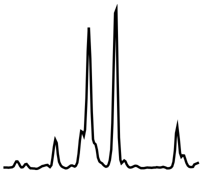

PyMS User Guide

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PyMS version 1.0

A Python toolkit for processing of chromatography–mass spectrometry data

Contents

1 Introduction **1**

1.1 The PyMS project 2

1.2 PyMS installation 2

1.2.1 Downloading PyMS source code 3

1.3 External Libraries 4

1.3.1 Package 'NumPy' (required for all aspects of PyMS) 4

1.3.2 Package 'pycdf' (required for reading ANDI-MS files) 4

1.3.3 Package 'Pycluster' (required for peak alignment by dynamic programming) 4

1.3.4 Package 'scipy.ndimage' (required for TopHat baseline corrector) 4

1.3.5 Package 'matplotlib' (required for plotting) 5

1.3.6 Package 'mpi4py' (required for parallel processing) 5

1.4 PyMS installation on Linux 5

1.5 Troubleshooting 7

1.5.1 Pycdf import error 7

1.6 PyMS tutorial and examples 8

1.7 Using PyMS on Windows 9

1.7.1 PyMS installation on Windows 9

1.7.2 Example processing GC-MS data with PyMS on Windows . . . 10

2 GC-MS Raw Data Model **13**

2.1 Introduction 13
2.2 Reading the raw GC-MS data 14
2.2.1 Reading JCAMP GC-MS data 14
2.2.2 Reading ANDI GC-MS data 14
2.3 A GCMS_data object 15
2.3.1 Methods 15
2.3.2 A Scan data object 15
2.3.3 Exporting data and printing information about a data set . . . 16
2.3.4 Comparing two GC-MS data sets 17
3 GC-MS data derived objects 19
3.1 IntensityMatrix Object 19
3.1.1 Discussion of Binning Boundaries 20
3.1.2 Build intensity matrix 20
3.1.3 Build intensity matrix parameters 21
3.1.4 Build integer mass intensity matrix 22
3.2 MassSpectrum Object 22
3.3 IonChromatogram Object 23
3.3.1 Writing IonChromatogram data to a file 23
3.4 Saving data 24
3.5 Importing ASCII data 25
4 Data filtering 27
4.1 Introduction 27
4.2 Time strings 27
4.3 Intensity Matrix resizing 27
4.3.1 Retention time range 27
4.3.2 Mass spectrum range and entries 28
4.4 Noise smoothing 28

4.4.1 Window averaging 29
4.4.2 Savitzky–Golay noise filter 29
4.5 Baseline correction 30
4.6 Pre-processing the IntensityMatrix 31

5 Peak detection and representation 33
5.1 Peak Object 33
5.1.1 Creating a Peak Object 33
5.1.2 Peak Object properties 34
5.1.3 Modifying a Peak Object 34
5.2 Peak detection 35
5.2.1 Sample processing and Peak detection 35
5.3 Filtering Peak Lists 36
5.4 Peak area estimation 37

6 Peak alignment by dynamic programming 39
6.1 Preparation of multiple experiments for peak alignment by dynamic programming 39
6.1.1 Creating an Experiment 39
6.1.2 Multiple Experiments 41
6.2 Dynamic programming alignment of peak lists from multiple experiments 42
6.3 Between-state alignment of peak lists from multiple experiments . . . 44

7 The Display module 47
7.1 Displaying the TIC 47
7.2 Displaying multiple ion chromatogram objects 48
7.3 Displaying the mass spectrum 49
7.4 Displaying detected peaks on the TIC plot 49
7.4.1 User interaction with the plot window 51

8 Analysis of ^{13}C labelled data with PyMS 53

8.1 Mass Isotopomer Distribution Extraction in ^{13}C Labelling Experiments 53

8.2 MID extraction algorithm 56

8.2.1 Diagnostic Ion Value and Number of Mass Isotopomers 57

8.2.2 TIC Retention Time 58

8.2.3 Peak Shape 58

8.2.4 Mass Spectra 62

8.2.5 Setting the two parameters 65

8.3 Mass Isotopomer Distribution Correction in ^{13}C Experiments 65

9 Parallel processing with PyMS 69

9.1 Requirements 69

9.1.1 Installation of 'mpich2' 69

9.1.2 Installation of 'mpi4py' 70

9.2 Background to using PyMS in parallel 71

9.3 Using PyMS in parallel 72

10 GCMS Simulator 79

10.1 Setting up input data for the simulator 79

10.1.1 Providing input to the simulator 79

10.2 Running the simulator 80

10.3 Adding noise to the simulated data 81

10.3.1 Constant scale noise 81

10.3.2 Variable scale noise 81

10.4 Adding noise to the whole simulated dataset 82

10.4.1 Constant scale gaussian noise 82

10.4.2 Variable scale gaussian noise 83

10.5 Detecting Peaks in the Simulated data 84

Appendix **84**

A Processing GC-MS data using PyMS **87**

A.1 Processing GC-Quad data 87

A.1.1 Instrument 87

A.1.2 Raw Data 87

A.1.3 Processing using PyMS 88

A.2 Processing GC-TOF data 88

A.2.1 Instrument 88

A.2.2 Raw Data 88

A.2.3 Processing using PyMS 89

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 example, 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 (relevant 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 towards PyMS installation 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 to use the subversions client. Several subversion clients are available, some are open source, some freeware, and some are commercial (for more information see http://subversion.tigris.org/). The svn client programs are available for all operating systems. For example, on Linux we use the svn client program which ships with most Linux systems called simply 'svn'. The 'svn' exists for all mainstream operating systems[1]. A well known svn client for Windows is TortoiseSVN (http://tortoisesvn.tigris.org/). TortoiseSVN provides graphical user interface, and is tightly integrated with Windows. TortoiseSVN is open source and can be downloaded from the project web page (http://tortoisesvn.tigris.org/). There are also several commercial svn clients for Windows.

Subversion has extensive functionality, however only the very basic functionality is needed to download PyMS source code. For more information about subversion please consult the book freely available at http://svnbook.red-bean.com/.

If the computer is connected to the internet, and the subversion client 'svn' is installed. On Linux, 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 ] ...
```

---

[1] For example, on Linux CentOS 4 it ships as a part of the RPM package 'subversion-1.3.2-1.rhel4.i386.rpm'

```
Checked out revision 71.
```

1.3 External Libraries

In addition to the source code, for the full PyMS functionality several external libraries are required.

1.3.1 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 instructions and the link to the source code.

1.3.2 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.

1.3.3 Package 'Pycluster' (required for peak alignment by dynamic programming)

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

1.3.4 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).

1.3.5 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/`

1.3.6 Package 'mpi4py' (required for parallel processing)

This package is required for parallel processing with PyMS. It can be downloaded from:
`http://code.google.com/p/mpi4py/`

1.4 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 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 before before it is possible to import PyMS modules. For more on this please consult the Python command sys.path.append().

PyMS is currently being developed on Linux with the following packages:

```
Python-2.5.2
numpy-1.1.1
netcdf-4.0
pycdf-0.6-3b
ndimage.zip
Pycluster-1.41
matplotlib-0.99.1.2
mpi4py-1.2.1.tar.gz
mpich2-1.2.1p1.tar.gz
```

For easy installation, we provide 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

In addition to the dependencies bundle, one also needs to dowload the PyMS source code as explained in the section 1.2.1). Below we give a quick installation guide of packages required by PyMS on Linux.

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’.

7. ’mpi4py’ installation:

This package is required for running PyMS processing on multiple processors (CPUs). Instructions how to install this package and run PyMS processing in parallel are given in Section 9.1.

1.5 Troubleshooting

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

1.5.1 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.

1.6 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.

1.7 Using PyMS on Windows

Python is highly cross-platform compatible, and PyMS works seamlessly on Windows. The only exception is reading of data in ANDI-MS (NetCDF) format, widely used standard format for storing raw chromatography–mass spectrometry data (see 2.1) This capability in PyMS depends on the 'pycdf' library, which is not supported on Windows (see the Subsection 1.3.2). Therefore at present the ability to read ANDI-MS files is limited to Linux. All other PyMS functionality is available under Windows.

We use Linux for the development and deployment PyMS, and this User Guide largely assumes that PyMS is used under Linux. In this Subsection we give some pointers on how to use PyMS under Windows.

1.7.1 PyMS installation on Windows

1. Install Python, NumPy, SciPy, and matplotlib for Windows. The bundle of these software packages tested under Windows XP and Windows 7 can be downloaded from the PyMS project page at the Bio21 Institute, University of Melbourne
   `http://bioinformatics.bio21.unimelb.edu.au/pyms.html`
2. Download the latest PyMS code from the PyMS Google Code project page
   `http://code.google.com/p/pyms/`

3. Unpack the PyMS code and place it in a standard place for Python libraries, or adjust the PYTHONPATH variable to include the path to PyMS. If Python is installed in C:
   Python25, the standard place for Python libraries is C:
   Python25
   Libs
   site-packages

4. Start IDLE, or other Python shell. If PyMS is installed properly, the following command will not return any warning or error messages:

   ```
   >>> import pyms
   ```

1.7.2 Example processing GC-MS data with PyMS on Windows

The example data can be downloaded from `http://bioinformatics.bio21.unimelb.edu.au/pyms/data/`. We will use the data file in the JCAMP-DX format, named `gc01_0812_066.jdx`. Once downloaded this data file will be placed in the folder `C:`
`Data`.

The Python environment can be accessed from several Python shells. The default shell that comes with the Python 2.5 installation is IDLE. In this example we first load the raw data,

```
>>> from pyms.GCMS.IO.JCAMP.Function import JCAMP_reader
>>> jcamp_file = "C:\Data\gc01_0812_066.jdx"
>>> data = JCAMP_reader(jcamp_file)
 -> Reading JCAMP file 'C:\Data\gc01_0812_066.jdx'
```

Then build the intensity matrix object by binning:

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

We obtain the dimensions of the intensity matrix, then loop over all ion chromatograms, and for each ion chromatogram apply Savitzky-Golay noise filter and tophat baseline correction. The resulting noise and baseline corrected ion chromatogram is saved back into the intensity matrix:

```
>>> n_scan, n_mz = im.get_size()
>>> from pyms.Noise.SavitzkyGolay import savitzky_golay
```

