Record of feature finding in MZmine and molecular networking

**I. MzMine:**

1. Raw data import <-raw data methods
2. Open one of the files to manually investigate
3. Processing wizard-> LC-MS workflow->choose mode and instrument
4. MS presets:

-MS noise level: zoom in on actual sample and find where the noise is

-MS2 noise level: choose several MS2 peaks from peak table (more intense and higher m/z) and check noise level

-min feature heigh: 3x higher than MS/noise level

1. HPLC presets:

-ret. time: adjust to program

-max peaks in chromatogram: 10-15 (hom namy of the same peaks are in chromatogram)

-min samples per allign feature: depends on how many repeatable samples are in batch

-min # of data points: usually 4

-approx feature FWHM: check several goos peaks

-intra-sample RT tolerance

-inter-sample RT tolearnce

-features with 13C

1. Export->V->choose location, put a date in a name
2. Build batch->remove smoothing

-ion identity networking->ion identity library->setup->choose all adducts

\*to make , for example, Fe3+:add->put mass monoisotopic->click combine->[M+Fe]+3 + [M-H]- + [M-H]- ->add->[M+Fe-2H]+

**II. Molecular Networking-GNPS**

-Classical:only MS2 data, need to load mzMLfile additionally

-Feature-based (FBMN): MS1+MS (perform MzMine feature finding beforehand)

*FBMN requirement files:*

1. Feature table:.txt or .cvs (with intensities of LC-MS)
2. .mgf file: MS2 spectral summary
3. Metadata table (text(tab delimited))

Metadata table format: open the result file from feature finding (cvs)->copy all of mzML peak area names

->transpose->delete "\_peak area"→filename

|  |  |  |
| --- | --- | --- |
| filename | Samplename | Attribute\_DIET |
| xxx1.mzML |  | normal |
| xxx2.mzML |  | deficient |
| xxx3.mzML |  | accessive |

Column 1: filename (xxx1.mzML, xxx2.mzML, ...)

Column 2: Samplename (input actual sample names that were randomized for MS)

Column 3-x: Attribute\_shorthand (attribute:wt/mutant/media etc)

! Do both pos and neg modes.

Transfer all files through CyberDuck to aronlabshared account.

*CyberDuck* (host: massive.ucsd.edu, username: aronlabshared)

GNPS submission:

Advanced Analysis Tool->Feature Networking->insert 3 files->basic options (0.02 Da tolerance for both)->

->Advanced Network Options(min pairs cos : 0.7, min mathced fr.ions:6)->normalization->

->PCoA Distance Metric: braycurtis (run dereplicator-annotates known Peptide Nat Products)->Submit

feature\_table\_filtered\_no\_null\_AK.csv - summed up intensities and deleted ones that are 0- brought a lot of unknowns to visualisatio in cytoscape

aronlabshared/mouse\_studies/refined.mgf -refinement using code provided by Allegra in python