fitMAN Suite
Version 1.5

User Manual

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Table of Contents

Chapter 1

fitMAN Installation Instructions

1. fitMAN runs using the IDL Virtual Machine, which is freely available for download for LINUX, UNIX, and Mac OSX operating systems. The download is found at the following web site:

   www.rsinc.com/idlvm

   On the left of the screen there is a link to download the IDL Virtual Machine.

   Please complete the installation of the IDL Virtual Machine prior to installation of the fitMAN software.

2. fitMAN comes archived as a tar file. Copy the file to the appropriate directory and type:

   tar –xvf fitMAN.tar

3. Four directories are extracted:
   - fitMAN_v1.0
   - Unix
   - Linux
   - MacOSX

The fitMAN_v1.0 directory contains all the files necessary to run the GUI. You can put this anywhere you want on a particular system.

To run the fitMAN program, change directory to fitMAN_v1.0 directory from a terminal window and type: idl -vm

The IDL virtual machine should start and request a file. Select the file called fitMAN_Suite1.5.sav

An Acknowledgement screen appears followed by the GUI displaying three horizontal panes along with some other options on the left side of the screen.

The other directories contain Operating System (OS) dependent files. Each directory has a file called 4t_cv, ge2fitman, and ultra_fitman. These files should be copied to the fitMAN_v1.0/bin directory as required for each OS.

Please email any questions to:

FitMAN_help@imaging.robarts.ca

Chapter 2

Practical Short Echo-Time ^{1}H MR Spectroscopy: A Method of Data Acquisition and Analysis

This Chapter contains a guide to:

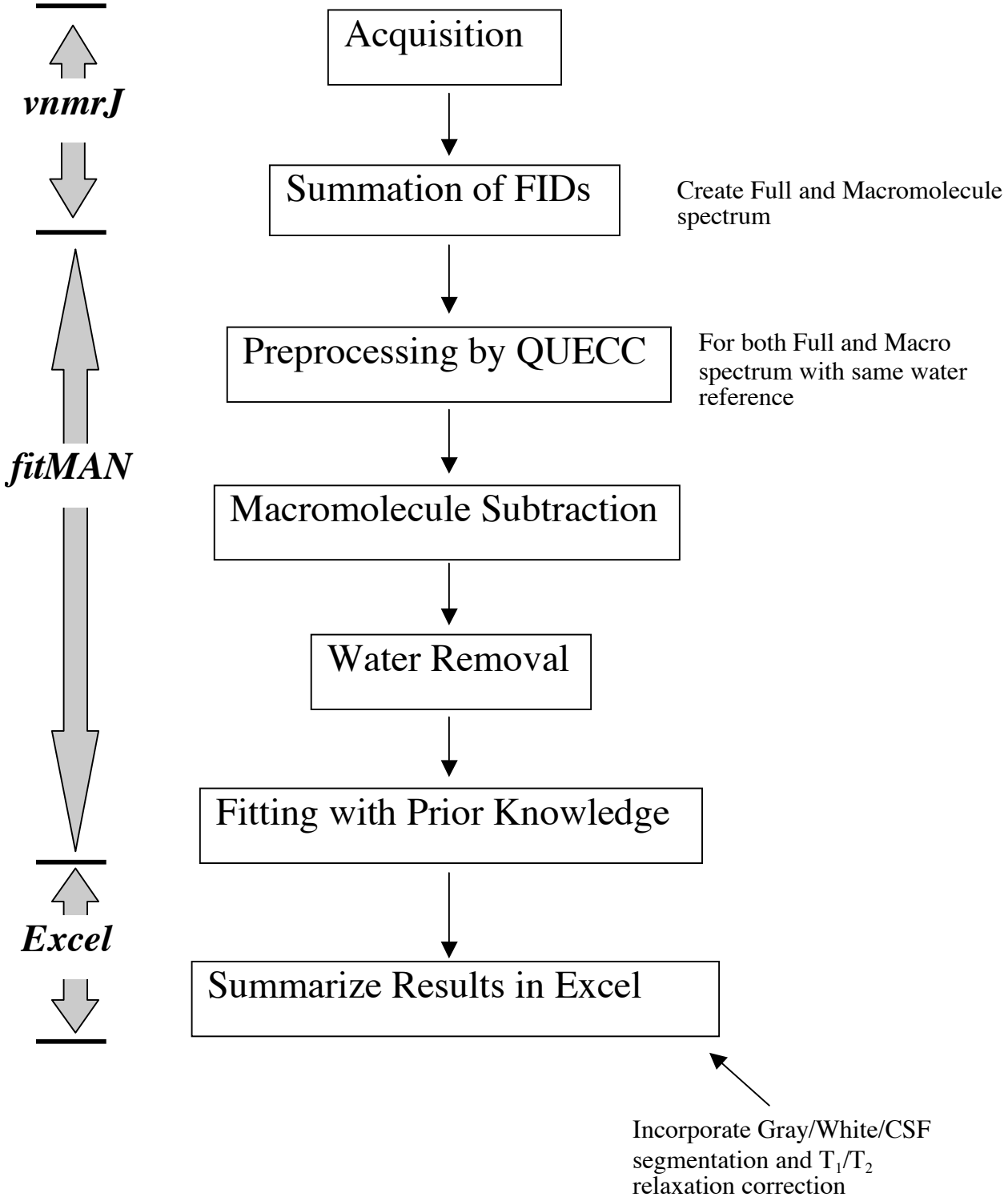
1) Acquiring LASER (1) localized short echo-time MRS data on the 4T Varian MRI system.
2) Reconstruction of full spectra and metabolite suppressed macromolecule spectra
3) Post-processing of MR spectra to remove eddy current artifacts and restore a Lorentzian lineshape.
4) Subtraction of macromolecule resonances.
5) Removal of residual water.
6) Fitting of MR spectra using fitMAN software (including fitting of water suppressed and unsuppressed data).
7) Consolidation of results in Microsoft Excel.
8) Prior-knowledge acquisition and development of new prior knowledge templates.

At the completion of this tutorial, participants should be able to perform all steps necessary to acquire, process, and fit short (and long) echo-time 1H MR spectroscopy data.