```
>>> from pyms.Baseline.TopHat import tophat
>>> for ii in range(n_mz):
print "working on IC", ii
ic = im.get_ic_at_index(ii)
ic1 = savitzky_golay(ic)
ic_smooth = savitzky_golay(ic1)
ic_base = tophat(ic_smooth, struct="1.5m")
im.set_ic_at_index(ii, ic_base)
```

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.

---

[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 IntensityMatrix 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 Discussion of Binning Boundaries

For any chemical element X, let $w(x)$ be the atomic weight of X. Now

$$\delta(X) = \frac{w(X) - \{w(X)\}}{w(X)}, \tag{3.1}$$

where $\{a\}$ is the integer value of a(rounded to the nearest integer)

Then, for Hydrogen $\delta(H) = \frac{1.007825032-1}{1.007825032} = 0.0076$. Similarly $\delta(^{12}C) = 0$, $\delta(^{14}N) = 0.00022$, $\delta(^{16}O) = -0.00032$, etc.

Let also $\Delta(X) = w(X) - \{w(x)\}$. Then $-0.023 < \Delta(P), \Delta(Si) < 0$.

Now let a compound undergo GC-MS and let Y be one of it's fragments. If Y consists of $k_1$atoms of type X_1, k_2 atoms of type X_2,....., k_r atoms of type X_r, then $\Delta(Y) = k_1 * \Delta(X_1) + k_2 * \Delta(X_2) + ... + k_r * \Delta(X_r)$.

The fragment will usually not contain more than 2 or 3 P or Si atoms and if it's molecular weight is less than 550 it may not contain more than 35 O atoms, so $\Delta(Y) \geq -0.023 * 5 - 0.00051 * 35 = -0.133$.

On the other hand, of Y contains k H atoms and m N atoms, then $\Delta(Y) \leq k * 0.00783 + m * 0.00051$. Since for each two Hydrogen atoms we will need at least one Carbon (or heavier)atom, we may have no more than 80 Hydrogen atoms, so in this case (i.e. H and C atoms only)$\Delta(Y) \leq 80 * 0.00783 = 0.63$. If we replace Carbon by any heavier atom we will have to sacrifice at least 2 hydrogen atoms so $\Delta(Y)$ becomes even smaller.

If the molecular weight of Y does not exceed 550 (typically the largest mass scanned for in a GC-MS setup) then $\mathbf{-0.133 \leq \Delta(Y) \leq 0.63}$. This means that if we set our binning boundaries to $(-0.3, 0.7)$ or $(-0.2, 0.8)$ there is no danger of having a fragment whose molecular weight is very close to the boundary, as long as it's molecular weight does not exceed 550.

Since the resolution of MS is at least 0.1 dalton, we may assume that it's error does not exceed 0.05, so even MS accuracy will not cause any problem.

3.1.2 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.

3.1.3 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.

3.1.4 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 1.3.4.

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.

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

from pyms.GCMS.IO.ANDI.Function import ANDI_reader
from pyms.GCMS.Function import build_intensity_matrix_i
from pyms.Noise.SavitzkyGolay import savitzky_golay
from pyms.Baseline.TopHat import tophat
from pyms.Peak.Class import Peak
from pyms.Peak.Function import peak_sum_area

from pyms.Deconvolution.BillerBiemann.Function import BillerBiemann, \
    rel_threshold, num_ions_threshold

# deconvolution and peak list filtering parameters
points = 9; scans = 2; n = 3; t = 3000; r = 2;

andi_file = "/x/PyMS/data/a0806_077.cdf"

data = ANDI_reader(andi_file)

# integer mass
im = build_intensity_matrix_i(data)

# get the size of the intensity matrix
n_scan, n_mz = im.get_size()

# smooth data
for ii in range(n_mz):
    ic = im.get_ic_at_index(ii)
    ic1 = savitzky_golay(ic)
    ic_smooth = savitzky_golay(ic1)
    ic_base = tophat(ic_smooth, struct="1.5m")
    im.set_ic_at_index(ii, ic_base)

# do peak detection on pre-trimmed data

# get the list of Peak objects
pl = BillerBiemann(im, points, scans)

# trim by relative intensity
apl = rel_threshold(pl, r)

# trim by threshold
peak_list = num_ions_threshold(apl, n, t)
```

```

print "Number of Peaks found:", len(peak_list)

# ignore TMS ions and set mass range
for peak in peak_list:
    peak.crop_mass(50,540)
    peak.null_mass(73)
    peak.null_mass(147)
    # find area
    area = peak_sum_area(im, peak)
    peak.set_area(area)

```

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 1.3.3.*

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 1.3.5.

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.

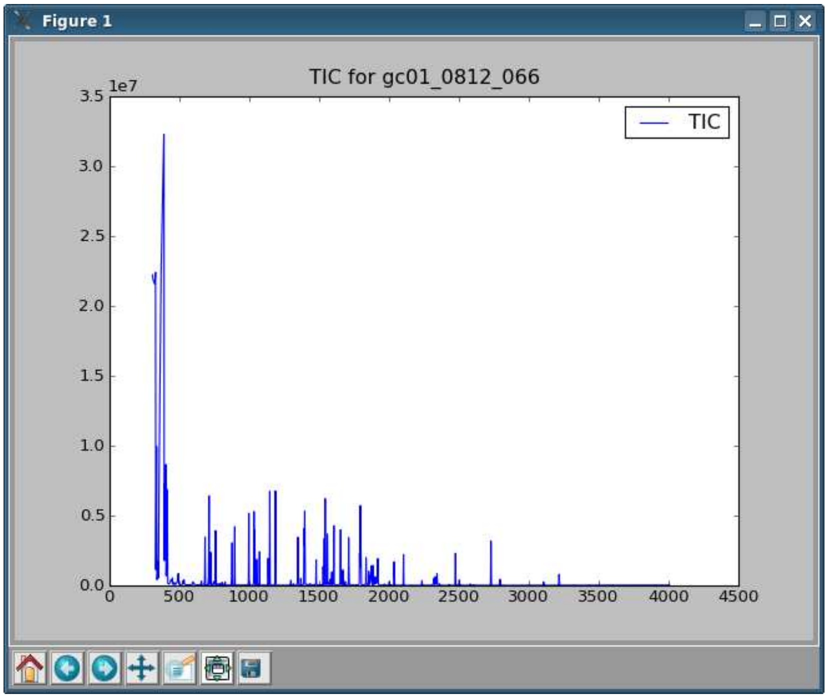


Figure 1
TIC for gc01_0812_066
1e7
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
TIC

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:

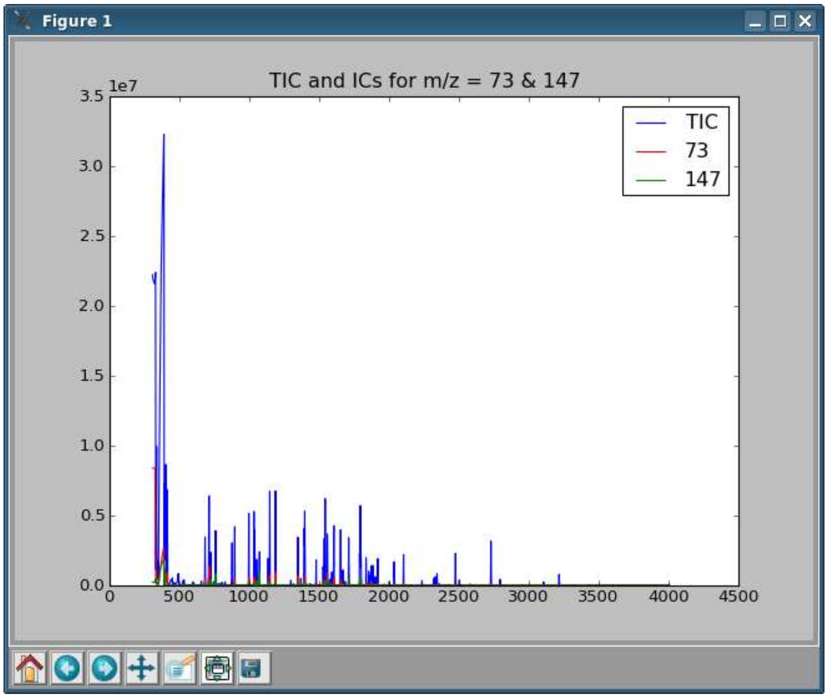


Figure 1
TIC and ICs for m/z = 73 & 147
1e7
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
TIC
73
147

Figure 7.2: Graphics window displayed by the script 70b/proc.py

7.3 Displaying the mass spectrum

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

The pyms Display module can also be used to display individual mass spectra. The following selects the mass spectrum of interest:

```
... im is an intensity matrix
>>> ms = im.get_ms_at_index(1024)
```

The pyms function `plot_ms()` can then be called to display the mass spectrum to the screen.

```
>>> plot_ms(ms, 'Mass Spectrum at index 1024')
```

The resulting window is shown below in figure 7.3

7.4 Displaying detected peaks on the TIC plot

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

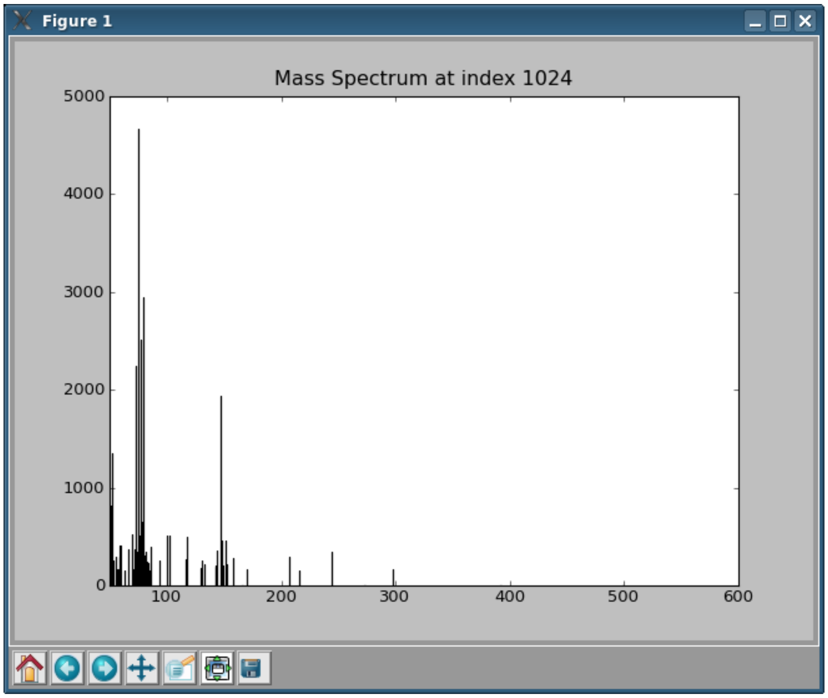


Figure 7.3: Graphics window displayed by the script 70c/proc.py

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 overall figure label is not supplied at this time.

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

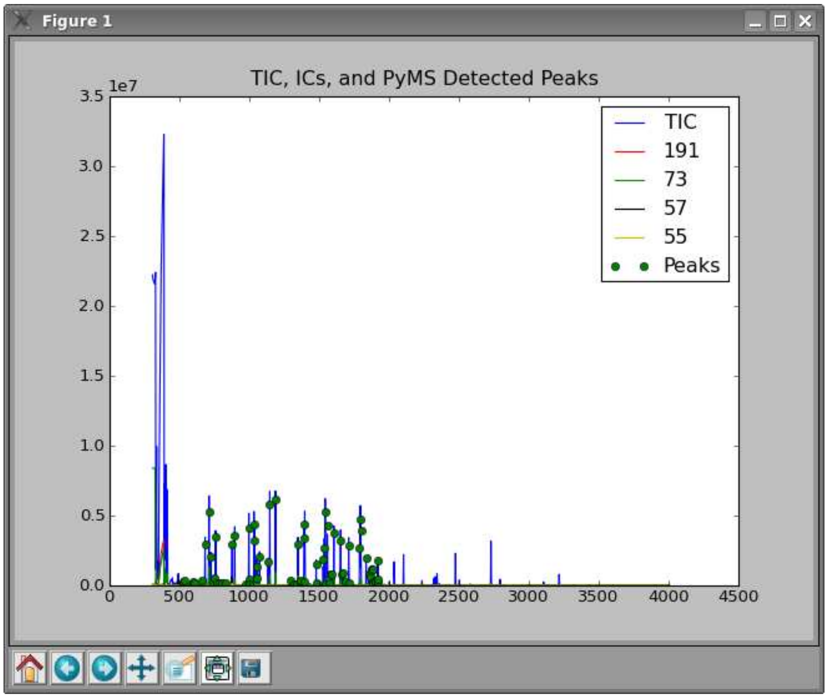


Figure 1
TIC, ICs, and PyMS Detected Peaks
1e7
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
TIC
191
73
57
55
Peaks

Figure 7.4: Graphics window displayed by the script 71/proc.py

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

```
>>>display.plot_peaks(peak_list, 'Peaks')
\end{verbatgoogle.com.au/im}

Finally, the function {\tt do\_plotting()} is called to draw the figure with
labels and display the plot.

\begin{verbatim}
>>> display.do_plotting('TIC, ICs, and PyMS Detected Peaks')
```

This should result in figure 7.4 being displayed:

7.4.1 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.

