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PyMS version 1.0

A Python toolkit for processing of chromatography–mass spectrometry data

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Introduction

1.1 About PyMS

PyMS is a Python toolkit for processing of chromatography–mass spectrometry data. The main idea behind PyMS is to provide a framework and a set of components for rapid development and testing of methods for processing of chromatography–mass spectrometry data. An important objective of PyMS is to decouple processing methods from visualization and the concept of interactive processing. This is useful for high-throughput processing tasks and when there is a need to run calculations in the batch mode.

PyMS is modular and consists of several sub-packages written in Python programming language [1]. PyMS is released as open source, under the GNU Public License version 2.

There are four parts of the pyms project:

- pyms – The PyMS code
- pyms-docs – The PyMS documentation
- pyms-test – Examples of PyMS use
- pyms-data – Data used in pyms-test

Each part is a separate project on Google Code that can be downloaded separately.

1.2 PyMS installation

There are many ways to install PyMS depending on your computer configuration and preferences. The recommended way to install PyMS is to compile Python from sources and install PyMS within the local Python installation. This procedure is described in detail below.

PyMS has been developed on Linux, and detailed installation instructions for Linux are given below. Installation on any Unix-like system should be similar. We have not tested PyMS under Microsoft Windows.

1.2.1 Downloading PyMS source code

PyMS source code resides on Google Code servers, and can be accessed from the following URL: <http://code.google.com/p/pyms/>. Under the section "Source" one can find the instructions for downloading the source code. The same page provides the link under "This project's Subversion repository can be viewed in your web browser" which allows one to browse the source code on the server without actually downloading it.

Google Code maintains the source code by the program called 'subversion' (an open-source version control system). To download the source code one needs to use the subversion client program called 'svn'. The 'svn' client exists for all mainstream operating systems¹, for more information see <http://subversion.tigris.org/>. The book about subversion is freely available on-line at <http://svnbook.red-bean.com/>. Subversion has extensive functionality. However only the very basic functionality is needed to download PyMS source code.

If the computer is connected to the internet and the subversion client is installed, the following command will download the latest PyMS source code:

```
$ svn checkout http://pyms.googlecode.com/svn/trunk/ pyms
A    pyms/Peak
A    pyms/Peak/__init__.py
A    pyms/Peak/List
A    pyms/Peak/List/__init__.py
.....
Checked out revision 71.
$ ls -CF
pyms/
$
```

1.2.2 Minimal PyMS installation

The minimal PyMS installation involves downloading the PyMS code and installing the pycdf package. Download the pycdf package from <http://pysclint.sourceforge.net/pycdf/>, and follow the installation instructions that come with it. To test your pycdf installation:

```
$ python
Python 2.5.1 (r251:54863, Jul 10 2007, 08:23:47)
[GCC 4.1.1 20070105 (Red Hat 4.1.1-52)] on linux2
Type "help", "copyright", "credits" or "license" for more information.
>>> import pycdf
>>>
```

¹For example, on Linux CentOS 4 we have installed the RPM package 'subversion-1.3.2-1.rhel4.i386.rpm' to provide us with the subversion client 'svn'.

If the pycdf is installed properly the above import statement will produce no error messages.

The PyMS code (ie. directory pyms/) can be placed anywhere as long as the Python interpreter is made aware where it is (see Python documentation for details on how to make packages visible to Python interpreter). In example, we keep the pyms code in `/home/current/proj/PyMS/` (ie. the PyMS code is `/home/current/proj/PyMS/pyms/`). Since this is a non-standard location for Python packages, before running PyMS we set the following in Python:

```
import sys
sys.path.append("/home/current/proj/PyMS/")
```

This makes Python aware that some packages may be in `/home/current/proj/PyMS/`. Therefore to check the PyMS installation one might try the following:

```
$ python
Python 2.5.1 (r251:54863, Jul 10 2007, 08:23:47)
[GCC 4.1.1 20070105 (Red Hat 4.1.1-52)] on linux2
Type "help", "copyright", "credits" or "license" for more information.
>>> import sys
>>> sys.path.append("/home/current/proj/PyMS/")
>>> from pyms.IO.ANDI.Class import ChemStation
>>>
```

The last command has loaded the class 'ChemStation' from PyMS. Since this did not produce any error messages the PyMS was accessible (and moreover, that pycdf is properly installed, since the class 'ChemStation' uses pycdf).

