PyMS User Guide

A Python toolkit for processing of chromatography–mass spectrometry data

Contents

Chapter 1

Introduction

PyMS is software for processing of chromatography–mass spectrometry data. PyMS is written in Python programming language [1], and is released as open source, under the GNU Public License version 2.

The driving 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. PyMS provides interactive processing capabilities through any of the various interactive Python front ends ("shells"). PyMS is essentially a Python library of chromatography–mass spectrometry processing functions, and individual commands can be collected into scripts which then can be run non-interactively when it is preferable to run data processing in the batch mode.

PyMS functionality consists of modules which are loaded when needed. For exlample, one such module provides display capabilities, and can be used to display time dependent data (e.g. total ion chromatogram), mass spectra, and signal peaks. This module is loaded only when visualisation is needed. If one is interested only in noise smoothing, only noise filtering functions are loaded into the Python environment and used to smooth the data, while the visualisation module (and other module) need not be loaded at all.

This modularity is supported on all levels of PyMS. For example, if one is interested in noise filtering with the Savitsky-Golay filter, only sub-module for Savitsky-Golay filter need to be loaded from the noise smoothing module, disregarding other modules, as well as other noise smoothing sub-modules. This organisation consisting of hierarchical modules ensures that the processing pipeline is put together from well defined modules each responsible for specific functions; and furthermore different functionalities are completely decoupled from one another, providing that implementing a new functionality (such as test or prototype of a new algorithm) can be implemented efficiently and ensuring that this will not break any existing functionality.

1.1   The PyMS project

The PyMS project consists of three parts, and each of which exists as a project in the Google Code repository that can be downloaded separately. These three parts are:

- pyms – The PyMS code (http://code.google.com/p/pyms/)

- pyms-docs – The PyMS documentation (http://code.google.com/p/pyms-docs/)

- pyms-test – Examples of how to use PyMS (http://code.google.com/p/pyms-test/)

The project 'pyms' contains the source code of the Python package PyMS. The project 'pyms-docs' contains PyMS style guide (relevant for those who contribute to the PyMS code) and User Guide (this document). The project 'pyms-test' contains tests and examples showing how to use various PyMS features. These examples are explained in detail in subsequent chapters of this User Guide.

In addition, the current PyMS API documentation (releant for those who are interested in PyMS internals) is available from here:
`http://bioinformatics.bio21.unimelb.edu.au/pyms.api/index.html`

1.2   PyMS installation

PyMS is written in Python, and extensible and modular object-oriented scripting language [1]. Python is highly portable, cross-platform programming language which works well on all major modern operating systems (Linux, MacOS X, Windows). PyMS is written in pure Python, and therefore works on all platforms on which Python has been ported.

The PyMS is essentially a python library (a 'package' in python parlance, which consists of several 'sub-packages'), and some of its functionality depends on several Python libraries, such as 'numpy' (the Python library for numerical computing), or 'matplotlib' (the Python library for plotting). These also need to work on the operating system of your choice for the functionality they provide to PyMS to be available. In general, the libraries that PyMS uses are available for all operating systems. The exception is 'pycdf' - a python interface to Unidata netCDF library written by Andre Gosselin of the Institut Maurice-Lamontagne, Canada (http://pysclint.sourceforge.net/pycdf/). *This library works only under Linux/Unix and therefore PyMS functionality which depends on it works only under the Unix operating system.*

There are several ways to install PyMS depending your computer configuration and personal preferences. PyMS has been developed on Linux, and a detailed installation instructions for Linux are given below. PyMS should work on all major Linux distributions, and has been tested extensively on Red Hat Linux.

1.2.1 Downloading PyMS source code

PyMS source code resides on publicly accessible 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. Regardless of the target operating system, the first step is to download the PyMS source code.

Google Code server maintains the source code with the program 'subversion' (an open-source version control system). To download the source code one needs we use the subversions client. Several subversion clients are available, some are open source, some freeware, and some are commerical (for more information see http://subversion.tigris.org/). We use the client program which ships will most Linux systems called 'svn'. The 'svn' client exists for all mainstream operating systems[1]. Subversion has extensive functionality, however only the very basic functionality is needed to download PyMS source code (the book about subversion is freely available on-line at http://svnbook.red-bean.com/).

If the computer is connected to the internet, and the subversion client 'svn' 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
... [ further output deleted ] ...
Checked out revision 71.
```

1.2.2 PyMS installation on Linux

We recommend compiling your own Python installation before installing PyMS. PyMS installation involves placing the PyMS code directory (pyms/) into a location visible to the Python interpreter. This can be in the standard place for 3rd

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

party software (the directory site-packages/), or alternatively if PyMS code is placed
in a non-standard location the Python interpreter needs to be made aware of it be-
fore before it is possible to import PyMS modules. For more on this please consult
the Python command sys.path.append().

In addition to the source code, PyMS uses several external packages. These are
explained below.

1.2.3   Package 'NumPy' (required for all aspects of PyMS)

The package NumPy is provides numerical capabilities to Python. This package is
used throughout PyMS (and also required for some external packages used in PyMS),
to its installation is mandatory.

The NumPy web site `http://numpy.scipy.org/` provides the installation instruc-
tions and the link to the source code.

1.2.4   Package 'pycdf' (required for reading ANDI-MS files)

The pycdf (a python interface to Unidata netCDF library) source and installation
instructions can be downloaded from
`http://pysclint.sourceforge.net/pycdf/`. Follow the installation instructions
to install pycdf.

1.2.5   Package 'Pycluster' (required for peak alignment by dy-
namic programming)

The peak alignment by dynamic programming is located in the subpackage pyms.Peak.List.DPA.
This subpackage used the Python package 'Pycluster' as the clustering engine. Py-
cluster with its installation instructions can be found here:
`http://bonsai.ims.u-tokyo.ac.jp/ mdehoon/software/cluster/index.html`.

1.2.6   Package 'scipy.ndimage' (required for TopHat baseline
corrector)

If the full SciPy package is installed the 'ndimage' will be available. However the
SciPy contains extensive functionality, and its installation is somewhat involved.
Sometimes it is preferable to install only the subpackage 'ndimage'. This subpackage
is provided as the PyMS-dependencies gzipped file available for download from the
PyMS webpage (see below).

1.2.7 Package 'matplotlib' (required for plotting)

The displaying of information such as Ion Chromatograms and detected peaks requires the package matplotlib. The matplotlib package can be downloaded from:
`http://matplotlib.sourceforge.net/`

1.3 Current PyMS development environment

PyMS is currently being developed with the following packages:

```
Python-2.5.2
numpy-1.1.1
netcdf-4.0
pycdf-0.6-3b
Pycluster-1.41
matplotlib-0.99.1.2
```

These packages bundled together into the archive 'PyMS-Linux-deps-1.1.tar.gz' which can be downloaded from the Bio21 Institute web server at the University of Melbourne:
http://bioinformatics.bio21.unimelb.edu.au/pyms.html
This bundle provides the dependencies required to install PyMS on Linux (one also needs to dowload the PyMS source code as explained in the section 1.2.1).

A quick installation guide for packages required by PyMS on Linux is given below.

1. 'Python' installation:

   ```
   $ tar xvfz Python-2.5.2.tgz
   $ cd Python-2.5.2
   $ ./configure
   $ make
   $ make install
   ```

   This installs python in /usr/local/lib/python2.5. It is recommended to make sure that python called from the command line is the one just compiled and installed.

2. 'NumPy' installation:

   ```
   $ tar xvfz numpy-1.1.1.tar.gz
   $ cd numpy-1.1.1
   $ python setup.py install
   ```

3. 'pycdf' installation

   Pycdf has two dependencies: the Unidata netcdf library and NumPy. The
   NumPy installation is described above. To install pycdf, the netcdf library
   must be downloaded
   (`http://www.unidata.ucar.edu/software/netcdf/index.html`),
   compiled and installed first:

   ```
   $ tar xvfz netcdf.tar.gz
   $ cd netcdf-4.0
   $ ./configure
   $ make
   $ make install
   ```

   The last step will create several binary 'libnetcdf*' files in /usr/local/lib. Then
   'pycdf' should be installed as follows:

   ```
   $ tar xvfz pycdf-0.6-3b
   $ cd pycdf-0.6-3b
   $ python setup.py install
   ```

4. 'Pycluster' installation

   ```
   $ tar xvfz Pycluster-1.42.tar.gz
   $ cd Pycluster-1.42
   $ python setup.py install
   ```

5. 'ndimage' installation:

   ```
   $ unzip ndimage.zip
   $ cd ndimage
   $ python setup.py install --prefix=/usr/local
   ```

   Since 'ndimage' was installed outside the scipy package, this requires some
   manual tweaking:

   ```
   $ cd /usr/local/lib/python2.5/site-packages
   $ mkdir scipy
   $ touch scipy/__init__.py
   $ mv ndimage scipy
   ```

6. 'matplotlib' installation:

   ```
   $ tar xvfz matplotlib-0.99.1.2
   $ cd matplotlib-0.99.1.1
   $ python setup.py build
   $ python setup.py install
   ```

The 'pyms.Display' module uses the TKAgg backend for matplotlib. If this is
not your default backend, the matplotlibrc file may be edited. To locate the
file 'matplotlibrc', in a python interactive session:

```
>>> import matplotlib
>>> matplotlib.matplotlib_fname()
```

Open the matplotlibrc file in a text editor and adjust the 'backend' parameter
to 'TKAgg'.

1.4 Troubleshooting

The most likely problem with PyMS installation is a problem with installing one of
the PyMS dependencies.

1.4.1 Pycdf import error

On Red Hat Linux 5 the SELinux is enabled by default, and this causes the following
error while trying to import properly installed pycdf:

```
$ python
Python 2.5.2 (r252:60911, Nov  5 2008, 16:25:39)
[GCC 4.1.1 20070105 (Red Hat 4.1.1-52)] on linux2
Type "help", "copyright", "credits" or "license" for more information.
>>> import pycdf
Traceback (most recent call last):
  File "<stdin>", line 1, in <module>
  File "/usr/local/lib/python2.5/site-packages/pycdf/__init__.py", line 22, in <module>
    from pycdf import *
  File "/usr/local/lib/python2.5/site-packages/pycdf/pycdf.py", line 1096, in <module>
    import pycdfext as _C
  File "/usr/local/lib/python2.5/site-packages/pycdf/pycdfext.py", line 5, in <module>
    import _pycdfext
ImportError: /usr/local/lib/python2.5/site-packages/pycdf/_pycdfext.so:
    cannot restore segment prot after reloc: Permission denied
```

This problem is removed simply by disabling SELinux (login as 'root', open the menu
Administration → Security Level and Firewall, tab SELinux, change settings from
'Enforcing' to 'Disabled').

This problem is likely to occur on Red Hat Linux derivative distributions such as
CentOS.

1.5   PyMS tutorial and examples

This document provides extensive tutorial on the use of PyMS, and the corresponding examples can be downloaded from the publicly accessible Google code project 'pyms-test' (http://code.google.com/p/pyms-test/). The data used in PyMS documentation and examples is available from the Bio21 Institute server at the University of Melbourne. Please follow the link from the PyMS web page:
`http://bioinformatics.bio21.unimelb.edu.au/pyms`
or go directly to
`http://bioinformatics.bio21.unimelb.edu.au/pyms-data/`

A tutorial illustrating various PyMS features in detail is provided in subsequent chapters of this User Guide. The commands executed interactively are grouped together by example, and provided as Python scripts in the project 'pyms-test' (this is a Google code project, similar to the project 'pyms' which contains the PyMS source code).

The setup used in the examples below is as follows. The projects 'pyms', 'pyms-test', 'pyms-docs', and 'data' are all in the same directory, '/x/PyMS'. In the project 'pyms-test' there is a directory corresponding to each example, coded with the chapter number (ie. `pyms-test/21a/` corresponds to the Example 21a, from Chapter 2).

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 with the following command:

```
$ 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("/x/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 may need to be adjusted to match your own directory structure.

Chapter 2

GC-MS Raw Data Model

2.1 Introduction

PyMS can read gas chromatography-mass spectrometry (GC-MS) data stored in Analytical Data Interchange for Mass Spectrometry (ANDI-MS),[1] and Joint Committee on Atomic and Molecular Physical Data (JCAMP-DX)[2] formats. These formats are essentially recommendations, and it is up to individual vendors of mass spectrometry processing software to implement "export to ANDI-MS" or "export to JCAMP-DX" features in their software. It is also possible to get third party converters. The information contained in the exported data files can vary significantly, depending on the instrument, vendor's software, or conversion utility.

For PyMS, the minimum set of assumptions about the information contained in the data file are:

- The data contain the m/z and intensity value pairs across a scan.

- Each scan has a retention time.

Internally, PyMS stores the raw data from ANDI files or JCAMP files as a GCMS_data object.

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[1] ANDI-MS was developed by the Analytical Instrument Association.
[2] JCAMP-DX is maintained by the International Union of Pure and Applied Chemistry.

2.2   Reading the raw GC-MS data

2.2.1   Reading JCAMP GC-MS data

[*This example is in pyms-test/20a*]

The PyMS package pyms.GCMS.IO.JCAMP provides capabilities to read the raw GC-MS data stored in the JCAMP-DX format.

The file 'gc01_0812_066.jdx' (located in 'data') is a GC-MS experiment converted from Agilent ChemStation format to JCAMP format using File Translator Pro.[3] This file can be loaded in Python as follows:

```
>>> from pyms.GCMS.IO.JCAMP.Function import JCAMP_reader
>>> jcamp_file = "/x/PyMS/data/gc01_0812_066.jdx"
>>> data = JCAMP_reader(jcamp_file)
 -> Reading JCAMP file '/x/PyMS/pyms-data/gc01_0812_066.jdx'
>>>
```

The above command creates the object 'data' which is an *instance* of the class GCMS_data.

2.2.2   Reading ANDI GC-MS data

[*This example is in pyms-test/20b*]

The PyMS package pyms.GCMS.IO.ANDI provides capabilities to read the raw GC-MS data stored in the ANDI-MS format.

The file 'gc01_0812_066.cdf' (located in 'data') is a GC-MS experiment converted to ANDI-MS format from Agilent ChemStation (from the same data as in example 20a above). This file can be loaded as follows:

```
>>> from pyms.GCMS.IO.ANDI.Function import ANDI_reader
>>> ANDI_file = "/x/PyMS/data/gc01_0812_066.cdf"
>>> data = ANDI_reader(ANDI_file)
 -> Reading netCDF file '/x/PyMS/pyms-data/gc01_0812_066.cdf'
>>>
```

The above command creates the object 'data' which is an *instance* of the class GCMS_data.

---

[3]ChemSW, Inc.

2.3 A GCMS_data object

2.3.1 Methods

[*The following examples are the same in pyms-test/20a and pyms-test/20b*]

The object 'data' (from the two previous examples) stores the raw data as a *GCMS_data* object. Within the GCMS_data object, raw data are stored as a list of *Scan* objects and a list of retention times. There are several methods available to access data and attributes of the GCMS_data and Scan objects.

The GCMS_data object's methods relate to the raw data. The main properties relate to the masses, retention times and scans. For example, the minimum and maximum mass from all of the raw data can be returned by the following:

```
>>> data.get_min_mass()
>>> data.get_max_mass()
```

A list of all retention times can be returned by:

```
>>> time = data.get_time_list()
```

The index of a specific retention time (in seconds) can be returned by:

```
>>> data.get_index_at_time(400.0)
```

Note that this returns the index of the retention time in the data closest to the given retention time of 400.0 seconds.

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

```
>>> tic = data.get_tic()
```

The IonChromatogram object is explained in a later chapter.

2.3.2 A Scan data object

A Scan object contains a list of masses and a corresponding list of intensity values from a single mass-spectrum scan in the raw data. Typically only non-zero (or non-threshold) intensities and corresponding masses are stored in the raw data.

[*The following examples are the same in pyms-test/20a and pyms-test/20b*]

A list of all the raw Scan objects can be returned by:

```
>>> scans = data.get_scan_list()
```

A list of all masses in a scan (e.g. the 1st scan) is returned by:

```
>>> scans[0].get_mass_list()
```

A list of all corresponding intensities in a scan is returned by:

```
>>> scans[0].get_intensity_list()
```

The minimum and maximum mass in an individual scan (e.g. the 1st scan) are
returned by:

```
>>> scans[0].get_min_mass()
>>> scans[0].get_max_mass()
```

2.3.3   Exporting data and printing information about a data set

[*This example is in pyms-test/20c*]

Often it is of interest to find out some basic information about the data set, e.g.
the number of scans, the retention time range, and m/z range and so on. The
GCMS_data class provides a method info() that can be used for this purpose.

```
>>> from pyms.GCMS.IO.ANDI.Function import ANDI_reader
>>> andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
>>> data = ANDI_reader(andi_file)
 -> Reading netCDF file '/x/PyMS/data/gc01_0812_066.cdf'
>>> data.info()
 Data retention time range: 5.093 min -- 66.795 min
 Time step: 0.375 s (std=0.000 s)
 Number of scans: 9865
 Minimum m/z measured: 50.000
 Maximum m/z measured: 599.900
 Mean number of m/z values per scan: 56
 Median number of m/z values per scan: 40
>>>
```

To export the entire raw data to a file, use the method write():

```
>>> data.write("output/data")
 -> Writing intensities to 'output/data.I.csv'
 -> Writing m/z values to 'output/data.mz.csv'
```

This method takes the string ("output/data", in this example) and writes two CSV files. One has extention ".I.csv" and contains the intensities ("output/data.I.csv" in this example), and the other has the extension ".mz" and contains the corresponding table of m/z value ("output/data.mz.csv" in this example). In general these are not two-dimensional matrices, because different scans may have different number of m/z values recorded.