Additional questions or comments can be sent to:

FitMAN_help@imaging.robarts.ca

Overview of Acquisition and Fitting



Summation of FIDs

Background: In-vivo short echo-time 1H MRS spectra of the brain have a contain information relating to large macromolecule and lipid resonances in addition to the usual metabolites of interest. The implementation of the LASER sequence in combination with metabolite suppression pulses can be used to null the metabolite signals and acquire a macromolecule spectrum. To reduce the effects of patient motion during scanning, single averages of the Full spectrum and metabolite suppressed Macromolecule spectrum are interleaved. An additional benefit of saving each average is that if a patient can not tolerate the entire length of the scan, the acquisition can be halted and data can be reconstructed up to the point of termination. Following acquisition, these the single averages must be summed for each spectrum prior to post-processing.

Implementation: (ensure that spec_sum and spec_sum_incomplete are in the system maclib or your own vnmrsys/maclib)

In vnmrJ, there are two macros that are used to sum individual FIDs.

spec_sum
spec_sum_incomplete

spec_sum:

1. Load data into an experiment other than Experiment 5
2. Ensure that there is nothing in Experiment 5 that can not be erased as all information in Experiment 5 will be deleted during summation
3. Join to Experiment that has spectroscopy data
4. Type: ***wft***
5. Type: ***spec_sum***
    a. Enter which spectrum to reconstruct (1 = Full, 2 = Macromolecule)
    b. Enter 1 for shift adjustment or 0 for none: specify 0 only as shift adjustment is not implemented.

Spectra are now summed and the result is saved in Experiment 5

6. Join to Experiment 5.
7. Save resultant spectrum with a new file name.

Spec_sum_incomplete:

This macro should be used when data acquisition is halted prior to full completion

1. Repeat steps 1-4 from above – following ***wft***, note the number of spectra acquired.
2. Type: spec_sum_incomplete
    a. Enter which spectrum to reconstruct (1 = Full, 2 = Macromolecule)
    b. Enter 1 for shift adjustment or 0 for none: specify 0 only as shift adjustment is not implemented.
    c. Enter the total number of elements *actually* acquired from step 1
3. Repeat steps 6 and 7 from above

Preprocessing Lineshape Correction

Background: MR spectra must be converted from vnmr (vnmrJ) format to fitMAN format. In addition MR spectra normally contain lineshape distortions due to eddy currents, gradient coil vibration, and B0 inhomogeneity, that distort the pure Lorentzian lineshape that is normally expected in NMR. Since the fitting procedure used for spectral quantification incorporates a Lorentzian lineshape, it is best to restore the Lorentzian lineshape of the spectrum prior to quantification.

Implementation:
Conversion of 4T vnmrJ format spectra to fitMAN format is accomplished by a c++ program called 4t_cv. This program can be executed from the command line or through the fitMANSuite program.

Command line, go to the directory where fitMAN is installed and into the /bin directory. Type ***4t_cv*** to see the usage options.

```
Usage: 4t_cv in_file out_file
<-scale> <-scaleby scale_factor> <-bc> <-zf #_points>

<-ecc> reference_file
<-scale> <-scaleby scale_factor> <-bc> <-zf #_points>

<-quality> delay_time(us) reference_file
<-norm> <-scale> <-scaleby scale_factor> <-bc> <-zf #_points> <-f FWHM>

<-quecc> QUALITY_points(real+imag) delay_time(us) reference_file
<-norm> <-scale> <-scaleby scale_factor> <-bc> <-zf #_points> <-(i)f
FWHM>
Note: <-if> must be specified for suppressed and unsuppressed file
separately

WARNING: Filtering applied with negative delay time assumes data is an
echo.
```

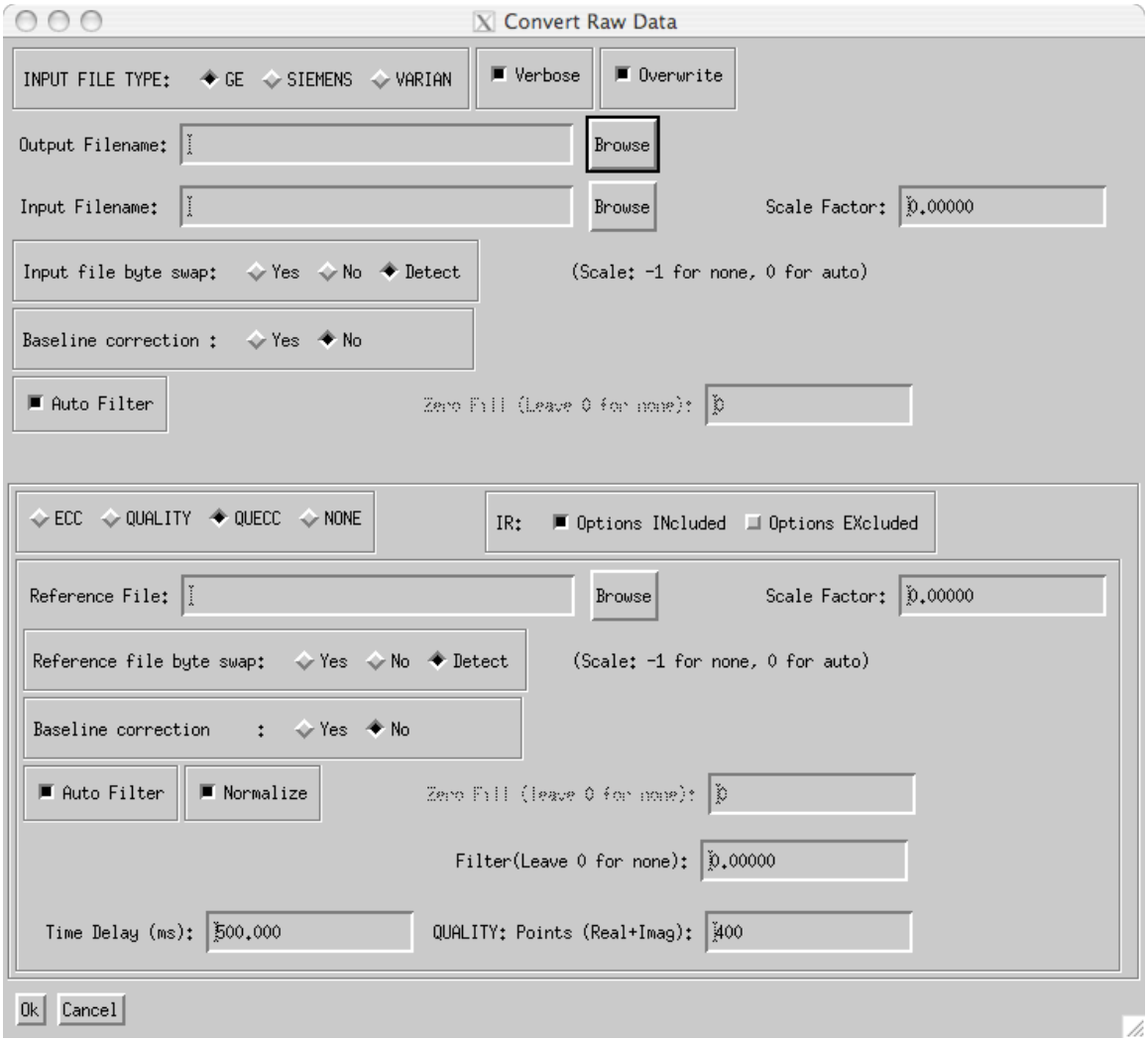
To convert the vnmrJ spectrum to fitMAN format and complete lineshape correction with QUECC, (2) type something similar to the following:

4t_cv full_spectrum_filename.fid converted_filename –scale –bc –if –quecc 400 500 full_water_reference_filename.fid –norm –scale –bc –if

fitMANSuite

To start fitMANSuite, go to the directory where that contains the latest version of the fitMANSuite.sav file (i.e. fitMAN_v1) and type: idl –vm

Select the latest fitMANSuitex.x.sav file
Select: File → Convert Raw Data



1) Select output file name
2) Select Input file (click into directory if browsing). For Varian, select directory (*.fid)
3) Select: Use Baseline Correction, Auto Filter, QUECC
4) Select water reference file. For Varian, select directory (*.fid)
5) Select: Use Baseline Correction, Normalize, Auto filter
6) Enter Delay time and number of points at the beginning of FID to perform QUALITY deconvolution

Output:

In both cases, either command line implementation, or fitMANSuite implementation, two files will be generated:

Converted_filename_s.dat → contains the water suppressed spectrum
Converted_filename_uns.dat → contains the water unsuppressed spectrum

Macromolecule Removal

Background: Macromolecule resonances are broad lines that overlap with the sharper metabolite resonances of interest. During acquisition, both a full spectrum containing all metabolites and macromolecules, as well as a macromolecule spectrum is acquired. The macromolecule spectrum must be subtracted from the full spectrum prior to quantification of metabolite signals.

Implementation: in fitMANSuite

Load Full spectrum into top window (right click on top window to activate)
File→ Data→ and choose the file

Load Macro spectrum into middle window (right click on middle window to activate)
File→ Data→ and choose the file

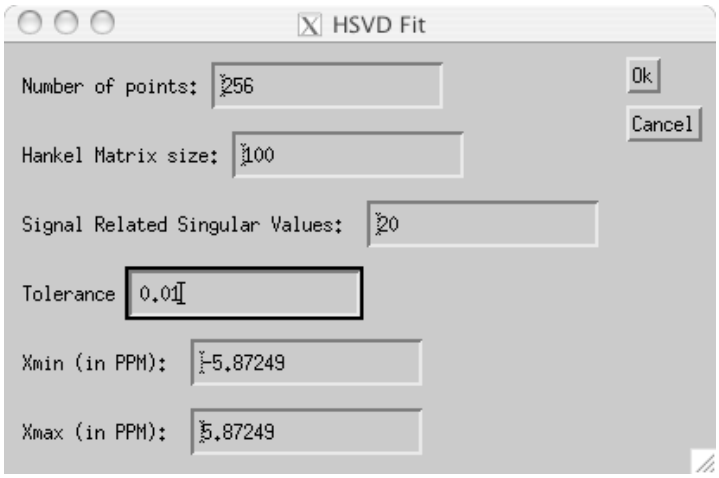
Direct Subtraction
Arithmetic → Subtract: Manual/No Scale → Specify the correct scaling value
(Scale factor for Varian 4T acquisition is 1.2)

The resultant spectrum appears in the bottom window. Save the data:
Activate the third window by right clicking
File → Save Active Window

(The macromolecule spectrum may be scaled prior to subtraction to account for the differences in T1 saturation if different repetition times are used to acquire the Full and Macromolecule spectra. At 4T, with TR = 4.2 for Macro and TR = 2.2 for Full acquisition, the scaling factor should be 1.2)

**Advanced Option:
Prior to subtracting the macromolecule spectrum, it can be fit by SVD. The result can then be subtracted from the Full spectrum. The advantage of this method is that there is no additional random noise added into the resultant spectrum. However, there is uncertainty associated with the SVD fit, resulting in no net benefit to metabolite quantification precision in the final result.

SVD Fit
Arithmetic → HLSVD Fit



Number of Points: The number of initial points along the FID to use in forming the Hankel Matrix
Hankel Matrix size: The number of columns of the Hankel Matrix
Signal Related Singular Values: The number of Lorentzian peaks to fit
Tolerance: The number of peaks to retain based on the value of the singular values – the specified value represents a fraction of the largest singular value. In this example, either 20 peaks are retained, fewer peaks if there are less that have singular values > 1% of the maximum singular value.
Xmin, Xmax: the range of frequencies to use for data reconstruction

Water Removal

Background: Following macromolecule removal a residual water peak is often still present. Since fitting occurs in the time domain, signal representing each spectral component is present throughout the time domain signal. Therefore it is necessary to remove the residual water signal prior to metabolite fitting.

Implementation: in fitMANSuite

Water removal is done using a similar method to that described above for advanced macromolecule removal.

After macromolecule removal, load the spectrum into the middle window in fitMANSuite

Use HSVD to fit the data – any peaks existing within the region of the spectrum occupied by water can then be removed:

File → Remove Water
Specify parameters as required.

Following water removal save the data in the middle window:
File → Save Active Window

Fitting Spectra

<u>Background</u>: Following water removal, the spectrum is now ready for fitting. Fitting is accomplished incorporating prior knowledge of metabolite lineshapes. Nineteen metabolite lineshapes are currently included in the in-vivo brain prior-knowledge template.

<u>Implementation</u>: Fitting can be done on the command line or within fitMANSuite.

