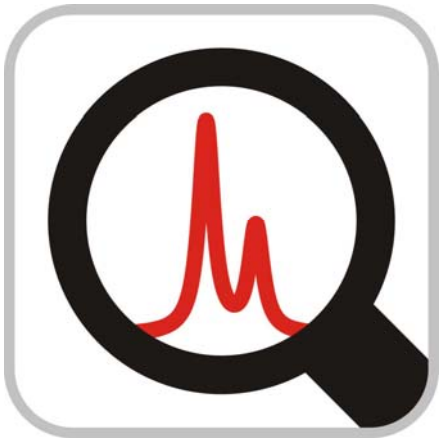

# INSPECTOR

## Magnetic Resonance Spectroscopy Software

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# Abstract

Magnetic resonance spectroscopy (MRS) allows the non-invasive measurement and quantification of chemical compounds from living tissues. This makes MRS a powerful tool for biomedical research and clinical diagnostics of virtually all disorders that possess distinct metabolic signatures. Optimal processing and quantification procedures are crucial, however, for the reliable extraction of biochemical information.

*In vivo* MRS is well established and a variety of software packages exist for the processing and quantification of spectral data. However, the potential for in-depth quality management and access to the details of the processing stream are typically limited. Also, some MRS software packages focus on only processing or quantification, necessitating the addition of external interfacing between the two.

The INSPECTOR software comprises comprehensive processing and analysis functionality for *in vivo* MRS in a one-stop-shop solution. Extensive data handling, in-depth quality management and visualization options are provided, enabling maximum transparency at every step in the processing chain. Advanced analysis tools are integrated into INSPECTOR, making it ideally suited for *in vivo* MRS analysis. The details of the quantification algorithm can be flexibly chosen and tailored to the problem at hand, and extended confidence information is provided with the quantification. The software stands out for its potential for automation and its user-friendly work flow. All parameters of the software are automatically maintained in protocol files that can be loaded to restore parameter settings to previously selected values. Besides convenience, functionalities like this ensure reliable and consistent processing throughout multi-experiment studies. INSPECTOR has been developed for biomedical research and is specifically tailored to its needs. If you truly want to know what is in your data, this software will become your first choice. Give it a try!

# Contents

# Introduction

The INSPECTOR software[1] comprises processing and analysis solutions for magnetic resonance spectroscopy (MRS). The software provides automated processing options and tools for in-depth visualization and analysis of MRS data. Though MRS is a powerful method, every deviation from the perfect experiment reduces the spectral quality and the information content that can be derived from it. As such, the pursuit of optimal experimental quality should be complemented by similarly optimal data processing and analysis routines. The INSPECTOR software, therefore, aims to provide comprehensive access to every detail of the data at hand. All intermediate results of the processing stream can be visualized, and the corresponding tools are provided for meaningful correction.

### *Overall Structure and Application Pages*

Overall, the software is organized by application pages for loading and preprocessing experimentally acquired data (*Data*), spectral processing of individual spectra or the comparison of two spectra, e.g. two *J*-difference edited (JDE) conditions (*Processing*), various types of $T_1$ and $T_2$ characterization (*T1/T2*), compound quantification via a linear combination of model spectra (*LCM*) and the spectral simulation (*Simulation*). Selecting the *Manual* page opens a copy of the manual you are currently reading. The *Exit* button saves all INSPECTOR settings to a default parameter file and closes the software properly. As such, the *Manual* and *Exit* functionalities do not represent entry points to full pages beyond those previously enumerated.

The software structure resembles the chronological order of the typical processing workflow and is organized when possible, from top to bottom and left to right. For instance, the analysis of experimentally acquired JDE spectra requires the loading and preprocessing of the data, potentially including the combination of signals from individual receivers, the correction of eddy-current-induced phase effects, and the frequency alignment of individual traces on the *Data* page. The resultant free induction decay (FID) of the edited and non-edited conditions are then processed and subtracted from each other on the *Processing* page before the resultant difference signal is quantified on the *LCM* page. Similarly, individual pages typically contain options for data selection on the top of the page, followed by processing and analysis options in the center, and finally tools for results assessment and visualization on the bottom.

### *Protocol Files*

All INSPECTOR parameters are saved to file when the software is closed properly via the *Exit* button and automatically reloaded when the software is restarted. This functionality allows users to close the software at any time and to return to the exact same situation very quickly at a later time. Notably, this also includes the INSPECTOR page itself, e.g., *Data* or *LCM*. In addition, the full set of INSPECTOR parameters can be saved to a user-specified protocol file (see the *Data* page for details).

### *Units*

All spatial dimensions are expressed in millimeters, and angles are expressed in degrees. Frequencies are in Hertz (Hz) or parts-per-million (ppm) depending on the application and indicated with every use. Further units are defined as needed.

### *Tool Tips*

This manual provides detailed explanations on the INSPECTOR software. Also, short summaries and reminders on the functionality at hand are added to most entry fields, flags, and buttons as tool tips. This information can be visualized by mousing over the button without clicking it. For further information, feel free to email me.

### *Download, Installation and Use*

The INSPECTOR software has been developed in MATLAB (MathWorks, Natick, MA, USA) and is currently distributed as a stand-alone executable program compiled in MATLAB version R2013b for 64-bit Windows©, Linux© and Apple Macintosh© operational systems. It can be downloaded from the Columbia Technology Venture website under a limited-term academic license:

*Juchem, C., INSPECTOR: Magnetic resonance spectroscopy software for optimized data extraction. Columbia Technology Venture license 17130 (2016). innovation.columbia.edu/technologies/cu17130_inspector*

The corresponding MATLAB Runtime environment needs to exist to provide the software with the required libraries. Detailed instructions on the installation of the MATLAB Runtime can be found at: https://www.mathworks.com/help/compiler/mcrinstaller.html.

It is important for Linux and Mac users to start the software via the provided shell script, i.e. the file ending with .sh, instead of e.g. double-clicking the INSPECTOR icon directly. This script opens a terminal window and dynamically links the necessary libraries before the software is started. All these settings are temporary and disappear when the software and the terminal window are closed. The terminal window corresponds to the Matlab command window and all info printouts are delivered to this window.

Step-by-step instructions for starting the INSPECTOR software via a shell script on Linux/Mac:

1) Place a copy of the provided INSPECTOR directory to your computer and make sure you have the permissions to access and execute the files. If needed, change the permissions with the Linux/Mac command *chmod*.

2) Identify the system's MCR root directory. This is typically something like */usr/local/MATLAB/R2013b* on Linux or */Applications/MATLAB/MATLAB_Runtime/v82* on Apple Mac

3) Run the shell script file with the MCR root directory as function argument, e.g.

*./run_INSPECTOR.sh /usr/local/MATLAB/R2013b* or similar for linux or

*./run_INSPECTOR.sh /Applications/MATLAB/MATLAB_Runtime/v82* or similar for mac if you are currently in the INSPECTOR directory or from anywhere via the absolute path:

*/home/juchem/matlab_exe/INSPECTOR/run_INSPECTOR.sh /usr/local/MATLAB/R2013b*

If the assigned MCR root directory has been correct, the INSPECTOR software will start. You will then find a GUI of the software itself and a terminal window containing information printouts.

4) For convenience, I recommend including the MCR root directory information in the shell script itself, thereby rendering its assignment as function argument obsolete and allowing to start INSPECTOR by double-clicking the shell script file or a link to it.
To do so, open (a copy of) the shell script and confirm the existence of the paths containing the variable MCRROOT. If the paths exist, the software will be able to find the libraries and nothing needs to be changed. If not, adopt the path details after the MCRROOT variable to account for the MCR installation details on your computer. Once all paths are correct, calling the shell script alone without function argument will start the software correctly. For Mac OS, you will need to edit the file to something like this:

```
#!/bin/sh
# script for execution of deployed applications
#
# Sets up the MATLAB Runtime environment for the current $ARCH and
executes
# the specified command.
#
exe_name=$0
exe_dir=`dirname "$0"`
echo "------------------------------------------"
if [ "x$1" = "x" ]; then
  echo Usage:
  echo    $0 \<deployedMCRroot\> args
else
  echo Setting up environment variables
  MCRROOT="$1"
  MCRROOT=/Applications/MATLAB/MATLAB_Runtime/v82;
  echo ---
  DYLD_LIBRARY_PATH=.:${MCRROOT}/runtime/maci64 ;
  DYLD_LIBRARY_PATH=${DYLD_LIBRARY_PATH}:${MCRROOT}/bin/maci64 ;
  DYLD_LIBRARY_PATH=${DYLD_LIBRARY_PATH}:${MCRROOT}/sys/os/maci64;
  export DYLD_LIBRARY_PATH;
  echo DYLD_LIBRARY_PATH is ${DYLD_LIBRARY_PATH};
  shift 1
  args=
  while [ $# -gt 0 ]; do
      token=$1
      args="${args} \"${token}\""
      shift
  done
  eval "\"${exe_dir}/INSPECTOR.app/Contents/MacOS/INSPECTOR\"" $args
```

```
fi
exit
```

Note that you need to tell your Mac to execute the shell script if you double-click it. Otherwise, the file will be opened in an editor for editing. To do so, go to *Open with* and select an iTerminal.

5) If you link the shell script to an icon or a command name, it can be executed very easily to start up the software. For Linux, this can be achieved by adding a symbolic link to the */usr/bin* directory connecting a command name to the location of the script:

*ln -s /usr/local/juchem/matlab_exe/INSPECTOR/run_INSPECTOR.sh INSPECTOR*

Note that root permissions are required for the creation of such link, therefore please ask your system administrator for help if needed. Once the link has been created, you can (run the script and thereby) start the software from any terminal window simply by typing *INSPECTOR* followed by the return key.

### *Before you start*

The manual is organized in chapters that resemble the page structure of the software. General aspects and core features, e.g. spectral processing and analysis options, are shared across pages. They are described in full with their first use, and it is, therefore, advisable to read the manual from the beginning. Also of note is the information provided in the last few paragraphs of the manual, especially the call to join us in the fight against multiple sclerosis (MS) as well as some remarks on academic merit and legal aspects of software use.

# I The *Data* Page

## 1.1 General

The *Data* page is used to load and pre-process experimentally acquired MRS data. The page also includes tools for data visualization, data correction and quality management.