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:

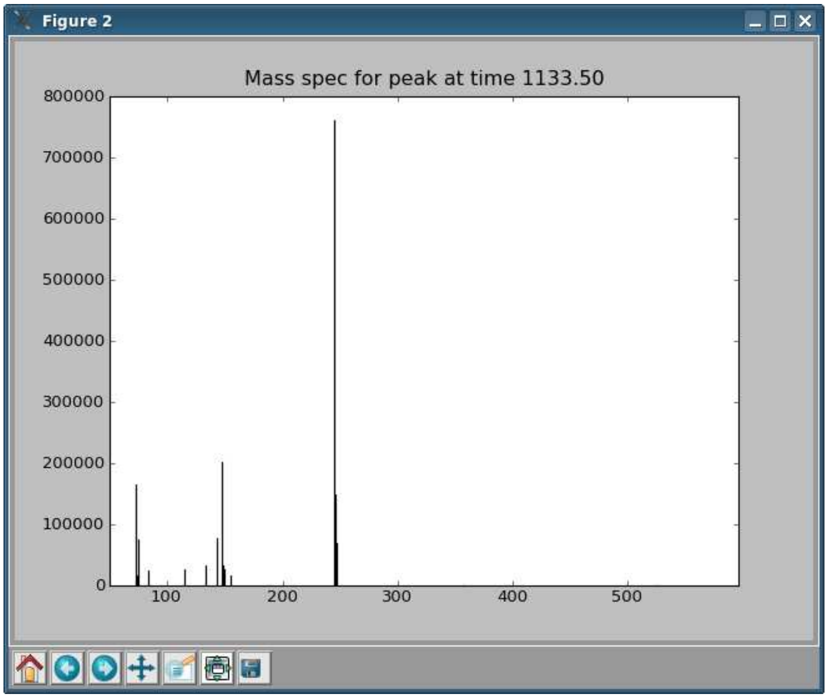


Figure 2
Mass spec for peak at time 1133.50
800000
700000
600000
500000
400000
300000
200000
100000
0
100
200
300
400
500

Figure 7.5: The mass spectrum displayed by PyMS when a peak in the graphics window of Figure 7.4 is clicked on

```
>>> 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 7.5):

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

Chapter 8

Analysis of ^{13}C labelled data with PyMS

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

[*Example in pyms-test/91*]

The aim of the algorithm is to alleviate manual data processing bottleneck by semi-automatically computing the mass isotopomer distribution (MID) of each metabolite of interest from raw GC-MS data files. MID is a direct measure of the metabolite's labelling pattern, and thus of great interest in carbon labelling experiments as well as mathematical modelling of metabolic fluxes.

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, or any other fragment ion incorporating whole carbon backbone, gives the mass isotopomer distribution (i.e. normalised M, M+1, M+2, M+3 values) of the compound.

GC-MS data pre-processing (high and low frequency noise removal) as well as the construction of Intensity Matrix is accomplished via functionality of the GCMS module(Figure 8.1). The new algorithm takes a list of each metabolites fragment ions, TIC retention time and a required number of isotopomers (MID vector size) and extracts the mass isotopomer distribution for each metabolite. The algorithms performance for a particular data set is optimised via two parameters, namely the intensity threshold and a time window.

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

```
'/x/PyMS/data/'
```

The extension file numbers 'a20090_3297', 'a200903_298' and 'a200903_299' are stored inside 'data_files' file. The compound is alanine, the retention time is 6.93 minutes and the diagnostic ions are 190 and 116 with MID vector size of 6 (i.e. ions to be extracted are m/z 190, 191, 192, 193, 194, and 195; and 116, 117, 118, 119, 120 and 121 respectively). This information is stored inside 'ion_definitions' file.

Firstly, import the relevant modules:

```
>>> from pyms.GCMS.IO.ANDI.Function import ANDI_reader
>>> from pyms.GCMS.Function import build_intensity_matrix_i
>>> from pyms.MIDs.Function import parse_ion_defs, parse_data_defs, \
...    write_mid_tables
>>> from pyms.MIDs.MassExtraction.Function import extract_mid
```

To enter the input data:

```
>>> data_file_root = '/x/PyMS/data/'
>>> ion_defs = 'input/ion_definitions'
>>> data_defs = 'input/data_files'
>>> out_file = 'output/out.csv'
```

Time window size used is 4 seconds, and intensity threshold is 4000. To enter both parameters:

```
>>> time_win = 4
>>> int_tresh = 4000
```

Then read in both input files:

```
>>> mid_table_list = parse_ion_defs(ion_defs)
>>> data_files = parse_data_defs(data_defs
```

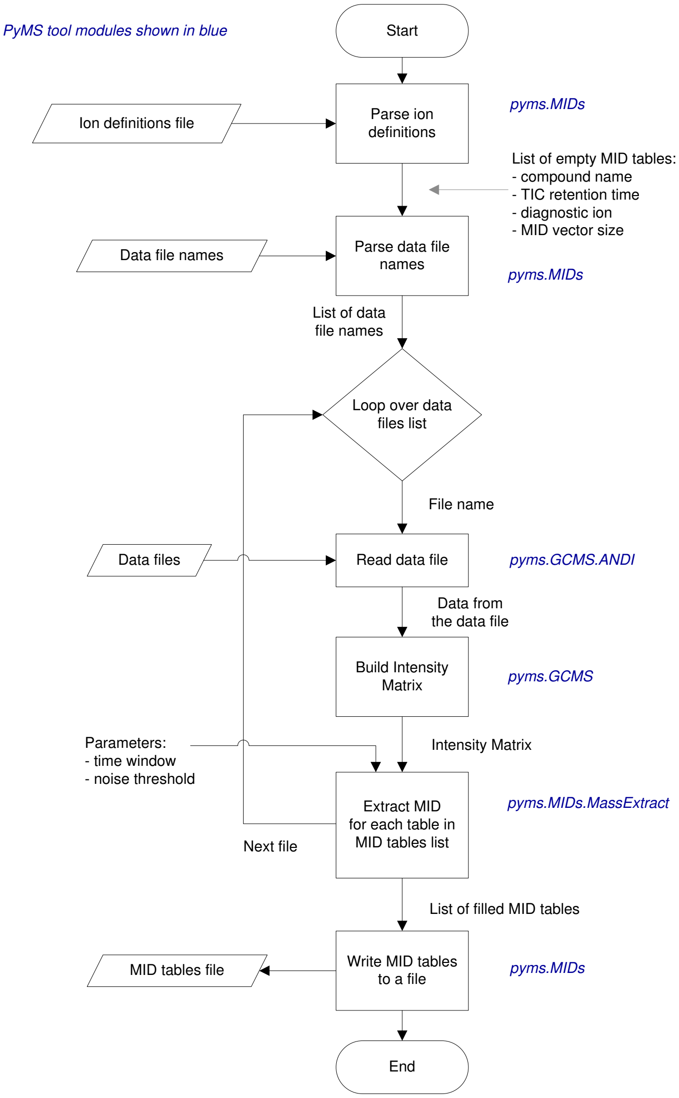

PyMS tool modules shown in blue
Start
Ion definitions file
Parse ion definitions
pyms.MIDs
List of empty MID tables:
- compound name
- TIC retention time
- diagnostic ion
- MID vector size
Data file names
Parse data file names
pyms.MIDs
List of data file names
Loop over data files list
File name
Data files
Read data file
pyms.GCMS.ANDI
Data from the data file
Build Intensity Matrix
pyms.GCMS
Parameters:
- time window
- noise threshold
Intensity Matrix
Extract MID for each table in MID tables list
pyms.MIDs.MassExtract
Next file
List of filled MID tables
MID tables file
Write MID tables to a file
pyms.MIDs
End

Figure 8.1: Data Flow Chart

To loop over data files and extract MID:

```
>>> for file_name in data_files:
...     andi_file = data_file_root + file_name + ".CDF"
...     data = ANDI_reader(andi_file)
...     im = build_intensity_matrix_i(data)
...     for mid_table in mid_table_list:
...         extract_mid(mid_table, file_name, im, time_win, int_tresh)
```

Finally, write MID data tables to previously defined out_file:

```
>>> write_mid_tables(mid_table_list, out_file)
```

The out_file should contain:

```
Alanine
rt = 415.8secs
rt = 6.93mins
ion 190
mdv size = 6
file name,M+0,M+1,M+2,M+3,M+4,M+5,
a0903_297,0.757476593654,0.161643594022,0.0686776427591,...
a0903_298,0.755688322848,0.164943331054,0.0675399871565,...
a0903_299,0.754408795882,0.166596996121,0.0671474860614,...