<u>Command line</u>, go to the directory where fitMAN is installed and into the /bin directory. Type ***ultra_fitman*** to see the usage options. Further detailed information on the ultra_fitman program is contained in a separate document called fitman_help_v1.pdf

```
Fitman Compiled: Jul 14 2004 22:52:07
Copyright    John J. Potwarka and Robert Bartha
usage: fitman <data file> <guess file> <constraints file> <guess out>
[parameters]
       Date: Jul 14 2004

  Parameters
  ----------
-b                      - input data file is NMR286 binary format
-t                      - input data file is NMR286 text format
-fr                     - perform fit in frequency domain
-ti                     - perform fit in time domain
-t1                     - perform fit of T1 data
-t2                     - perform fit of T2 data
-z <n>                  - zero fill to point <n> (Frequency fitting
only)
-ew <n>                 - apply <n> Hz FWHM exponential weighting
-gw <n>                 - apply <n> Hz FWHM Gaussian weighting
-v <label> <n>          - set variable
-vf <filename>          - read variable file
-fp <parameter> [args]   - set or override fitting parameter
-c <filename>           - output file for covariance matrix
```

To fit the spectrum type something similar to the following:

ultra_fitman full_spectrum_filename.dat Human_vivo.ges Human_vivo.cst Outputfile.out

Human_vivo.ges – a file that contains all the initial parameter value estimates
Human_vivo.cst – a file that contains all the linking information for each metabolite

Both files can be found in the fitMAN_DOCUMENTATION folder.

fitMANSuite

If using fitMANSuite to fit data, load data into middle window.

File → Data → Choose file

Load constraints file:
File → Constraints → Choose file

Load guess file:
File → Guess → Choose file

Middle window will now display three lines:

1) Original data spectrum (yellow)
2) The initial guess spectrum (red)
3) The residual (blue)

Prior to fitting we must ensure that the initial parameter value estimates are "close" to the data. This is particularly important for the shift parameter. Amplitude, and width parameters are significantly more robust.

To alter the initial parameter estimates, we must open the guess file in a text editor.

The top of the guess file looks like:

```
****_Guess_File_Begins_****  <--- Do not remove this line

[Parameters]
number_peaks     228
shift_units      ppm

[Variables]
amp    0.3
pos    0.01
wl     -1
d     0.0012
pha     3.2
posCho    0.56
```

The variables section contains all the parameters that may need to be adjusted prior to fitting:
amp – scales the amplitude of all metabolites
pos – shifts the frequency of all metabolites
wl – changes the width of all metabolites
d – changes the delay time of all metabolites
pha – changes the phase of all metabolites
posCho – is used to move the position of choline relative to other metabolites

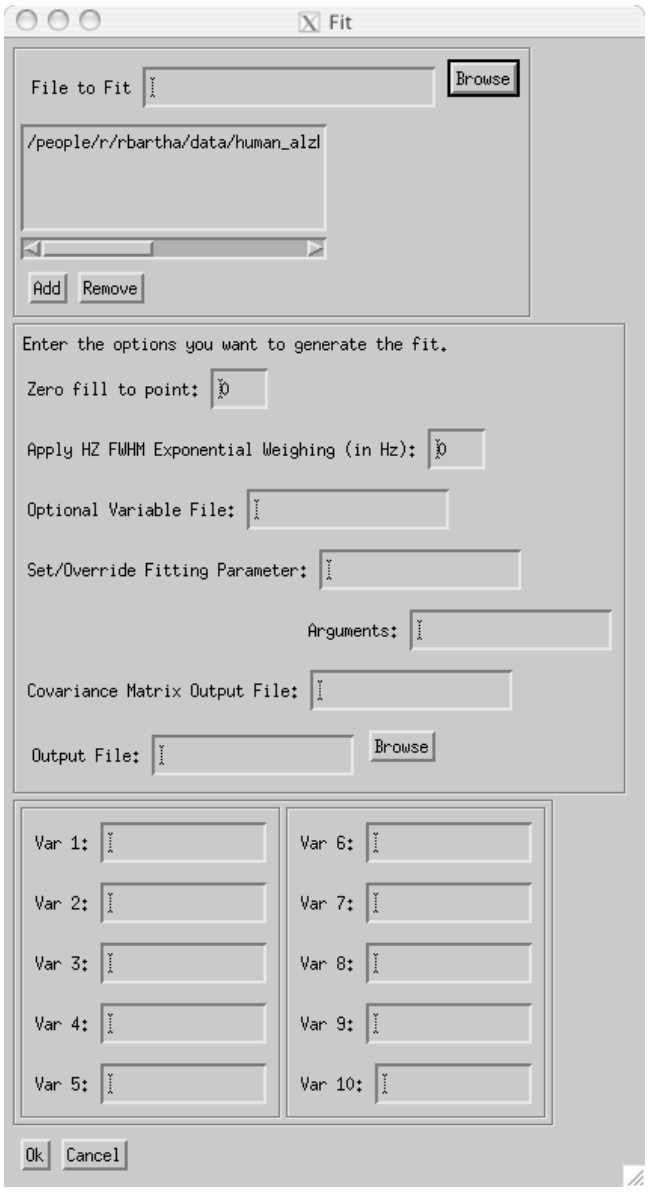
Adjust values as needed and then reload the guess file into fitMANSuite

File → Reload Guess File

Continue to adjust parameters until an adequate match is obtained.

The to fit the spectrum:

File → Generate Fit



Enter file to fit (by default, the file in the middle window appears). Multiple files can be specified to be fit sequentially.

Enter name of file for covariance matrix

Enter name of output file

If variable values are known to be different from that contained within the guess file, the can be specified here.

Fill in the required information and click OK… fitting will begin

The terminal window will show progress of the fit.

When fit is complete, the specified output file will be created and can be visually inspected by loading into fitMANSuite

File → Output

Fitting Unsuppressed Water

<u>Background</u>: Fitting the unsuppressed water signal is required if the water signal is to be used as an internal reference standard. Water is fit to a single exponential decay.

<u>Implementation</u>: Similar to the steps above, water is fit can be fit on the command line or in fitMANSuite.

Load water unsuppressed spectrum. This spectrum is generated during the conversion process and has a file name with _uns appended.

File → Data → Select unsuppressed water file

Load in the appropriate constratins and guess files:

File → Constraints → Select constraints file
File → Guess → Select guess file

It is best to visualize the parameter estimates in the time domain to alter the Guess file until an appropriate starting point is achieved.

Edit and reload Guess file as needed.