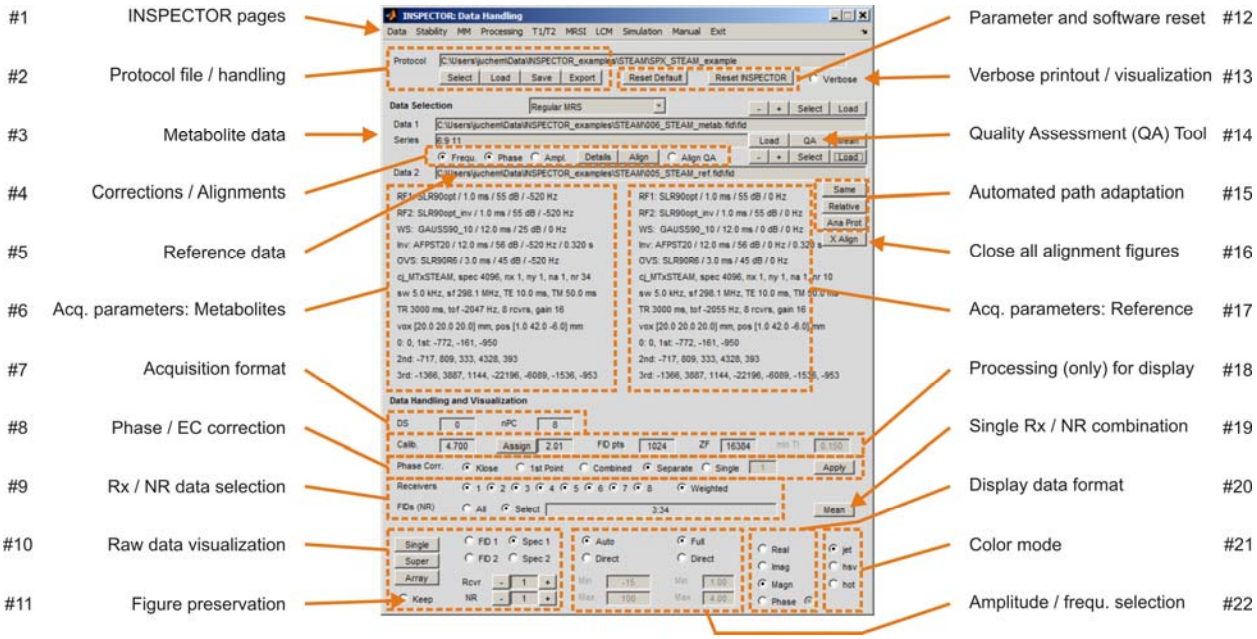



Fig. 1.1: Main *Data* page.

### *Protocol*

Spectroscopic processing relies on a wealth of parameter settings and choices. The INSPECTOR software accounts for this by saving the entire parameter selection to protocol files. These protocols can be loaded to restore all parameter settings to previously selected values. While all software and processing parameters are written to file, the decision has been made to exclude larger data structures, thereby keeping the size of the resultant protocol files to a minimum, typically to a few kilobytes. Explicit export options are available to save the data to file. Notably, protocols are saved in MATLAB (.mat) format. Besides convenience, the reloading of tailored protocol files can be used to assure consistent data processing of various experiments throughout a study.

There are two different protocol options: 1) A general default file and 2) user-defined protocols. Parameter settings are inherently saved to a general default file when the software is properly closed via the *Exit* button (#1, rightmost page option) as it is strongly advised to do so. One default parameter file exists for every INSPECTOR installation. This background file is shared across users and overwritten every time the software is closed. In addition, parameter settings can be saved

to protocol files designated by the user in the protocol section (#2). The *Select*, *Load*, *Save*, and *Delete* functionalities provide handling options for the selected protocol file.

Note that closing INSPECTOR through the operating system's Close button (*x*) in the upper right corner of the window is not advised as parameter settings are thereby lost.

### *Reset Defaults*

This functionality resets all parameters to their default values (#12, left), thereby returning to a meaningful set of starting conditions. In most cases, however, it is more convenient to start from a protocol file of a similar experiment type.

### *Reset INSPECTOR*

Processing MRS data requires a multitude of intermediate steps. Sometimes the status of the current data in memory becomes unclear, such as when certain corrections have been applied. The *Reset INSPECTOR* button restarts the software (#12, right). All parameter settings are kept, but the data memory is freed, thereby returning to a well-defined starting point.

### *Verbose*

INSPECTOR can provide detailed information on every processing and analysis step to enable the user to access the processing stream and to evaluate the results obtained at each step. While this information is always available, its display might not always be warranted. A situation-dependent balance exists between the provided level of detail and the risk of overloading the result display. If the *Verbose* flag (#13) is selected, detailed information is provided, e.g. for more comprehensive data analysis and experiment debugging. If the flag is not selected, only overview and summary data are shown instead.

### *Experiment Type*

The drop-down menu to the right of the *Data Selection* label allows a user to define the experiment type to be loaded. The data format *Regular MRS* refers to a single MRS experiment such as STEAM or LASER. *J*-difference editing (JDE) in which one or two editing conditions are compared to a non-edited reference, is also are supported. Notably, the unedited metabolite reference is always expected to be found in the last condition. When three steps are used, the selection *1$^{st}$ and last* extracts the first (edited) and the last (reference) condition. Similarly, the *2$^{nd}$ and last* selection is used to compare the second (edited) with the last (reference) condition. Regular JDE experiments with one editing (*ON*) and non-edited (*OFF*) condition can be analyzed by selecting the *1$^{st}$ and last* option. The *JDE Array* option allows the user to load and process a series of two-condition (*ON*/*OFF*) JDE experiments that are arrayed with respect to an independent parameter such as echo time or repetition time. Arrays of *T1*-encoded MRS are read with the *Saturation-Recovery* option. Further modes include *Stability* for the MR spectroscopic

analysis of technical aspects of the MR scanner performance, *T1/T2* for the quantification of metabolite relaxation times, and *MRSI* for MR spectroscopic imaging data. Note that the *Saturation-Recovery*, *Stability* and *MRSI* modes require data from specific experiment types that we developed in our laboratory for use on a Varian MR scanner. We are in the process of establishing these methods on MR systems of other vendors or, alternatively, enable the INSPECTOR software to allow processing such data derived with built-in sequences (e.g. for MRSI). We plan to make these extensions available as soon as they are complete, thereby allowing the full functionality of INSPECTOR.

### *Data 1/2*

MRS experiments typically consist of metabolite data and a water reference. The MRS experiment at hand, i.e. the metabolite MRS, is expected as data set 1 (#3), whereas the reference should be assigned as data set 2 (#5). The data assignment refers to the FID file and can be achieved as an absolute path via the entry field or by selecting the file via the *Select* button. If the data directories of individual scans are numbered (e.g. *005_STEAM_metabolite\fid* in Varian or Bruker), the *+* and – buttons can be used to scroll through the scan series. For instance, pressing the *+* button once updates the metabolite scan to the next highest scan number (e.g. *006_STEAM_metabolite\fid*) if a unique, corresponding data set can be found. The *Load* buttons associated with data sets 1 and 2 each loads a single metabolite data set and single reference as data sets 1 and 2, respectively. Data from other vendors such as Siemens or General Electric are saved in individual files. If these MRS data files do not contain a serial number at the beginning of the file name, it is advisable to add such number followed by an underscore (e.g., rename 'filename.p' to '01_filename.p'), thereby allowing the above data handling functionality. For Philips data, please assign the equivalent numbers to both the .SDAT and the .SPAR files as well as the .sin and .lab files that go with Philips' .raw data files. Notably, I have not been able to identify the Larmor frequency and acquisition bandwidth (or dwell time) in Philips' .raw data and parameter files. The software therefore checks for an .SPAR file with the same number or, if no such file can be found, any .SPAR file in the data directory and retrieves that information from there.

A set of relevant acquisition parameters is automatically read from the parameter files when MRS data are loaded. These parameters are displayed in INSPECTOR for convenience and provide the basis for experiment-specific data handling. User-input for the definition of the data set at hand is not required.

If both metabolite spectra and the corresponding water references are included in the same data file, as can be the case e.g. with General Electric, Siemens or Philips data, the same file needs to be assigned as *Spec 1* (metabolite) and *Spec 2* (water); INSPECTOR will then extract the corresponding portions. For instance, the below example printout reports the successful reading of 4 water references + 64 metabolite scans acquired as 2048 complex data points with an 8-channel RF coil and saved to a single file. If this data set is assigned as data set 1, the metabolite scans will be extracted and used, whereas selection of the same data file as data set 2 results in extraction and use of the 4 water references.

```
Loading file 1 (of 2):
C:\Users\juchem\Data\NYSPI\002_NYSPI_sLASER\01_PSLASERMetabs.7
Data format: General Electric
Reading <C:\Users\juchem\Data\NYSPI\002_NYSPI_sLASER\01_PSLASERMetabs.7>
nr/nspecC/nRcvrs: 4+64/2048/8
```

It is highly advisable to process at least part of the experimentally acquired MRS data while scanning to confirm the overall data quality and the success of the experiment. Saving the resultant INSPECTOR settings as a protocol furthermore provides an excellent starting point for later in-depth processing and analyses. The latter typically involves 1) data transfer to a different machine, potentially having a different operating system, 2) adoption of the corresponding path structures of the protocol file and the data files, as well as 3) renaming the protocol file to preserve the original parameters settings employed during the experimental session. INSPECTOR therefore includes a series of options providing selected features or combinations thereof. The *Same* button adopts the paths of both data sets 1 and 2 on the analysis computer assuming that the data share the same directory with the protocol. The *Relative* button reassigns the paths of both data sets to maintain the directory structure relative to the current protocol file. These functionalities not only come in handy when data are transferred from the acquisition computer to an analysis computer but also provide a convenient means to adopt the overall path structure when studies including data and INSPECTOR protocol files are transferred between hard drives or memory locations. The button *Ana Prot* performs all 3 steps outlined above in a serial fashion and thereby represents the common starting point for off-line processing of experimentally acquired MRS data.

### *Basic Data Review*

A basic review of the available experimental data is always advised to confirm the success of the underlying experiment, the appropriate data formatting, and loading as well as essential quality parameters. The *Data* page, therefore, contains a series of options to display various aspects of the raw data sets 1 and 2 (#10). These include individual (*Single*) and multiple (*Array*) FIDs or spectra of selected receivers (*Rcvr*) or repetitions (*NR*).