For examples on PyMS use see Chapter 2.

1.3 Minmax peak detector

1.3.1 Introduction

Minmax peak detector is the simplest kind of a peak finding algoritam for TIC. It operates by finding peak maxima, and then attempting to determine peak boundaries. Cursory evidence suggests that gives results similar to the ChemStation peak detection, but this was not examined rigorously. The purpose of this algorithm is to provide an example of how 1D peak pickin can be implemented in PyMS. *At present the Minmax algorithm was not properly tested, do not use it for critical publication quality results.*

1.3.2 A brief description of the algorithm

Many peak detection algorithms are used in practice to process GC/LC-MS data, but only a few are fully documented, most notable those of open source projects MZmine [2] and XCMS [3]. MZmine detects peaks by finding local maxima of a certain width [2]. In XCMS peaks are detected by using an empirical signal-to-noise cutoff after matched filtration with a second-derivative Gaussian [3]. PyMS peak detection procedure was developed in-house, and relies on finding local maxima and local minima in the signal, followed by a subsequent refinement of peak left and right boundaries. Peak detection depends on two input parameters: window width over which a peak is expected to be a global maximum, and the scaling factor S used to calculate the intensity threshold $S\sigma$ which must be exceeded at the peak apex. The noise level σ is estimated prior to peak detection by repeatedly calculating median absolute deviation (MAD – a robust estimate of the average deviation) over randomly placed windows and taking the minimum. A detailed description of procedures for peak detection follows.

1. **Extracting local maxima.** Initially, an ordered list of local maxima in the signal with an intensity larger than a threshold is compiled. Two input parameters are specified by the user: the width of a window over which the peak is required to be a global maximum (W); and (2) the scaling factor S used to calculate intensity threshold $S\sigma$, where S is the noise level estimated previously (defaults: $W = 2$ data points, $S = 10$). User specified window is centered on each point of the signal, and the point is deemed to be a local maximum if the following is satisfied:

- (a) It is equal or greater than all of the points within the window W .
- (b) It is greater than at least one point in the half-window interval to the left, and at least one point in the half-window interval to the right.²
- (c) Any point closer to the edge of the signal than half-window is rejected.

Intensity at each local maxima is tested, and those that have the intensity below the threshold $N*S$ are rejected. Accepted local maxima are compiled into a list.

2. **Determination of peak left/right boundaries.** For each local maxima (base maximum) the stretch of the signal between itself and the next local maximum on either side is extracted. These two signal slices are searched for the first local minimum in the direction away from the base maximum point itself. The local maxima are defined in a very similar manner as the local maxima in the previous step. A point is deemed to be a local maximum if:

- (a) It is equal or smaller than all the points within the window W .
- (b) It is smaller than at least one point in the half-window interval to the left, and at least one point in the half-window interval on the right.
- (c) Any point closer to the edge of the slice than half-window is rejected. This has the effect that the boundary point cannot approach next peak's apex closer than half-window.
- (d) If no minimum point is found, set the boundary point to the point furthestest away from the base maximum, but outside to the half-window range of the adjacent peak.

²This is to reject points within intervals of uniform intensity

3. **Elimination of peak overlaps.** In spectra dense with peaks peak boundaries as found in the step (2) may overlap due to the effect of user supplied window. The list of pre-peaks is searched for overlapping peaks. In overlapping peaks the right boundary of the lower retention time peak overlaps with the left boundary of the higher retention time peak. The overlapping boundaries are resolved by finding the point of minimum intensity between the two peaks (the split point). The peak boundaries are set to one point to the left from the split point for the right boundary of the lower retention time peak, and to one point to the right from the split point for the left boundary of the higher retention time peak.
4. **Correction for long tails.** In this step peak boundaries are adjusted to remove stretches of near-uniform intensities (i.e. long tails). Each peak is divided at the apex into two halves, and each half is processed individually in the boundary-to-apex direction. A line is fitted through M points from the boundary in the least-squares sense. Prior to calculating the angle between the line and the retention time axis, the rise in intensity is normalized with the intensity at the peak apex. If this angle is below the user specified cutoff (Q) the boundary point is dropped, and the process is repeated. This adjustment is repeated until the best fit through M points from the boundary gives an angle greater than the cutoff. The parameters M and Q are user specified (defaults: $M = 3$, $Q = 1.0^\circ$).