Finally, generate the fit:

File → Generate the fit → specify an appropriate output file name.

The output file will be generated with information for each iteration of the fitting displayed in the terminal window.

Summarize Results in Excel

Background: After fitting, the results of the output file can be viewed in fitMANSuite, and metabolite levels quantified in Microsoft Excel. The conversion of absolute units incorporates metabolite spectral area, the area of the unsuppressed water peak, corrections for tissue partial volume, T1 and T2 relaxation time, and acquisition parameters such as receiver gain and the number of acquisitions.

Implementation: Two separate Excel spreadsheets are used.

Data_spreadsheet.xls → A spreadsheet that contains all metabolite/subject information related to the scan and is used to calculate absolute metabolite concentrations

Macro_spreadsheet.xls → A spreadsheet that contains a macro to read information directly from the output file (*.out) generated from fitMANSuite. Only the suppressed water file is read. Information from the water unsuppressed fit must be entered manually or a new macro written to read automatically. A different macro spreadsheet is provided for MAC and PC.

To compile output data in the Data_spreadsheet.xls, open the spreadsheet and place the cursor in the cell where the first raw value for NAA should be placed (BL31 in the example provided). Open the Macro spreadsheet and ensure that all parameters are specified correctly. First look at the portion of the spreadsheet that specifies which peaks should be attributed to each metabolite (a portion of the table is shown below). Each metabolite is specified in the first column, and then subsequent columns attribute peaks to each metabolite:

Metabolite	Peak 1	Peak 2
NAA	1	2
Glu	10	11
Gln	36	37
GABA	54	55
Asp	72	73
NAAG	84	85

Below this table are additional outputs may be specified:

Reference Peak #	9	
Reference Value	2.01	**ppm**
Display Lorenzian Widths	0	**1=Yes, 0=No**
Display Gaussian Widths	0	
Display Amplitudes	1	
Display Shifts	0	
Display Phase	0	
Display Delay Time	0	
Display Noise STD	1	
Display Headings	2	**1=Yes, 0=No, 2=Auto**
Number groups	19	

Reference Peak → peak number corresponding to a known chemical shift (in this case the NAA_{CH3} peak is specified at 2.01 ppm
Reference Value → chemical shift (in ppm) corresponding to Reference Peak
Display → Set corresponding value to 1 to display the specified parameter (only amplitudes and noise standard deviation are currently set)
Display Headings → Headings will be printed above metabolite values if set to 1 and only printed if cell above current cursor position are empty if set to 2

The macro may be started in Excel using:

Tools → Macro → Macros

Select macro 'fitman' and → Run

Select fitman output file in browser window

The macro may also be started using the shortcut keys:

option + command + 'f'

Raw metabolite amplitudes and standard deviations will be loaded. Metabolite levels are calculated using the following formula:

=(((((RawMetaboliteArea/NumberAcquisitions)/10^(ReceiverGain/20))/fitMANScaling)
/ (((RawWaterArea/NumberAcquisitions)/10^(ReceiverGain/20))/fitMANScaling))
* 55.5556*WaterPartialVolumeScaling*1000)*2/NumberMetaboliteProtons
* RelaxationCorrectionFactor

Several assumptions are incorporated into the calculation of the RelaxationCorrectionFactor that are described in (3)

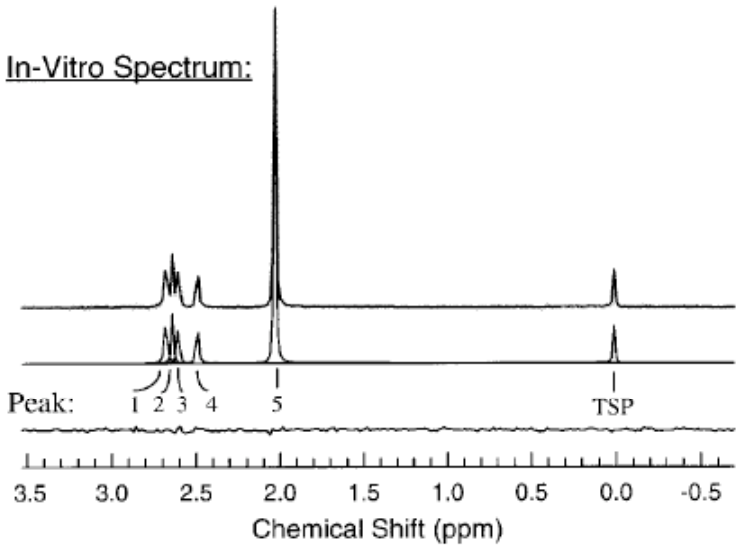
New Metabolite Templates: **aPriori-*Knowledge***

<u>Background</u>: The current prior knowledge template was established for a particular set of experimental conditions (LASER localization, TE=46 ms) and modeling the known metabolites in the human brain. This prior-knowledge can be changed as required and should be updated when new metabolite information is obtained – particularly related to the observation of new metabolites in pathological conditions.

When incorporating new metabolite information, it is imperative that very high quality data be used – either obtained experimentally or simulated. Experimental data can be obtained by mixing phantoms of the metabolite and acquiring high resolution, high signal-to-noise spectra under the correct physiological conditions (pH and temperature). Information regarding the metabolites used in the current template of prior knowledge is included in a separate addendum to this document. Simulation of metabolite spectra is also a viable option with programs such as GAMMA.

<u>Implementation</u>: The following details are provided for the development of new prior knowledge and incorporating this information into the template of prior-knowledge provided. Details of this procedure can also be found in Bartha et al (4)

R. BARTHA *ET AL.*



<u>Constraints:</u>

Peak #	Chemical Shift	Exponential Width	Area	Phase	Delay Time	Gaussian Width
1	$\omega+(\omega_{TSP}-\omega_1)$	$\alpha+(\alpha_{TSP}-\alpha_1)$	$c_{NAA}\cdot c_1/c_5$	ϕ_0	t_0	β
2	$\omega+(\omega_{TSP}-\omega_2)$	$\alpha+(\alpha_{TSP}-\alpha_2)$	$c_{NAA}\cdot c_2/c_5$	ϕ_0	t_0	β
3	$\omega+(\omega_{TSP}-\omega_3)$	$\alpha+(\alpha_{TSP}-\alpha_3)$	$c_{NAA}\cdot c_3/c_5$	ϕ_0	t_0	β
4	$\omega+(\omega_{TSP}-\omega_4)$	$\alpha+(\alpha_{TSP}-\alpha_4)$	$c_{NAA}\cdot c_4/c_5$	ϕ_0	t_0	β
5	$\omega+(\omega_{TSP}-\omega_5)$	$\alpha+(\alpha_{TSP}-\alpha_5)$	$c_{NAA}\cdot c_5/c_5$	ϕ_0	t_0	β

Fitting Long Echo-Time 1H MRS Spectra

Background: Although the focus of this tutorial has been on the acquisition and analysis of short echo-time 1H MRS data, fitMANSuite may also be used for fitting long echo-time MRS data or other spectra from other nuclei (31P, 13C etc.).