The amplitude range to be visualized can be set directly (*Direct*) or determined automatically to have the spectral signals cover 90% of the vertical axis (*Auto*, #22). Similarly, a frequency range can be assigned (*Direct*); otherwise the *Full* bandwidth is shown (#22). Various aspects of the available data can be selected as visualization format (*Real*/*Imag*inary/*Magn*itude/*Phase*, #20) and displayed in different color modes (*jet*/*hsv*/*hot*, #21). A linear detrending of the FID phase, corresponding to a frequency correction, is available to allow an unobstructed assessment of the potential phase non-linearities.

Figure 1.2 shows an example visualization of selected FIDs 3, 5, 7 and 9 of a water reference for all 8 receivers employed in the experiment. All spectra use the identical amplitude scaling (achieved via the *Direct* option). Notably, the receivers were arranged in a single horizontal ring around the subject's head. The distance of the MRS voxel relative to the individual elements of

the RF phased array thereby leads to differences in coil sensitivities and larger signals in coils that were closer to the MRS voxel (around receiver 4).

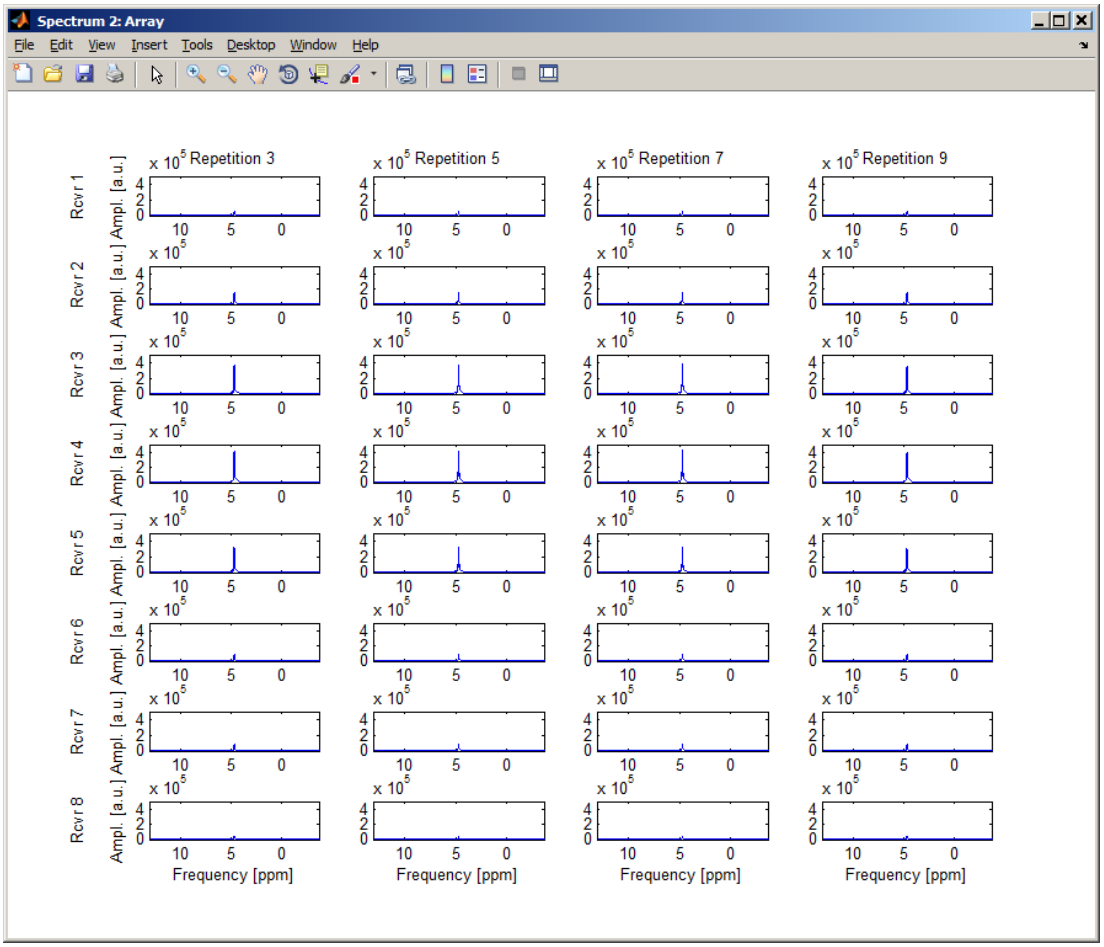



Fig. 1.2: Review of raw data: Array of selected FIDs (NR) 3:2:9 of water spectrum 2 for all 8 receivers.

The above functionalities display individual FIDs or spectra from selected repetitions (NR) and receivers, i.e., the true raw data before any NR and/or receivers are combined. A series of basic processing parameters can be selected to focus on specific aspects. For instance, apodization in the time-domain is applied to the vector length determined by *FID pts*, before zero-filling is applied up to the *ZF* number of points. Focusing on the initial points of an FID is therefore achieved by choosing the number of FID points small, and ZF small enough, not to exceed that choice.

Referencing of the spectral scale is possible by frequency assignment to a selected spectral position. For $^{1}$H MRS of the brain, this is typically achieved through dominant singlets like N-acetyl aspartate. Entering the 2.01 ppm frequency into the text field and subsequent manual peak selection via the *Assign* button will calculate and update the spectrum's calibration frequency (*Calib.*).

Dummy scans are commonly applied in MRS to mitigate potential *T1*-mediated amplitude effects. If the data acquired during these dummy scans are not discarded, but part of the data set as saved, the number of dummy scans needs to be assigned to the parameter *DS* to assure appropriate selection of water references for eddy current correction. This is not a common scenario and a DS of zero is typically the appropriate choice when 1) dummy scans have not been saved or 2) have not been acquired. Note that DS has no effect on the FID data selection which is achieved via the *FID (NR)* instead.

The number of phase cycling steps *nPC* can be assigned if a step-specific correction of eddy currents is intended (compare Klose correction below). This functionality, however, is rarely applied and under most circumstances the default value of one is appropriate.

The figures representing the uncombined, raw data are updated automatically when processing or visualization parameters are changed, e.g. receivers are added or the frequency calibration is changed. The comparison of spectral features at varying parameter settings, e.g. the impact of zero-filling, or the assessment of multiple independent data sets, requires the opportunity to preserve figures. The selection of the *Keep* flag will freeze a given figure and add date and time information for convenience.

### *Single MRS Experiment versus MRS Scan Series*

MRS typically suffers from limited detection sensitivity. The resultant limitations in signal-to-noise (SNR) ratio per acquisition can be mitigated by signal averaging, and a series of identical MRS acquisitions is, therefore, the rule for MRS. The *Data* page allows the consideration of individual metabolite scans with a number of repetitions (NR) of one or more per scan as well as a series of metabolites scans (multiple *NR*) separately. By assigning the same scan twice, a single scan can therefore also be considered and processed as a specific case of a metabolite scan series. Such assignment enables the use of the *Align*ment (#4) and the quality assessment (*QA*, #14) routines that are available for the multi-scan experiments (see below).

Note that while the processing of individual metabolite scans (potentially involving multiple *NR*) is still supported, it does not represent the way MRS experiments are typically executed. In practice, it is advisable not to acquire all repetitions of a given MRS experiment in one run but to break the scanning down to smaller blocks. Doing so, experimental corrections, e.g. of the scanner drift, can be applied more regularly; moreover, fewer data are lost if the session has to be aborted and online processing during experimental execution is possible. This approach leads to a *Series* of smaller data files of identical format instead of a single data file containing all data. Again, while the processing of single data files is still supported and therefore described in the next paragraph, it is not the typical mode of operation.

### *Data Processing of a Single Metabolite Scan*

The overall goal of the *Data* page is to convert the multitude of data associated with an MRS scan to a single FID that can be further processed or analyzed. First, data and parameters are read from file, and basic experiment information is displayed for review via the *Load* button. Then,

frequency and phase corrections are applied (#8), signals from multiple receivers are combined, and selected acquisition traces are considered for data averaging based on the selected processing options that are located below the parameter window (#9).

Typical processing of a single metabolite scan includes eddy current correction using the method proposed by *Klose* et al. [2] that is executed when the *Apply* button in #8 is pressed. This correction employs the eddy-current-induced phase behavior of the high-SNR water reference to rewind the corresponding erroneous phase evolution of the metabolite FID at hand. The subsequent averaging of signals from different receivers and repetitions is done when the *Mean* button is pressed based on the receiver and NR selections defined. If *Weighted* in #9 is selected, the average of signals from different receivers is weighted by individual receiver sensitivities as determined by signal ratios of the corresponding water references [3].

#### *Data Processing of a Series of Metabolite Scans*

The single scan assigned in *Data 1* (#3) is still used to define the overall directory of the experiment series and needs to contain one of the metabolite scans. The appropriate assignment is achieved, for instance, by selecting the first scan of the considered series as *Data 1*. The *Series* of specific metabolite scans is defined in the corresponding entry field below. Note that MATLAB's indexing format is supported: For instance, every other scan between 4 and 20 can be assigned by entering 4:2:20. The *Series* mode provides dedicated options for the visualization, assessment, and correction of serially acquired spectral data. These functions are described below and are not available for single metabolite scans.

## 1.2 Quality Assessment ('QA')

The basic data loading and formatting and receiver-specific fundamental aspects of the MRS data quality can be visualized on the *Data* page directly. The summation of spectral data for improved SNR rests on the assumption that the elements of the sum represent identical experiments. In reality, however, frequency drifts, scanner instability, subject motion/breathing, and other effects lead to variations across scan repetitions. It is, therefore, advisable to access the spectral data quality after the signals from individual receivers of a multi-receiver array have been combined to form one FID (or spectrum) per experimental trace and repetition time (TR). The quality assessment (*QA*) tool provides this functionality. The QA tool can visualize, quantify, and manipulate the array of individual traces both before and after phase, frequency or amplitude corrections are applied (compare next section). It therefore allows an assessment of the data quality obtained experimentally along with means to confirm and quantify the functionality of various alignment routines.