Alanine
rt = 415.8secs
rt = 6.93mins
ion 116
mdv size = 6
file name,M+0,M+1,M+2,M+3,M+4,M+5,
a0903_297,0.742205716152,0.185529670258,0.0623422956963,...
a0903_298,0.709695079502,0.209264922281,0.0701801446155,...
a0903_299,0.696956557166,0.21788080549,0.0737847830385,...
```

8.2 MID extraction algorithm

The overall information, and therefore possible inputs, available for each metabolite consists of:

- Diagnostic fragment ion value (e.g. m/z 116 for alanine) and a number of subsequent isotopomers

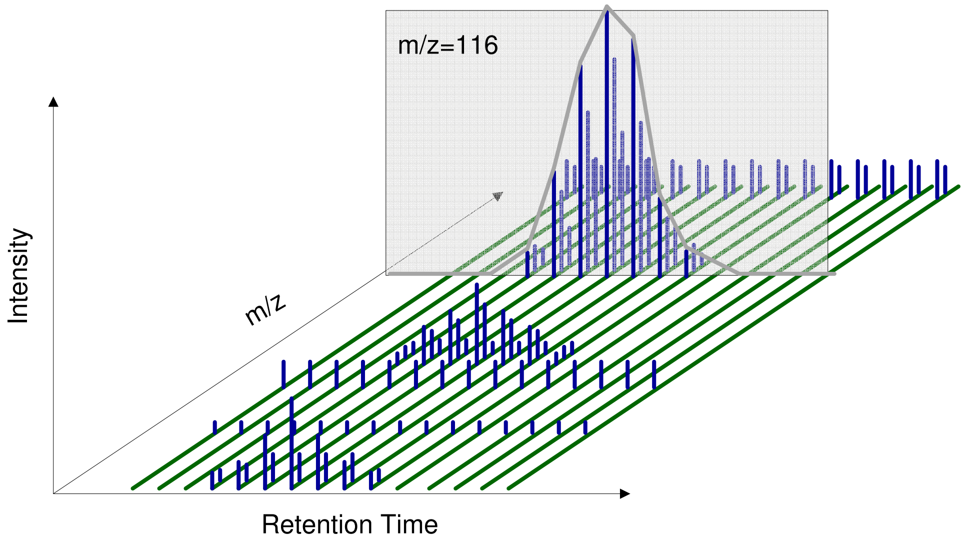


Figure 8.2: Ion Chromatogram Extraction at m/z 116

- TIC retention time from an unlabelled sample (e.g. 6.93 minutes)
- Chromatographic peak shape
- Unlabelled mass spectra

Next each of these is explained in detail.

8.2.1 Diagnostic Ion Value and Number of Mass Isotopomers

The fragment ion value is used to extract ion chromatogram of the unlabelled isotopomer as well as subsequent, labelled, isotopomers, the number of which is predetermined. This information is constant and does not change across files. For example, in case of alanine fragment m/z 116, which contains 2 backbone carbons, there are 2 subsequent isotopomers m/z 117 (contains singly labelled carbon) and m/z 118 (contains two labelled carbons). Since we are dealing with metabolically labelled data, carbons belonging to the derivatisation agent are considered unlabelled (this is not strictly true due to natural isotopic abundance - dealt with in the next section). Another important point is that each compound will have many fragments containing both different and the same parts of the carbon skeleton, thus redundant data will be available for consistency checks. These checks are currently not implemented.

Example in the figure 8.2 gives a visual representation of the extraction of m/z 116 diagnostic ion for TMS derivatised alanine. The subsequent isotopomer ICs are extracted in the likewise manner.

8.2.2 TIC Retention Time

Unlike the fragment ion value, the use of TIC retention time is less dependable. In the current processing procedure, retention time value must be manually obtained from an unlabelled standard (once for each metabolite) at the beginning of each experiment run. TIC retention time is prone to change with the settings of the GC-MS instrument (e.g. pressure, temperature) as well as normal operating conditions (e.g. cutting of the GC column). Retention time locking and use of retention indices have both been developed to counteract this inherent time shift.

The GC-MS instrument is capable of adjusting pressure to lock one compound to a particular retention time. This means that a particular compound can be chosen to elute at an exact time. For example, mannitol can be chosen to elute at 10.5mins every time. Mannitol is chosen for the fact that its retention time is in the middle of most metabolomic TICs, thus giving the maximum benefit (as opposed to choosing the compound at the beginning or end of a TIC). This procedure is referred to as retention time locking.

While the retention time locking aims to eliminate the effect of the linear component of the retention time drift, indices aim to compensate for the non-linear time shift (i.e. TIC expansion and contraction). Ideally, a group of alkines each differing by 2 carbon atoms (e.g. C10 to C32) is added to samples. The alkines elute in an equidistant pattern, and are given indices of 1000, 1200, , 3200. The components of interest are then assigned indices depending on their elution time relative to their two neighbouring alkines. For example, if a compound elutes of the way between alkine with 10 carbon atoms and alkine with 12 carbon atoms then its index is 1050. This is referred to as indices procedure.

Finally, as TIC retention time at peak apex will, most of the time, not be i dentical to an IC retention time anyway, the algorithm has been designed to cope with a small variation in supplied retention time value. Thus, the retention time locking, currently employed in the local lab, is sufficient for its correct operation, and indices, while certainly beneficial, are not necessary.

8.2.3 Peak Shape

Detecting Peak Apex

Following from the TIC retention time discussion in the previous section, the two anticipated scenarios are represented in figure 8.3. The TIC retention time will be located immediately before (on the left of) or after (on the right of) ICs peak apex.

ICs peak apex is then detected as a local maximum within a specified window. Time window size is a parameter set to just below average peak width. Too narrow window will fail to account for the time drift present and encompass the actual peak apex,

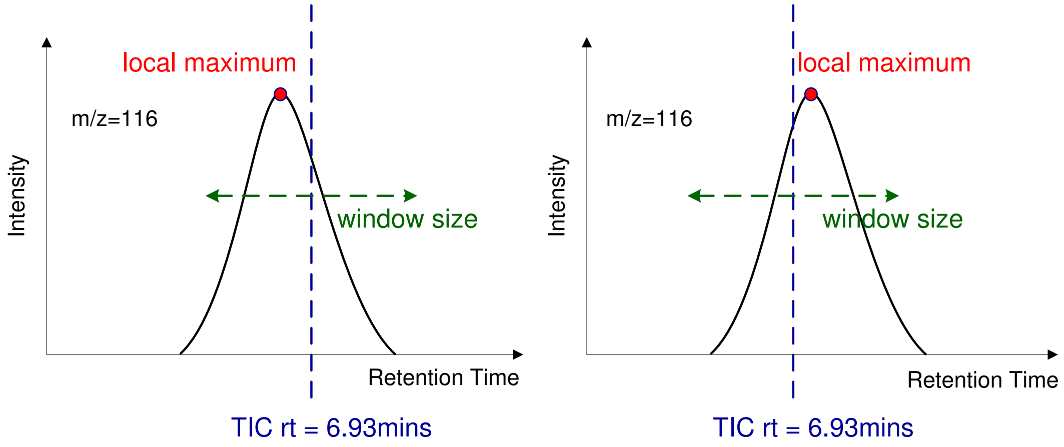


Figure 8.3: Peak Apex Detection

and too wide window size will raise the probability of reaching the neighbouring peak apex instead. Both scenarios are discussed in detail next.

As we are not guaranteed that the TIC retention time will be located within a specified window of ICs peak apex, and by examining the two dimensional IC space by placing our detected local maximum at the origin of a Cartesian coordinate system, we can draw some conclusions on the success of finding the true peak apex (figure 8.4). Firstly, if either left or right neighbouring intensities are greater than our detected local maximum one can safely deduce that the IC peak apex detection has failed. In the case that both left and right neighbour intensities are equal a warning will be raised as GC-MS peaks are not expected to be flat, and this could indicate unusually high baseline or MS detector reaching its limit. This can be further checked by comparing peak apex intensity with the instrument detection limit value (not implemented in the current version).

The above analysis also covers the scenarios where TIC retention time is located too close to a much larger peak, or the peak is simply missed by the particular IC retention time being too far to the left or too far to the right from the supplied overall TIC retention time (figure 8.5).

The four scenarios pictured in figure 8.5 suggest a middle ground scenario shown in figure 8.6. The TIC retention time can happen to be in the middle of two peaks with similar intensities. In this case, with only a slight time shift between ICs the detected peak apex would oscillate between the two peaks. The algorithm will detect this and raise a warning, as it is still possible to have an unusually wide peak with legitimate peak apex shift of plus/minus half the time window size (also pictured to the right). Note that the peaks in the first diagram in 8.6 belong to the same IC, while the two peaks in the diagram on the right belong to two different ICs.

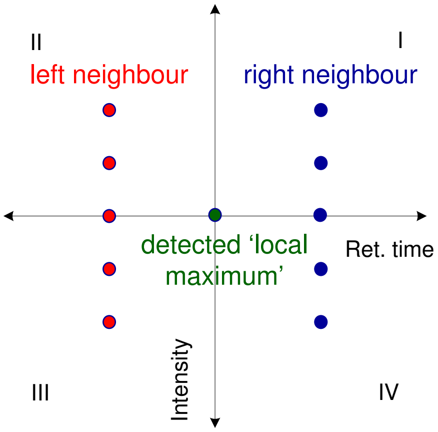

II
I
left neighbour
right neighbour
detected 'local maximum'
Ret. time
Intensity
III
IV

Figure 8.4: Left and right peak neighbour check

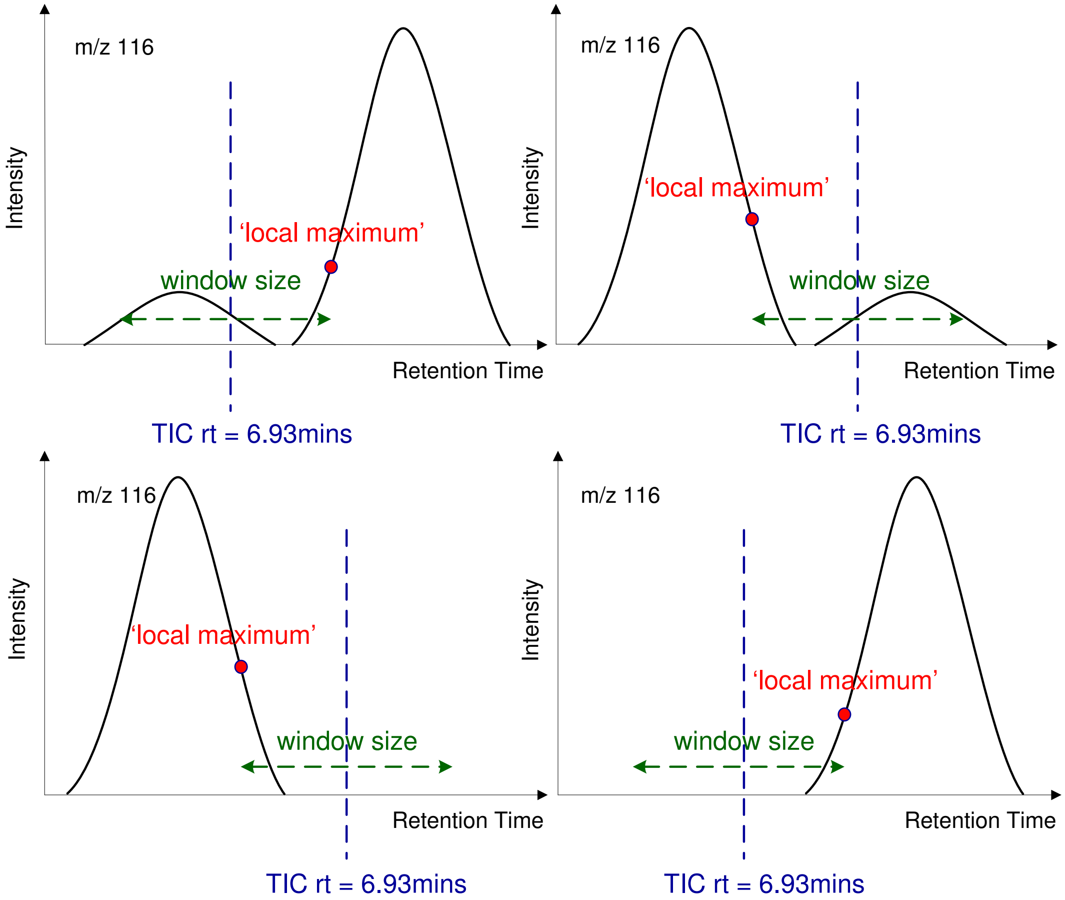

m/z 116
Intensity
'local maximum'
window size
Retention Time
TIC rt = 6.93mins
m/z 116
Intensity
'local maximum'
window size
Retention Time
TIC rt = 6.93mins
m/z 116
Intensity
'local maximum'
window size
Retention Time
TIC rt = 6.93mins
m/z 116
Intensity
'local maximum'
window size
Retention Time
TIC rt = 6.93mins

Figure 8.5: Missed or large peak check

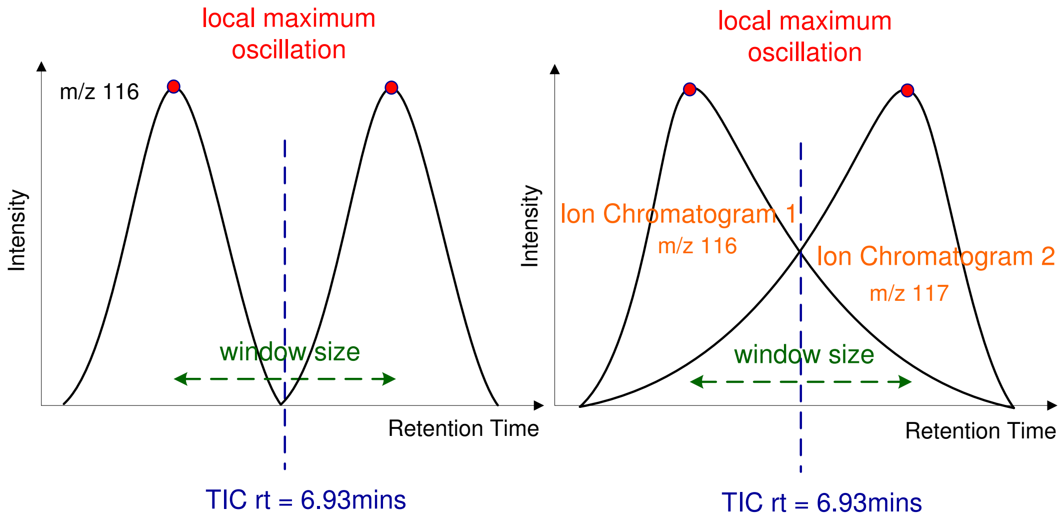

local maximum
oscillation
m/z 116
Intensity
window size
Retention Time
TIC rt = 6.93mins
local maximum
oscillation
Intensity
Ion Chromatogram 1
m/z 116
Ion Chromatogram 2
m/z 117
window size
Retention Time
TIC rt = 6.93mins

Figure 8.6: Close or wide peak

Detecting left and right boundary

Having analysed the possible scenarios for the relationship between TIC retention time and detected peak apex, this brings us to the detection of left and right peak boundary (figure 8.7). If our peak detection was successful this should be as easy as detecting first local minimum on the right and first local minimum on the left of detected peak apex. The integration then proceeds via summing up of all intensities between the two boundaries.

In addition integrating multiple ICs belonging to a single compound inside a single file can help integrate low intensity irregular shape peaks. This is shown in figure 8.8, and accomplished via setting an intensity threshold parameter which is equal to the intensity below which peaks are too close to the noise, irregular shape and impossible to integrate via above described method. In the drawing below the procedure is illustrated for the peak inside the IC belonging to the m/z of 119. Should there be no peaks with intensity above the threshold no integration occurs, should there be only a single peak above the threshold a warning is issued. The diagram also illustrates the importance of baseline correction for these low intensity peaks.

The introduction of an intensity threshold parameter introduces one more undesirable scenario. Namely, as peaks are not detected below threshold and the algorithm operates in a blind mode it is possible for a neighbouring peak (not present or not as high intensity in other ICs) to interfere with the integration result (figure 8.9). Should this happen a warning is raised.

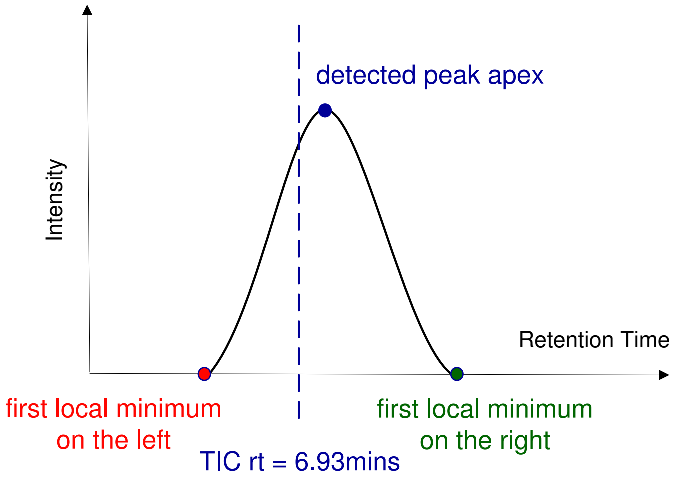


Figure 8.7: Feature detection at mass-to-charge ratio of 116 and retention time of 6.93 minutes

8.2.4 Mass Spectra

The diagram in Figure 8.9 introduces another important question. When is a neighbouring peak a co-elution and when just a shoulder? The only way to reliably distinguish the two scenarios is via the use of mass spectra, which acts as a signature for a particular compound in GC-MS data. In fact TIC retention time itself is determined via mass spectral library, which can be used inside unlabelled data. Metabolic labelling, however introduces uncertainty into this signature, i.e. components can no longer be compared with the standard unlabelled mass spectra. Indeed, a test run of an existing similarity algorithm [6] shows that labelled mass spectra can be more similar to spectra of other labelled compounds, than their own unlabelled spectra.

However, it might be possible to use a mass spectrum at peak apex inside labelled data to at least run a check between, and just outside, the integration boundaries. This might give an answer to a question if we have missed a shoulder or included a neighbouring peak in the overall integration.

The above will, of course, not solve the question of certainty that the right compound was integrated. In theory it should be possible to design an elaborate algorithm, which would compute all possibilities of metabolic labelling, as well as take natural isotopic abundance into account, and then score the probability of integrated peak belonging to the compound we were interested in. This is indeed what analysts do in practice. In the manual procedure unlabelled chromatogram is compared to a labelled chromatogram. At the apex of a peak, the mass spectral fingerprint of both labelled and unlabelled metabolites is compared by eye. Labelled metabolites are expected to result in an increase in the mass to charge ratio of ions in the labelled chromatogram, and based on the retention time and mass spectra similarity analyst

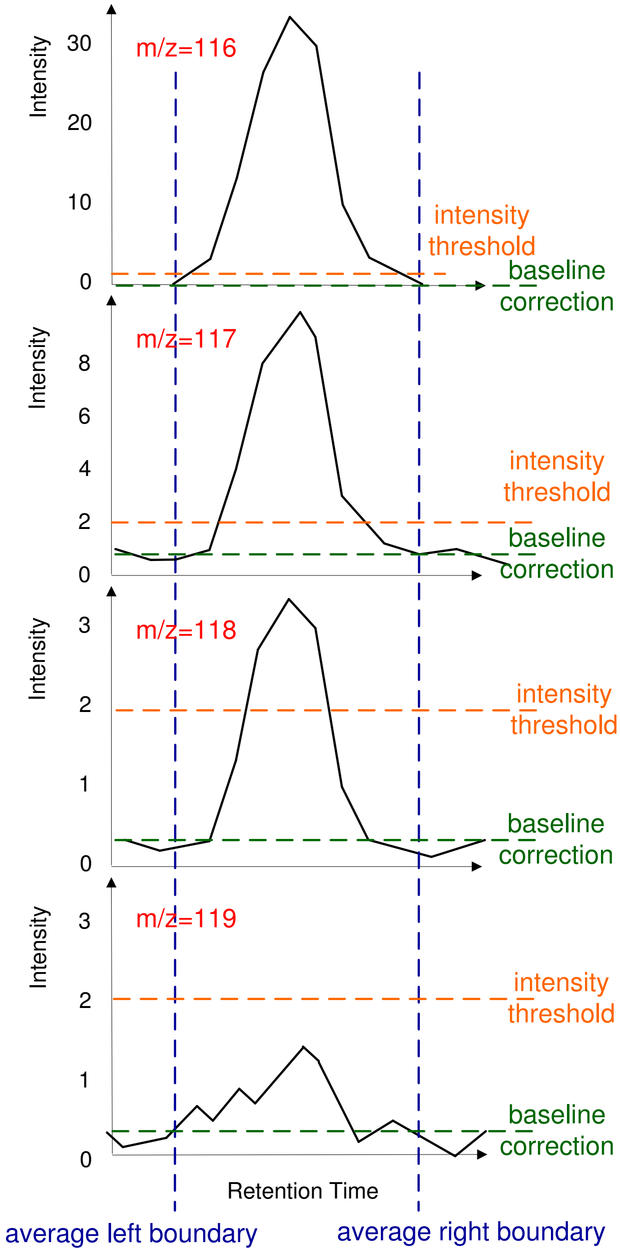


Figure 8.8: Averaging of left and right boundary values inside an ion chromatogram, and baseline correction (inspired by [7])

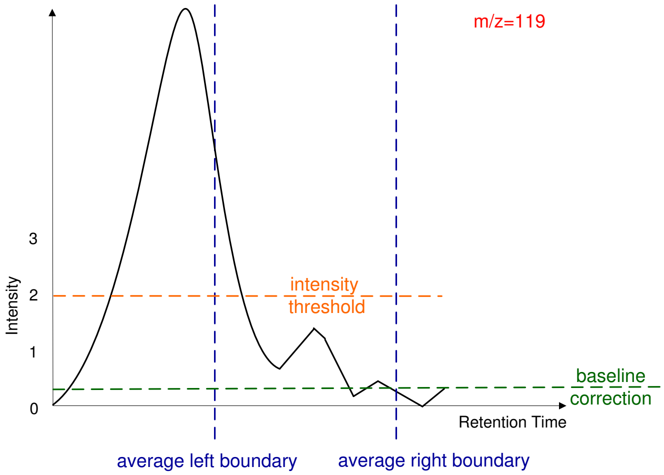

m/z=119
3
2
1
0
Intensity
intensity threshold
baseline correction
Retention Time
average left boundary
average right boundary

Figure 8.9: Large peak co-eluting with a peak below the intensity threshold

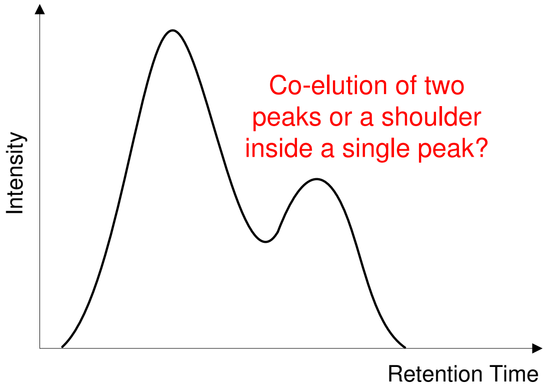

Co-elution of two peaks or a shoulder inside a single peak?
Intensity
Retention Time

Figure 8.10: Co-elution or a shoulder of a single peak ambiguity

decides if the peaks belong to the same compound or not.

8.2.5 Setting the two parameters

Setting the threshold too high will result in large number of unintegrated peaks. Setting the threshold too low will result in the algorithm attempting to integrate irregular shaped peaks thus setting off a large amount of warnings indicative of false positives. Due to a large variety in the abundance of metabolites one could almost perform two runs: one for high abundance peaks with higher threshold (to avoid peak shape detection of dubious peaks), and one for low abundance peaks which would benefit from lower threshold. This of course is not necessary and a well-chosen single threshold is sufficient.

As discussed earlier, setting the time window too narrow will result in inability to detect peak apex (as it will not be present within the window) setting the time window too wide will increase the chance of neighbouring peak being detected instead. Thus a window size of just below average peak width will give optimum performance.

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

[*Example in pyms-test/92*]

Method implemented is that of Wittman and Heinzle [8], and the details of the example are given in Nanchen et al [9].

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)+ 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 [9]. 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
>>> from pyms.MIDs.MassCorrection.Function import fract_labelling, \
...     corr_unlabelled, overall_correction_matrix, \
...     correction_matrix, c_mass_isotope_distr, mass_dist_vector
```

