PIQED Installation and Usage Instructions

Release version 1.0

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Getting started:

- Download the github repository, un-zip, and move the folder to C drive.
- Download the example raw MS/MS data (.wiff and .wiff.scan files) from Massive by entering the following into your browser:
  ftp://MSV000080189@massive.ucsd.edu/
  ID: MSV000080189
  password: $diapip3
  Click on the 'raw' folder and download all the .wiff and .wiff.scan files

Download and install all of the following programs (*denotes executables that must be added to the system path variable):

- msconvert.exe (Proteowizard)
- DIA Umpire, DIA_Umpire_SE.jar
- MS-GF+, msgfplus.jar
- mapDIA, copy mapDIA.exe into C:\...\DIA-pipe\bin\
- Skyline and SkylineRunner.exe
- *Python 2.7
- *Java AND *Javac, java Compiler (included in JDK), added to the system path variable
- *R version > 3.1.0, added to the system path variable
- comet binaries – must use 64-bit version, NOT the 32-bit included with the TPP

Download and install TPP 5.0 programs:

- xinteract.exe
- InterProphetParser.exe
- tandem.exe
- Tandem2XML.exe
- indexmzXML.exe
- PTMProphetParser.exe development version required

Suggested hardware:

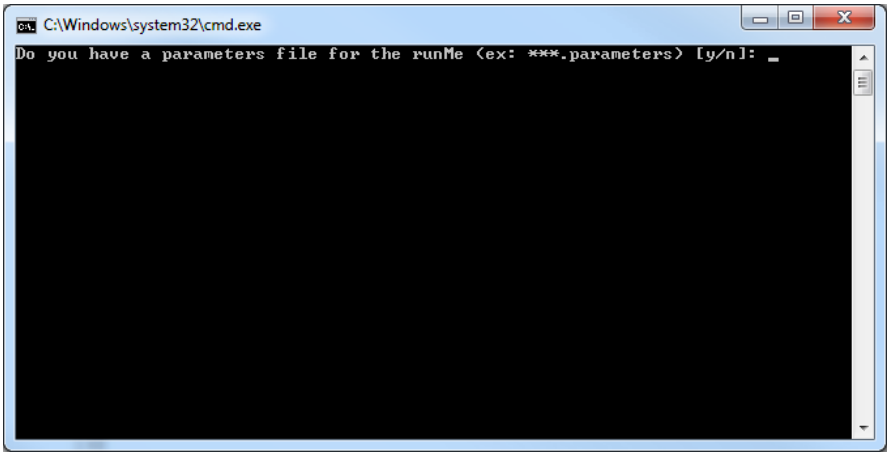
- Windows 7
- Intel i5 quad-core minimum, Xeon >16 core recommended
- 4GB RAM minimum, >32 GB DDR4 RAM recommended
- 1TB 7200 rpm hard drive minimum, 1TB Solid-state hard drive recommended

IMPORTANT NOTES before you begin:

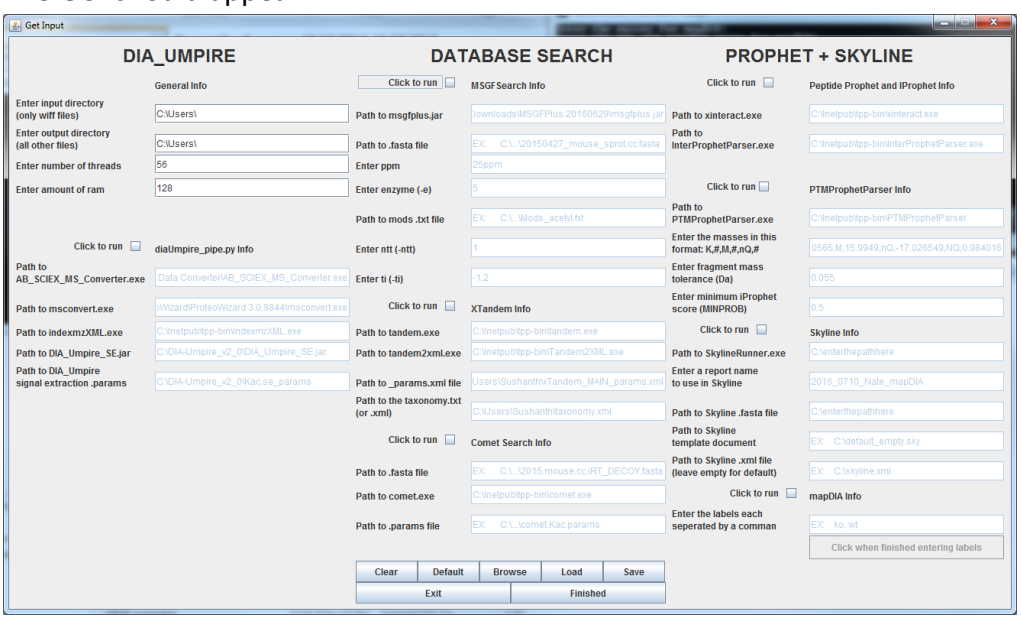
- **Prepare your parameter files** before starting the pipeline, for example:
  - DIA-Umpire Signal Extraction parameters file
  - Database search parameter files, e.g. FASTA, modifications, taxonomy.xml, etc.
  - Skyline template document
- **Modify example parameter files whenever possible** instead of using your own because some steps in the program look for specific lines within these files.
- **Place all raw mass spectrometry files into a folder on your C:\ drive**. From SWATH that means your ".wiff" and ".wiff.scan" files, or from Thermo DIA that means all ".RAW" files. You don't need to explicitly specify whether the data is from Sciex or Thermo, the pipeline will automatically detect the file type and convert the files to .mzXML as appropriate. The parameters used for downstream steps for each type of data are quite different.
- **Use two different fasta files for the database searches (module 2)**. The MSGF+ search uses a database not containing decoys (e.g. 20150810.mouse.cc.iRT.fasta), as they will be created automatically by the search engine. The X! Tandem and COMET searches use a database containing decoys (e.g. 20150810.mouse.cc.iRT_DECOY.fasta).
- **For the mapDIA section to work properly** through mapDIA quantification, files from each condition must all contain the same unique text identifier, e.g. when comparing two replicates each of wild-type (WT) and knockout (KO) samples, files could be named: "DATE_WT1.wiff, DATE_WT2.wiff, DATE_KO1.wiff, DATE_KO2.wiff". **Alternatively,** all file names must either contain text with their group name, or the directory must contain a tab-delimited text file named "name_mapping.txt" where the first row contains names of the group names in the same order entered into the main PIQED GUI before generating the pop-up pictured below, and the rows below list the names of the files in each condition.
- **To enable protein-level correction,** include a file in the output directory named "proteinlevel.txt", which will contain uniport identifiers that match those in your FASTA databases in the first column, followed by the measured areas for each PTM analysis replicate in subsequent columns that are in the same order as the skyline document (alphabetical).

Starting DIA-Pipe and setting up files:

1. Right click on “runME.bat” and choose “Run as Administrator.”
2. Command prompt should appear with the question, ”Do you have a parameters file for the runME (ex: ***.parameters) [y/n]:” type “n” and press enter.