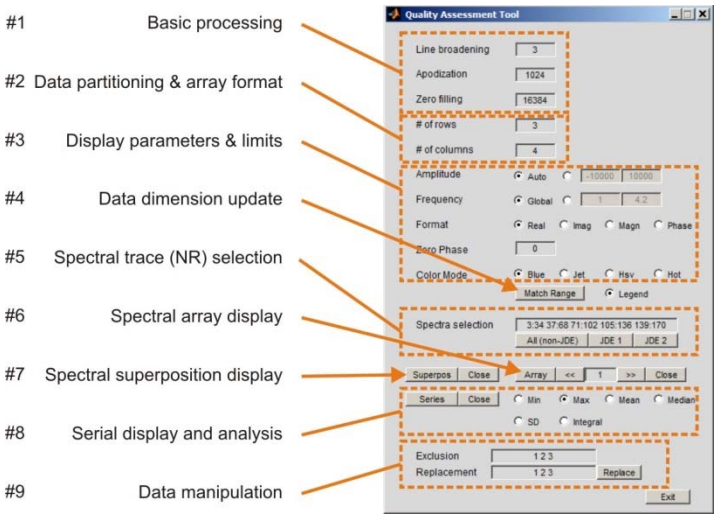



Fig. 1.3: Quality Assessment (QA) tool.

### *Parameter and Data Selection*

Spectral processing options (#1) include exponential *Line broadening* (in [Hz]), *Apodization* to shorten the FID (in [pts]), *Zero-filling* in the time domain (in [pts]), and an overall *Zero Phase* correction (in [degrees]). Arrayed displays are shown with the *number of rows* and the *number of columns* defined by the user (#2). The amplitude range to be visualized can be set directly (*Direct*) or determined automatically (*Auto*, #3). Similarly, a frequency range can be assigned (*Direct*); otherwise the *Full* bandwidth is shown. Various *Formats* of the considered data can be selected (*Real* / *Imag*inary / *Magn*itude / *Phase*) and displayed in different color modes (*Blue* / *Jet* / *Hsv* / *Hot*). Spectral selection allows selecting the set of individual traces (or repetitions NR) to be displayed and can be achieved directly via listing them in the entry field (#6). Note that MATLAB's colon format is supported, e.g., to select every third repetition between scan 10 and 100 as 10:3:100. Buttons have been added for user convenience for trace selection of typical MRS experiments: *All (non-JDE)* for all repetitions of an MRS experiment, *JDE 1* for all editing ON spectra and *JDE 2* for all traces of the editing OFF condition. Note that the button functionality considers the overall trace selection on the main *Data* page as an easy means for further trace handling.

### *Data Visualization and Analysis*

The selected spectral traces can be visualized as *Super*position, as rectangular *Array* of individual traces or longitudinally as a *Series* of traces. The related flags allow basic analysis options for the *Series* mode. Note that the resultant figures are updated (and potentially recreated) automatically when processing or visualization parameters are changed. Closing one or more of the above three display options properly via the *Close* button will prevent their automated recreation.

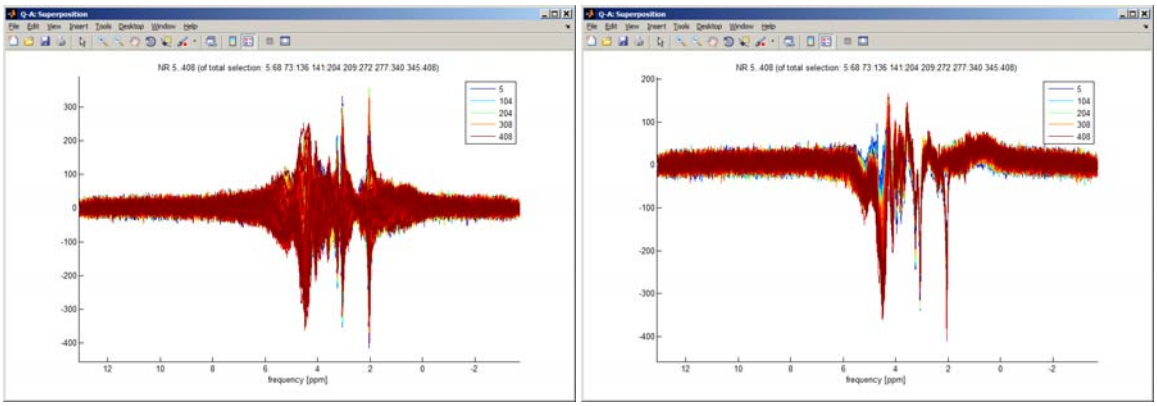



Fig. 1.4: QA Superposition of individual traces before (A) and after (B) frequency and phase alignment.

$B_0$ field alteration during the MRS acquisition induces phase changes in the obtained spectra. While this effect is less relevant for very short echo times, the resultant phase variations can be substantial if echo times are long. Figure 1.4A shows the superposition of the non-edited spectra from individual repetitions (NR) of a JDE experiment of GABA (TE 72 ms) acquired at 7 Tesla. Note that the phase variation is as large as 180 degrees, resulting in a full polarity switch in some cases. If these spectra were summed up without phase alignment, the consequences would be major peak distortion and signal cancellation. Phase alignment is therefore necessary before individual traces are combined to assure maximally constructive summation (Figure 1.4B).

Note that updates to any of the parameters of the QA tool will be immediately reflected in the superposition display. The color coding of the selected spectra is the only exception to support two different types of assignment. In general, the color coding is consistent for all modes of the QA tool in that any given spectral traces possess the same color in all displays including the arrayed and the serial modes described below. The number of colors is determined as the product of the number of rows times the number of columns. By default, the full color range is used between the first and the last spectrum of the array mode (compare Figure 1.5, blue to red). In some situations it is preferable, however, to scale the color to a specific NR selection. This is achieved by the matching the colors to the range of selected NR (#4).

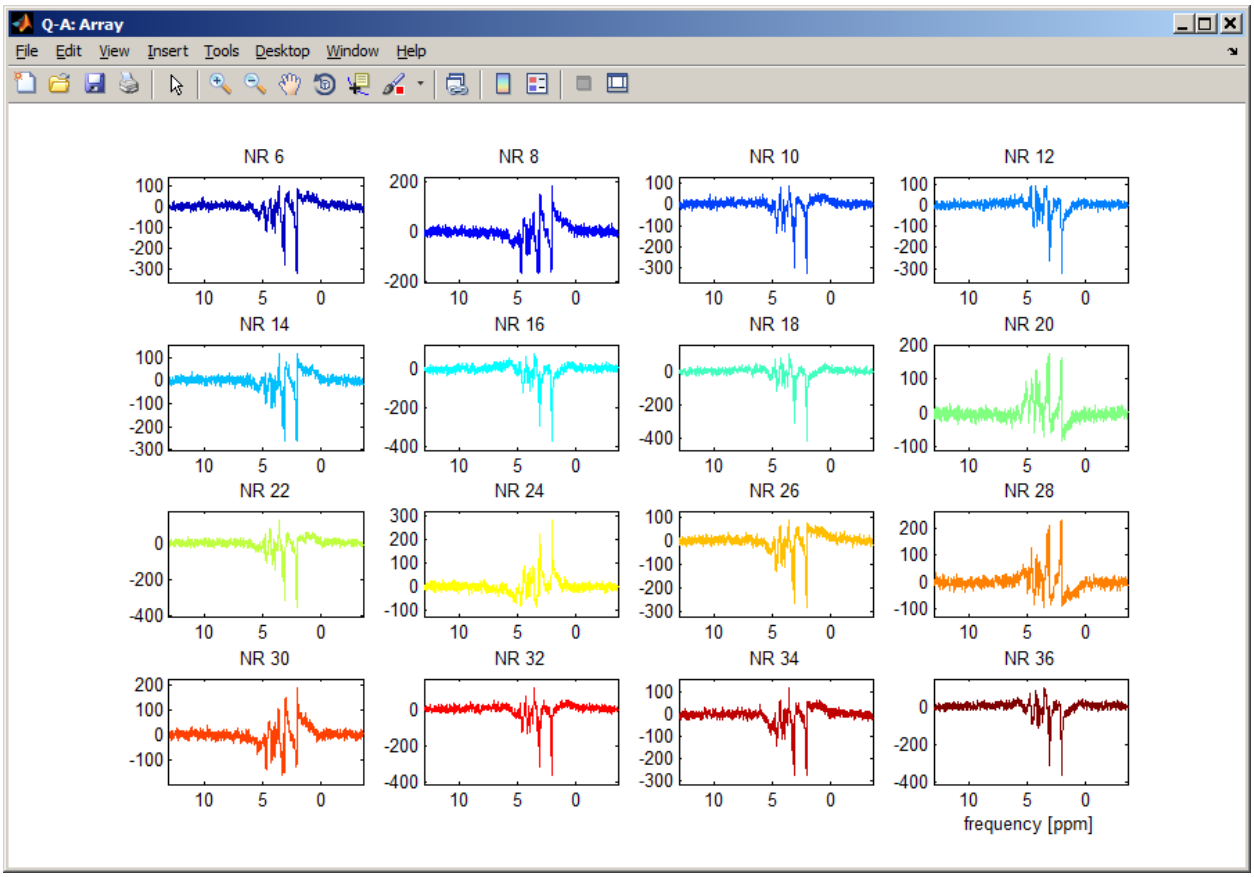



Fig. 1.5: QA Array of first 16 repetitions of non-edited condition from GABA JDE experiment before alignment.

The selected spectra can furthermore be visualized in an array. The side-by-side unobstructed display of individual traces sometime aids in assessing the apparent data quality and, moreover, the identification of problems or outliers. The numeric entry field and surrounding arrow buttons can be used to select a block of # columns times # rows to be displayed given the overall NR selection. If the above case, for instance, corresponded to the first block, block 2 would replace the first 4x4=16 spectra with the next 16 spectra for the NR selection at hand.

