Sherlock PLFA Tools Users' Guide version 1.1 May 2013

A Summary of Use

The following document allows you to get started using the Sherlock PLFA Tools. The features of the PLFA tools are:

- Adjusting for the molarity of different fatty acid compounds
- Scaling by a known amount of internal standard
- Categorizing results based on Fatty Acid types (e. g. iso/anteiso, omega)
- Categorizing results based on microbial types (e. g. gram positive bacteria, fungi)

Before starting, make sure the tools are installed in c:\Sherlock\Exe\PLFA. The tools should include the default text files used to create methods and to weight fatty acids.

Also, make sure that the c:\Sherlock\Exe\License.txt file has a license for the tools. (The last part of the PLFA Tools license line should read XFORM=1.)

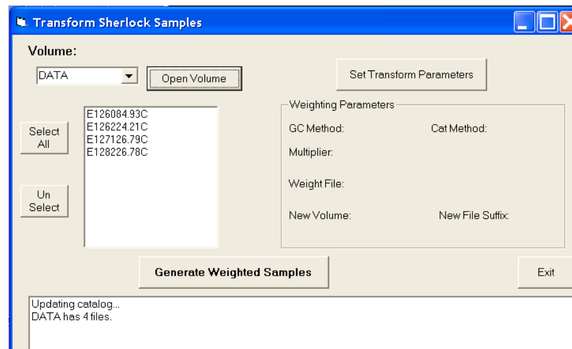
Adjusting for Molarity Differences

The program ***TransformSamps*** takes a Sherlock data file and creates a new data file based on a variety of criteria. In this simplest use, adjusting for molarity differences is achieved by taking the named compounds in each sample and adjusting their amounts, taking into account the molarity differences between compounds. This normalization yields peaks that are in correct molarity percentages. To normalize for molarity, the program reads a text file that correlates the compounds to weight information. Note that these are essentially weight **inverses**, because to get molar percentages we need to **divide** by the molecular weight, while *TransformSamps* is designed to multiply each named compound by the amount in the table.

```
PLFAMole.txt:
Peak Name        Scaled Weight
9:0              1.814
10:0             1.678
11:0 iso         1.560
11:0 anteiso     1.560
11:0             1.560
…
19:0 cyclo w6c   1.013
19:0             1.000
…
24:1 w7c         0.821
24:1 w3c         0.821
24:0             0.817
```

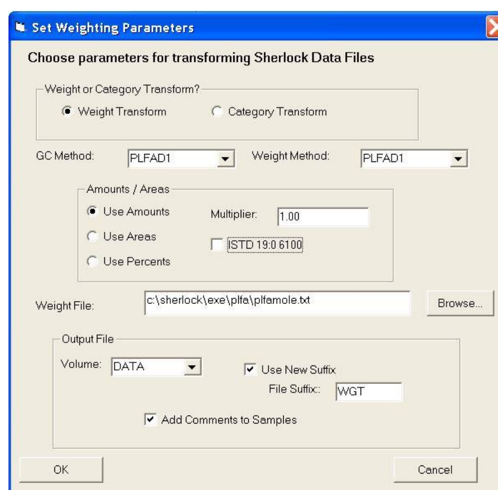
The default file, PLFAMole.txt, uses a multiplier of 1.000 for 19:0; multipliers for compounds of less weight (and thus higher molarity) are greater than 1.000, of greater weight are less than 1.000. Note also that one may choose to use Sherlock Areas or Sherlock Amounts. The difference is that the

Sherlock Amounts have been corrected based on the response factor calculated for the FID. One should use Areas only if one applying a different weight file that takes into account both molecular weight and also FID selectivity.



The procedure is as follows. After starting *TransformSamps,* select the data volume (directory) in which the file resides and press the *Open Volume* button.