All steps in the quantification process are similar to that described, with the omission of the macromolecule subtraction and lineshape correction for nuclei other than 1H. New Guess and Constraints files must also be produced based on the number of metabolites in the spectrum of interest. Fitting of long echo-time 1H MRS data may be possible with more parameter freedom (i.e. shifts, widths, and amplitudes can be fit as independent parameters).

References

1. Garwood M, DelaBarre L. The return of the frequency sweep: designing adiabatic pulses for contemporary NMR. J Magn Reson 2001;153(2):155-177.
2. Bartha R, Drost DJ, Menon RS, Williamson PC. Spectroscopic lineshape correction by QUECC: combined QUALITY deconvolution and eddy current correction. Magn Reson Med 2000;44(4):641-645.
3. Kassem MN, Bartha R. Quantitative proton short-echo-time LASER spectroscopy of normal human white matter and hippocampus at 4 Tesla incorporating macromolecule subtraction. Magn Reson Med 2003;49(5):918-927.
4. Bartha R, Drost DJ, Williamson PC. Factors affecting the quantification of short echo in-vivo 1H MR spectra: prior knowledge, peak elimination, and filtering. NMR Biomed 1999;12(4):205-216.

Chapter 3

FitMAN Spectral Fitting

Welcome to FitMANSuite!

FitMANSuite is a comprehensive processing and analysis package for quantification of in-vivo NMR spectroscopy data in either the time domain or frequency domain.

This HELP document will outline the basic elements which are required to run FitMAN and describe the various features which can aid in spectroscopic fitting. Although the included fitting routines have been used in our laboratory now for several years, the software is still undergoing occasional changes and improvements. If you have any suggestions or would like to see additional features added please email to rob.bartha@imaging.robarts.ca.

In the past, spectroscopic fitting has been possible with intensity estimates, or area integration. However, in-vivo where spectral resolution and signal to noise is limited, mathematical modeling is essential (1-11).

The model used depends on the characteristics of the data, as well as the post-processing steps that transform the data.

FitMAN incorporates two basic model functions. One describes spectroscopic data in the measurement (time) domain, while the other describes spectroscopic data in the frequency domain (after Fast Fourier Transform). Real and imaginary data must both be present for fitting. The fitting routine uses a Levenberg-Marquart minimization routine.

The time domain model function used by the FitMAN minimization routine is given by equation [1]

$$\hat{y}(n) = \sum_{k=1}^{K} c_k \cdot \mathrm{e}^{i(2\pi\cdot\omega_k(n\cdot\Delta t+t_0)+\varphi_k)} \cdot \mathrm{e}^{-\pi\cdot\alpha_k\cdot|n\cdot\Delta t+t_0|} \cdot \mathrm{e}^{-(\pi^2/(4\cdot\log 2))\beta_k^2\cdot(n\cdot\Delta t+t_0)^2} \qquad [1]$$

$\hat{y}(n)$ ≡ Sampled points along the estimated time domain signal at points n (n = 1, 2, …, N).

K ≡ Maximum number of resonances.

N ≡ Maximum number of discrete samples.

k ≡ Resonance index (k = 1, 2, …, K).

ω_k ≡ Chemical shift (frequency) of resonance k.

c_k ≡ Amplitude of resonance k.

α_k ≡ Lorentzian damping of resonance k.

β_k ≡ Gaussian damping of resonance k.

φ_k ≡ Phase of resonance k (allows peaks outside the SRI to have independent phase).

t_0 ≡ Delay time (time between center of stimulated echo and first readout point).

Δt ≡ Dwell time.

The frequency domain model function used by the FitMAN minimization routine is given in equation [2]

$$\hat{F}(s) = \sum_{k=1}^{K} \frac{c_k}{2\pi(i(\omega_k - s) - \alpha_k)} \cdot e^{i(2\pi\omega_k t_0 + \vartheta_k) - 2\pi\alpha_k t_0} \qquad [2]$$

where $\hat{F}(s)$ ≡ sampled points along spectrum at frequency s

t_0 ≡ delay time

c_k , α_k , ω_k , φ_k ≡ real valued amplitude, Lorentzian damping factor, angular frequency, and phase of sinusoid number k

Both time and frequency domain model functions incorporate phase and delay time as parameters to be fit. The Frequency domain model function does not include a term for Gaussian damping.

Data Format

Input data files must be in ascii file format and should be labeled with a .dat extension. Files are organized as follows:

Line1	2048	// number of points
Line2	1	// unused
Line3	0.001	// dwell time in seconds
Line4	63.8	// acquisition frequency in MHz
Line5	128	// number of acquisitions
Line6	comment	// comment
Line7	comment	// comment
Line8	comment	// comment
Line9	comment	// comment
Line10	SIMULTANEOUS	// method of data acquisition
Line11	0.0	// for future use
Line12	EMPTY	// for future use
Line13	1.234	// real component of first data point
Line14	-2.234	// imaginary component of first data point
Line15	0.932	// real component of second data point
Line16	-2.111	// imaginary component of second data point
.		
.		
.		
.		
Line2059	0.123	// real component of last data point
Line2069	0.011	// imaginary component of last data point

A sample data file called “sample_1H_spec_data.dat” can be found in the /FitMANSuite/samples directory.

Guess File

The initial guesses for the peak parameters are placed in a separate file or section of a file. The guess portion of a file must begin with:

```
****_Guess_File_Begins_****
```

and end with:

```
****_Guess_File_Ends_****
```

Guess File Sections

The guess file contains three distinct sections called:

[Parameters]
[Variables]
[Peaks]

Each section contains specific information that is interpreted by the fitting routine.