Using PyMS

2.1 Introduction

This chapter demonstrates main functions of PyMS in a tutorial like manner. The data files used in the examples are provided in the project 'pyms-data'. The commands executed interactively are grouped together by example, and provided as Python scripts in the project 'pyms-test'.

The setup used in the examples below is as follows. The projects 'pyms', 'pyms-test', 'pyms-docs', and 'pyms-data' were downloaded in the directory `/home/current/proj/PyMS`. In the project 'pyms-test' there is a directory corresponding to each example coded with the example number (ie. `pyms-test/01/` corresponds to Example 1). In each example directory there is a script named 'proc.py' which contains the commands given in the example. Provided that the paths to 'pyms' and 'pyms-data' are set properly, these scripts could be run by simply:

```
$ python proc.py
```

Before running each example the Python interpreter was made aware of the PyMS location with the following commands:

```
import sys
sys.path.append("/home/current/proj/PyMS/")
```

For brevity these commands will not be shown in the examples below, but they are included in 'pyms-test' example scripts. The above path need to be adjusted to match your own location of pyms.

All data files (raw data files, peak lists etc) used in the example below can be found in 'pyms-data'.

2.2 Example 1: Reading of GC-MS data and basic manipulation of data

2.2.1 Reading ChemStation GC-MS data into PyMS

This example is in `pyms-test/01`

The PyMS package `pyms.IO` provides capabilities to read the raw GC-MS data stored in the ANDI-MS format. The function `IO.ANDI.ChemStation()` provides the interface to ANDI-MS data files saved from Agilent ChemStation software.³

The file `'a0806_140.CDF'` is a GC-MS experiment exported from Agilent ChemStation (located in `'pyms-data'`). This file can be loaded in the memory as follows:

```
>>> from pyms.IO.ANDI.Class import ChemStation
>>> andi_file = "/home/current/proj/PyMS/pyms-data/a0806_140.CDF"
>>> andi_data = ChemStation(andi_file)
-> Processing netCDF file '/home/current/proj/PyMS/pyms-data/a0806_140.CDF'
    [ 3236 scans, masses from 50 to 550 ]
>>>
```

The above command creates the object `'andi_data'` which is an *instance* of the class `IO.ANDI.ChemStation`.

2.2.2 Exploring an ANDI-MS data object

The object `'andi_data'` has several attributes and methods associated with it.

```
>>> print "ANDI-MS data filename:", andi_data.get_filename()
ANDI-MS data filename: /home/current/proj/PyMS/pyms-data/a0806_140.CDF
```

The method `get_tic()` return total ion chromatogram (TIC) of the data as an `IonChromatogram` object:

```
tic = andi_data.get_tic()
```

An `IonChromatogram` object is a one dimensional vector containing mass intensities as a function of retention time. This can be either m/z channel intensities (for example, ion chromatograms at $m/z = 65$), or cumulative intensities over all measured m/z (TIC).

The method `get_ic_at_index(i)` returns i -th ion chromatogram, as an `IonChromatogram` object. For example, to get the first ion chromatogram from the data:

³ANDI-MS data format stands for Analytical Data Interchange for Mass Spectrometry, and was developed for the description of mass spectrometric data developed in 1994 by Analytical Instrument Association. ANDI-MS is essentially a recommendation, and it is up to individual vendors of mass spectrometry processing software to implement "export to ANDI-MS" feature in their software.

```
ic = andi_data.get_ic_at_index(1)
```

The method `get_ic_at_mass(MZ)` returns the ion chromatogram for $m/z = MZ$. For example, to get the ion chromatogram that corresponds to $m/z = 73$:

An ion chromatogram object has a method `is_tic()` which returns True if the ion chromatogram is TIC, False otherwise:

```
>>> print "'tic' is a TIC:", tic.is_tic()
'tic' is a TIC: True
>>> print "'ic' is a TIC:", ic.is_tic()
'ic' is a TIC: False
```