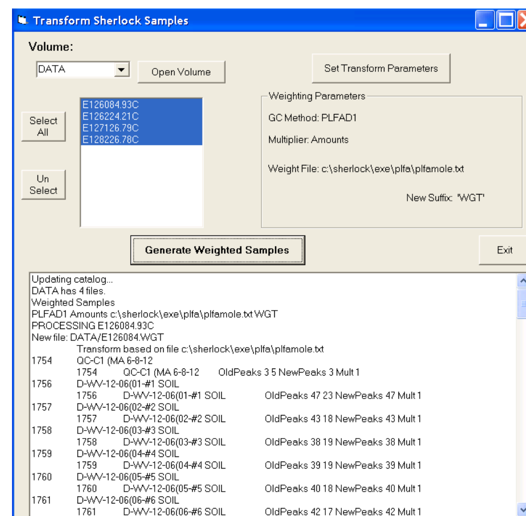
Next, select the *Set Transformation Parameters* button. The default setting is for a *Weight Transform.* Select the GC Method that was used to run the samples (typically, PLFAD1). For a simple transform, leave the Weight Method the same as the GC Method. See below for advanced features including Multiplier and ISTD. (Note that if an internal standard has been defined for the method, it will be selected automatically. If one does not wish to scale to an internal standard, simply unclick the ISTD checkbox.) Make sure the Weight file points to the correct file, in this case the PLFAMole.txt file.



The new data file can be placed in the current directory but then it must have a different suffix. (There cannot be two files with the same name in the same directory.) Alternatively, one can select a different volume, in which case the file's suffix can be maintained.

Pressing OK takes returns to the main screen and shows the selections made in the box to the right of the data files.

Select one or more data files to be transformed. (*Select All* will select all files.) Press the *Generate Weighted Samples* button and the file will be transformed, with the results listed in the box below.



The transform also creates a log file TransformSampsLog.txt in c:/Sherlock/Exe/PLFA which lists the results of each transform.

In Sherlock's CommandCenter, there will now be a .WGT file associated with each file that was transformed. One way to tell that it has done what was expected is to look at the first and last named peak. The first peak, being one of the lightest compounds, will have the most moles given its response. So the effect of the transform will be that its percentage is slightly higher in the .WGT file than in the original file. Equivalently, the last peak, being among the heaviest compounds, will have a slightly lower percentage. This proves that the technique was successful.

To see detailed information concerning the changes made to each sample, inspect the Comments section of the sample. This section will describe the specific transformation(s) applied to the sample.

Because the result of running *TransformSamps* is a new Sherlock data file, all of the Sherlock tools may be used on the new file. One can reprint reports, compare samples to each other, or compare before and after transformation on the same sample. Dendrograms, two-dimensional plots, library generation, and data export to spreadsheet or database are all available options.

Scaling to an Internal Standard

The *TransformSamps* program can apply Internal Standard calculations if specified. To use an Internal Standard, the name of the compound used and its amount must be defined in the Weight Method. If the *TransformSamps* program finds an ***ISTDNAME*** and ***ISTDAMT*** key in the [Method] section of the method's .INI file, it will use these values to set the ISTD peak to that amount. This file is in c:\Sherlock\Sysfiles\Methods. To set the Internal Standard for PLFAD1, for example, one would edit PLFAD1.INI in that directory. Under the line…

```
[Method]
```

…adding these two lines…

```
ISTDNAME=19:0
ISTDAMT=6100
```

…will cause the program to scale the data so that 19:0 compound is set to a total amount of 6,100. If the 19:0 was known to have a molarity of 6,100 picomoles, then all of the numbers for all of the compounds will be picomoles.

(Note: Sherlock is most comfortable with numbers that are in thousands. Thus, though one could set the value to 6.1 to make numbers in nanomoles, this approach is not advised.)

To create a data file scaled for molarity, the exact same procedure is used as in the last section. Because the method now has a defined internal standard, the ISTD information will be listed when *Set Transform Parameters* is pressed and the PLFAD1 method is selected. A different file suffix can be chosen, perhaps .MOL for molarity, but the same scaling file (PLFAMole.txt) can still be applied.

The result of this scaling is that the values will be in moles (picomoles in our example) and can be read directly off the Sherlock Profile. Thus if the 15:0 ISO peak is listed as 18,700 then it is 18,700 picomoles (18.7 nanomoles) if the ISTD is given in picomoles.