[Parameters] Section

The [Parameters] section contains the following:

number_peaks <n> Specifies the number of peaks use in the fitting.
Default: 0

shift_units <s> Specifies the units for the initial guesses of shift values in the guess file. Two choices are available hz or ppm.
Default: ppm

[Variables] Section

The [Variables] section contains the following:

<s> <n> Where <s> specifies the variable name and <n> specifies the value assigned to it.

[Peaks] Section

Assigning Variables to Peaks

The [Peaks] section contains the following:

```
;PEAK#   SHIFT   LORENTZIAN WIDTH    AMPLITUDE    PHASE    DELAY    GAUSSIAN WIDTH
1        2.11          6.0             1.0        0.0      0.0          0.0
```

repeated for every peak.

The semi-colen identifies a remark and forces the parsing routine to ignore this line.

Units

The following units are used for each peak parameter

for SHIFT parameters	shift_units (in parameter section)
for LORENTZIAN WIDTH parameters	Hz (FWHM of Lorentzian line)
for AMPLITUDE parameters	Absolute Units
for PHASE parameters	Radians
for DELAY parameters	Seconds
for GAUSSIAN WIDTH parameters	Hz (FWHM Gaussain line)

Parameters with Offsets

Variables that are specified in the constraints file and contain offsets are resolved so that the initial guess specified in the guess file is the initial value for a particular parameter. For example suppose the following combination of guess file and constraints file occurred:

```
****_Constraints_File_Begins_****
.
.
.

;PEAK#   SHIFT       LORENTZIAN WIDTH    AMPLITUDE    PHASE    DELAY    GAUSSIAN WIDTH
1     {shift1}+0.1  {lorentzian_width1} {amplitude1} {phase1} {delay1} {Gaussian_width1}

****_Constraints_File_Ends_****

****_Guess_File_Begins_****
.
.
.

;PEAK#   SHIFT   LORENTZIAN WIDTH    AMPLITUDE    PHASE    DELAY    GAUSSIAN WIDTH
1         2.11          6.0             1.0        0.0      0.0           0.0

****_Guess_File_Ends_****
```

In this case, the first peak would have a starting shift of 2.11 ppm. Therefore, the parameter shift1 would be evaluated as shift1=2.01.

Linked Parameters

In the above example further references to shift1 anywhere else in the constraints file would cause the initial guess of that peak's shift to be evaluated base on the value of shift1=2.01. For example suppose the next peak in the constraints file was specified according to:

```
2      {shift1}+0.3 {lorentzian_width2} {amplitude2}  {phase2} {delay2} {Gaussian_width2}
```

The initial guess for the shift of this peak would be evaluated as shift1+0.3 = 2.31 regardless of what the initial guess file specifies for this peak. Since all the other parameters occur for the first time, they would be resolved based on the information in the guess file for that peak.

Constraints File

The constraints which are placed on the fitting procedure and individual peaks must be specified in a separate file or section of a file. The constraints section of a file must begin with:

```
****_Constraints_File_Begins_****
```

and end with:

```
****_Constraints_File_Ends_****
```

Constraints File Sections

The constraints file contains three distinct sections called:

[Parameters]
[Variables]
[Peaks]

Each section contains specific information which is interpretted by the fitting routine.

[Parameters] Section

The [Parameters] section contains the following:

shift_units <s> Specifies the units for offsets to shift values in the constraints file. Two choices are available hz or ppm.
Default: ppm

output_shift_units <s> Specifies the units of the output shift values. The constraints portion of th output file will appear as in the original constraints file. The output_shift_units parameter affects only the guess portion of the output file. Two choices are available hz or ppm.
Default: Hz

tolerance <n> Specifies the % change in chi squared value between iterations which must be achieved before the fit is completed. The must be repeated "minimum_iterations" before the fit completes.

Default: 0.001

noise_points <n> Specifies the number of points at end of file used to calculate the standard deviation of the noise. The default is set at 32.

Default: 32

fixed_noise Specifies a fixed value for the standard deviation of the noise. If this is encountered the noise is not calculated from the data file but is taken to be the value specified.

Default: inactive

alambda_increment <n> Specifies the multiplicative factor used to increment alambda.

Default: 10

alambda_decrement <n> Specifies the multiplicative factor used to decrement alambda.

Default: 0.1

domain <s> Specifies in which domain data will be fit. Choices are time_domain or frequency_domain.

Default: time_domain

number_peaks <n> Specifies the number of peaks use in the fitting.

Default: 0

fwhm_exp_weighting <n> Specifies the full-width half-maximum (FWHM) of the exponential weighting function. This can be applied to data fit in the time or frequency domain (although it is handled differently in each domain).

Default: 0

maximum_iterations <n> Specifies the maximum number of iterations which will be executed before the fit is terminated. If this is achieved the current paramter values are retained in the output file.

Default: 50

minimum_iterations <n> Specifies the minimum number of iterations which must meet the tolerance level in order for the fit to be completed.

Default: 5

positive_amplitudes This flag force all amplitudes to be positive. This is identical to using the >0 boundary condition following each amplitude parameter.

Default: inactive

noise_equal This flag forces the noise in each the real and imaginary channel to be equal. Since the noise is calculated independantly for each channel they may be slightly different. The noise in each channel is taken to be the average of the noise in the two channels calculated independantly.

Default: inactive

fix_all_shift This flag fixs all the shifts to values specified in the guess file. This is equivalent to placeing the @ symbol in front of the first curly bracket defining each shift parameter.

Default: inactive

fix_all_delay_time This flag fixs all the delay times to values specified in the guess file. This is equivalent to placeing the @ symbol in front of the first curly bracket defining each delay time parameter.

Default: inactive

fix_all_l_width This flag fixs all the lorentzian widths to values specified in the guess file. This is equivalent to placeing the @ symbol in front of the first curly bracket defining each lorentzian width parameter.

Default: inactive

fix_all_g_width This flag fixs all the gaussian widths to values specified in the guess file. This is equivalent to placeing the @ symbol in front of the first curly bracket defining each gaussian width parameter.

Default: inactive

fix_all_phase This flag fixs all the phases to values specified in the guess file. This is equivalent to placeing the @ symbol in front of the first curly bracket defining each phase parameter.

Default: inactive

fix_all_amplitude This flag fixs all the amplitudes to values specified in the guess file. This is equivalent to placeing the @ symbol in front of the first curly bracket defining each amplitude parameter.