2.2.3 Writing data to a file

The method `write()` of IonChromatogram object allows one to save the ion chromatogram object to a file:

```
>>> tic.write("output/tic.dat", minutes=True)
>>> ic.write("output/ic.dat", minutes=True)
```

The flag `minutes=True` indicates that retention time will be saved in minutes. The ion chromatogram object saved with the `write` method is a plain ASCII file which contains a pair of (retention time, intensity) per line:

```
$ head tic.dat
5.0944      745997.0000
5.1002      726566.0000
5.1059      717704.0000
5.1116      684214.0000
5.1173      701866.0000
5.1230      893306.0000
5.1287     1278099.0000
5.1345     1290984.0000
5.1402      925558.0000
5.1459      644122.0000
```

Figure 2.1 shows the plot of the file 'tic.dat' produced with the program Gnuplot. The Gnuplot script used to produce this plot is provided as `pym-s-test/01/output/plot.gnu`.

The method `get_intensity_matrix()` of ChemStation object returns the entire matrix of intensities:

```
>>> im = andi_data.get_intensity_matrix()
```

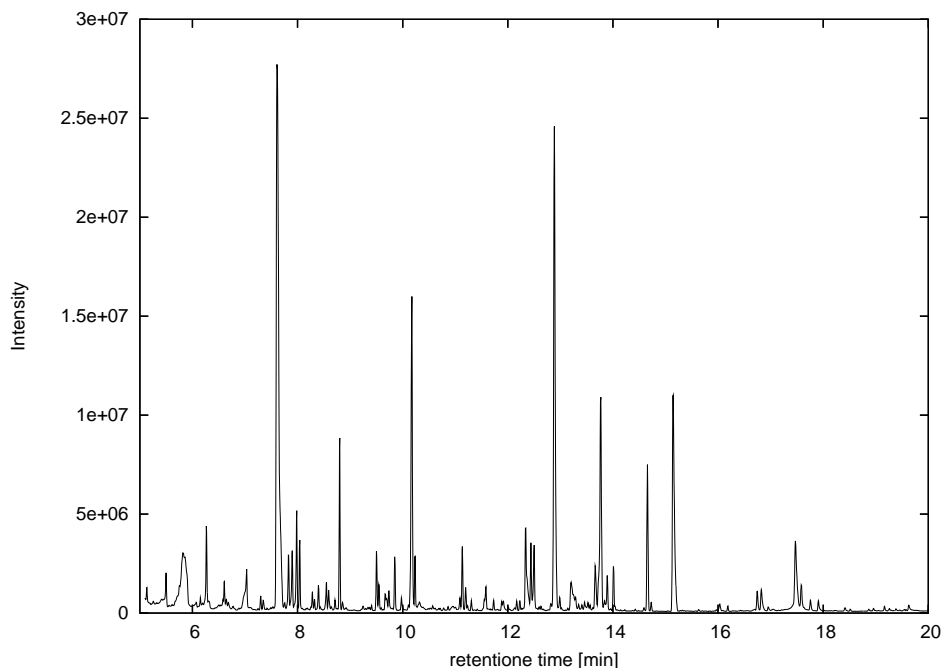


Figure 2.1: The Gnuplot plot of the file 'tic.dat'

```
>>> print "Dimensions of the intensity matrix are:",len(im),"x",len(im[0])
Dimensions of the intensity matrix are: 3236 x 501
```

This data matrix contains 3236 time points (MS scans), and each time point corresponds to a mass spectrum of 501 m/z points.

The intensity matrix can be saved to a file with the function 'save_data()':

```
save_data("output/im.dat", im)
```

The entire data (ie. ChemStation object) can be saved as CSV with the method `export_csv()`. For example,

```
>>> andi_data.export_csv("output/data")
```

will create 'data.im.csv', 'data.mz.csv', and 'data.rt.csv' where these are the intensity matrix, retention time vector, and m/z vector in the CSV format.

