Getting Started with *iMS2Flux*

This document provides a quick getting started guide for the *iMS2Flux* software on a computer running Microsoft Windows (XP, Vista and 7). The software should run on older versions, but the instructions have not been verified.

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 not natively supported by Windows, you can get download links for current Perl binaries or source code from `Perl.org`:

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

iMS2Flux has been tested on Windows (XP, Vista and 7) using both the 32 bit and 64 bit versions of ActiveStates' ActivePerl.

Install Perl before continuing.

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 (`...\`**`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 Windows Command Prompt:

   Windows XP

   ```
   Click Start.
   Click Run.
   Type cmd and press enter.
   ```

   Windows Vista and 7

   ```
   Click Start.
   Type cmd and press enter.
   ```

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

Windows XP

```
cd C:\Documents and Settings\user\Desktop\iMS2Flux\Example_AA\ <enter>
```

Windows Vista and 7

```
cd Desktop\iMS2Flux\Example_AA\ <enter>
```

3. 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:

Windows XP

```
C:\Documents and Settings\user\Desktop\iMS2Flux\iMS2Flux.pl <enter>
```

Windows Vista and 7

```
C:\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 specify the path

1. Open the environment variable settings:

Windows XP

```
Right-click My Computer, and then click Properties.
Click the Advanced tab.
```

Windows Vista and 7

```
Click on the Start menu.
Right-click Computer, and then click Properties.
Click the Advanced system settings.
```

2. Edit the environment variables:

Continue for all Windows versions

```
Click Environment variables.
Select the Environment variable PATH (in the User variables), and then click Edit.
At the end of the Variable add the path to the main program, in the above example you would add:
      ;C:\Documents and Settings\user\Desktop\iMS2Flux
Click OK on both windows to close and save.
```

Note: Each different path specified by the environment variable is separated from the others by the semi-colon, “;”, thus the leading semi-colon in the path addition is to separate this new path from the last path in the list.