Getting Started with *iMS2Flux*

This document provides a quick getting started guide for the *iMS2Flux* software on a computer running the Mac OS X operating system.

Download the Software:

If you have this file, but not the *iMS2Flux* source code, it can be downloaded from:

`http://sourceforge.net/projects/ims2flux/`*iMS2Flux*`.zip`

iMS2Flux is written in Perl, thus in addition to this software you will need to have a Perl interpreter installed on your computer. Perl is natively supported by OS X, however if it is not installed you can get download links for current Perl binaries or source code from `Perl.org`:

`http://www.perl.org/get.html`

Install Perl before continuing - if not already installed.

Installing *iMS2Flux*:

iMS2Flux together with its example files are zipped up in the single file `iMS2Flux.zip`. It only requires to be unzipped in the desired install directory.

Throughout this document it will be assumed that the software is being installed in the directory `iMS2Flux` located on your desktop (`/Users/`**`user`**`/Desktop/iMS2Flux`).

Running the Example:

The iMS2Flux folder should contain 3 files, including the main program file (`iMS2Flux.pl`), and 4 sub-directories. Two of the sub-directories, `Math` and `FluxY_Lib`, contain perl modules (library files) for use with the program. The third sub-directory, `Getting_Started`, contains this and other instructive documents for getting started with the software. The last sub-directory, `Example_AA`, contains a complete example with mass spectrometry (MS) data measurements for amino acid data (derivatized with TBDMS). Although command line oriented, *iMS2Flux* gets most of its information provided by means of auxiliary files. Besides the MS data file, there is a required configuration file (config.txt) in the example directory, and an optional headers file (which provides names for each experiment). Lastly there is an additional sub-directory, Results, which contains a copy of the results you should get when running *iMS2Flux* with this example.

1. To run the example start by opening the Terminal application, located in the Utilities folder.

2. Next change directories to the folder `iMS2Flux`, and make the program executable:

```
cd Desktop/iMS2Flux/ <enter>

chmod 755 iMS2Flux.pl <ENTER>
```

3. Next change directories to the folder `Example_AA`:

```
cd Example_AA/ <enter>
```

4. Finally, with all of the data and auxiliary files prepared, run the correction program:

```
../iMS2Flux.pl <ENTER>
```

Note: You must run the program from the directory containing the data files, but you don't need to be in the same folder as the program. Instead you can specify the path to where the program is located, as in this example (specified relatively as: ".." which indicates the parent directory, i.e. `iMS2Flux`), or add the program to the OS search path so that it may be directly called from anywhere, see 'Run From Anywhere' below.

Note: You can also run the program by specifying the absolute path to where it is installed:

```
/Users/user/Desktop/iMS2Flux/iMS2Flux.pl <enter>
```

If everything has been installed properly the program should run and finish while providing no output to the terminal. In the `Example_AA` folder there will now be 8 additional files, one each for the different output requested in the configuration file.

The Results:

To get an idea of the basic format, Tab Separated Values (TSV), you may examine the original MS measurement file. For ease of viewing, files of this type may be imported to and exported from spreadsheet software.

To get a better idea about the data itself and what was done to it, look at the configuration file in any text editor. For more information on the configuration file you can use the help command:

```
..\iMS2Flux.pl -h <ENTER>
```

Skimming through the configuration file you can see the data is identified as amino acid data (AA) derivatized using TBDMS. The data file contains 24 sets of chromatogram data, representing 3 experiments with 8, 7 and 9 replicates respectively. Names for the 3 experiments are provided in the file headers.txt. Furthermore, each data fragment has one additional mass measurement at the end of the fragment masses.

No pre-correction data checks are performed, but the average carbon labelling check is performed after the data is corrected for natural abundance.

Finally the following output is generated:

- the raw measurement data (TSV), useful when not already in TSV format,
- the processed data (TSV), in this case corrected and normalized,
- the average of the processed data (TSV), over experimental replicates,
- the standard deviation of the processed data (TSV), over experimental replicates,
- the average carbon labelling (modified TSV), over experimental replicates,
- three measurement files, each containing one set of experimental data formatted to be cut and pasted into the MASS_SPECTROMETRY section of an FTBL file for processing with the 13CFLUX software.

What To Do Now:

Of course you can try changing the options in the configuration file, or data in the MS measurement file and try running the program to see what happens. To get more information on the configuration file you can use the help command:

```
../iMS2Flux.pl -h <ENTER>
```

For detailed information on what the program does and how to use it please see the accompanying manual:

```
iMS2Flux-Manual.pdf
```

For a complete tutorial on using *iMS2Flux* with the automated data extraction tools in QuanLynx see the accompanying PDF document:

```
MSto13C with QuanLynx and iMS2Flux.pdf
```

Run From Anywhere:

To run the program from any directory without having to explicitly specify the path each time, you can either add the install directory to your PATH environment variable, or more simply install the program in a directory that is already included in your PATH, such as your bin directory.

To add the path to your PATH environment variable it is necessary to edit your shell profile. For example, to add the example install directory to the path using the Bash shell then edit the file .bash_profile located in your home directory:

1. Make sure you are in your home directory:

```
cd <ENTER>
```

2. Edit the .bash_profile and add the following line:

```
PATH=${PATH}:/Users/user/Desktop/iMS2Flux
```

3. Save the editted file.