Different internal standard peaks and amounts can be specified in different Weight Methods. The *Set Weighing Parameters* dialog box will show the selection for that Weight Method.

Advanced Features

Calculating Weights

Typically one desires the number of moles per gram of soil. If 1 gram of soil is used, no changes need be made. If a different weight is used for an entire set of data, the *Multiplier* in the Set Transform Parameters dialog can be set. Note that one would use the inverse of the weight of the soil. If one uses 2 grams of soil, set the *Multiplier* to 0.5.

If different weights are used for different samples, the system can take those weights into account. Simply put the weight in the sample's Sample Id in the form: "G=weight". Thus if a sample was weighed out as 3.7 grams of soil (after drying), the Sample Id may look like:

SOIL-PLOT4-SAMPLE16(G=3.7

The software will automatically divide the Fatty Acid responses by the number listed to correct for soil weights.

Alternative Weight Methods

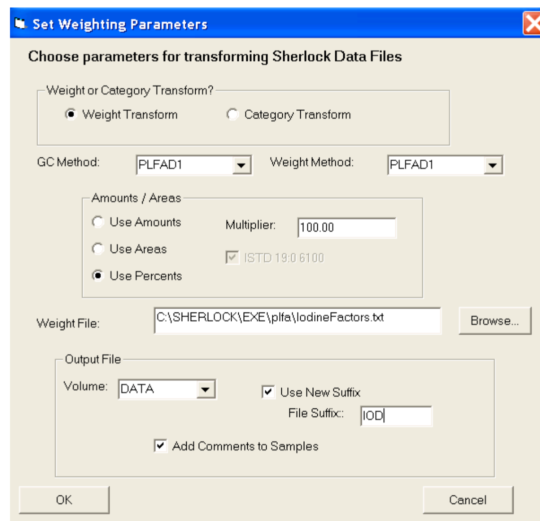
For the most part, the Weight Method will either be identical to the GC Method or vary only in the Internal Standard specified. One may, however, use a different Weight Method (as long as its calibration peaks are the same). For example, the PLFAD1 method zeros out the 19:0 peak because it is commonly used as the internal standard. One can create a method that is identical to PLFAD1 but treats the 19:0 peak as an individual fatty acid peak, giving it an amount and a percentage. This method could be used as the Weight Method. The new file generated will have method PLFA19 for each sample.

Calculation of Iodine Value

The Iodine Value for a sample is an indicator of the unsaturation of that sample. AOCS method Cd 1c-85 describes the mechanics of manually calculating the value given the fatty acid composition of a sample. Using the *TransformSamps* allows automation of this tedious calculation.

The Iodine Value calculation multiplies the **percentage** of each fatty acid by a factor that combines both the molecular weight and the number of double bonds present in that fatty acid. For example, the value for 18:1 fatty acids is 0.8599, while 18:2 fatty acids have value 1.7315 and 18:3 fatty acids value 2.6151. The full table of fatty acid factors is stored in the file IodineFactors.txt.

To transform a sample so that the iodine value is calculated, use *TransformSamps* selecting the following parameters: under Amounts/Areas select *Use Percents*; for Multiplier, select 100.00, and for Weight File choose the IodineFactors.txt file. A File Suffix such as .IOD seems reasonable as well. (The advantage of using a multiplier of 100 is that Sherlock tends to display responses with decimal digits. So a value of 1.22 would typically be displayed as “1”. With a multiplier of 100, two digits are displayed and the value will be “122”.)



The effect of using this transform is that each peak’s response will be replaced with its portion of the Iodine Value, and the Total Response will yield the Iodine Value itself (multiplied by 100). In a simple example (of olive oil) the results were:

Response	Peak Name	Percent
11	16:1 w9c	0.13
99	16:1 w7c	1.19
23	17:1 w8c	0.27
1318	18:2 w6c	15.88
6580	18:1 w9c	79.23
250	18:1 w7c	3.02
24	20:1 w9c	0.28

The Total Response listed for this sample was “8295”, reflecting and Iodine Value of 82.95. The typical range for olive oil is 80 – 88.