2.3.4 Comparing two GC-MS data sets

[*This example is in pyms-test/20d*]

Occasionally it is useful to compare two data sets. For example, one may want to check the consistency between the data set exported in netCDF format from the manufacturer's software, and the JCAMP format exported from a third party software.

For example:

```
>>> from pyms.GCMS.IO.JCAMP.Function import JCAMP_reader
>>> from pyms.GCMS.IO.ANDI.Function import ANDI_reader
>>> andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
>>> jcamp_file = "/x/PyMS/data/gc01_0812_066.jdx"
>>> data1 = ANDI_reader(andi_file)
 -> Reading netCDF file '/x/PyMS/data/gc01_0812_066.cdf'
>>> data2 = JCAMP_reader(jcamp_file)
 -> Reading JCAMP file '/x/PyMS/data/gc01_0812_066.jdx'
```

To compare the two data sets:

```
>>> from pyms.GCMS.Function import diff
>>> diff(data1,data2)
 Data sets have the same number of time points.
   Time RMSD: 1.80e-13
 Checking for consistency in scan lengths ... OK
 Calculating maximum RMSD for m/z values and intensities ...
   Max m/z RMSD: 1.03e-05
   Max intensity RMSD: 0.00e+00
```

If the data is not possible to compare, for example because of different number of
scans, or inconsistent number of m/z values in between two scans, diff() will report
the difference. For example:

```
>>> data2.trim(begin=1000,end=2000)
Trimming data to between 1000 and 2000 scans
>>> diff(data1,data2)
 -> The number of retention time points different.
 First data set: 9865 time points
 Second data set: 1001 time points
 Data sets are different.
```

Chapter 3

GC-MS data derived objects

In this chapter the methods for converting the raw GC-MS data to an Intensity Matrix object are illustrated.

In the raw GC-MS data, consecutive scans do not necessarily contain the same mass per charge (mass) values. For data processing, it is often necessary to convert the data to a matrix with a set number of masses and scans. In PyMS there are functions to explicitly convert the raw mass values to consistent values across all scans.

3.1   IntensityMatrix Object

The general scheme for converting raw mass values is to bin intensity values based on the interval the corresponding mass belongs to. The general procedure is as follows:

- Set the interval between bins, lower and upper bin boundaries

- Calculate the number of bins to cover the range of all masses.

- Centre the first bin at the minimum mass found for all the raw data.

- Sum intensities whose masses are in a given bin.

A mass, m, is considered to belong to a bin when $c - l \leq m < c + u$, where c is the centre of the bin, l is the lower boundary and u is the upper boundary of the bin. The default bin interval is one with a lower and upper boundary of ± 0.5.

A function to bin masses to the nearest integer is also available. The default bin interval is one with a lower boundary of -0.3 and upper boundary of +0.7 (as per the NIST library).

3.1.1   Build intensity matrix

[*This example is in pyms-test/30a*]

An intensity matrix on the raw GC-MS data can be built using the following function.
First the raw data is imported as before.

```
>>> from pyms.GCMS.IO.JCAMP.Function import JCAMP_reader
>>> jcamp_file = "/x/PyMS/data/gc01_0812_066.jdx"
>>> data = JCAMP_reader(jcamp_file)
 -> Reading JCAMP file '/x/PyMS/pyms-data/gc01_0812_066.jdx'
>>>
```

Then the data can be converted to an intensity matrix using the functions available in
"pyms.GCMS.Function", namely `build_intensity_matrix()` and `build_intensity_matrix_i()`.

The default operation of `build_intensity_matrix()` is to use a bin interval of one
and treat the masses as floating point numbers. The default intensity matrix can be
built as follows:

```
>>> from pyms.GCMS.Function import build_intensity_matrix
>>> im = build_intensity_matrix(data)
```

The size as the number of scans and the number of bins is returned by:

```
>>> im.get_size()
```

There are 9865 scans and 551 bins in this example.

The raw masses have been binned into new mass units based on the minimum mass
in the raw data and the bin size. A list of the new masses are returned by:

```
>>> masses = im.get_mass_list()
```

It is also possible to search for a particular mass, by finding the index of the binned
mass closest to the desired mass. For example, the index of the closest binned mass
to a mass of 73.3 m/z is returned by:

```
>>> index = im.get_index_of_mass(73.3)
```

The value of the closest mass can be returned by:

```
>>> print im.get_mass_at_index(index)
```

A mass of 73.0 is returned in this example.

3.1.2 Build intensity matrix parameters

[*This example is in pyms-test/30b*]

The bin interval can be set to values other than one. For example, the bin interval can be set to 0.5. The boundaries can also be adjusted. In this example, to fit the 0.5 bin interval, the upper and lower boundaries are set to ±0.25.

```
im = build_intensity_matrix(data, 0.5, 0.25, 0.25)
```

The size of the intensity matrix will reflect the change in the number of bins.

```
>>> im.get_size()
```

There are 9865 scans (as before) and 1101 bins in this example.

The index and binned mass of the mass closest to 73.3 should also reflect the different binning.

```
>>> index = im.get_index_of_mass(73.3)
>>> print im.get_mass_at_index(index)
```

A mass of 73.5 is returned in this example.

3.1.3 Build integer mass intensity matrix

[*This example is in pyms-test/30c*]

It is also possible to build an intensity matrix with integer masses and a bin interval of one. The default range for the binning is -0.3 and +0.7 mass units. The function is imported from "pyms.GCMS.Function".

```
>>> from pyms.GCMS.Function import build_intensity_matrix_i
>>> im = build_intensity_matrix_i(data)
```

The masses are now all integers.

```
>>> index = im.get_index_of_mass(73.3)
>>> print im.get_mass_at_index(index)
```

A mass of 73 is returned in this example.

The lower and upper bounds can be adjusted by build_intensity_matrix_i(data, lower, upper).

3.2   MassSpectrum Object

[*This example is in pyms-test/31*]

A MassSpectrum object contains two attributes, `mass_list` and `mass_spec`, a list of the mass values and corresponding intensities, respectively. MassSpectrum is returned by the IntensityMatrix method of `get_ms_at_index(index)`.

For example, the properties of the first MassSpectrum object of an IntensityMatrix, *im*, can be investigated by;

```
>>> ms = im.get_ms_at_index(0)
>>> print len(ms)
>>> print len(ms.mass_list)
>>> print len(ms.mass_spec)
```

The length of all attributes should be the same.

3.3   IonChromatogram Object

[*This example is in pyms-test/31*]

An IonChromatogram object is a one dimensional vector containing mass intensities as a function of retention time. This can can be either m/z channel intensities (for example, the ion chromatogram at m/z = 73), or cumulative intensities over all measured m/z (TIC).

An IonChromatogram for the TIC and a given mass or index can be obtained by;

```
>>> tic = data.get_tic()
>>> ic = im.get_ic_at_index(0)
>>> ic = im.get_ic_at_mass(73)
```

This will return, respectively: the TIC; the ion chromatogram of the first mass; and the ion chromatogram of the mass closest to 73.

An ion chromatogram object has a method `is_tic()` which returns True if the ion chromatogram is a 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
```

3.3.1 Writing IonChromatogram data to a file

[*This example is in pyms-test/31*]

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 with the `write` method is a plain ASCII file which contains a pair of (retention time, intensity) per line.

```
$ head tic.dat
  5.0930 2.222021e+07
  5.0993 2.212489e+07
  5.1056 2.208650e+07
  5.1118 2.208815e+07
  5.1181 2.200635e+07
  5.1243 2.200326e+07
  5.1306 2.202363e+07
  5.1368 2.198357e+07
  5.1431 2.197408e+07
  5.1493 2.193351e+07
```

3.4 Saving data

[*This example is in pyms-test/32*]

A matrix of intensity values can be saved to a file with the function `save_data()` from `pyms.Utils.IO`. A matrix of intensity values can be returned from an IntensityMatrix with the method `get_matrix_list()`. For example,

```
>>> from pyms.Utils.IO import save_data
>>> mat = im.get_matrix_list()
>>> save_data("output/im.dat", mat)
```

It is also possible to save the list of masses (from `im.get_mass_list()`) and the list of retention times (from `im.get_time_list()`) using the `save_data()` function. For convenience, the intensity values, mass list and time list, can be saved with the method `export_ascii()`. For example,

```
>>> im.export_ascii("output/data")
```

will create "data.im.dat", "data.mz.dat", and "data.rt.dat" where these are the intensity matrix, retention time vector, and m/z vector. By default the data is saved as space separated data with a ".dat" extension. It is also possible to save the data as comma separated data with a ".csv" extension by the command "im.export_ascii("output/data", "csv")".

Additionally, the entire IntensityMatrix can be exported as LECO CSV. This is useful for import into other analytical software packages. The format is a header line specifying the column heading information as: "scan, retention time, mass1, mass2, . . .", and then each row as the intensity data.