2.3 Example 2: Creating signal peaks

This example is in `pyms-test/02 G` In PyMS a signal peak is represented as 'Peak' object defined in `pyms.Peak.Class.py`. A peak object is initialized with two arguments: peak retention time and peak raw area. The following commands create a peak named 'p' with the retention time of 5.553 min and a peak area of 2759280 (this is the peak no. 3 in the ChemStation peak area report file 'a0806_140.txt'):

```
>>> from pyms.Peak.Class import Peak
>>> p = Peak(5.553*60.0,2759280)
```

As a matter of convention PyMS internally stores retention times in seconds, hence above the retention time is multiplied by 60. Peak raw area is in arbitrary units.

Peak properties can be accessed through its attributes:

```
>>> print "Peak retention time is", p.rt
Peak retention time is 333.18
>>> print "Peak raw area is", p.raw_area
Peak raw area is 2759280.0
```

Other important properties of a peak object are peak normalized area and peak mass spectrum. The peak created in the above example does not have values associated with these two attributes, and they are merely initialized to 'None':

```
>>> print "Peak normalized area is", p.norm_area
Peak normalized area is None
>>> print "Peak mass spectrum is", p.mass_spectrum
Peak mass spectrum is None
```

The peak mass spectrum can be set by calling the method `set_mass_spectrum()`. This method requires the raw data, and fetches mass spectrum at peak retention time:

```
>>> p.set_mass_spectrum(andi_data)
```

This will set the mass spectrum attribute:

```
>>> print p.mass_spectrum
49976  54520 102752 15570   1872  18392   8765  14966  46136  16141
  1635   1743   686   1019   712   1199   1641   3182   1234  30400
  4261   3746   3348 82392  8354  24824   3797   6086  23312 140480
[--output deleted--]
```

These are m/z channel intensities in arbitrary units. The m/z values themselves are in the mass list attribute:

```
>>> print p.mass_list
[50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65,
66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81,
[--outout deleted--]]
```

The length of the two arrays must match:

```
>>> print len(p.mass_spectrum)
501
>>> print len(p.mass_list)
501
```

The mass spectrum can be written to a file by calling the peak `write_mass_spectrum()` method:

```
>>> p.write_mass_spectrum("output/ms.dat")
```

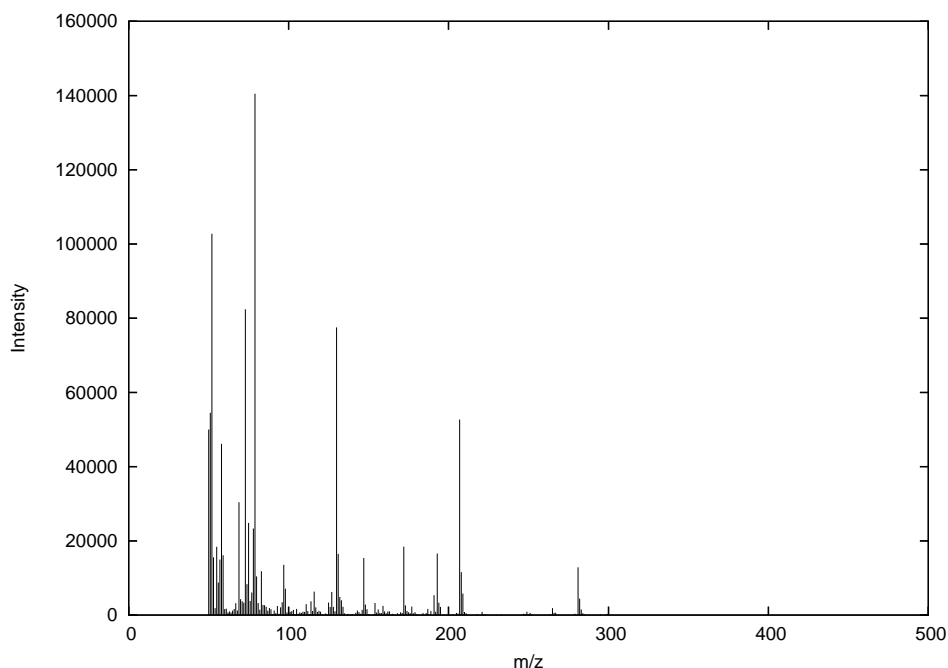
The file 'output/ms.dat' contains the pairs (mz , intensity), one pair per line:

```
$ head output/ms.dat
50.000      49976.000
51.000      54520.000
52.000     102752.000
53.000      15570.000
54.000       1872.000
55.000     18392.000
56.000       8765.000
57.000     14966.000
58.000     46136.000
59.000     16141.000
```

Figure 2.2 shows the plot of ms.dat created with the program Gnuplot. The gnuplot script used to create this plot is provided as `pyms-test/02/output/plot.gnu`.