Calculate the overall correction matrix:

```
>>> c_corr = 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 = 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 = corr_unlabelled(n, mdv_alpha_star, f_unlabelled)
>>> mdv_aa
array([[ 0.77102099],
       [ 0.03725005],
       [ 0.01848709],
       [ 0.17324187]])
```

Finally, the fractional labelling is calculated:

```
>>> fl = fract_labelling(n, mdv_aa)
Fractional labelling FL: 0.197983278219
```

Chapter 9

Parallel processing with PyMS

9.1 Requirements

Using PyMS parallel capabilities requires installation of the package 'mpi4py', which provides bindings of the Message Passing Interface (MPI) for the Python programming language. This package can be downloaded from `http://code.google.com/p/mpi4py/`. Since 'mpi4py' provides only Python bindings, it requires an MPI implementation. We recommend using mpich2:
`http://www.mcs.anl.gov/research/projects/mpich2/`
We show the installation of 'mpich2' and 'mpi2py' on Linux system from software distributions downloaded from the projects' web site.

9.1.1 Installation of 'mpich2'

1. From the mpich2 project web site download the current distribution of mpich2 (in our case the file 'mpich2-1.2.1p1.tar.gz').

2. Prepare the directory for mpich2 installation. In this example we have chosen to use /usr/local/mpich2/. Our version of mpitch2 is 1.2.1, and to allow for the installation of different version later, we create a subdirectory "1.2.1",

   ```
   $ mkdir -vp /usr/local/mpich2/1.2.1
   ```

   The above command will make the directory /usr/local/mpich2/ and also /usr/local/mpich2/1.2.1. Note that /usr/local is usually owned by root, and the above commands may require root privileges.

3. Unpack this file and change to the source code directory:

```
$ tar xvfz mpich2-1.2.1p1.tar.gz
$ cd  mpich2-1.2.1p1
```

4. Configure, compile, and install mpich2:

```
$ ./configure --prefix=/usr/local/mpich2/1.2.1 --enable-sharedlibs=gcc
$ make
$ make install
```

   If /usr/local/mpich2/1.2.1 is owned by rood, the above command may require root privileges.

