time-point. The metric defines the similarity across the time-points of two runs. Few common metrics are: dotProduct, cosineAngle, dotProductMasked (default).
SgolayFiltOrd	Polynomial order of the Savitzky-Golay filter. It is suggested to be the same as *sgolay_polynomial_order* parameter of OpenSwathWorkflow.
SgolayFiltLen	Length of the the Savitzky-Golay filter. It is suggested to be the same as *sgolay_frame_length* parameter of OpenSwathWorkflow.
samplingTime	Cycle time used for DIA data. This is the time-difference between two neighboring points of MS2 chromatograms.
analyteFDR	Maximum q-value (between 0 and 1) for peaks to be eligible for quantification.
maxFdrLoess	Maximum q-value (between 0 and 1) for peaks to be eligible for global fit.


DIA spectra
MSConvert
Assay library
(.pqp)
DIA spectra
(.mzML)
OpenSWATH
Spiked-in
standards
Library RT alignment
Run retention time
Library retention time
m/z
Transition 1
Transition 2
Transition 3
Retention time
Extracted-ion
chromatograms
(XICs)
Intensity
Peak 1
Peak 2
Peak 3
Peak 4
Peak 5
Retention time
pyProphet
Discriminant score
against decoy
p-value and q-value
calculation
qvalue
= 0.3
qvalue
= 0.7
qvalue
= 1e-05
qvalue
= 0.35
qvalue
= 0.8
Intensity
Retention time


a
run2
run3
run1
run4
run5
b
run2
run3
run1
DIAlignR


MSConvertGUI (64-bit)
List of Files
File of file names
File:
Browse
About MSConvert
Add
Remove
808_Strep0%PlasmaBiolRepl1_R01_SW.wiff
808_Strep0%PlasmaBiolRepl1_R02_SW.wiff
0808_Strep10%PlasmaBiolRepl2_R03_SW.wiff
Filters
Peak Picking
Algorithm:
Vendor (does not work for Waters, and it MUST be the first filter!
MS Levels:
1
2
Min SNR:
0.1
Min peak spacing:
0.1
Output Directory:
F:\PASS01508\hroest_K120808_Strep0%F
Browse
Add
Remove
Options
Output format:
mzML
Extension:
Binary encoding precision:
64-bit
32-bit
Write index:
Use zlib compression:
TPP compatibility:
Package in gzip:
Use numpress linear compression:
Use numpress short logged float compression:
Use numpress short positive integer compression:
Filter
Parameters
peakPicking
vendor msLevel=1-2
Use these settings next time I start MSConvertGUI with
Start


group d-score densities
target
decoy
density
d-score
0.5
0.4
0.3
0.2
0.1
0.0
−2
0
2
4
6