```
>>> im.export_leco_csv("output/data_leco.csv")
```

3.5   Importing ASCII data

[*This example is in pyms-test/32*]

The LECO CSV data format can be used to import ASCII data directly into an IntensityMatrix object. The data must follow the format outlined above. For example, the file saved above can be read and compared to the original:

```
>>> from pyms.GCMS.Class import IntensityMatrix
>>>
>>> iim = IntensityMatrix([0],[0],[[0]])
>>>
>>> iim.import_leco_csv("output/data_leco.csv")
>>>
>>> print im.get_size()
>>> print iim.get_size()
```

The line "IntensityMatrix([0],[0],[[0]])" is required to create an empty IntensityMatrix object.

Chapter 4

Data filtering

4.1 Introduction

In this chapter filtering techniques that allow pre-processing of GC-MS data for analysis and comparison to other pre-processed GC-MS data are covered.

4.2 Time strings

Before considering the filtering techniques, the mechanism for representing retention times is outlined here.

A time string is the specification of a time interval, that takes the format 'NUMBERs' or 'NUMBERm' for time interval in seconds or minutes. For example, these are valid time strings: '10s' (10 seconds) and '0.2m' (0.2 minutes).

4.3 Intensity Matrix resizing

Once an IntensityMatrix has been constructed from the raw GC-MS data, the entries of the matrix can be modified. These modifications can operate on the entire matrix, or individual masses or scans.

4.3.1 Retention time range

[*This example is in pyms-test/40a*]

A basic operation on the GC-MS data is to select a specific time range for processing.

In PyMS, any data outside the chosen time range is discarded. The `trim()` method operates on the raw data, so any subsequent processing only refers to the trimmed data.

Given a previously loaded raw GC-MS data file, *data*, the data can be trimmed to specific scans;

```
>>> data.trim(1000, 2000)
>>> print data.info()
```

or specific retention times (in "seconds" or "minutes");

```
>>> data.trim("6.5m", "21m")
>>> print data.info()
```

4.3.2   Mass spectrum range and entries

[*This example is in pyms-test/40b*]

An IntensityMatrix object has a set mass range and interval that is derived from the data at the time of building the intensity matrix. The range of mass values can be modified. This is done, primarily, to ensure that the range of masses used are consistent when comparing samples.

Given a previously loaded raw GC-MS data file that has been converted into an IntensityMatrix, *im*, the mass range can be "cropped" to a new (smaller) range;

```
>>> im.crop_mass(60, 400)
>>> print im.get_min_mass(), im.get_max_mass()
```

It is also possible to set all intensities for a given mass to zero. This is useful for ignoring masses associated with sample preparation. The mass can be "nulled" via;

```
>>> data.null_mass(73)
>>> print sum(im.get_ic_at_mass(73).get_intensity_array())
```

4.4   Noise smoothing

The purpose of noise smoothing is to remove high-frequency noise from data, and thereby increase the contribution of the signal relative to the contribution of the noise.

4.4.1 Window averaging

[*This example is in pyms-test/41a*]

A simple approach to noise smoothing is moving average window smoothing. In this approach the window of a fixed size ($2N + 1$ points) is moved across the ion chromatogram, and the intensity value at each point is replaced with the mean intensity calculated over the window size. The example below illustrates smoothing of TIC by window averaging.

Load the data and get the TIC:

```
>>> andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
>>> data = ANDI_reader(andi_file)
 -> Reading netCDF file '/x/PyMS/data/gc01_0812_066.cdf'
>>> tic = data.get_tic()
```

Apply the mean window smoothing with the 5-point window:

```
from pyms.Noise.SavitzkyGolay import window_smooth
tic1 = window_smooth(tic, window=5)
 -> Window smoothing (mean): the wing is 2 point(s)
```

Apply the median window smoothing with the 5-point window:

```
>>> tic2 = window_smooth(tic, window=5, median=True)
 -> Window smoothing (median): the wing is 2 point(s)
```

Apply the mean windows smoothing, but specify the window as a time string (in this example, 7 seconds):

```
>>> tic3 = window_smooth(tic, window='7s')
 -> Window smoothing (mean): the wing is 9 point(s)
```

Time strings are explained in the Section 4.2.

4.4.2 Savitzky–Golay noise filter

[*This example is in pyms-test/41b*]

A more sophisticated noise filter is the Savitzky-Golay filter. Given the data loaded as above, this filter can be applied as follows:

```
>>> from pyms.Noise.SavitzkyGolay import savitzky_golay
>>> tic1 = savitzky_golay(tic)
 -> Applying Savitzky-Golay filter
      Window width (points): 7
      Polynomial degree: 2
```

In this example the default parameters were used.

4.5   Baseline correction

[*This example is in pyms-test/42*]

Baseline distortion originating from instrument imperfections and experimental setup is often observed in mass spectrometry data, and off-line baseline correction is often an important step in data pre-processing. There are many approaches for baseline correction. One advanced approach is based top-hat transform developed in mathematical morphology [2], and used extensively in digital image processing for tasks such as image enhancement. Top-hat baseline correction was previously applied in proteomics based mass spectrometry [3].

PyMS currently implements only top-hat baseline corrector, using the SciPy package 'ndimage'. For this feature to be available either SciPy (Scientific Tools for Python [4]) must be installed, or the local versions of scipy's ndimage must be installed. For the SciPy/ndimage installation instructions please see the section 1.2.6.

Application of the top-hat baseline corrector requires the size of the structural element to be specified. The structural element needs to be larger than the features one wants to retain in the spectrum after the top-hat transform. In the example below, the top-hat baseline corrector is applied to the TIC of the data set 'gc01_0812_066.cdf', with the structural element of 1.5 minutes:

```
>>> from pyms.GCMS.IO.ANDI.Function import ANDI_reader
>>> andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
>>> data = ANDI_reader(andi_file)
 -> Reading netCDF file '/x/PyMS/data/gc01_0812_066.cdf'
>>> tic = data.get_tic()
>>> from pyms.Noise.SavitzkyGolay import savitzky_golay
>>> tic1 = savitzky_golay(tic)
 -> Applying Savitzky-Golay filter
      Window width (points): 7
      Polynomial degree: 2
>>> from pyms.Baseline.TopHat import tophat
>>> tic2 = tophat(tic1, struct="1.5m")
 -> Top-hat: structural element is 239 point(s)
```

```
>>> tic.write("output/tic.dat",minutes=True)
>>> tic1.write("output/tic_smooth.dat",minutes=True)
>>> tic2.write("output/tic_smooth_bc.dat",minutes=True)
```

In the interactive session shown above, the data set if first loaded, Savitzky-Golay smoothing was applied, followed by baseline correction. Finally the original, smoothed, and smoothed and baseline corrected TIC were saved in the directory 'output/'.

4.6 Pre-processing the IntensityMatrix

[*This example is in pyms-test/43*]

The entire noise smoothing and baseline correction can be applied to each ion chromatogram in the intensity matrix;

```
>>> jcamp_file = "/x/PyMS/data/gc01_0812_066.jdx"
>>> data = JCAMP_reader(jcamp_file)
>>> im = build_intensity_matrix(data)
>>> n_scan, n_mz = im.get_size()
>>> for ii in range(n_mz):
...     print "Working on IC#", ii+1
...     ic = im.get_ic_at_index(ii)
...     ic_smooth = savitzky_golay(ic)
...     ic_bc = tophat(ic_smooth, struct="1.5m")
...     im.set_ic_at_index(ii, ic_bc)
...
```

The resulting IntensityMatrix object can be "dumped" to a file for later retrieval. There are general perpose object file handling methods in `pyms.Utils.IO`. For example;

```
>>> from pyms.Utils.IO import dump_object
>>> dump_object(im, "output/im-proc.dump")
```

Chapter 5

Peak detection and representation

5.1   Peak Object

Fundamental to GC-MS analysis is the identification of individual components of the sample mix. The basic component unit is represented as a signal Peak. In PyMS a signal peak is represented as 'Peak' object defined in `pyms.Peak.Class`, and functions to detect the peaks are also provided (discussed at the end of the chapter).

A peak object stores a minimal set of information about a signal peak, namely, the retention time at which the peak apex occurs and the mass spectra at the apex. Additional information, such as, peak width, TIC and individual ion areas can be filtered from the GC-MS data and added to the Peak object information.

5.1.1   Creating a Peak Object

[*This example is in pyms-test/50*]

A peak object can be created for a scan at a given retention time by providing the retention time (in minutes or seconds) and the MassSpectrum object of the scan. For example, first a file is loaded and an IntensityMatrix, *im*, built, then a MassSpectrum, *ms*, can be selected at a given time (31.17 minutes in this example).