9.1.2 Installation of 'mpi4py'

1. From the mpi4py project web site download the current distribution of mpi4py (in our case the file 'mpi4py-1.2.1.tar.gz').
2. Unpack this file and change to the source code directory:

```
$ tar xvfz mpi4py-1.2.1.tar.gz
$ cd mpi4py-1.2.1
```

3. Edit the file 'mpi.cfg' to reflect the location of mpich2. In our case this file after editing contained the following:

```
# MPICH2
[mpi]
mpi_dir              = /usr/local/mpich2/1.2.1
mpicc                = %(mpi_dir)s/bin/mpicc
mpicxx               = %(mpi_dir)s/bin/mpicxx
```

4. Install mpi4py:

```
$ python setup.py install
```

5. Check that mpi4py works:

```
$ python
Python 2.5.2 (r252:60911, Sep 10 2008, 14:39:22)
[GCC 4.1.1 20070105 (Red Hat 4.1.1-52)] on linux2
Type "help", "copyright", "credits" or "license" for more information.
>>> import mpi4py
>>>
```

   If the above command import produced no output, mpi4py is installed properly and ready to use.

9.2 Background to using PyMS in parallel

Any processing that loops through ion chromatograms or mass spectra can be performed in parallel, by distributing the processing of individual ion chromatograms or mass spectra to different CPUs by using the efficient MPI mechanism.

Before the parallel processing can be deployed, data needs to be binned to produce an IntensityMatrix object, as described in the Section 3.1. This is essentially a two dimensional matrix, with ion chromatograms along one dimension and mass spectra along the other dimension.

Consider the processing which applies a noise smoothing function to each ion chromatogram. We first read the raw data:

```
andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
data = ANDI_reader(andi_file)
```

Then build the intensity matrix, and get its dimensions:

```
im = build_intensity_matrix_i(data)
n_scan, n_mz = im.get_size()
```

The last command sets the variables n_scan and n_mz to the number scans and number of m/z values present in data, respectively. Processing of ion chromatograms with the noise smoothing function requires fetching of each ion chromatogram from the data, and application of the noise smoothing function. This can be achieved with a simple loop:

```
for ii in n_mz:
    print ii+1,
    ic = im.get_ic_at_index(ii)
    ic_smooth = window_smooth(ic, window=7)
```

This example epitomizes the typical processing required on the GC-MS data. Another, equally important processing, is that of individual mass spectra. In this case the same logic can be applied, except that one would loop over the other dimension of the IntensityMatrix object 'im'. That is, one would loop over all the scan indices, and use the method get_ms_at_index() to fetch individual mass spectra:

```
for ii in n_scan:
    print ii+1,
    ms = im.get_ms_at_index(ii)
    # here do something the the mass spectra 'ms'
```

Processing of data in this fashion is computationally intensive. A typical data set may consist of 3,000-10,000 scans and 500 m/z values. If complex processing algorithms are applied to each ion chromatogram (or mass spectra), the processing will quickly become computationally prohibitive.

The type of calculation illustrated above is an ideal candidate for parallelization because each ion chromatogram (or mass spectrum) are processed independently. PyMS takes advantage of this and allows one to harvest the power of multiple CPUs to speed-up the processing. To achieve this PyMS can distributes the loop from the above (either type, ie. over ion chromatograms or mass spectra) over the available CPUs, achieving a linear speed-up with the number of CPUs.

9.3 Using PyMS in parallel

Using PyMS in parallel requires a minimal intervention, only that special method of the IntensityMatrix object is invoked in the for loop described above. For looping over all ion chromatograms in parallel,

```
for ii in im.iter_ic_indices():
    print ii+1,
    ic = im.get_ic_at_index(ii)
    ic_smooth = window_smooth(ic, window=7)
```

The only change is that

```
for ii in n_mz:
```

is replaced with

```
for ii in im.iter_ic_indices()
```

The corresponding method for looping over all mass spectra would involve replacing:

```
for ii in n_scan:
```

with

```
for ii in im.iter_ms_indices()
```

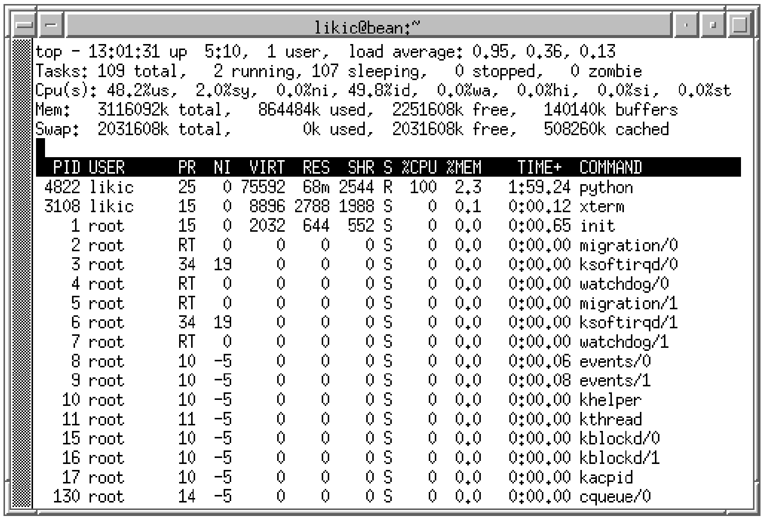
The special constructs `for ii in im.iter_ic_indices():` and `for ii in im.iter_ms_indices()` will distribute the calculation in parallel if MPI capability is available (ie. mpi4py is installed on the system, and multiple CPUs are available). If MPI capability is not available, the processing will be performed in a serial mode. Running in parallel also requires some prior preparations, as explained below.

Consider how the following script that performs noise smoothing example described above (named 'proc.py'). This script is can be run in serial or parallel mode.

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

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

from pyms.GCMS.IO.ANDI.Function import ANDI_reader
from pyms.GCMS.Function import build_intensity_matrix_i
from pyms.Noise.Window import window_smooth

# read the raw data as a GCMS_data object
andi_file = "/x/PyMS/data/gc01_0812_066.cdf"
data = ANDI_reader(andi_file)

# build the intensity matrix
im = build_intensity_matrix_i(data)

# get the size of the intensity matrix
n_scan, n_mz = im.get_size()
print "Size of the intensity matrix is (n_scans, n_mz):", n_scan, n_mz

# loop over all m/z values, fetch the corresponding IC, and perform
# noise smoothing
for ii in im.iter_ic_indices():
    print ii+1,
    ic = im.get_ic_at_index(ii)
    ic_smooth = window_smooth(ic, window=7)
```

A simple running of this script will produce a serial run without any warning messages:

```
$ python proc.py
 -> Reading netCDF file '/x/PyMS/data/gc01_0812_066.cdf'
Size of the intensity matrix is (n_scans, n_mz): 9865 551
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47
... [ further output deleted ] ...
```



```
top - 13:01:31 up  5:10,  1 user,  load average: 0.95, 0.36, 0.13
Tasks: 109 total,   2 running, 107 sleeping,   0 stopped,   0 zombie
Cpu(s): 48.2%us,  2.0%sy,  0.0%ni, 49.8%id,  0.0%wa,  0.0%hi,  0.0%si,  0.0%st
Mem:   3116092k total,   864484k used,  2251608k free,   140140k buffers
Swap:  2031608k total,        0k used,  2031608k free,   508260k cached

  PID USER      PR  NI  VIRT  RES  SHR S %CPU %MEM    TIME+  COMMAND
 4822 likic     25   0 75592  68m 2544 R  100  2.3   1:59.24 python
 3108 likic     15   0  8896 2788 1988 S    0  0.1   0:00.12 xterm
    1 root      15   0  2032  644  552 S    0  0.0   0:00.65 init
    2 root      RT   0     0    0    0 S    0  0.0   0:00.00 migration/0
    3 root      34  19     0    0    0 S    0  0.0   0:00.00 ksoftirqd/0
    4 root      RT   0     0    0    0 S    0  0.0   0:00.00 watchdog/0
    5 root      RT   0     0    0    0 S    0  0.0   0:00.00 migration/1
    6 root      34  19     0    0    0 S    0  0.0   0:00.00 ksoftirqd/1
    7 root      RT   0     0    0    0 S    0  0.0   0:00.00 watchdog/1
    8 root      10  -5     0    0    0 S    0  0.0   0:00.06 events/0
    9 root      10  -5     0    0    0 S    0  0.0   0:00.08 events/1
   10 root      10  -5     0    0    0 S    0  0.0   0:00.00 khelper
   11 root      11  -5     0    0    0 S    0  0.0   0:00.00 kthread
   15 root      10  -5     0    0    0 S    0  0.0   0:00.00 kblockd/0
   16 root      10  -5     0    0    0 S    0  0.0   0:00.00 kblockd/1
   17 root      10  -5     0    0    0 S    0  0.0   0:00.00 kacpid
  130 root      14  -5     0    0    0 S    0  0.0   0:00.00 cqueue/0
```

Figure 9.1: The xterm output of the program ’top’ with PyMS running in serial mode on the computer with multiple CPUs

Inspection of the CPU usage during the execution of the program shows that only one CPU is utilised 100% (although multiple CPUs are available) as shown in Figure 9.2.

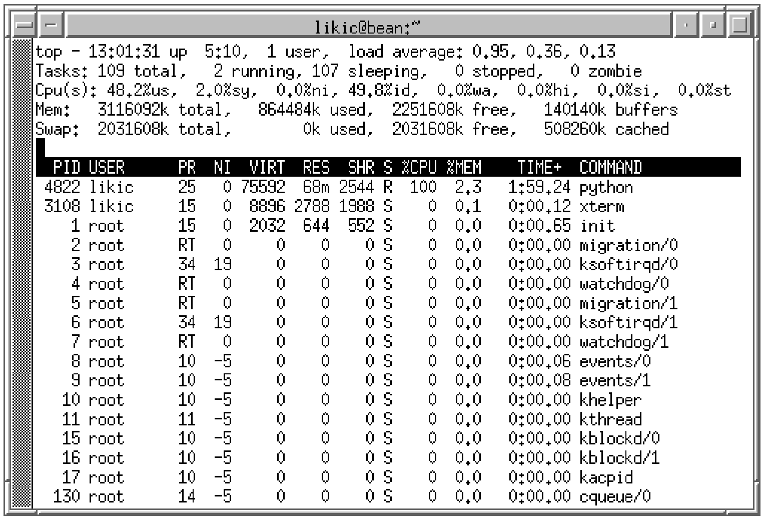
To run the above script in parallel, one needs first to start the mpitch2 process launcher, called ’mpd’ (this is a program, in this example located in
`/usr/local/mpich2/1.2.1/bin/mpd`,
see the section). This can be achieved as follows:

```
$ /usr/local/mpich2/1.2.1/bin/mpd --daemon
```

The above command start ’mpd’ as a daemon (the program runs in the background, without a controlling terminal). A common problem that causes the above command is fail is the absence of the `.mpd.conf` file which ’mpd’ requires to be present in the home directory of the user who is starting the process. Here is an excerpt from the ’mpd’ help page:

```
A file named .mpd.conf file must be present in the user’s home directory
with read and write access only for the user, and must contain at least
a line with MPD_SECRETWORD=<secretword>
To run mpd as root, install it while root and instead of a .mpd.conf file
use mpd.conf (no leading dot) in the /etc directory.’
```

Fixing this problem is simple, and requires creating the file `/.mpd.conf`, which in our case contains only one line:



```
top - 13:01:31 up  5:10,  1 user,  load average: 0.95, 0.36, 0.13
Tasks: 109 total,   2 running, 107 sleeping,   0 stopped,   0 zombie
Cpu(s): 48.2%us,  2.0%sy,  0.0%ni, 49.8%id,  0.0%wa,  0.0%hi,  0.0%si,  0.0%st
Mem:   3116092k total,   864484k used,  2251608k free,   140140k buffers
Swap:  2031608k total,        0k used,  2031608k free,   508260k cached