Default: inactive

range <n> <n> Specifies the range of points over which to fit if fitting is done in the time domain. If fitting is done in the frequency domain, this specifies the range of points of time domain data which are fourier transformed to the frequency domain.

Default: 1 256

qrt_sin_weighting_range <n> <n> Specifies the range over which to apply the quarter sine weighting function at the beginning of the data.

Default: 0 0

zero_fill <n> Specifies the number of points to zero fill to if fitting in the frequency domain.

Default: 1024

frequency_range <n> <n> Specifies the frequency range over which to fit when fitting in the frequency domain. This value is specified in Hz. Values must be entered in "reverse" order since by convention the spectral frequency axis is positive on the left and negative on the right. Therefore to fit the majority of the ^{1}H spectrum at 4T the following would be specified: frequency_range 0 –400.

Default: 0 0

[Variables] Section

The [Variables] section contains the following:

<s> <n> Where <s> specifies the variable name and <n> specifies the value assigned to it.

[Peaks] Section

Assigning Variables to Peaks

The [Peaks] section contains the following:

```
;PEAK#   SHIFT    LORENTZIAN WIDTH    AMPLITUDE    PHASE    DELAY    GAUSSIAN WIDTH
1       {shift1} {lorentzian_width1} {amplitude1} {phase1} {delay1} {Gaussian_width1}
```

repeated for every peak.

The semi-colen identifies a remark and forces the parsing routine to ignore this line.

Linking to Other Peaks

The strings within the curly brackets identify the variable name used for linking. Therefore any other peak can be linked to the above peak parameters by specifying the same string. Offsets can also be used for linking. For example the second peak and third peaks could be constrained as follows:

```
2      {shift2} {lorentzian_width2}  {amplitude1}*2 {phase1} {delay1}   {Gaussian_width2}
3      {shift3} {lorentzian_width3}  {amplitude1}   {phase1} {delay1}   {Gaussian_width3}
```

This is interpreted as follows. Since each peak has a different string to constrain SHIFT these will be fit as independent parameters. The same is true for LORENTZIAN WIDTH and GAUSSIAN WIDTH parameters. The amplitude strings for all three peaks are the same, although the amplitude for peak two is scaled by a factor of 2. Therefore this identifies a triplet with the amplitude ratios 1:2:1. Similarly, since the PHASE and DELAY strings are the same for all three peaks, they are all linked. Since no offets are provided for these parameters, they will all have the same value.

Linking Offsets

When linking parameters the following functions can be used:

for SHIFT parameters	+ or -
for LORENTZIAN WIDTH parameters	+ or -
for AMPLITUDE parameters	* or /
for PHASE parameters	+ or -
for DELAY parameters	+ or -
for GAUSSIAN WIDTH parameters	+ or -

Multiple arguments can also be use as long as no spaces separate them...for example:

```
{shift1}+0.1-0.5
```

This will be resolved to {shift1}-0.4

Fixing Peak Values

Any peak parameter can be fixed to the value specified for the corresponding peak in the guess file by placing an @ sign in front of the first curly bracket of the parameter constraint. For example:

```
4   @{shift3} @{lorentzian_width3} @{amplitude1} @{phase1} @{delay1} @{Gaussian_width3}
```

This fixed the value of each paramter to the corresponding guess in the guess file. It makex no difference what the strings are within the curly brackets...these are ignored.

Imposing Limits on Parameter Values

Limits may also be specified for specific parameters using the > or < symbols. This ensures that parameters will not exceed the specified values in the fitting. If a limit is exceeded, the parameter is removed from the fit and the entire fit is restarted using the current set of parameter values as the initial guess. A message to this effect is given during the fit (i.e. peak 1 paramter 3 has exceeded minimum bounds). Parameters are removed from the fit according to the following rules:

```
4   @{shift3} @{lorentzian_width3} {amplitude1} >0 @{phase1} @{delay1} @{Gaussian_width3}
```

for SHIFT parameters	The entire peak is removed from the fit
for AMPLITUDE parameters	The entire peak is removed from the fit
for PHASE parameters	The entire peak is removed from the fit
for DELAY parameters	The entire peak is removed from the fit
for LORENTZIAN WIDTH parameters	Lorentzian width is fixed to zero...if gaussian width is also fixed to zero the peak is removed
for GAUSSIAN WIDTH parameters	Gaussian width is fixed to zero...if lorentzian width is also fixed to zero the peak is removed

Output File

The output file contains all the information required to repeat a fit and obtain identical results. As such it is divided into two portions, one portion contains the guess file information while a second portion contains the constraints information. Therefore each output file will have the following sections:

```
****_Guess_File_Begins_****


****_Guess_File_Ends_****
****_Constraints_File_Begins_****


****_Constraints_File_Ends_****
```

The Guess File Portion

The guess file portion contains the results of the fit with the following units.

for SHIFT parameters	shift_units (in parameter section) Where shift_units = output_shift_units from the constrains file.
for LORENTZIAN WIDTH parameters	Hz (FWHM of Lorentzian line)
for AMPLITUDE parameters	Absolute Units
for PHASE parameters	Radians
for DELAY parameters	Seconds
for GAUSSIAN WIDTH parameters	Hz (FWHM Gaussain line)

The Constraints File Portion

The constraints portion contains all the information needed to repeat the identical fit. All parameters that affect the fitting of the data are output. All linking information in the [Peaks] section is copied from the original constraints file.

The Flexibility of the Output File

Since the output file contains a guess portion and a constraints portion it can be used as a guess file, a constraints file, or both for any fit. This allows for recursive fitting where the output of one fit can be used as the input to a second fit.

Fitting Data

Once data has been converted to the correct format, and Guess and Constraints files have been developed, the fitting program can be initiated from within FitMANSuite.

Following each iteration of the fitting process, information will be displayed in the terminal window describing the fitting status (i.e. the iteration number, the current value of alambda, and the current Chi Squared value).

Once fitting is complete, the routine checks to make sure that all parameters are within the bounds specified in the constraints file. If any parameters exceed their specified bounds, the parameter is removed from the fitting and the file is refit with the current parameter estimates as the starting point. The parameters which have exceeded their bounds and are responsible for the re-fitting are displayed.

Once convergence has been achieved, parameter values are written to the output file name specified on the command line. The Cramer-Rao estimates of minimum variance are also written to the output file for each parameter.

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