```
>>> from pyms.GCMS.Function import build_intensity_matrix_i
>>> from pyms.GCMS.IO.ANDI.Function import ANDI_reader
>>> andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
```

```
>>> data = ANDI_reader(andi_file)
>>> im = build_intensity_matrix_i(data)
>>> index = im.get_index_at_time(31.17*60.0)
>>> ms = im.get_ms_at_index(index)
```

Now a Peak object can be created for the given retention time and MassSpectrum.

```
>>> from pyms.Peak.Class import Peak
>>> peak = Peak(31.17, ms, minutes=True)
```

By default the retention time is assumed to be in seconds. The parameter `minutes` can be set to True if the retention time is given in minutes. As a matter of convention, PyMS internally stores retention times in seconds, so the `minutes` parameter ensures the input and output of the retention time are in the same units.

5.1.2   Peak Object properties

[*This example is in pyms-test/50*]

The retention time of the peak can be returned with `get_rt()`. The retention time is returned in seconds with this method. The mass spectrum can be returned with `get_mass_spectrum()`.

The Peak object constructs a unique identification (UID) based on the spectrum and retention time. This helps in managing lists of peaks (covered in the next chapter). The UID can be returned with `get_UID()`. The format of the UID is the masses of the two most abundant ions in the spectrum, the ratio of the abundances of the two ions, and the retention time (in the same units as given when the Peak object was created). The format is `Mass1-Mass2-Ratio-RT`. For example,

```
>>> print peak.get_rt()
1870.2
>>> print peak.get_UID()
319-73-74-31.17
```

5.1.3   Modifying a Peak Object

[*This example is in pyms-test/51*]

The Peak object has methods for modifying the mass spectrum. The mass range can be cropped to a smaller range with `crop_mass()`, and the intensity values for a single ion can be set to zero with `null_mass()`. For example, the mass range can be set from 60 to 450 m/z, and the ions related to sample preparation can be ignored by setting their intensities to zero;

```
>>> peak.crop_mass(60, 450)
>>> peak.null_mass(73)
>>> peak.null_mass(147)
```

The UID is automatically updated to reflect the changes;

```
>>> print peak.get_UID()
319-205-54-31.17
```

It is also possible to change the peak mass spectrum by calling the method set_mass_spectrum().

5.2 Peak detection

The general use of a Peak object is to extract them from the GC-MS data and build a list of peaks. In PyMS, the function for peak detection is based on the method of Biller and Biemann (1974)[5]. The basic process is to find all maximising ions in a pre-set window of scans, for a given scan. The ions that maximise at a given scan are taken to belong to the same peak.

The function is BillerBiemann() in pyms.Deconvolution.BillerBiemann.Function. The function has parameters for the window width for detecting the local maxima (points), and the number of scans across which neighbouring, apexing, ions are combined and considered as belonging to the same peak. The number of neighbouring scans to combine is related to the likelyhood of detecting a peak apex at a single scan or several neighbouring scans. This is more likely when there are many scans across the peak. It is also possible, however, when there are very few scans across the peak. The scans are combined by taking all apexing ions to have occurred at the scan that had to greatest TIC prior to combining scans.

5.2.1 Sample processing and Peak detection

[*This example is in pyms-test/52*]

The process for detecting peaks is to pre-process the data by performing noise smoothing and baseline correction on each ion (as in *pyms-test/52*). The first steps then are;

```
>>> from pyms.GCMS.IO.ANDI.Function import ANDI_reader
>>> from pyms.GCMS.Function import build_intensity_matrix
>>> from pyms.Noise.SavitzkyGolay import savitzky_golay
>>> from pyms.Baseline.TopHat import tophat
>>>
```

```
>>> andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
>>> data = ANDI_reader(andi_file)
>>>
>>> im = build_intensity_matrix(data)
>>> n_scan, n_mz = im.get_size()
>>>
>>> for ii in range(n_mz):
...     ic = im.get_ic_at_index(ii)
...     ic_smooth = savitzky_golay(ic)
...     ic_bc = tophat(ic_smooth, struct="1.5m")
...     im.set_ic_at_index(ii, ic_bc)
...
```

Now the Biller and Biemann based technique can be applied to detect peaks.

```
>>> from pyms.Deconvolution.BillerBiemann.Function import BillerBiemann
>>> peak_list = BillerBiemann(im)
>>> print len(peak_list)
9845
```

Note that this is nearly as many peaks as there are scans in the data (9865 scans). This is due to noise and the simplicity of the technique.

The number of detected peaks can be constrained by the selection of better parameters. Parameters can be determined by counting the number of points across a peak, and examining where peaks are found. For example, the peak list can be found with the parameters of a window of 9 points and by combining 2 neighbouring scans if they apex next to each other;

```
>>> peak_list = BillerBiemann(im, points=9, scans=2)
>>> print len(peak_list)
3698
```

The number of detected peaks has been reduced, but there are still many more than would be expected from the sample. Functions to filter the peak list are covered in the next section.

5.3   Filtering Peak Lists

[*This example is in pyms-test/53*]

There are two functions to filter the list of Peak objects. The first, rel_threshold(), modifies the mass spectrum stored in each peak so any intensity that is less than

a given percentage of the maximum intensity for the peak is removed. The second, `num_ions_threshold()` removes any peak that has less than a given number of ions above a given threshold. Once the peak list has been constructed, the filters can be applied by;

```
>>> from pyms.Deconvolution.BillerBiemann.Function import \
... rel_threshold, num_ions_threshold
>>> pl = rel_threshold(peak_list, percent=2)
>>> new_peak_list = num_ions_threshold(pl, n=3, cutoff=10000)
>>> print len(new_peak_list)
146
```

The number of detected peaks is now more realistic of what would be expected in the test sample.

5.4   Peak area estimation

[*This example is in pyms-test/54*]

The Peak object does not contain any information about the width or area of the peak when it is created. This information can be added after the instantiation of a Peak object. The area of the peak can be set by the `set_area()` method of the peak object.

The peak area can by obtained by the `peak_sum_area()` function in `pyms.Peak.Function`. The function determines the total area as the sum of the ion intensities for all masses that apex at the given peak. To calculate the peak area of a single mass, the intensities are added from the apex of the mass peak outwards. Edge values are added until the following conditions are met: the added intensity adds less than 0.5% to the accumulated area; or the added intensity starts increasing (i.e. when the ion is common to co-eluting compounds). To avoid noise effects, the edge value is taken at the midpoint of three consecutive edge values.

Given a list of peaks, areas can be determined and added as follows:

```
>>> from pyms.Peak.Function import peak_sum_area
>>> for peak in peak_list:
...     area = peak_sum_area(intensity_matrix, peak)
...     peak.set_area(area)
...
```

Chapter 6

Peak alignment by dynamic programming

PyMS provides functions to align GC-MS peaks by dynamic programming [6]. The peak alignment by dynamic programming uses both peak apex retention time and mass spectra. This information is determined from the raw GC-MS data by applying a series of processing steps to produce data that can then be aligned and used for statistical analysis. The details are described in this chapter.

6.1 Preparation of multiple experiments for peak alignment by dynamic programming

6.1.1 Creating an Experiment

[*This example is in pyms-test/60*]

Before aligning peaks from multiple experiments, the peak objects need to be created and encapsulated into PyMS experiment objects. During this process it is often useful to pre-process the peaks in some way, for example to null certain m/z channels and/or to select a certain retention time range.

To capture the data and related information prior to peak alignment, an `Experiment` object is used. The `Experiment` object is defined in `pyms.Experiment.Class`.

The procedure is to proceed as described in the previous chapter. Namely: read a file; bin the data into fixed mass values; smooth the data; remove the baseline; deconvolute peaks; filter the peaks; set the mass range; remove uninformative ions; and estimate peak areas. The process is given in the following program listing.

```
01   import sys, os
02   sys.path.append("/x/PyMS")
03
04   from pyms.GCMS.IO.ANDI.Function import ANDI_reader
05   from pyms.GCMS.Function import build_intensity_matrix_i
06   from pyms.Noise.SavitzkyGolay import savitzky_golay
07   from pyms.Baseline.TopHat import tophat
08   from pyms.Peak.Class import Peak
09   from pyms.Peak.Function import peak_sum_area
10
11   from pyms.Deconvolution.BillerBiemann.Function import BillerBiemann, \
12       rel_threshold, num_ions_threshold
13
14   # deconvolution and peak list filtering parameters
15   points = 9; scans = 2; n = 3; t = 3000; r = 2;
16
17   andi_file = "/x/PyMS/data/a0806_077.cdf"
18
19   data = ANDI_reader(andi_file)
20
21   # integer mass
22   im = build_intensity_matrix_i(data)
23
24   # get the size of the intensity matrix
25   n_scan, n_mz = im.get_size()
26
27   # smooth data
28   for ii in range(n_mz):
29       ic = im.get_ic_at_index(ii)
30       ic1 = savitzky_golay(ic)
31       ic_smooth = savitzky_golay(ic1)
32       ic_base = tophat(ic_smooth, struct="1.5m")
33       im.set_ic_at_index(ii, ic_base)
34
35   # do peak detection on pre-trimmed data
36
37   # get the list of Peak objects
38   pl = BillerBiemann(im, points, scans)
39
40   # trim by relative intensity
41   apl = rel_threshold(pl, r)
42
43   # trim by threshold
44   peak_list = num_ions_threshold(apl, n, t)
```

```
45
46  print "Number of Peaks found:", len(peak_list)
47
48  # ignore TMS ions and set mass range
49  for peak in peak_list:
50      peak.crop_mass(50,540)
51      peak.null_mass(73)
52      peak.null_mass(147)
53      # find area
54      area = peak_sum_area(im, peak)
55      peak.set_area(area)
56
```

The resulting list of peaks can now be stored as an `Experiment` object.