Categorizing Samples

The other primary capability of the Sherlock PLFA tools is to categorize compounds in samples by their types. One can categorize by Fatty Acid types or by Microbial types. (Advanced capabilities allow for creating complex categorization functions.) Categories for Fatty Acid types would include Straight, Branched, Hydroxy and so on. Categories for Microbial types would include Gram Positive Bacteria, Gram Negative Bacteria, Fungi, etc.

The software comes with default files for these two categorizations. The file **PLFAD1FA.txt** categorizes by fatty acid type:

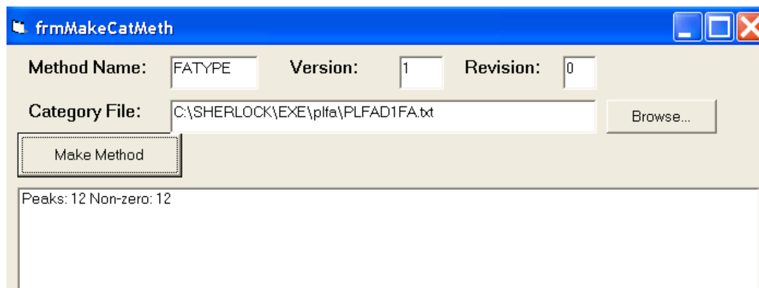
```
Category        Index  Multiplier    Peaks
…
Straight        1      1       10:0   11:0   12:0   13:0   14:0   15:0   16:0   17:0
                       18:0    20:0   21:0   22:0   23:0   24:0
…
Branched        1      1       11:0 iso      11:0 anteiso 12:0 iso      12:0 anteiso
                       13:0 iso      13:0 anteiso 14:1 iso w7c 14:0 iso
…
MUFA    1       1      12:1 w8c      12:1 w4c      13:1 w5c      13:1 w4c
                       13:1 w3c      14:1 w9c      14:1 w8c      14:1 w7c
                       14:1 w5c      15:1 w9c      15:1 w8c      15:1 w7c
…
```

The file **PLFAD1SoilMic.txt** categorizes by microbes found in soil:

```
Category        Index  Multiplier    Peaks
…
AM Fungi        1      1       16:1 w5c
Gram Negative 1        1       10:0 2OH      10:0 3OH      12:1 w8c      12:1 w4c
                       13:1 w5c      13:1 w4c      13:1 w3c      12:0 2OH
                       14:1 w9c      14:1 w8c      14:1 w7c      14:1 w5c
…
Methanobacter 1        1       16:1 w8c
Eukaryote       1      1       15:4 w3c      15:3 w3c      16:4 w3c      16:3 w6c
                       18:3 w6c      19:4 w6c      19:3 w6c      19:3 w3c
…
Fungi   1       1      18:2 w6c
…
```

In order to use the categorization capability of the Sherlock PLFA tools, one must first create Sherlock methods that contain the list of categories. The tool ***MakeCatMeth*** is used to make methods for categorizing Sherlock samples.

A method name, no more than 8 characters, needs to be given. The description file must also be selected. To create a method for Fatty Acid types the method name FATYPE seems adequate. Type that into the *Method Name* text box on the screen. The Fatty Acid file as shown above is named **PLFAD1FA.txt** resides in c:/Sherlock/exe/PLFA. Use the *Browse* button to select that file. With the method named FATYPE and the PLFAD1FA.txt file selected, pressing *Make Method* will create this method.