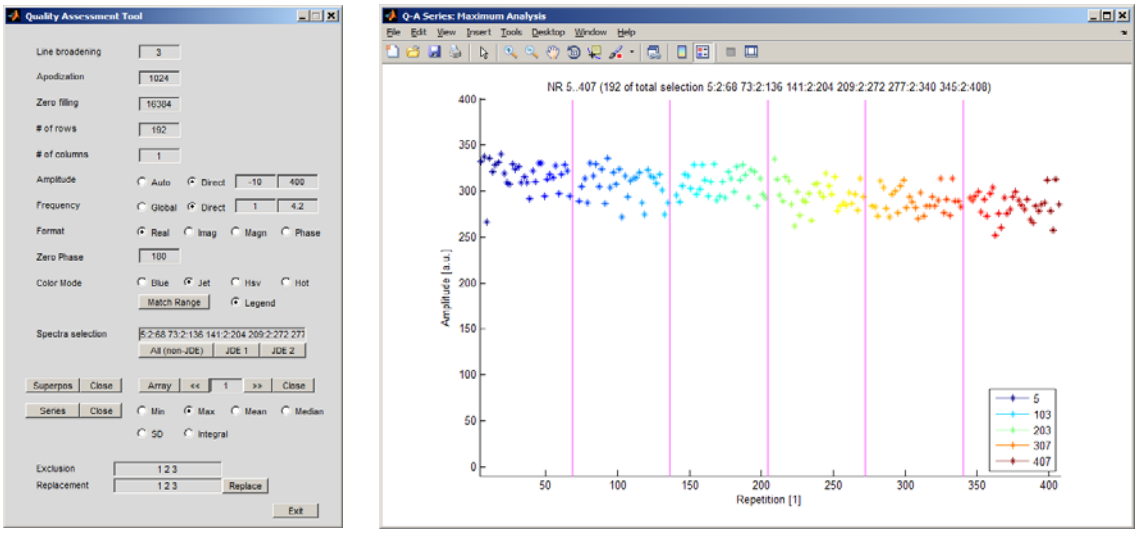



Fig. 1.6: QA Series of NAA peak heights of non-edited condition from GABA JDE experiment after alignment.

It is sometimes useful to confirm the stability or reproducibility of the acquired data stream. This functionality is provided by the *Series* mode that allows the serial analysis and display of 1) the minimum, 2) the maximum, 3) the mean, 4) the median, 5) the standard deviation and 6) the signal sum over the selected spectral frequency window. Common applications include the assessment of the water suppression efficiency or the reproducibility of the metabolite measurement by maximum values in the 4:5.5 ppm and 1.7:2.3 ppm windows, respectively.

## *Data Processing*

*In vivo* MRS can be challenging and individual traces might be corrupted by, for example, excessive subject movement. The quality assessment tool supports the replacement of individual (corrupted) traces of a spectroscopy experiment at hand by other (non-corrupted) traces of the same experiment. After QA, the corrected data set remains available in the original series format for processing and quantification. The traces to be replaced are assigned as a space-separated list in the *Exclusion* entry field along with a list of equal length in the *Replacement* field for the spectral traces to be used instead of the corresponding entries above.

Note that this functionality should be used with *extreme care* as the regular appearance of distorted MRS traces is indicative of suboptimal subject performance or study design. Replacing, and thereby duplicating, individual traces maintains the correct signal strength at the expense of some noise correlation. In other words, the SNR benefit of summing of independent acquisitions of uncorrelated noise contributions is not achieved via trace duplication.

The parameter window of the quality assessment tool should be closed by the *Exit* button of the graphical interface, not the close button of the window.

## 1.3 Spectral Alignment

INSPECTOR's alignment tool allows the evaluation and mitigation of frequency, phase and amplitude effects before individual traces are combined. The various corrections are modular and selected by the corresponding flags *Frequ.*, *Phase* and *Ampl.* on the main *Data* page (#4). The parameter settings for the alignment tool are selected on a dedicated parameter page that is opened by pressing the *Details* button. The alignment is then performed by pressing the *Align* button. The *Align QA* flag allows the display of detailed information of the alignment procedures along with the visualization of the results. Typical MRS processing involves mitigation of frequency and phase effects. The correction of amplitude effects is possible but should be applied with care. The existence of significant amplitude variations implies experimental conditions that probably warrant further investigation.

The verbose mode of the spectral alignment optimization (*Align QA*) opens figures for the first *# Verbose* frequency, phase and amplitude corrections for every block. While the serial appearance of these windows allows an efficient and convenient evaluation of the alignment functionality and the underlying parameter settings, it would be cumbersome if all these windows would need to be closed manually. A button (*X Align*) has therefore been included that selectively closes all windows related to the spectral alignment procedure.

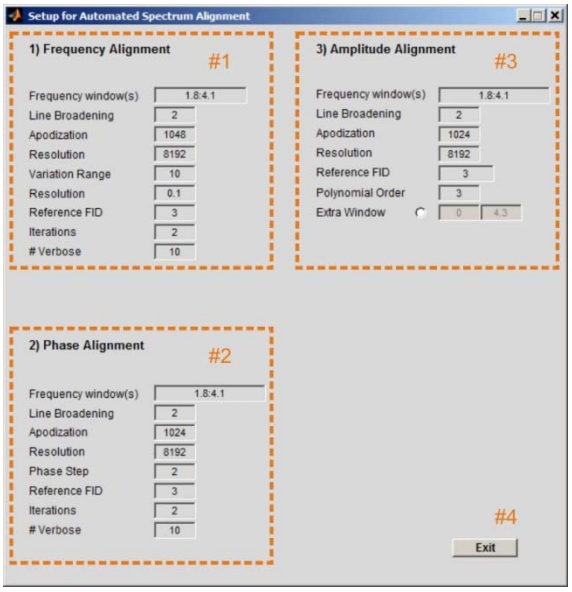



Fig. 1.6: Spectral alignment page.

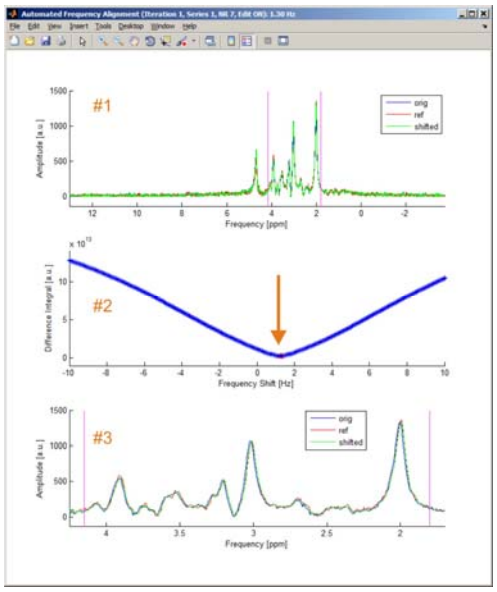



Fig. 1.7: Frequency alignment summary.

### *Frequency Alignment*

The frequency section of the alignment tool (#1) contains the parameters defining the processing of spectral information necessary to perform frequency alignment as well as the details of the alignment algorithm. Frequency alignment relies on the comparison of a MRS trace with a reference, and a cross-correlation algorithm is applied to minimize the deviation of the two. The

frequency correction is applied to one or more *Frequency window(s)*, defined as string in MATLAB format and ppm units. For instance, 1.8:2.2 7.5:8.5 considers the two target ranges between 1.8 and 2.2 ppm and between 7.5 and 8.5 ppm. Spectral processing options include exponential *Line broadening* (in Hertz), *Apodization* to shorten the FID (in points), zero-filling to define the frequency *Resolution* (in points), the maximum expected range of frequency alterations (*Variation Range*, in Hertz) and targeted *Resolution* of the frequency alignment (in Hertz), the *Reference FID* for comparison, and the number of iterations of the optimization algorithm.

The visualization of intermediate results (Figure 1.7) is enabled by the *Align QA* flag on the *Data* page. The figures contain 3 parts: 1) An overview display covering the full bandwidth (#1) indicating the selected frequency ranges for optimization by vertical pink lines. This window allows an assessment of the overall data quality including baseline behavior, water suppression efficiency and potential lipid contaminations. 2) The optimization curve covering the designated *Frequency Range* is shown in the central part of the figure (#2). The optimal frequency shift corresponding to a minimum deviation integral, i.e. maximal spectral overlap, is then applied as correction. 3) A zoomed window (#3) focusing on the frequency range(s) selected for optimization is included for the comparison of the original spectral trace (blue), the reference used for alignment (red) and the resultant spectrum after frequency correction (green). The underlying algorithm is insensitive to potential amplitude differences; however, the assignment of frequency limits to peak positions should be avoided. The latter can be confirmed in the zoomed display and corrections can be applied if necessary. In addition, identifying information on the selected trace is added as a label to the top of the figure.

Note that the single *Reference FID* is used for the first iteration, whereas all spectra (that have been frequency-corrected in the first run) are combined for improved SNR and used as a reference for the second and further iterations. The use of condition-specific references is supported for JDE experiments to account for potential systematic differences such as improved water suppression efficiency with the application of editing at the 4.56 ppm position for JDE of the GSH cysteine moiety.

### *Phase Alignment*

The phase alignment (#2) is based on a set of parameters that largely resemble the processing options for frequency alignment. Note, however, that this set of parameters is fully independent of those for the frequency and amplitude alignment routines to potentially account for different processing requirements. Parameters specific to phase alignment include the *Phase Step* size to define the targeted phase *Resolution* (in degrees).

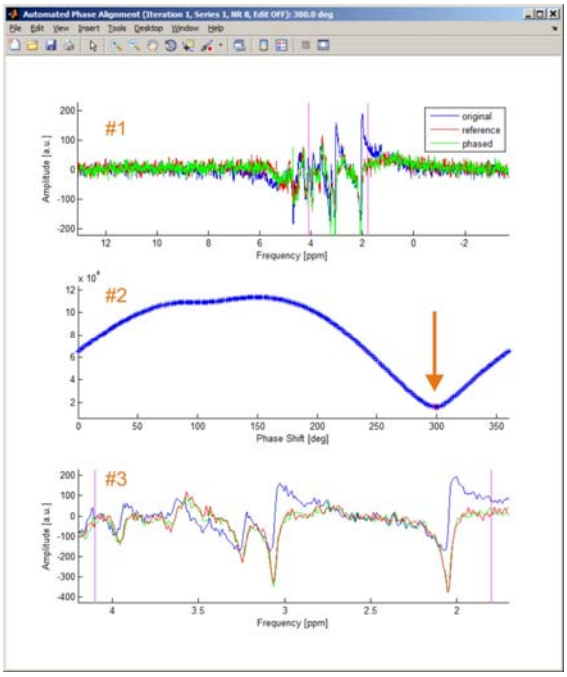



Fig. 1.8: Phase alignment summary.