```
from pyms.Experiment.Class import Experiment
from pyms.Experiment.IO import store_expr

# create an experiment
expr = Experiment("a0806_077", peak_list)

# set time range for all experiments
expr.sele_rt_range(["6.5m", "21m"])

store_expr("output/a0806_077.expr", expr)
```

Once an experiment has been defined, it is possible to limit the peak list to a desired range using `sele_rt_range()`. The resulting experiment object can then be stored for later alignment.

6.1.2 Multiple Experiments

[*This example is in pyms-test/61a*]

This example considers the preparation of three GC-MS experiments for peak alignment. The experiments are named 'a0806_077', 'a0806_078', 'a0806_079', and represent separate GC-MS sample runs from the same biological sample.

The procedure is the same as above, and repeated for each experiment. For example:

```
...
# define path to data files
base_path = "/x/PyMS/data/"
```

```
# define experiments to process
expr_codes = [ "a0806_077", "a0806_078", "a0806_079" ]

# loop over all experiments
for expr_code in expr_codes:

    print "Processing", expr_code

    # define the names of the peak file and the corresponding ANDI-MS file
    andi_file = os.path.join(base_path, expr_code + ".cdf")
...


...
    # create an experiment
    expr = Experiment(expr_code, peak_list)

    # use same time range for all experiments
    expr.sele_rt_range(["6.5m", "21m"])

    store_expr("output/"+expr_code+".expr", expr)
```

[*This example is in pyms-test/61b*]

The previous set of data all belong to the same experimental condition. That is,
they represent one group and any comparison between the data is a within group
comparison. For the original experiment, another set of GC-MS data was collected
for a different experimental condition. This group must also be stored as a set of
experiments, and can be used for between group comparison.

The experiments are named 'a0806_140', 'a0806_141', 'a0806_142', and are processed
and stored as above (see pyms-test/61b).

6.2   Dynamic programming alignment of peak lists from multiple experiments

- *This example is in pyms-test/62*

- *This example uses the subpackage pyms.Peak.List.DPA, which in turn uses the
  Python package 'Pycluster'. For 'Pycluster' installation instructions see the
  Section 1.2.5.*

In this example the experiments 'a0806_077', 'a0806_078', and 'a0806_079' prepared in pyms-test/61a will be aligned, and therefore the script pyms-test/61a/proc.py must be run first, to create the files 'a0806_077.expr', 'a0806_078.expr', 'a0806_079.expr' in the directory pyms-test/61a/output/. These files contain the post-processed peak lists from the three experiments.

A script for running the dynamic programming alignment on these experiments is given below.

```
"""proc.py
"""

import sys, os
sys.path.append("/x/PyMS/")

from pyms.Experiment.IO import load_expr
from pyms.Peak.List.DPA.Class import PairwiseAlignment
from pyms.Peak.List.DPA.Function import align_with_tree, exprl2alignment

# define the input experiments list
exprA_codes = [ "a0806_077", "a0806_078", "a0806_079" ]

# within replicates alignment parameters
Dw = 2.5  # rt modulation [s]
Gw = 0.30 # gap penalty

# do the alignment
print 'Aligning expt A'
expr_list = []
expr_dir = "../61a/output/"
for expr_code in exprA_codes:
    file_name = os.path.join(expr_dir, expr_code + ".expr")
    expr = load_expr(file_name)
    expr_list.append(expr)
F1 = exprl2alignment(expr_list)
T1 = PairwiseAlignment(F1, Dw, Gw)
A1 = align_with_tree(T1, min_peaks=2)

A1.write_csv('output/rt.csv', 'output/area.csv')
```

The script reads the experiment files from the directory where they were stored (`61a/output`), and creates a list of the loaded `Experiment` objects. Each experiment object is converted into an `Alignment` object with `exprl2alignment()`. In this example, there is only one experimental condition so the alignment object is only for

within group alignment (this special case is called 1-alignment). The variable F1 is a Python list containing three alignment objects.

The pairwise alignment is then performed. The parameters for the alignment by dynamic programming are: Dw, the retention time modulation in seconds; and Gw, the gap penalty. These parameters are explained in detail in [6]. `PairwiseAlignment()`, defined in `pyms.Peak.List.DPA.Class` is a class that calculates the similarity between a ll peaks in one sample with those of another sample. This is done for all possible pairwise alignments (2-alignments). The output of `PairwiseAlignment()` (T1) is an object which contains the dendrogram tree that maps the similarity relationship between the input 1-alignments, and also 1-alignments themselves.

The function `align_with_tree()` takes the object T1 and aligns the individual alignment objects according to the guide tree. In this example, the individual alignments are three 1-alignments, and the function `align_with_tree()` first creates a 2-alignment from the two most similar 1-alignments and then adds the third 1-alignment to this to create a 3-alignment. The parameter '`min_peaks=2`' specifies that any peak column of the data matrix that has less than two peaks in the final alignment will be dropped. This is useful to clean up the data matrix of accidental peaks that are not truly observed over the set of replicates.

Finally, the resulting 3-alignment is saved by writing alignment tables containing peak retention times ('rt1.csv') and the corresponding peak areas ('area1.csv'). These two files are plain ASCII files is CSV format, and are saved in the directory `62/output/`.

The file 'area1.csv' contains the data matrix where the corresponding peaks are aligned in the columns and each row corresponds to an experiment. The file 'rt1.csv' is useful for manually inspecting the alignment in some GUI driven program.

6.3   Between-state alignment of peak lists from multiple experiments

[This example is in pyms-test/63]

In the previous example the list of peaks were aligned within a single experiment with multiple replicates ("within-state alignment"). In practice, it is of more interest to compare the two experimental states. In a typical experimental setup there can be multiple replicate experiments on each experimental state or condition. To analyze the results of such an experiment statistically, the list of peaks need to be aligned within each experimental state and also between the states. The result of such an alignment would be the data matrix of integrated peak areas. The data matrix contains a row for each sample and the number of columns is determined by the number of unique peaks (metabolites) detected in all the experiments.

In principle, all experiments could be aligned across conditions and replicates in the one process. However, a more robust approach is to first align experiments within each set of replicates (within-state alignment), and then to align the resulting alignments (between-state alignment) [6].

This example demonstrates how the peak lists from two cell states are aligned. The cell state, A, consisting of three experiments aligned in *pyms-test/61a* ('a0806_077', 'a0806_078', 'a0806_079') and cell state, B, consisting of three experiments aligned in *pyms-test/61b* ('a0806_140', 'a0806_141', and 'a0806_142').

The between group alignment can be performed by the following alignment commands.

```
# between replicates alignment parameters
Db = 10.0 # rt modulation
Gb = 0.30 # gap penalty

print 'Aligning input {1,2}'
T9 = PairwiseAlignment([A1,A2], Db, Gb)
A9 = align_with_tree(T9)

A9.write_csv('output/rt.csv', 'output/area.csv')
```

where A1 and A2 are the results of the within group alignments (as above) for group A and B, respectively.

In this example the retention time tolerance for between-state alignment is greater compared to the retention time tolerance for the within-state alignment as we expect less fidelity in retention times between them. The same functions are used for the within-state and between-state alignment. The result of the alignment is saved to a file as the area and retention time matrices (described above).

Chapter 7

The Display module

PyMS has graphical capabilities to display information such as ion chromatogram objects (ICs), total ion chromatogram (TIC), and detected lists of peaks. This functionality is provided by the package matplotlib, which must be installed in order to use the Display module and to run the scripts referred to in this chapter. The instructions for this are provided in 1.2.7.

7.1 Displaying the TIC

[*This example is in pyms-test/70a*]

TIC must first be extracted from the data (see section 3.3). Once the TIC object is created, a simple call to the function `plot_ics()` displays the TIC in a graphical window. In addition to the TIC, two strings may be passed to plot_ics() which label the data (in this case 'TIC'), and the overall plot ('TIC for gc01_0812_066').