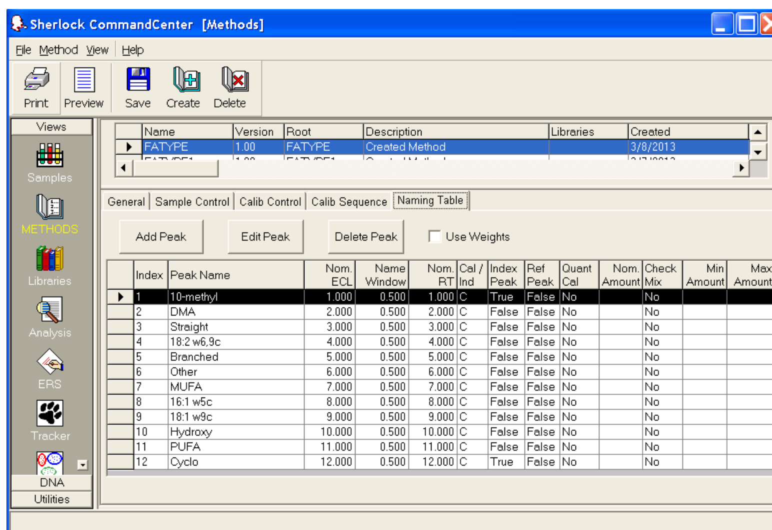


The program lists the number of peaks (features) in the created method.

This process can be repeated for the microbial types, selecting a different method name such as MICTYPE and the **PLFAD1SoilMic.txt** file. A log of transformations is kept in the TransformSampsLog.txt in c:/Sherlock/exe/PLFA.

Check the methods by running CommandCenter and going to Method's mode. The peak naming tables will reflect Fatty Acid types for the FATYPE method and microbial types for the MICTYPE method.

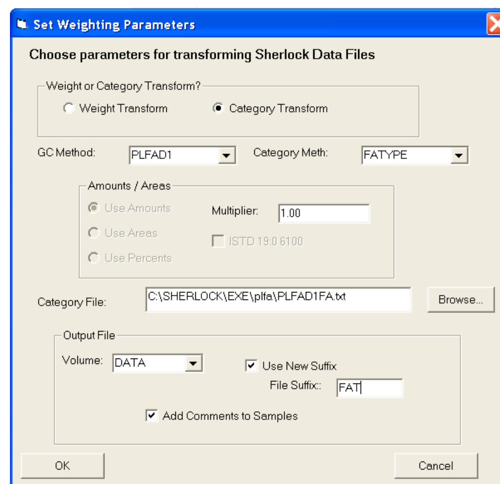
Note that this process need to be done only once. These methods may now be used for all future categorizations. Of course if one wishes to change categories, one simply changes the text file (or, better, copies the text file and then makes the change) and creates a new method with the categories of interest. One can also use CommandCenter's Method mode to make changes to the method.



TransformSamps for Categorization

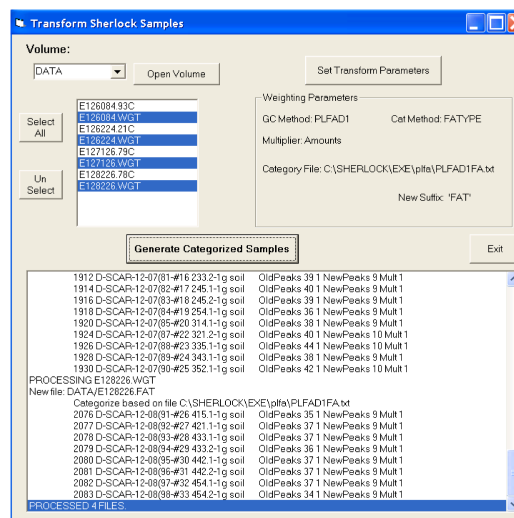
Just as *TransformSamps* can make a new Sherlock data file based on molarity, it can also be used to create a new data file based on a categorization method. The text file which describes the peaks are summed into categories acts as a roadmap to transforming samples.

Start *TransformSamps* and select the data, just as for the Weight transform. Then press the *Set Transformation Parameters* button.



At the top of the dialog box, select the radio button for *Category Transform* instead of the *Weight Transform* default that was previously used. The dialog box will change to include a second method. For *GC Method* select the original method used (typically PLFAD1). For *Category Method* select either FATYPE or MICTYPE (or other name of a categorization method that you have created).