### *Amplitude Alignment*

Similarly, most processing parameters for amplitude alignment resemble those of other corrections. Specifics are limited to the way the amplitude variation is modeled along the series of experimental traces (*Polynomial Order*, in [1]). An *Extra Window* can be shown for result visualization. Significant amplitude variations are typically a sign of suboptimal experimental conditions and this functionality should therefore be used with extreme care due its immediate impact on signal amplitudes and thus derived concentrations.

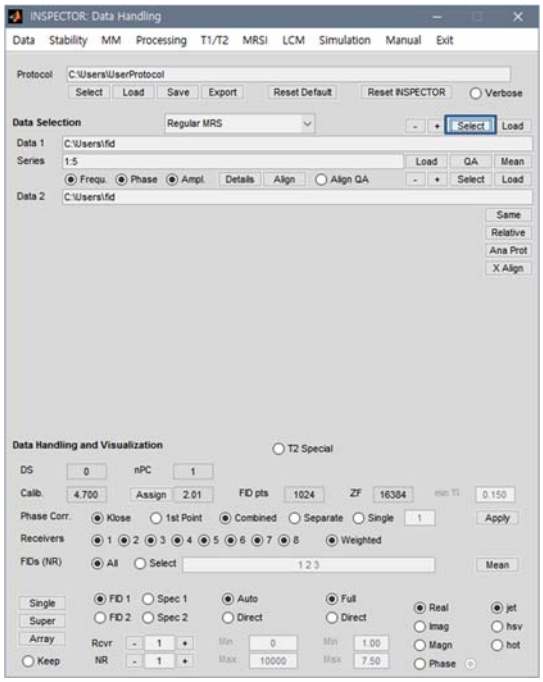
Note the order of the applied corrections: The frequency is aligned first for a given iteration, before the phase correction is applied. It is, therefore, advisable to select a number of phase corrections at least as high as the number of frequency alignments to minimize the risk of impacting the phasing behavior by suboptimal frequency positions. The amplitude correction is applied once after all iterations of the frequency and phase corrections have been completed.

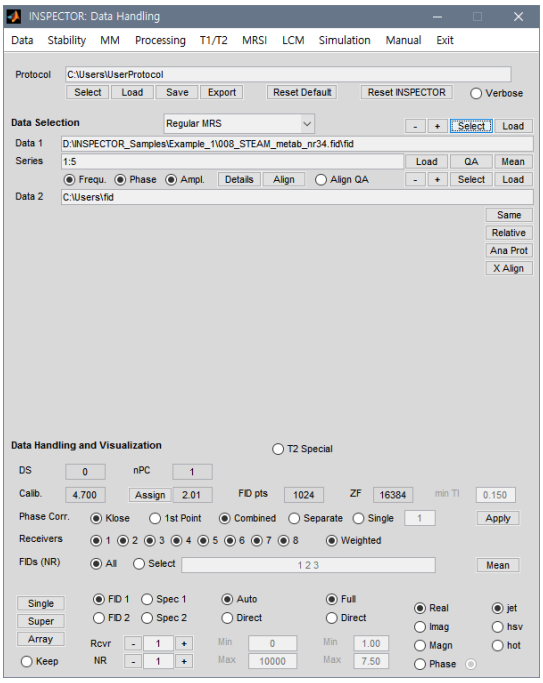
The spectral alignment tool should always be closed by the *Exit* button of the graphical interface, not the close button of the window itself.

## 1.4 Usage Example: Single-Voxel MRS

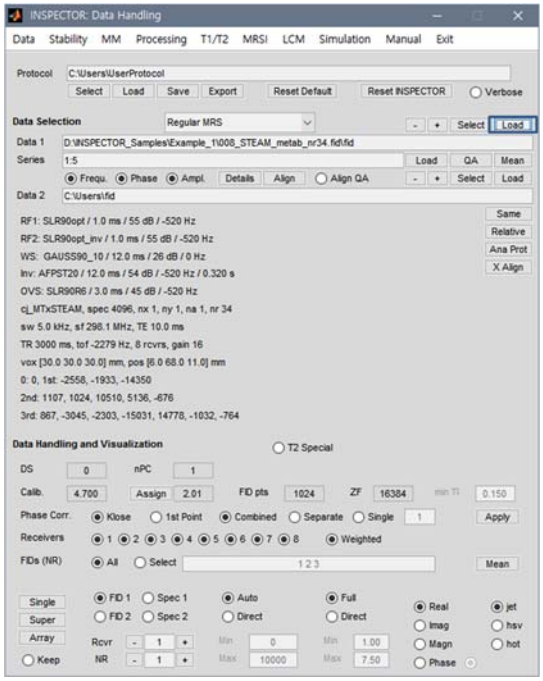
Typical processing of a single-voxel MR spectrum includes the following steps:

1) Select/assign first metabolite scan as *Data 1*.

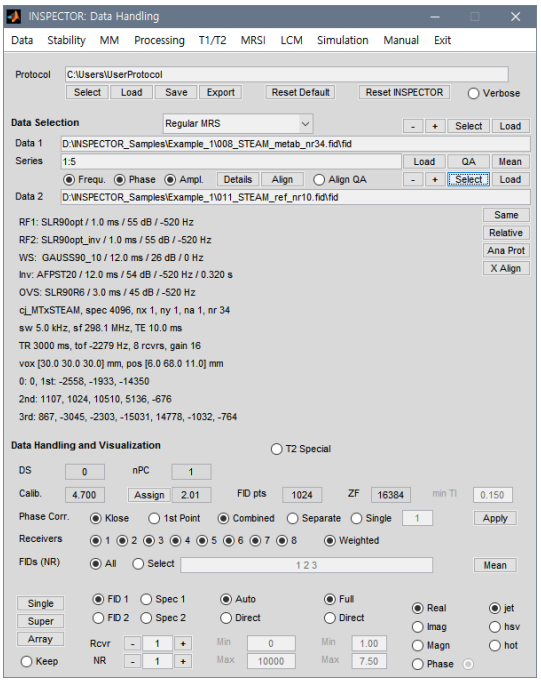


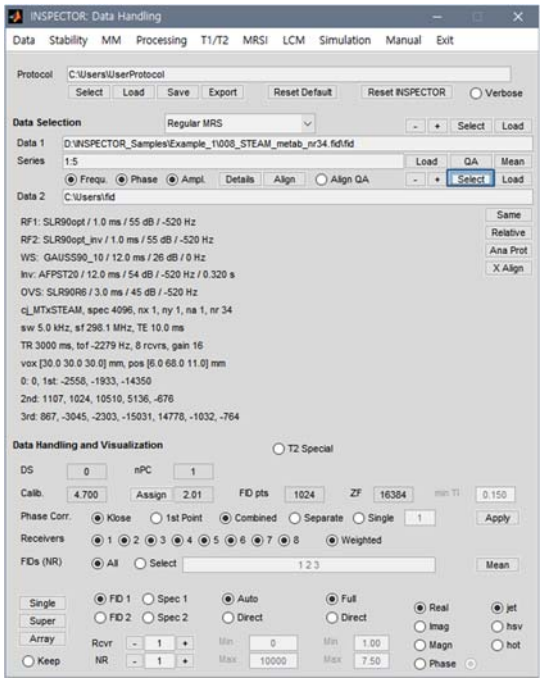




2) Load data set 1, review experimental parameters and check data formatting.

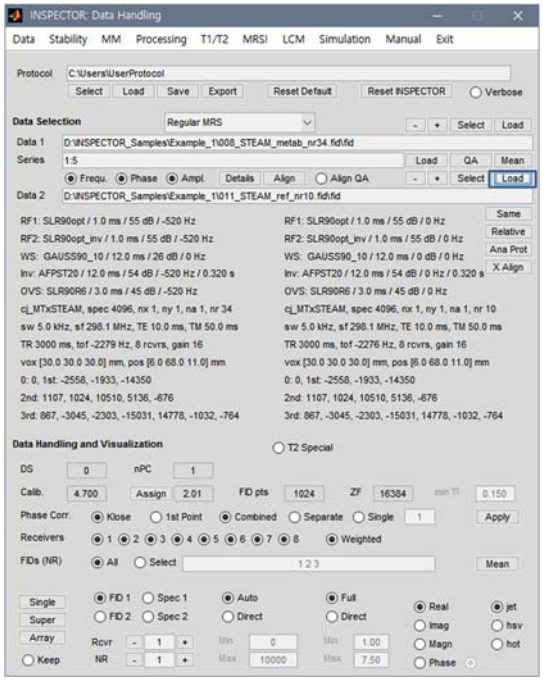


3) Select water reference as *Data 2*.

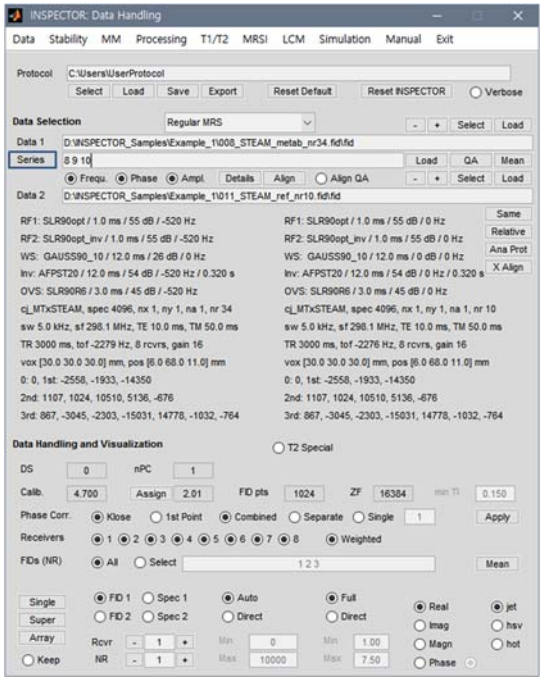


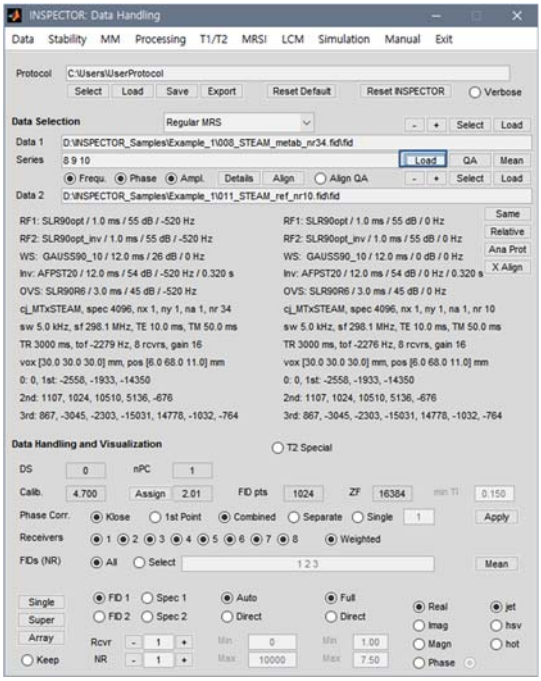




4) Load data set 2, review experimental parameters, and check data formatting.