```
>>> plot_ics(tic, 'TIC','TIC for gc01_0812_066')
```

The window in figure 7.1 should now be displayed:

To zoom in on a portion of the plot, select the button, hold down the left mouse button while dragging a rectangle over the area of interest. To return to the original view, click on the button.

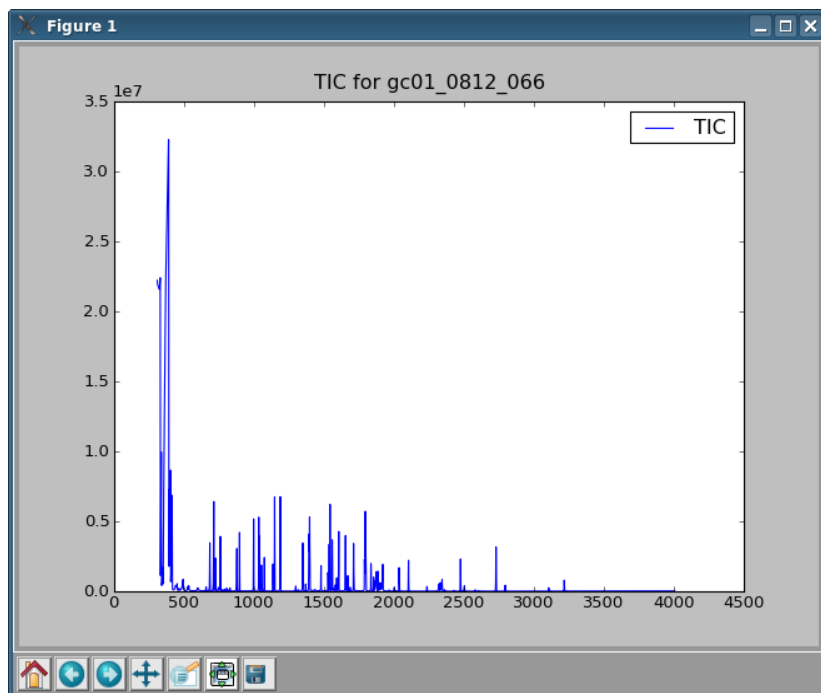The button allows panning across the zoomed plot.

Figure 7.1: Graphics window displayed by the script 70a/proc.py

7.2   Displaying multiple ion chromatogram objects

[*This example is in pyms-test/70b*]

The Display module can plot multiple ICs and the TIC on the same figure, as shown
in the following example. First, a list of ion chromatogram objects is created:

```
>>> tic = data.get_tic()
>>> ic = im.get_ic_at_mass(73)
>>> ic1 = im.get_ic_at_mass(147)
>>> ics = [tic, ic, ic1]
```

This list is passed to the function plot_ics(), along with a list of strings to label
each ion chromatogram:

```
>>> plot_ics(ics, ['TIC', '73','147'], 'TIC and ICs for m/z = 73 & 147')
```

This results in figure 7.2 being displayed:
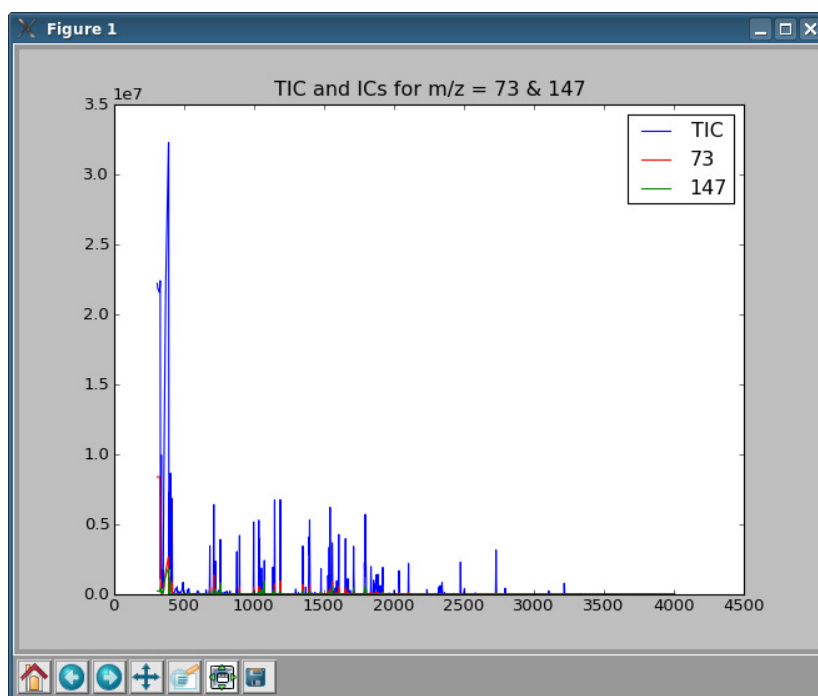
Figure 7.2: Graphics window displayed by the script 70b/proc.py

7.3 Displaying detected peaks on the TIC plot

[*This example is in pyms-test/71*]

The Display class is a more powerful implementation of plotting which allows plotting of detected peaks and user interaction with the plot figure.

In order to plot a list of peaks, the peaks must be created first. The example pyms-test/71 contains the script `proc_save_peaks.py` which produces such a peak list. For more information on detecting peaks see section 5.2. The function `store_peaks()` in `proc_save_peaks.py` stores the peaks, while `load_peaks()` in `proc.py` loads them for the Display class to use.

When using the Display Class, a series of plots is added to the figure one at a time before the final plot is displayed. The first step is always creating an instance of the Display class:

```
>>>display = Display()
```

Next some ics and the TIC are plotted. Unlike in simple plotting, the TIC is plotted using a separate function. This function ensures that the TIC is always plotted in blue for easy reference. The legend for each IC is supplied to the functions, but the
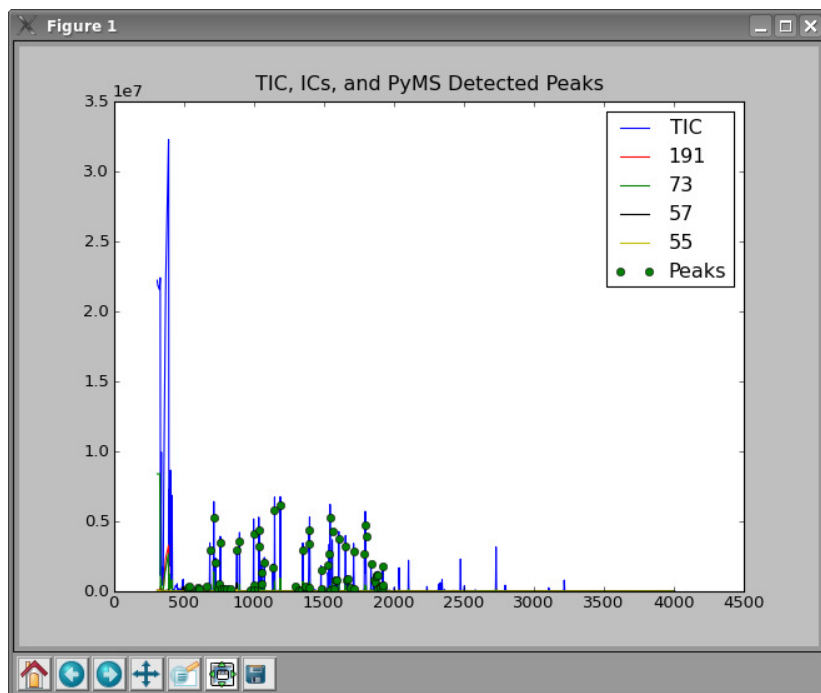
Figure 7.3: Graphics window displayed by the script 71/proc.py

overall figure label is not supplied at this time.

```
>>> display.plot_ics(ics, ['73','147'])
>>> displat.plot_tic(tic, 'TIC')
```

The function `plot_peaks()` adds the PyMS detected peaks to the figure.

```
>>>display.plot_peaks(peak_list, 'Peaks')
```

Finally, the function `do_plotting()` is called to draw the figure with labels and display the plot.