2.4 Example 3: Creating an experiment from ChemStation data

This example is in `pyms-test/03`



53	8.242	480	483	486	VV 4	99010	1301807	0.13%	0.051% BLANK
54	8.285	486	490	495	VV 2	124626	2241940	0.22%	0.088%
55	8.337	495	499	501	VV 2	44820	662145	0.06%	0.026%
56	8.365	501	504	506	VV 2	58181	789953	0.08%	0.031%
57	8.399	506	510	515	VV	713172	9893695	0.97%	0.389%
58	8.443	515	518	521	VV 5	143812	2590885	0.25%	0.102%
59	8.477	521	524	526	VV 4	149127	2438042	0.24%	0.096%
60	8.548	526	536	558	VV 2	55398426	1025005618	100.00%	40.335% BLANK

In the file 'a0806_140.txt.anno' the keyword 'BLANK' was manually added to peaks 53 and 60, which are known to originate from the derivatizing agent use in GC-MS data preparation. These peaks can be excluded from the analysis later.

The peak eluting at 15.590 min originated from scyllo-inositol reference compound added during sample preparation. In the file 'a0806_140.txt.anno' this peak was labelled as follows:

```
178  15.590  1758 1767 1773 VV  5307268  80504143  7.85%  3.168% RF-SI
```

There could be an arbitrary number of reference peaks in the peak list, and each must have a unique reference 'tag' starting with 'RF-' and following with a two letter code denoting a particular reference compound (in this case SI for scyllo-inositol).

The ChemStation peak list is loaded in PyMS with the function 'read_chem_station_peaks()':

```
>>> from pyms.Peak.List.IO import read_chem_station_peaks
>>> peak_file = "/home/current/proj/PyMS/pyms-data/a0806_140.txt.anno"
>>> peaks = read_chem_station_peaks(peak_file)
-> Reading ChemStation peak integration report
'/home/current/proj/PyMS/pyms-data/a0806_140.txt.anno'
```

The variable 'peaks' now contains the peaks from the file 'a0806_140.txt.anno' This is merely a Python list:

```
>>> type(peaks)
<type 'list'>
>>> print "The number of peaks is:", len(peaks)
The number of peaks is: 347
```

The next step is to set the mass spectrum is set for each peak. For this we need first to load the raw data:

```
>>> import sys
```



```
>>> sys.path.append("/home/current/proj/PyMS/")
>>> from pyms.IO.ANDI.Class import ChemStation
>>> andi_file = "/home/current/proj/PyMS/pyms-data/a0806_140.CDF"
>>> andi_data = ChemStation(andi_file)
-> Processing netCDF file '/home/current/proj/PyMS/pyms-data/a0806_140.CDF'
    [ 3236 scans, masses from 50 to 550 ]
```

The following command sets the mass spectrum for each peak,

```
>>> for peak in peaks:
...     peak.set_mass_spectrum(andi_data)
```

The experiment object is initiated with the list of peaks and the experiment label, in this case "a0806_140":

```
>>> from pyms.Experiment.Class import Experiment
>>> expr = Experiment("a0806_140", peaks)
```

In the next steps we call a series of methods associated with the experiment object to set the reference peak, remove blank peaks, create peak normalized area (in this case the same as peak raw area), purge negative peaks (if any), and finally select the retention time range for the experiment to between 6.5 and 21 minutes, discarding all peaks outside this range:

```
>>> expr.set_ref_peak("si")
[ Reference peak found: 'rf-si' @ 935.400 s ]
[ Removing reference peak 'rf-si' @ 935.400 s ]
>>> expr.remove_blank_peaks()
[ Designated blank peak at 438.660 s removed ]
[ Designated blank peak at 494.520 s removed ]
[ Designated blank peak at 512.880 s removed ]
[ Designated blank peak at 751.980 s removed ]
>>> expr.raw2norm_area()
>>> expr.purge_peaks()
Experiment a0806_140: 0 peaks purged (below threshold=0.00)
>>> expr.sele_rt_range(["6.5m", "21m"])
-> Selecting peaks by retention time (from 6.5m to 21m): 247 peaks selected
```