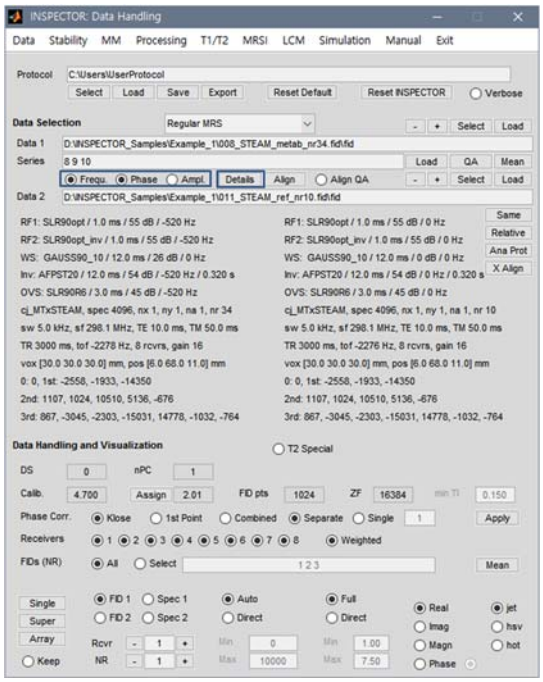


5) Assign a list of metabolite scans as *Series*. Load the entire series using the *Load* button next to the *Series* field.

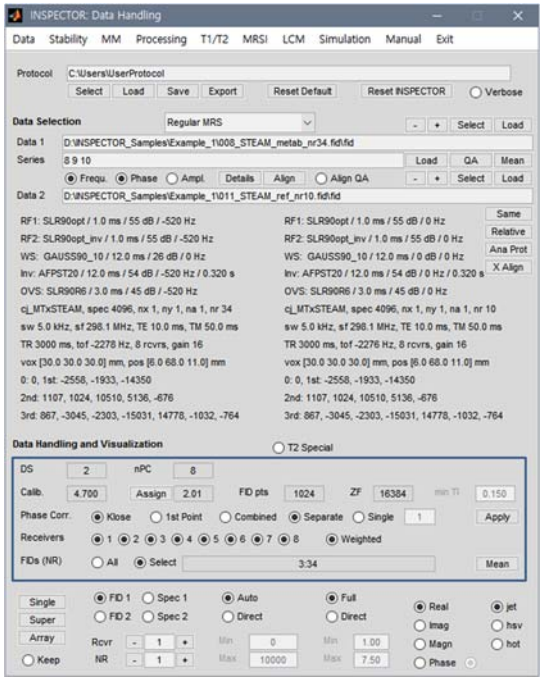


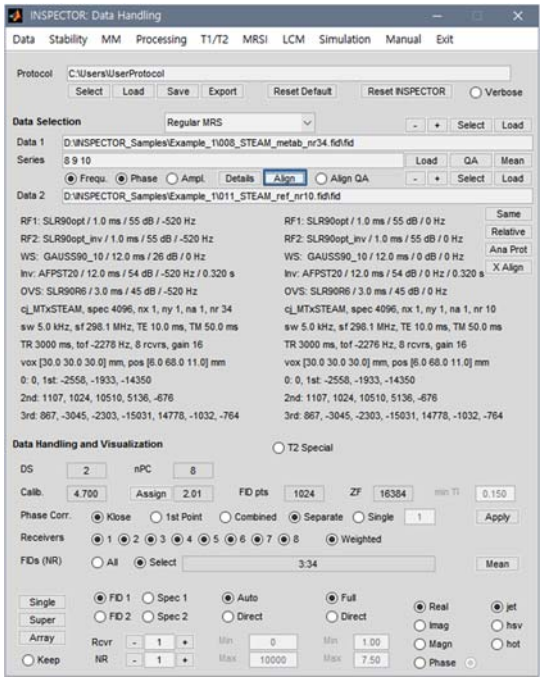

6) Enable direct alignment methods (Frequ., Phase, and/or Ampl.). Open the *Setup* for *Automated Spectral Alignment* by selecting the *Details* button (see Figure 1.6 for more information on this pop-up menu).



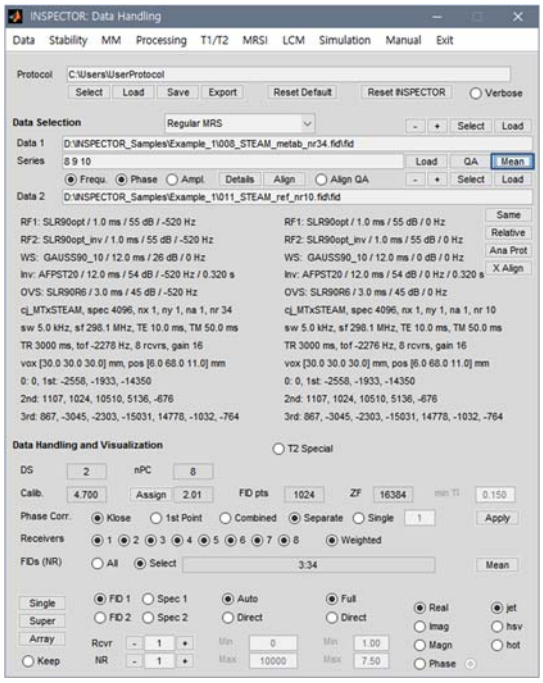
7) Specify additional appropriate data handling parameters like phase cycling, phase correction details, and included receivers and repetitions.



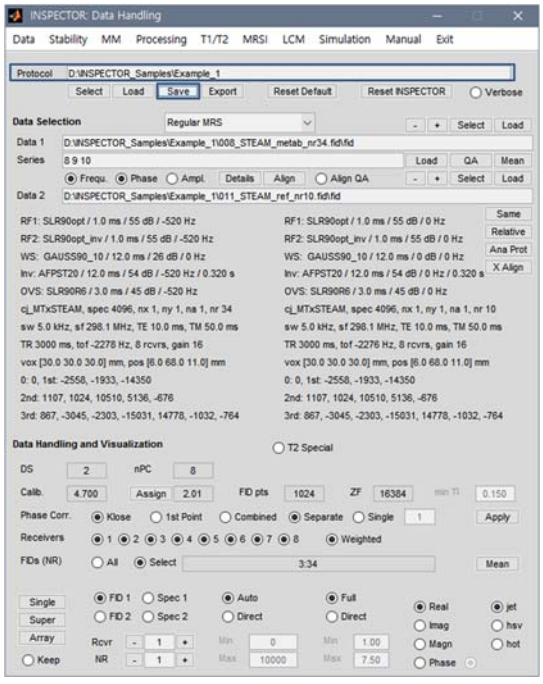

8) Perform alignment via *Align* button.



9) Average all metabolite traces via the *Mean* button.




10) If desired, save the alignment protocol as a .mat for future use by typing the desired file path into the *Protocol* field and clicking the *Save* button.




Note that INSPECTOR averages the experimental traces instead of the summing them together. As such, the resultant spectrum contains signals normalized per trace and is therefore

independent of the number of traces considered. In other words, experiments employing variable number of repetitions can be compared directly and no further scaling is necessary.

# II The *Processing* Page

## 2.1 General

The *Processing* page supports a variety of processing options for single spectra (e.g., an individual STEAM spectrum) or two spectra (e.g., from a *J*-difference edited or JDE experiment). One or two spectra can be assessed at once via the flags *1* or *2* on the upper left of the page. The data source is selected in the pull-down menu on top of the page. The *Data* selection retrieves experimental data that have been previously loaded and pre-processed on the *Data* page, whereas selecting *Proc* allows data loading from a file directly on the *Processing* page. Similarly, the *MRSI*, *LCM,* and *Sim* selections consider data from the MR spectroscopic imaging, the *LCM*odel quantification, or the *Simulation* pages, respectively (compare corresponding chapters). Note that the data have to be available from the respective page if not loaded directly from the *Processing* page. For instance, experimental data have to be loaded and pre-processed on the data page before they can be used on this page for further processing.

Supported format for the data to be loaded directly from the *Processing* page include INSPECTOR (.mat) format, the text (.txt) and parameter (.par) formats employed by R.A. de Graaf's *NMRWizard* software [4], as well as the data (.raw) and parameter (.coord) formats used by S. Provencher's *LCModel* software [5].