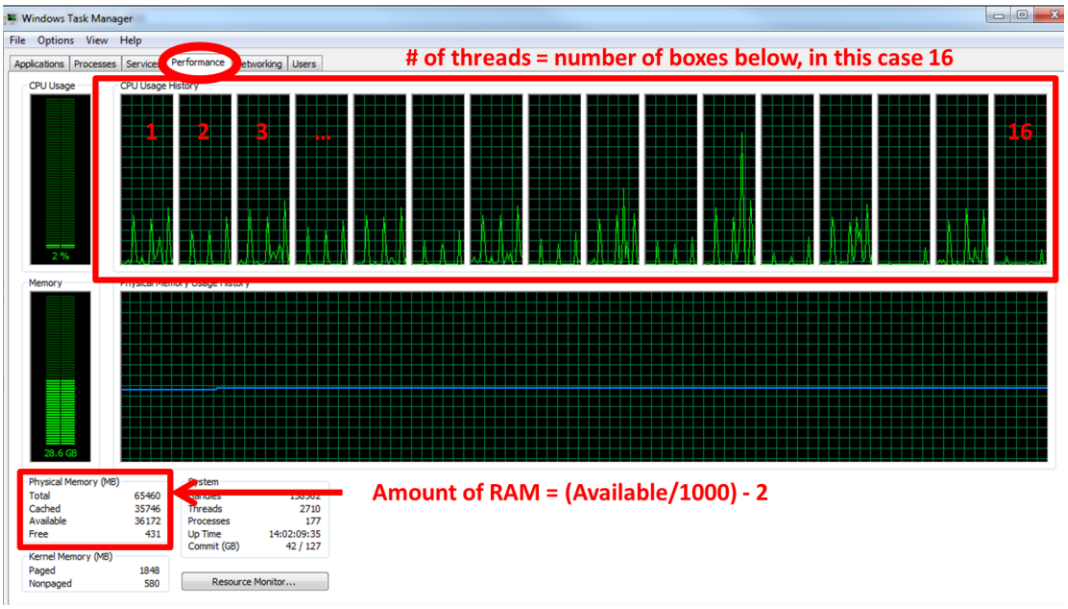


3. The GUI should appear:



4. At the top left under **General Info**, enter the full path to the directory where all .wiff + .wiff.scan files OR all .raw files are located as the input and output directories.
5. Enter the number of threads (equal to or less than the number of CPU cores), and enter the amount of ram (equal to or less than the amount of RAM installed). To determine the number of cores and the amount of RAM, press control+shift+Esc, which opens the task manager, then click on the performance tab, and count the boxes:

1. **Press Control+Shift+Esc**
2. **Select the "Performance" tab**



Module 1, File Conversions and DIA-Umpire signal extraction:

1. Check the box, "Click to run" under each section to activate the fields.
2. In the bottom-left section, you need to specify the locations for all the file conversion executables. **NOTE: Use of Sciex data converter is currently depreciated! You do not need to specify the actual location, but this box must contain text, e.g. "none".**
3. In the bottom box, enter the path to **your specific** DIA-Umpire signal extraction parameter file containing your desired settings and possibly your variable window definitions.

DIA-Umpire Signal extraction parameter notes: An example parameter file for Sciex data 'diaumpire_se.params' is available in the github repository under the "params" folder. An example parameter file for orbitrap data is available from github under the parameter folder: (1) "diaumpire_se_orbi_strict.txt" was used for the non-enriched urine data in the manuscript, or (2) "diaumpire_se_orbi.txt" for enriched samples. This example can be used with the "halfDIA" and "fullDIA" files from massive. These are default parameters that can be used for typical Sciex datasets. Using your own data, you likely need to change the isolation windows section of the parameters file to reflect those used on your instrument. You should also change the "Threads=" to reflect the threads available on your computer. For more details on these parameters, please see the DIA-Umpire documentation: http://diaumpire.sourceforge.net/?page_id=19

Module 2, Database Searches:

1. Check the boxes next to each of the database searches you want to run.
2. Enter the locations of the files for all the search program executables and the parameters.
   - MS-GF+ and X! Tandem searches are set to automatically convert the results to .pep.xml after completion, whereas the comet.params file should specify the output as .pep.xml

MSGF+ search: If using the example SWATH files from MASSIVE, under MSGF+ enter ntt = 1, enzyme= 1, ti= 0,1, ppm = 25ppm, fasta file = "20150810.mouse.cc.iRT.fasta", and mods.txt file = "tutorial_MSGFmods.txt" from the github repository.

X! tandem search: DIA-Pipe looks for specific lines of text in the X! Tandem Parameters file. **Do not edit the input and output file locations within the tandem.params file.** You must edit two lines minimum: (1) the path to your scoring parameter file (e.g. kscore downloaded with TPP), and (2) the full path to your taxonomy.xml file containing the database location. If using the example SWATH files from MASSIVE, use the params.xml file "xTandem_Kac_params.xml" and the taxonomy.xml file from github. Within the taxonomy.xml file, update the path to the database to reflect the full path to the .fasta database on your computer, e.g. "20150810.mouse.cc.iRT_DECOY.fasta".

COMET search: If using the example SWATH files from MASSIVE, use the "comet64.sciex.Kac.params" file from github, and open the file to update the first line "database_name =" to reflect the location of "20150810.mouse.cc.iRT_DECOY.fasta" on your computer.

Module 3, Prophet Refinement, Skyline Signal Extraction, and mapDIA:

PeptideProphet, iProphet, and PTMprophet

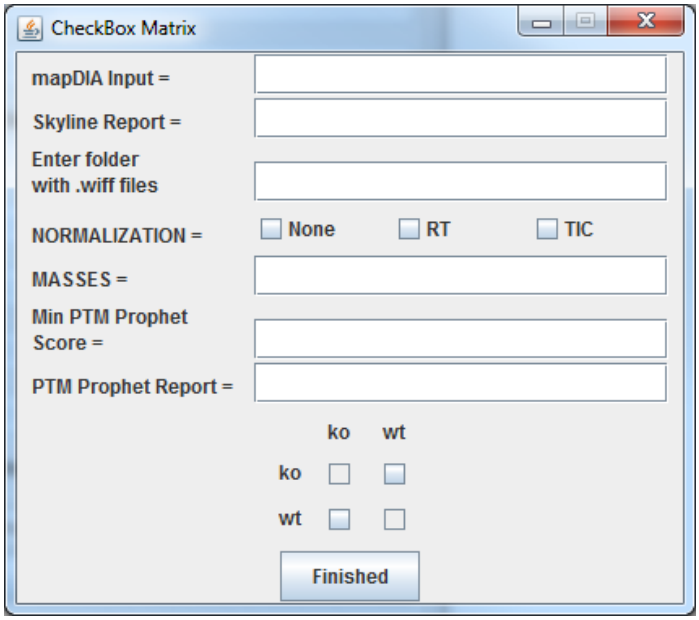
1. Check the boxes for all the desired steps and enter the requested information.
2. If using the tutorial data, the defaults under PTMProphetParser, except for the path to PTMProphetParser, are appropriate settings. Otherwise, enter the mass corresponding to your modifications of interest, fragment mass tolerance appropriate for your instrument, and the minimum iprobability used to consider peptides for site-localization scoring (0.9 should be safe).

Skyline Signal Extraction and report generation

1. Skyline Template document:
   a. Tutorial 5600 or Orbitrap data: Open Skyline and create a template or copy the appropriate skyline template document to your output directory, "..\DIA-Pipe\skyline\default_empty.sky" for data from ABsciex 5600, or "..\DIA-Pipe\skyline\orbi_empty.sky"
   b. User datasets: Open Skyline and create a new blank document containing the transition settings you want to use for signal extraction. Add the report "2016_0826_mapDIA.skyr" to your document report list. Save the empty document to the output directory containing your .mzXML files.
3. Copy the skyline report file from "..\DIA-Pipe\skyline\2016_0826_mapDIA.skyr" to your output directory
4. Under the Skyline section, if using the tutorial files from MASSIVE, use the skyline report name "2016_0826_mapDIA," and the skyline template document titled "default_empty.sky" from the github page.
5. If using the tutorial data accessible from Massive, use the .fasta file '20150810.mouse.cc.iRT.fasta' for the skyline .fasta file.

mapDIA

1. To setup mapDIA, enter the conditions-specific labels contained in the raw file names separated by commas, and then click, "Click when finished entering labels." If using the tutorial files, enter "fulldia" and "halfdia" as condition labels.
2. The following box should appear:



NOTE: The mapDIA module requires either the path to your mapDIA input file (if running this module independent of the other steps), OR the name of the skyline report created from the previous steps. Enter only one of these.

6. If only running mapDIA (not the previous steps in DIA-Pipe), enter the mapDIA input file path and leave the Skyline Report box blank.
7. If running mapDIA using results from previous modules, and using the skyline template file with template report, then enter "2016_0826_mapDIA.csv" as the **skyline report** and leave the **mapDIA Input** box blank.
8. Enter the starting directory containing your .wiff files (if completing the entire pipeline, this path will match the path under the first section "Enter Input directory)
9. Chose the type of normalization (see mapDIA documentation). If using the example SWATH files from MASSIVE, choose "None."
10. Enter the integer mass of the modification you want to quantify (enter 42 for acetylation if using the example SWATH files from MASSIVE).
11. Enter the minimum localization score you are willing to accept for your quantified results (0.75 suggested).
12. Enter the name of the PTM Prophet report to use for finding the localization scores. If this was produced using the PTMProphet section, use the name: "ptmProphet-output-file.ptm.pep.xml".
13. Make checks in the boxes where you want comparisons to be made. For each comparison, the column title will be the denominator. For example, to compute KO/WT in the above example, check the top-right box. To compute, WT/KO, check the bottom-left box. **NOTE:** The same comparison cannot be made twice with alternate denominators, e.g. WT/KO and KO/WT will cause an error from mapDIA.
14. Once everything is correctly filled out, click "finished" to return to the main window.
15. Save the parameters you entered (in case of an error) by clicking the save button and choosing one of the save slots:

16. To run the pipeline, click the "finished" button at the bottom of the page. A command prompt will pop up and display the status of the current commands as they run.
17. After clicking the finished button, a window will open saying that 'errors may occur'. This does not mean that the system paths were entered incorrectly. It is just a warning. This warning is telling you that if the database searches do not find your specified modification (acetylation if using tutorial data), then the Prophet refinement will fail.