Next, choose the *Category File* that describes the mapping from PLFAD1 peaks to categories. The file PLFAD1FA.txt has these mappings for FATYPE. An appropriate File Suffix can be set, such as FAT for bacterial categorization. Alternatively, one can select a new volume to place the transformed files into.

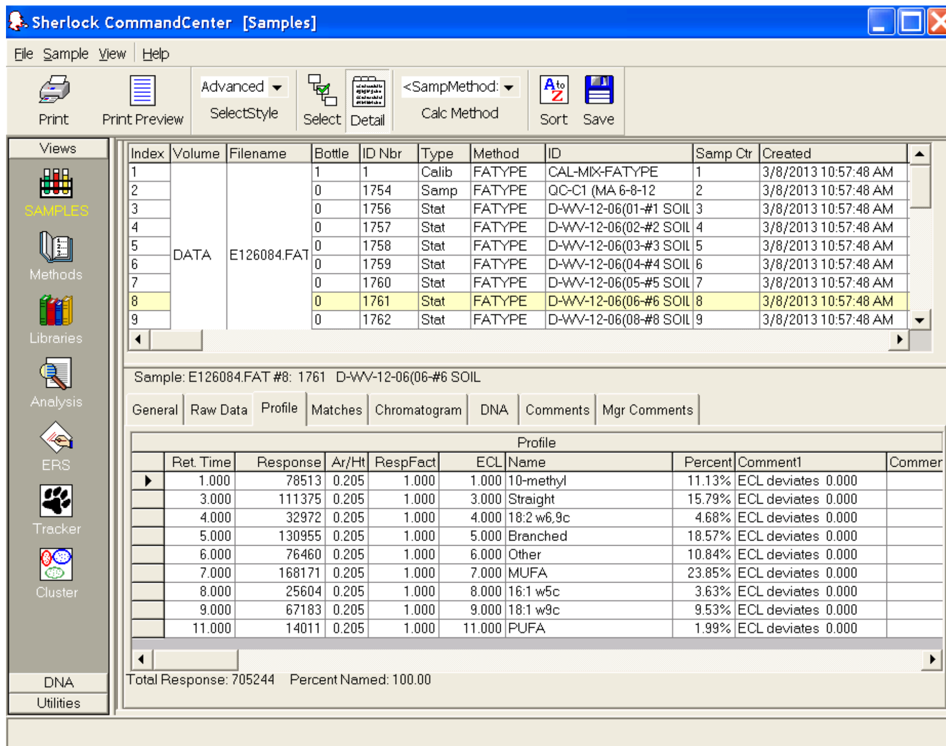


By pressing OK to exit this dialog box, the main execution button for *TransformSamps* will now read '*Generate Categorized Samples*'. Select one or more files and create the categorization file(s) by pressing the execution button. Other files can be transformed by selecting each in turn. Note in particular that the input to the categorized transform can be the weighted files instead of the original files. In this manner the categories will contain correct molarities for the fatty acids in each group.

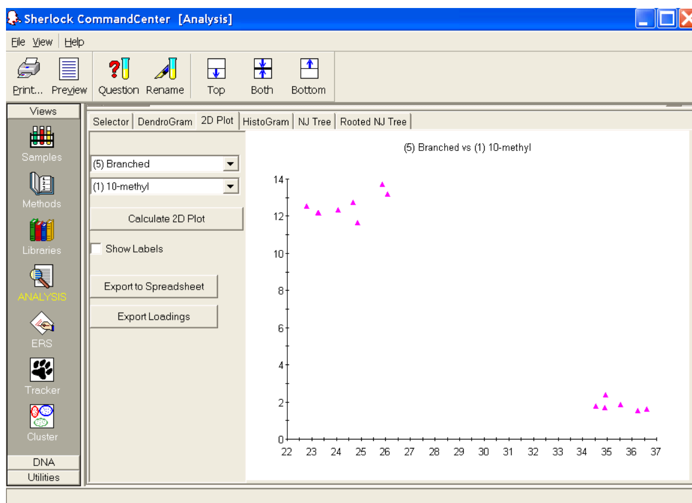
To create the Microbial categorization, run the program again, using Cat Method MICTYPE, and a file suffix such as MIC, and mapping file PLFAD1SoilMic.txt.