  PID USER      PR  NI  VIRT  RES  SHR S %CPU %MEM    TIME+  COMMAND
 4822 likic     25   0 75592  68m 2544 R  100  2.3   1:59.24 python
 3108 likic     15   0  8896 2788 1988 S    0  0.1   0:00.12 xterm
    1 root      15   0  2032  644  552 S    0  0.0   0:00.65 init
    2 root      RT   0     0    0    0 S    0  0.0   0:00.00 migration/0
    3 root      34  19     0    0    0 S    0  0.0   0:00.00 ksoftirqd/0
    4 root      RT   0     0    0    0 S    0  0.0   0:00.00 watchdog/0
    5 root      RT   0     0    0    0 S    0  0.0   0:00.00 migration/1
    6 root      34  19     0    0    0 S    0  0.0   0:00.00 ksoftirqd/1
    7 root      RT   0     0    0    0 S    0  0.0   0:00.00 watchdog/1
    8 root      10  -5     0    0    0 S    0  0.0   0:00.06 events/0
    9 root      10  -5     0    0    0 S    0  0.0   0:00.08 events/1
   10 root      10  -5     0    0    0 S    0  0.0   0:00.00 khelper
   11 root      11  -5     0    0    0 S    0  0.0   0:00.00 kthread
   15 root      10  -5     0    0    0 S    0  0.0   0:00.00 kblockd/0
   16 root      10  -5     0    0    0 S    0  0.0   0:00.00 kblockd/1
   17 root      10  -5     0    0    0 S    0  0.0   0:00.00 kacpid
  130 root      14  -5     0    0    0 S    0  0.0   0:00.00 cqueue/0
```

Figure 9.2: The xterm output of the program 'top' with PyMS running in serial mode on the computer with multiple CPUs

```
MPD_SECRETWORD=euSe0veo
```

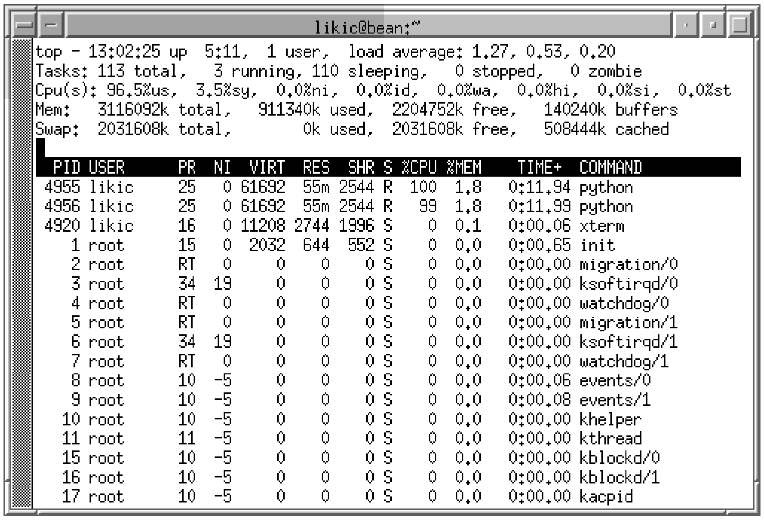
After this the 'mpd' can be launched. Running the PyMS script in the parallel mode requires the use of 'mpirun' command,

```
$ /usr/local/mpich2/1.2.1/bin/mpirun -np 2 python proc.py
```

The above command prepare 'python proc.py' to run in parallel, in this case by using two CPUS (`-np 2`). The execution produces the following output:

```
$ /usr/local/mpich2/1.2.1/bin/mpirun -np 2 python proc.py
 -> Reading netCDF file '/x/PyMS/data/gc01_0812_066.cdf'
 -> Reading netCDF file '/x/PyMS/data/gc01_0812_066.cdf'
Size of the intensity matrix is (n_scans, n_mz):Size of
the intensity matrix is (n_scans, n_mz): 9865 551
276 9865 551
1 277 2 278 3 279 4 280 5 281 6 282 7 283 8 284 9 285 10 286 11 287 12
288 13 289 14 290 15 291 16 292 17 293 18 294 19 295 20 296 21 297 22
298 23 299 24 300 25 301 26 302 27 303 28 304 2
... [ further output deleted ] ...
```

The above shows that two processes are active (each reading its own version of data). While the distribution of processing between the two processes has been achieved automatically by PyMS. Since both processes were started from the same terminal



```
top - 13:02:25 up  5:11,  1 user,  load average: 1.27, 0.53, 0.20
Tasks: 113 total,   3 running, 110 sleeping,   0 stopped,   0 zombie
Cpu(s): 96.5%us,  3.5%sy,  0.0%ni,  0.0%id,  0.0%wa,  0.0%hi,  0.0%si,  0.0%st
Mem:   3116092k total,   911340k used,  2204752k free,   140240k buffers
Swap:  2031608k total,        0k used,  2031608k free,   508444k cached

  PID USER      PR  NI  VIRT  RES  SHR S %CPU %MEM    TIME+  COMMAND
 4955 likic     25   0 61692  55m 2544 R  100  1.8   0:11.94 python
 4956 likic     25   0 61692  55m 2544 R   99  1.8   0:11.99 python
 4920 likic     16   0 11208 2744 1996 S    0  0.1   0:00.06 xterm
    1 root      15   0  2032  644  552 S    0  0.0   0:00.65 init
    2 root      RT   0     0    0    0 S    0  0.0   0:00.00 migration/0
    3 root      34  19     0    0    0 S    0  0.0   0:00.00 ksoftirqd/0
    4 root      RT   0     0    0    0 S    0  0.0   0:00.00 watchdog/0
    5 root      RT   0     0    0    0 S    0  0.0   0:00.00 migration/1
    6 root      34  19     0    0    0 S    0  0.0   0:00.00 ksoftirqd/1
    7 root      RT   0     0    0    0 S    0  0.0   0:00.00 watchdog/1
    8 root      10  -5     0    0    0 S    0  0.0   0:00.06 events/0
    9 root      10  -5     0    0    0 S    0  0.0   0:00.08 events/1
   10 root      10  -5     0    0    0 S    0  0.0   0:00.00 khelper
   11 root      11  -5     0    0    0 S    0  0.0   0:00.00 kthread
   15 root      10  -5     0    0    0 S    0  0.0   0:00.00 kblockd/0
   16 root      10  -5     0    0    0 S    0  0.0   0:00.00 kblockd/1
   17 root      10  -5     0    0    0 S    0  0.0   0:00.00 kacpid
```

Figure 9.3: The xterm output of the program 'top' with PyMS running in parallel mode with two CPUs

their output is intermingled. This time the processing is using two CPUs, and this can be seen from the inspection of CPU utilisation, as shown in Figure 9.3. Also the execution of the script 'proc.py' is now two times faster.

This simple example shows how to speed-up PyMS processing on a common workstation with two CPUs. The MPI also allows PyMS processing to run on specialised computers with many CPUs, such as Linux clusters. The MPI implementation allows PyMS to be easily used in such distributed computing environments, much like in the example above. We have tested PyMS on Linux clusters, and the resulting speed-up is nearly linear with the number of CPUs employed (Figure 9.4).

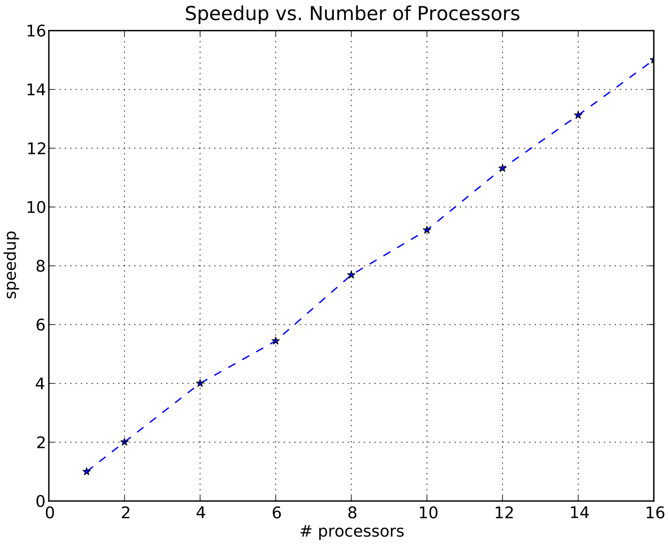


Figure 9.4: The speedup in PyMS processing when run in parallel on a Linux cluster as a function of a number of CPUs deployed

Chapter 10

GCMS Simulator

The PyMS GCMS data simulator is a useful tool for creating datasets with known peaks.

10.1 Setting up input data for the simulator

The PyMS GCMS data simulator package provides an Intensity Matrix object which can be processed by PyMS in the same way as any Intensity Matrix object derived from real data.

As input, the simulator requires a list of peaks, a list of times and a list of m/z channels. In this example we will use the time list, mass list and PyMS detected peak list from an existing real GCMS dataset.

For the purpose of demonstrating the simulator, only a portion of this dataset is examined. The following code selects the area of interest in the dataset and limits subsequent processing to this interval:

```
... data is a GCMS data object
>>> data.trim(4101, 4350)
```

10.1.1 Providing input to the simulator

[*This example is in pyms-test/100*]

The peaks are detected using the PyMS function `BillerBiemann()`, and subsequently filtered to remove those which do not meet certain requirements. This procedure is fully detailed in section 5.2.

Next the time list and mass list of the original data are copied to be used in the simulator

```
>>> mass_list = real_im.get_mass_list()
>>> time_list = real_im.get_time_list()
```

, where `real_im` is the Intensity Matrix object derived from the real data. (For more information about Intensity Matrix and data conversion refer to section (3.1).

10.2 Running the simulator

The function `gcms_sim()` takes three inputs: time list, mass list and peak list. It returns an Intensity Matrix object which can be manipulated in PyMS.

```
>>> sim_im = gcms_sim(time_list, mass_list, peak_list)
```

Using the PyMS display functionality described in chapter 7, the results of the simulator can be viewed. Figure 10.1 shows the IonChromatograms of the simulated Intensity Matrix.

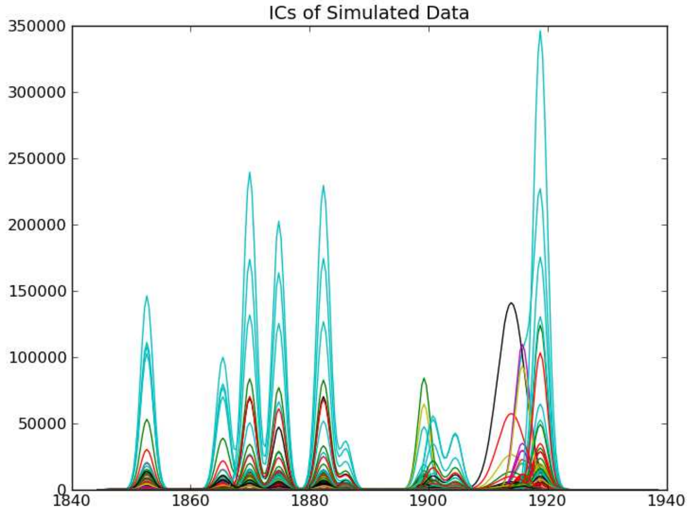


Figure 10.1: Graphics window displayed by the script 100/proc.py

10.3 Adding noise to the simulated data

Noise can be added to either the full Intenstity Matrix, or to individual Ion Chromatograms. Currently two noise models have been implemented in PyMS. These are:

1. Gaussian noise drawn from a normal distribution with constant scale across all scans.
2. Gaussian noise drawn from a normal distribution with scale varying with the intensity value at that point.

10.3.1 Constant scale noise

[*This example is in pyms-test/101*]

The Python package NumPy contains routines for pseudo random number generation. These "random" values are drawn from a normal distribution with a user defined scale over the same number of samples as the number of scans in a GC-MS experiment. This resulting list of numbers is a good approximation to gaussian noise on an m/z channel in the GC-MS experiment.

To add gaussian noise to an IC, the following code is used:

```
>>> ic = sim_im.get_ic_at_mass(73)
>>> scale = 1000
>>> add_gaussc_noise_ic(ic, scale)
```

The normal distribution from which the noise values are drawn in this example has a top value of 1000. This noisy IC can be displayed using the PyMS package Display, the resulting figure is shown in figure 10.2.

10.3.2 Variable scale noise

[*This example is in pyms-test/102*]

In reality, noise from a GC-MS experiment tends to have higher values in areas where peaks occur than in the valleys between peaks. To attempt to model this, a variable scale noise function has been implemented in PyMS. For a given time-point in an Ion Chromatogram, if the intensity at that time-point is greater than a user defined threshold value, the scale of the normal distribution from which the noise value is drawn will be proportional to the intensity at that point. The actual scale of the distribution at that point will be `scale * (intensity*proportion)`, if the intensity is above the `cutoff` value, and `scale` if below.

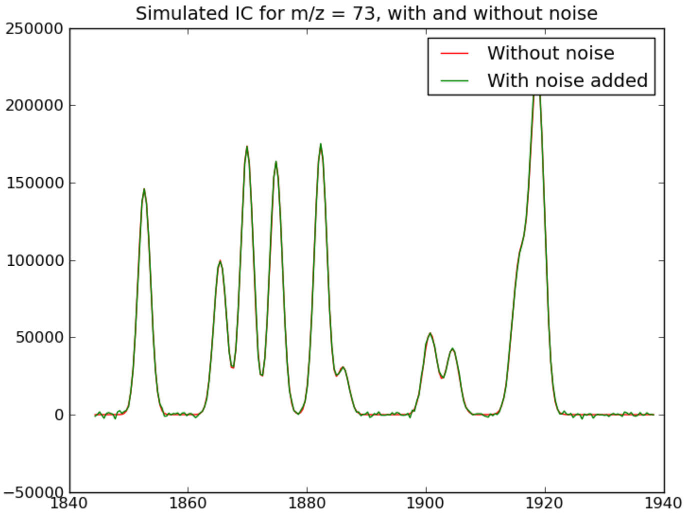


Figure 10.2: Graphics window displayed by the script 101/proc.py

To add variable scale gaussian noise to an IC, the following code is used:

```
>>> ic = sim_im.get_ic_at_mass(73)
>>> scale = 1000
>>> cutoff = 10000
>>> prop = 0.0003
>>> add_gaussv_noise_ic(ic, scale, cutoff, prop)
```

The resulting noisy IC is shown in figure 10.3

10.4 Adding noise to the whole simulated dataset

Often, the user may desire to add noise to all ICs in the dataset. This can be accomplished easily using the functions `add_gaussc_noise()` and `add_gaussv_noise()` The noise models in question are exactly the same as for the single IC noise functions above.

10.4.1 Constant scale gaussian noise

[*This example is in pyms-test/103*] For constant scale gaussian noise:

```
... sim_im is a simulated intensity matrix object
```

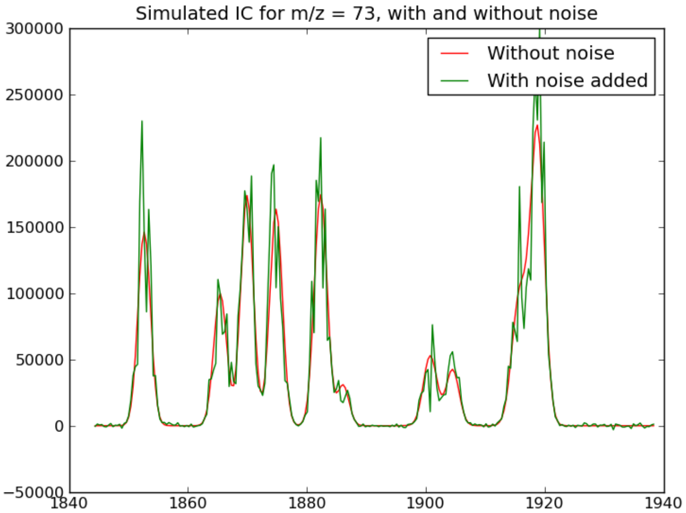


Figure 10.3: Graphics window displayed by the script 102/proc.py

```
>>> scale = 1000
>>> add_gaussc_noise(sim_im, scale)
```

This code adds noise to all Ion Chromatograms in the Intensity Matrix. These can be viewed in the PyMS package Display, with the resulting figure shown below (figure 10.4).

10.4.2 Variable scale gaussian noise

[*This example is in pyms-test/104*]

To apply variable scale gaussian noise to the whole simulated dataset:

```
>>> scale = 1000
>>> cutoff = 10000
>>> prop = 0.0003
>>> add_gaussv_noise(sim_im, scale, cutoff, prop)
```

The arguments supplied to `add_gaussv_noise()` relate to how the gaussian distribution supplying the noise values changes with the intensity. These parameters are further discussed in sub-section 10.3.2 above.

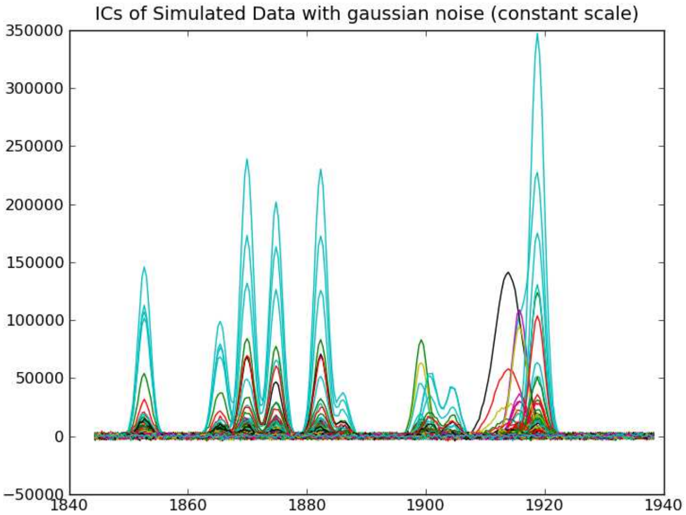


Figure 10.4: Graphics window displayed by the script 103/proc.py

10.5 Detecting Peaks in the Simulated data

[*This example is in pyms-test/105*]

The simulated intensity matrix may be processed in PyMS in the same way as an intensity matrix derived from real data. Applying the same techniques as outlined in section 5.2, the peaks of the simulated IM with added noise from 10.4.2 above can be found and displayed.

The found peaks are shown below in fig 10.6.

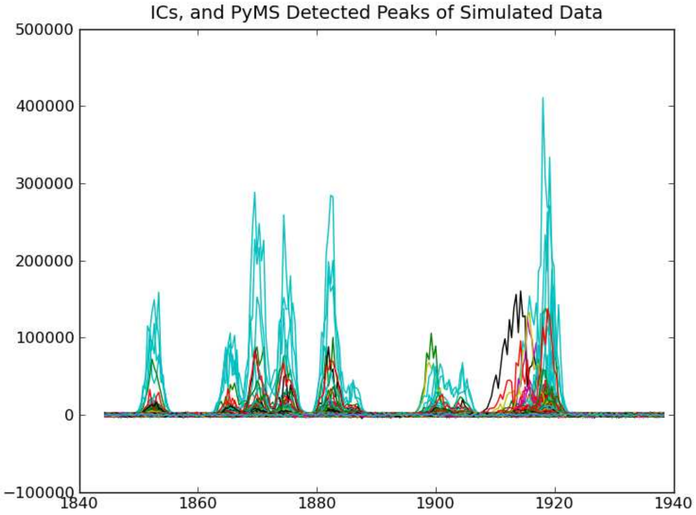


Figure 10.5: Graphics window displayed by the script 104/proc.py

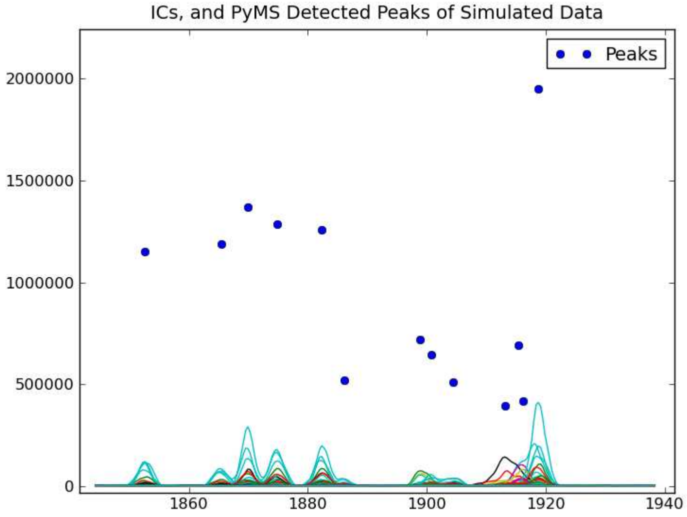


Figure 10.6: Graphics window displayed by the script 105/proc.py

Appendix A

Processing GC-MS data using PyMS

This Chapter presents different test examples where PyMS is used to process GC-MS data from different instruments.

A.1 Processing GC-Quad data

[*This example is in pyms-test/A1*]

A.1.1 Instrument

- Maker: Agilent
- Software: Chemstation

A.1.2 Raw Data

- File: gc01_0812_066.cdf
- Location: PyMS/data/
- Nature: Metabolite mix
- Processing: This is the raw cdf file generated by the instrument and no further processing was done using Chemstation.

A.1.3 Processing using PyMS

As detailed in the previous chapters, PyMS processes this data by binning the data, smoothing the data, removing the baseline, deconvolute peaks, filtering peaks, setting the mass range, removing uninformative ions and estimating peak areas.

GC-Quad data is made up of approximately 3 scans per second. As a result of this, the values of the following parameters in PyMS are unique for this dataset.

- *points* in Biller Biemann algorithm

  The parameter *points* in the Biller Biemann algorithm defines the width of the window that scans across the ion chromatogram in order to define a peak. The GC-Quad is made up of only a few scans per second and hence the peaks form over only a few number of scans resulting in a lower value defining the window width. In this example, this value is set to 3.

- *scans* in Biller Biemann algorithm

  The parameter *scans* in the Biller Biemann algorithm defines the number of consecutive scans across which a peak can be defined. The near optimal value of this parameter for GC-Quad data that is used in this example is 2.

- *threshold* in peak filtering

  In peak filtering, the parameter *threshold (t)* defines the intensity above which a certain number of ions needs to be represented to be accounted in and not to be filtered out. The near optimal value of this parameter for GC-Quad data that is used in this example is 10000.

A.2 Processing GC-TOF data

[*This example is in pyms-test/A2*]

A.2.1 Instrument

- Maker: Leco Pegasus
- Software: ChromaTOF

A.2.2 Raw Data

- File: MM-10.0_1_no_processing.cdf

- Location: PyMS/data/
- Nature: Metabolite mix
- Processing: This is the raw cdf file generated by the instrument and no further processing was done using ChromaTOF.

A.2.3 Processing using PyMS

PyMS processes this data through the following steps; binning the data, smoothing the data, removing the baseline, deconvolute peaks, filtering peaks, setting the mass range, removing uninformative ions and estimating peak areas.

GC-TOF data is made up of nearly 10 scans per second. As a result of this, the values of the following parameters in PyMS are unique for this dataset.

- *points* in Biller Biemann algorithm

  The GC-TOF is made up of more scans per second and as a result the data is more dense when compared to the GC-Quad data. Therefore the peaks form over more number of scans resulting in a higher value defining the window width. In this example, this value is set to 15.

- *scans* in Biller Biemann algorithm

  Due to the dense nature of the data, a peak can have its maximum value over more than 1 scan. In this example, this value is set to 3.

- *threshold* in peak filtering

  The near optimal value of this parameter for GC-TOF data that is used in this example is 4000.

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