

DCAnalysis v1.10

Installing and Opening DCAnalysis

1. Copy the folder DCAnalysis_v1.10 to your hard drive.
2. Within this folder there is a file titled "DCAnalysis_v1.10.exe".
3. Double click this file to open the program.

Data format

To analyse your data with DCAnalysis your data must be in the form of a CSV file and contain a single isotopomer in each row. An example of this format is produced when extracting data with XCMS. This file must be in the following format:

1. Column A must contain a unique number for the isotopomer.
 - a. The heading can be blank
 - b. The heading must not contain "mz", "m/z", "MZ" or "M/Z"
 - c. The heading must not contain "rt" or "RT"
2. Retention time data
 - a. The first column from the left which contains "rt" or "RT" in its heading is selected as the retention time data – If you have problems try **retention time data in minutes, and retentions less than 150 min**
3. Mass to charge data
 - a. The first column from the left which contains "mz", "m/z", "MZ" or "M/Z" in its heading is selected as the retention time data
4. Peak area data
 - a. Each column which data averages above 2000 is selected as a sample column
 - b. Multiple sample data can be within the same CSV, similar to CSV files produced by XCMS.
 - c. If your sample data averages less than 2000 it will not be selected by the programme as a sample. Consider to add a multiplier to your sample data.
 - d. The heading of the column which contains your peak area information is used as the sample name.

Please refer to the files CSVSampleData1.csv, and CSVSampleData2.csv for acceptable data formats. For increased program speeds it is recommended that isotopomers which are present in both samples and blanks are removed.

Uploading and Processing Data

1. Within the programs user interface click the button "Import CSV"
2. From the dialog box navigate to the folder containing you CSV file and select it.
3. Once you have opened your data file within DCAnalysis the program will begin to sort your data. Once the sorting it complete a message will appear in the text box to the bottom left which says "File loaded". In addition to this the drop down list labelled "Current sample:" will be populated with each of the samples. The samples within this drop down box are the headings of columns within the CSV file that have an average value of over 2000.
4. From the drop down list labelled "Current sample:" select the sample that you want to process.
5. In the text box labelled "RT drift:" enter the acceptable error in retention time between isotopomers of the same compound.
6. In the text box labelled "Mass error:" enter the acceptable error in mass to charge ratio in ppm.
7. In the drop down box labeled "Detector polarity", select which polarity your data was acquired in, positive or negative – this is used to compile molecular features.

8. Click the button "Find Features"
 - a. This will compile all isotopomers within your data into chemical and molecular features based on the RT and m/z errors selected above.
 - b. Once this process is finished a message will appear in the text box to the bottom left which says "Your current samples chemical and molecular features have been compiled".
9. In the drop down box labelled "Filter:" select which algorithms you would like to use to filter your data
 - a. DCA-Hal for filter your data for chlorine and bromine containing compounds
 - b. DCA-Sul for filtering your data for sulphur containing compounds
 - c. All Features for displaying all compiled features
10. In the text box labelled "Area cut-off:" enter the minimum peak area for your results. A molecular feature with a maximum isotopomer peak area less than this value will not be displayed in your results. Chemical features with peak areas less than this within a molecular feature with peak areas greater than this will still be displayed and assessed – This can be used to remove data with low signal to noise if not preformed from early in the data extraction.
11. In the drop down box labelled "Feature Mode:" select how you would like to filter your data.
 - a. By selecting "Molecular", each chemical feature within a molecular feature is assessed by the algorithms and if it exceeds the required rate set below, the entire molecular feature is filtered.
 - b. By selecting chemical feature, each chemical feature is assessed independently.
12. The text box labelled "Required Rate:" is only available in Molecular feature mode. This rate is the percentage that must be exceeded for a molecular feature to be filtered by the algorithms. For example: if a chlorine containing molecular feature contains the following three chemical features: $[M+H]^+$, $[M+Na]^+$, and $[M-HCl+H]^+$ only two of the three chemical features will be classified as halogenated (66.7 %) due to the loss of the chlorine atom in the $[M-HCl+H]^+$ chemical feature, therefore a required rate less than 66.7 % would be needed to classify this molecular feature correctly. The default of 50 % has been used effectively to classify halogenated metabolites from algae extracts.
13. Click the button "Apply Filter"
14. In the text box to the bottom right a summary of filtered features is made. In addition, in the drop down box labelled "Filtered Features:" is a list of all the molecular (if in Molecular feature mode) or chemical features (if in Chemical feature mode) filtered by the selected filter.
 - a. Selecting one of these will display the mass spectra of the feature.
15. To save your filtered data as a CSV click the button "Export Results"