In Sherlock's sample mode, there may now be four copies of each file: the original, the weight transform, the .MIC with microbial categorization, and the .FAT with Fatty Acid categorization. (Note that it is handy to sort by either Sample ID or ID Number to bring the three samples together.)

Shown below is the fatty acid categorization for a soil sample. Because a weight transform was performed, these percentages are correct molarity relationships.



As an example of how categorization can give insights into data, the two-dimensional plot below is based on the FATYPE results and shows two groups of samples separated by the amount of branched chain fatty acids (typically related to gram positive bacteria) and 10-methyl fatty acids (related to actinomycetes).



Advanced Categorization Techniques

The categorization techniques are not limited to summing specific fatty acids to make a category.

One option is to cause a category to be summed but not included in the percentages. Straight chain fatty acids, for example, occur in so many different microbes that they are not particularly indicative of any particular microbial type. The category input file has a column labeled “Index”. If the Index for a category is set to zero, then that category will be summed but its result will not be included in the percentages.

A related option is to create a sum of categories. For example, one might like to break out AM Fungi from Fungi and get totals for each separately. (AM Fungi are recognized by 16:1 w5c, while other Fungi by 18:2 w6,9c.) But in the final reckoning it might be helpful to sum these together. By giving AM Fungi and Fungi the same negative Index (-1), they will be listed separately but then summed together as summed feature 1.

The categorization file also contains an extra column titled *Multiplier*. By entering a number other than 1 in this column, that particular feature will be multiplied by its multiplier before reporting the result. This capability is particular useful with complex functions, described next.

Complex Functions

The categorization method is not limited to simple sums of fatty acids. In fact, it can calculate the following formula:

$$Multiplier \times \frac{\sum NumeratorPeaks - \sum NumSubPeaks}{\sum DenominatorPeaks - \sum DenSubPeaks}$$

A set of peaks can be added together in the numerator and a set of peaks subtracted from them. In the denominator a similar approach can be used. The net result is a complex function of the original fatty acid peaks.

The method for defining the equation is quite simple. All peaks up until a slash (“/”) is found are considered to be in the numerator; all peaks after the “/” are in the denominator. Any peak whose name is preceded by a minus sign (“-“) is subtracted from either the numerator or denominator.

Some examples might clarify:

Name	Ind	Mult	Peaks					
FungiMin	1	2	18:2 w6c	-16:1 w5c				
15/17	0	100	15:0 iso	15:0 anteiso	/	17:0 iso	17:0 anteiso	
OmegaCyclo	0	100	18:1 w9c	18:1 w8c	18:1 w7c	18:1 w6c		
			18:1 w5c	18:1 w3c	/	17:0 cyclo w7c	19:0 cyclo w9c	
			19:0 cyclo w7c	19:0 cyclo w6c				

The feature *FungiMin* is the 18:2w6c peak minus the 16:1 w5c, multiplied by 2.
The feature 15/17 is the ratio of 15:0 iso and anteiso to 17:0 iso and anteiso, with the result multiplied by 100.
The feature OmegaCyclo is the ration of some 18:1 w peaks divided by some cyclo omega peaks.

Note that the ratio features are multiplied by 100. Sherlock software generally assumes that features will be greater than one, so results are generated with that in mind. Note also that ratio features are zeroed so that they will not be included in the percentages. This allows adding ratio calculations to a standard category file (like PLFAD1FA.txt) without affecting the percentages of the non-ratio categories.

PLFA Tools Summary

The PLFA Tools are a powerful mechanism for transforming Sherlock fatty acid data. By returning the results as a Sherlock data file, all of the other tools available in the Sherlock program are available, including Data Export.

These tools are a great time saver for the PLFA researcher. The open format of input files allow the researcher to experiment with a variety of calculations based on their own data file.

For More Information

For more information about these tools, please contact MIDI, Inc. technical support:
(302) 737-4297
support@midi-inc.com