Finally, we dump the experiment object to a file allowing it to be used later, for example in the process of peak alignment (see the example pyms-test/04):

```
>>> from pyms.Experiment.IO import dump_expr
>>> dump_expr(expr, "output/a0806_140.pickle")
-> Experiment 'a0806_140' saved as 'output/a0806_140.pickle'
```

2.5 Example 4: Preparation of multiple experiments for peak alignment by dynamic programming

This example is in `pyms-test/04`

Before aligning peak lists from multiple experiments the peak objects need to be created in PyMS. It is often useful to pre-process in some way the peaks, for example to null certain m/z channels and to select a certain retentiontime range.

This example considers preparation of three GC-MS experiments for the peak alignment. The experiments are 'a0806_140', 'a0806_141', 'a0806_142', and the input for each experiment consists of two files: the peak list exported from Agilent ChemStation (peak area report file), and the corresponding ANDI-MS data file. For example, the input files for the experiment "a0806_140" are:

- a0806_140.txt.a – ChemStation peak area report, manually edited to denote blanks peaks and the reference peak
- a0806_140.CDF – ANDI-MS file corresponding to a0806_140.txt.a

The ANDI-MS data files are required for the assignment of peak mass spectra (mass spectra taken at the peak apex), since peak alignment by dynamic programming uses both peak retention times and peak mass spectra [4].

The listing below shows the Python code for the script `pyms-test/04/proc.py`:

```
01  """proc.py
02  """
03
04  import sys, os
05  sys.path.append("/home/current/proj/PyMS/")
06
07  from pyms.IO.ANDI.Class import ChemStation
08  from pyms.Experiment.Class import Experiment
09  from pyms.Peak.List.IO import read_chem_station_peaks
10  from pyms.Experiment.IO import dump_expr
11
12  base_path = "/home/current/proj/PyMS/pyms-data/"
13
14  expr_codes = [ "a0806_140", "a0806_141", "a0806_142" ]
15
16  for expr_code in expr_codes:
17
18      peak_file = os.path.join(base_path, expr_code + ".txt.a")
19      andi_file = os.path.join(base_path, expr_code + ".CDF")
```

2.5. Example 4: Preparation of multiple experiments for peak alignment by dynamic programming¹⁷

```
20
21     andi_data = ChemStation(andi_file)
22     peaks = read_chem_station_peaks(peak_file)
23
24     andi_data.null_mass(73)
25     andi_data.null_mass(147)
26
27     for peak in peaks:
28         peak.set_mass_spectrum(andi_data)
29         peak.crop_mass_spectrum(50,540)
30
31     expr = Experiment(expr_code, peaks)
32
33     expr.set_ref_peak("si")
34     expr.remove_blank_peaks("blank")
35     expr.raw2norm_area()
36     expr.purge_peaks()
37     expr.sele_rt_range(["6.5m", "21m"])
38
39     dump_expr(expr, "output/" + expr_code + ".pickle")
```

The line 14 defines three experiments, by defining only the root name for each experiment. In the line 16 a loop is initiated over all experiments defined in the list 'expr_codes'. The actions in the body of the loop are applied to each experiment in turn:

- Full path names of the peak file and ANDI-MS file are created (lines 18-19)
- ANDI-MS and peak report files are loaded (lines 21-22)
- The m/z channels 73 and 147 are nulled in the raw data files (lines 24-25). These two m/z channels contain strong trailing signals from the derivatizing agent across all retentiontimes, and therefore can potentially lower the sensitivity in mass spectra comparison
- For each peak in the experiment the mass spectrum is set, and the m/z range is restricted to 50-540 (lines 27-29)
- An experiment object is created from the input data (line 31)
- The reference peak is removed (line 33), non-informative peaks are removed (line 34), peak operation area is created from the raw peak area (line 35), peaks below the threshold are remove (here negative peaks, if any), and the retention time of between 6.5 minutes and 21 minutes is selected.
- The experiment is dumped onto a file in the sub-directory 'output'.

Bibliography

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