```
>>> display.do_plotting('TIC, ICs, and PyMS Detected Peaks')
```

This should result in figure 7.3 being displayed:

7.3.1   User interaction with the plot window

When using the Display class, the resulting figure window can be used to access data about the displayed peaks.
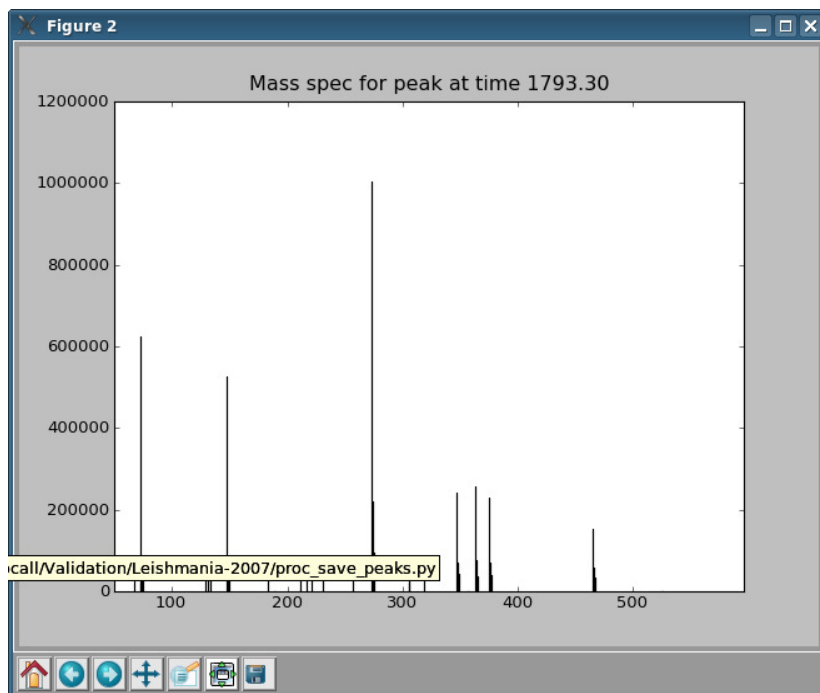
Figure 7.4: The mass spectrum displayed by PyMS when a peak in the graphics window of Figure 7.3 is clicked on

Clicking on a peak causes a list of the 5 highest intensity eluting ions at that peak to be written to the screen in order. Clicking a mouse button over one of the peaks should result in output similar to the following:

```
>>> mass      intensity
>>> 273       1003678.47619
>>> 73        625396.428571
>>> 147       526953.333333
>>> 363       255903.714286
>>> 347       241031.333333
```

In addition, clicking a mouse button other than the left button on a peak displays the mass spectrum at the peak in a new window (figure 7.4):

Clicking on other peaks will display further mass spectrums for those peaks in new windows.

Chapter 8

Labelled data related algorithms

8.1 Mass Isotopomer Distribution Extraction in ^{13}C Experiments

[*This example is in pyms-test/91*]

The aim of this method is to semi-automatically extract the isotopomer mass distribution (MID) of each metabolite of interest from raw GC-MS data files.

A metabolite with n carbon atoms can be labelled at any position resulting in 2^n different combinations, which are called isotopomers. For example, if a metabolite contains three carbon atoms and we denote unlabelled atom with 0 and labelled with 1, the possible labelling patterns for the carbon backbone are 000, 001, 010, 011, 100, 101, 110, 111. Mass spectrometry can only resolve isotopomer distribution completely if enough fragments can be detected (for a compound with n carbon atoms this means 2^n 1 different fragments containing different number and/or combinations of carbons).

The same metabolite has n+1 different masses: base mass M (000), singly labelled mass M+1 (001, 010, 100), doubly labelled mass M+2 (011, 101, 110) and fully labelled mass M+3 (111). The MS measurement of the molecular ion gives the mass isotopomer distribution (e.g. M, M+1, M+2, M+3) of the compound.

The algorithm developed relies on the value of the metabolites diagnostic fragment ion to extract the ion chromatograms from the data file. Metabolites retention time is then used for the feature extraction. Peak apex is defined as the local maximum within a specified window of the given retention time. Left and right boundaries are

defined as the first local minima on the left and right of the peak apex. The extraction of left and right boundary values is repeated for the characteristic fragment ion and its subsequent isotopomers.

The boundary values are then averaged for each file and the overall isotopomer intensity is quantified by summing all of the intensities between average left and right boundary values inside each ion chromatogram. The results are then normalised to obtain mass isotopomer distribution (i.e. percentage contributions of M, M+1, M+2, M+3, etc. ions). Where M represents the base mass of a non-labelled ion, M+1 represents the ion incorporating a single labelled atom, M+2 ion is the one with two labelled atoms, etc.

In the example, experimental data are saved as a series of .CDF files, and the base file name is:

```
 '/home/projects/PyMS/data-0903/a0903_'
```

with the extension file numbers '297, 298, 299'. The compound is Alanine, the retention time is 411 seconds and the diagnostic ion is 190 with MID size of 6 (i.e. ions to be extracted are 190, 191, 192, 193, 194, and 195). Window size used is 4 seconds.

To enter the above input data:

```
>>> file_base = '/home/projects/PyMS/data-0903/a0903_'
>>> file_numbers = (297, 298, 299)
>>> compound = 'Alanine'
>>> ion = 190
>>> rt = 411
```

and to enter the parameters:

```
>>> mid_size = 6
>>> win_size = 4
```

Finally, to extract MID:

```
>>> import pyms.LabelledData.MID.Extract.Function
>>> extract_mid(file_base, file_numbers, compound, ion, rt, mid_size, win_size)
```

This will give the following result:

```
\begin{verbatim}
Alanine
```

```
 -> Reading netCDF file '/home/projects/PyMS/data-0903/a0903_297.CDF'
Mass Isotopomer Distribution (MID): 615040.0 128760.0 58418.0 8837.0 1440.0 0.0

 -> Reading netCDF file '/home/projects/PyMS/data-0903/a0903_298.CDF'
Mass Isotopomer Distribution (MID): 738048.0 155456.0 68656.0 9833.0 1859.0 0.0

 -> Reading netCDF file '/home/projects/PyMS/data-0903/a0903_299.CDF'
Mass Isotopomer Distribution (MID): 982400.0 207456.0 85200.0 12021.0 3368.0 908.0
```

8.2 Mass Isotopomer Distribution Correction in ^{13}C Experiments

[*This example is in pyms-test/92*]

Method implemented is that of Wittman and Heinzle [7], and the details of the example are given in Nanchen et al [8].

Higher mass ion intensities (M, M + 1, M + 2, etc.) from ^{13}C tracer experiments will include natural abundance of non-C isotopes and also natural abundance of ^{13}C isotopes inside the GC-MS derivatization reagent, if applicable. The function takes the experimental ion intensities, which are obtained prior via integration of individual ion chromatograms, calculates the corresponding mass distribution vector and performs the correction for natural isotope abundances of C, O, N, H, Si, and S atoms, as well as any unlabelled biomass (this corrects for the fact that in practice substrate is never 100% labelled). The result is a corrected mass distribution vector. Fractional labelling value is also provided as a check, and it should be equal to the fractional labelling of the input substrate.

Experimental data is entered using the 'mdv' variable. In the example of alanine fragment (M-57)+ [8] there are n = 3 exogenous (non-natural abundance) C atoms, and the length of the mass distribution vector is chosen to be n+1=4. Hence only first four intensities (ion counts) from the mass spectrum, corresponding to M, M+1, M+2 and M+3, are entered.

```
>>> mdv = [737537, 179694, 88657, 178433]
>>> n = len(mdv) - 1
```

Determine the number of C, O, N, H, Si, and S atoms in the fragment, noting that the number of C atoms excludes C atoms which may contain exogenous ^{13}C atoms. For the alanine (M-57)+ fragment these numbers are C=8, O=2, N=1, H=26, Si=2, and S=0.

```
>>> atoms = { 'c':8, 'o':2, 'n':1, 'h':26, 'si':2, 's':0}
```

Import the relevant modules:

```
>>> import numpy
>>> import pyms.LabelledData.MID.Correct.Function
>>> import pyms.LabelledData.MID.Correct.Constants
```

Calculate the overall correction matrix:

```
>>> c_corr = pyms.LabelledData.MID.Correct.Function.overall_correction_matrix(n, mdv, atoms)
```

Calculating c correction matrix

Calculating h correction matrix

Calculating si correction matrix

Calculating o correction matrix

Calculating n correction matrix

Calculating s correction matrix

Calculated overall correction matrix.

```
>>> c_corr array([[0.77152972 , 0.          , 0.          , 0.         ],
                  [0.1508547  , 0.77152972 , 0.          , 0.         ],
                  [0.06721399 , 0.1508547  , 0.77152972 , 0.         ],
                  [0.00866575 , 0.06721399 , 0.1508547  , 0.77152972]])
```

Calculate the exclusive mass isotope distribution of the carbon skeleton:

```
>>> mdv_alpha_star = pyms.LabelledData.MID.Correct.Function.c_mass_isotope_distr(mdv, c_corr)
>>> mdv_alpha_star array([[ 0.7729932 ],
                          [ 0.03719172],
                          [ 0.01830562],
                          [ 0.17150946]])
```

Correct for unlabelled biomass. This example accounts for the contribution of 1%
unlabelled biomass.

```
>>> f_unlablelled = 0.01
>>> mdv_aa = pyms.LabelledData.MID.Correct.Function.corr_unlabelled(n, mdv_alpha_star, f_unlablelled)
```

```
>>> mdv_aa array([[ 0.77102099],
                  [ 0.03725005],
                  [ 0.01848709],
                  [ 0.17324187]])
```

Calculate the fractional labelling:

```
>>> fl = pyms.LabelledData.MID.Correct.Function.fract_labelling(n, mdv_aa)
Fractional labelling FL: 0.197983278219
```

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