## 2.2 Processing of a Single Spectrum

A series of processing options is available that can be selectively enabled or disabled based on the corresponding flag selection. The processing options of an MRS spectrum include:

| | |
|---|---|
| Cut | Apodization of FID data vector [pts] |
| ZF | Time-domain zero-filling [pts] |
| LB | Lorentzian / exponential line broadening [Hz] |
| GB | Gaussian line broadening [Hz] |
| PHC0 | Zero-order phasing [deg] |
| PHC1 | First-order phasing [deg/Hz] |
| Scale | Amplitude scaling [1] |
| Shift | Frequency shift [Hz] |
| Offset | Amplitude offset [a.u.] |
| Stretch | Frequency stretch [Hz/ppm] |

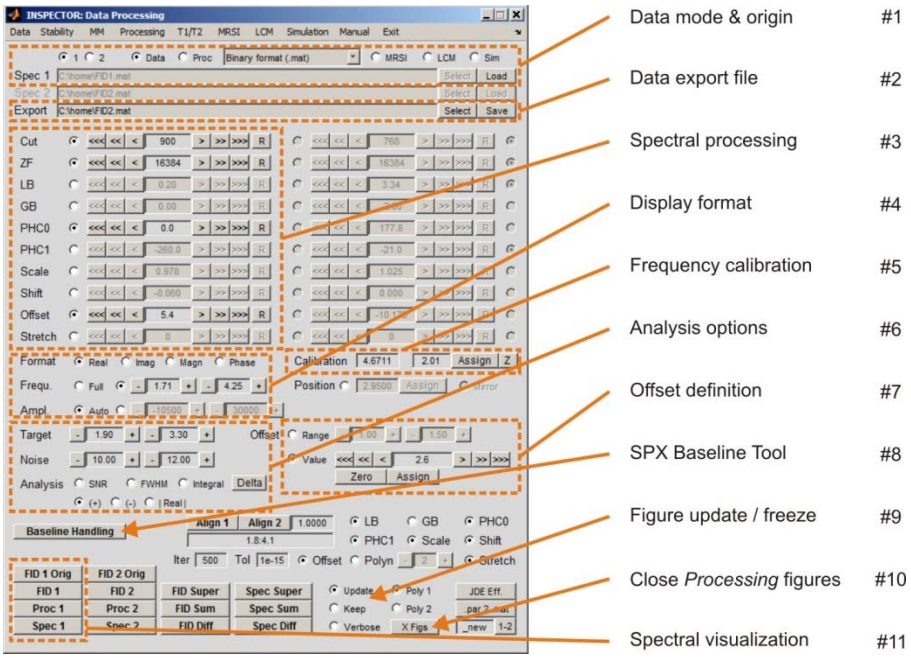



Fig. 2.1: Main *Processing* page for single spectrum.

### *Display of Original FID*

The visualization of the signal of the original free induction decay (FID) without any modification is useful to gain a general impression of the achieved data quality and properties (*FID 1 Orig*). It allows, for instance, determination of the point in time when the metabolite signals have decayed below the noise level as a starting point for the selection of apodization. The display is furthermore useful to appreciate the effects of further processing as described in the next section.

### *Display of Processed FID*

The visualization of the processed FID allows appreciating the effects of data processing directly on the FID signal (*FID 1*).

### *Automated vs. Explicit Processing*

In most cases, the user wants to see the effects of the altered processing parameters immediately. This functionality, i.e. the automated reprocessing with every parameter modification, is provided if the *Update* flag is selected (#10). If the flag is not selected, spectral processing and data update are performed only when the *Proc 1* button is pressed.

### *Display of Spectrum*

The spectrum resulting from the selected processing options is shown when this button is pressed (*Spec 1*).

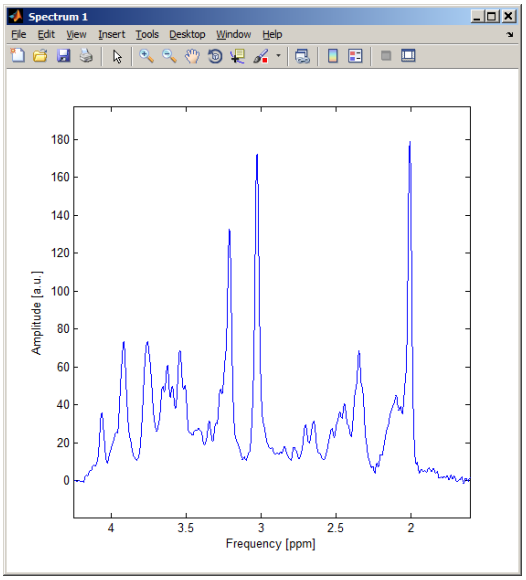



Fig. 2.2: Single spectrum.

### *Basic Spectrum Visualization and Analysis*

The processing panels are followed by a series of display and analysis options that are described in the following. Both FIDs and spectra can be displayed in different *Format*s, namely by their real parts, by their imaginary parts, as magnitude or as phase signals. Spectra are shown either with their full *Frequency* bandwidth or over a selected frequency window. Similarly, spectral amplitude can be *Auto*matically scaled to cover 90% of the figure display or a directly assigned range. The frequency *Calibration*, i.e. the reference frequency of the spectrum, is either assigned directly or a known frequency (e.g. the NAA $CH_3$ resonance at 2.01 ppm) is assigned manually to derive the calibration frequency. Notably, this functionality always employs the figure that has been opened last and thereby provides full flexibility for different experiment types. The *Z* button provides the same functionality, however, in a window zoomed to the frequency value to be assigned. Doing so, the selected display bandwidth is not affected and unnecessary clicking is avoided, thereby streamlining the processing workflow. In addition, a vertical line can be added to a given/assigned frequency to emphasize specific spectral aspects such as an expected peak *Position*. The visualization of a second vertical line mirrored around the calibration frequency can be added if needed. The latter might help distinguish true spectral signals from potential vibration artifacts.

Several *Analysis* options are provided for the assessment of basic spectroscopic data parameters, namely the signal to noise ratio (*SNR*), the full width at half maximum (*FWHM*) and the integral of a given spectral *Target* region. Peak picking is applied for the calculation of *SNR* and *FWHM*,

and selection of a *Noise* region is required for the SNR calculation. Notably, detrending of the noise area with a second order polynomial is applied to remove shallow baseline effects; however, it is the user's responsibility to avoid significant signal variations and spectral peaks. The *Offset* defining the vertical level of these *SNR*, *FWHM* and *Integral* analyses is selected 1) as mean value of an assigned frequency *Range*, 2) as numeric value, 3) set to zero, or 4) assigned manually in the figure that has been opened last. Note that both the SNR and FWHM analyses can be applied to both positive (*+*) and negative (-) peaks. In addition, the frequency difference between two spectral positions is accessible via the *Delta* button and subsequent manual assignment of the target frequencies.

#### *Figure Preservation*

In some situations, it is helpful to compare various spectra of the same kind with different processing details side by side. The explicit extraction of figures is supported by the *Keep* functionality (#10). When selected, displayed figures are disconnected from further updating, and new figures are created with every modification. Additionally, the current date and time are added to the figure label for further referencing.

#### *Data Export*

The *Processing* page allows a wealth of different spectral results that can be saved to the file assigned to #2 or transferred to other INSPECTOR pages. The software always saves or exports the last spectrum that has been visualized. Simple visualization thereby allows straightforward extraction of any given spectrum, including further modifications such as baseline corrections (compare section 2.3) or the difference spectrum of a *J*-difference experiment (example in section 2.5). Available export formats include INSPECTOR's own format (.mat), the text format (.txt) and LCModel's .raw format and is selected by the pulldown menu that is used to define the data format to be loaded from file on the top of the page. In all cases, the time-domain signal is saved to file.

## 2.3 Baseline Correction

A dedicated tool is available via the *Baseline Handling* button for *Polynomial*-, *Interpolation*- and *Hankel Singular Value Decomposition (SVD)*-based baseline determination and correction. In all cases, a variable sequence of frequency windows is assigned for the region-specific determination of global baselines (*Calc 1/2*). Note that the corresponding correction has been separated from the baseline determination. The correction is applied only if specifically selected (*Corr 1/2*) to allow optimization of the baseline processing without need for repetitive data loading. After the derived baseline correction has been applied to data set 1 (*Corr 1*) or data set 2 (*Corr 2*), the *Baseline Tool* can be closed to return to the main *Proc*essing page.

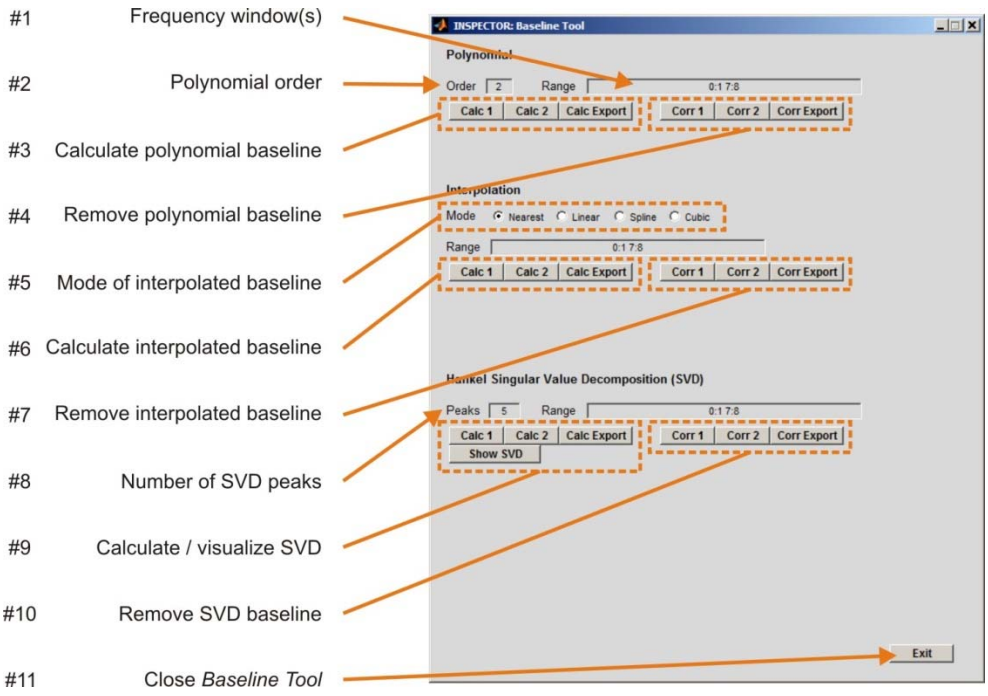



Fig. 2.3: Baseline Tool.

The baseline tool should be closed by the *Exit* button of the graphical interface, not the close button of the window.

Note that baseline processing is somewhat different from regular spectral processing on the main *Processing* page. There are two ways the corrected data can be considered for further processing. Immediately after the baseline correction has been applied, e.g. to data set 1, the resultant data are found 1) in data set 1 and 2) in the memory location that is passed on to other INSPECTOR pages or saved to file. As such, 1) the data can be immediately imported to other INSPECTOR pages or 2) explicitly saved to file via data export on the *Processing* page. Note that every parameter change on the *Processing* page will cause the original (uncorrected) data to be reprocessed if the *Update* option is selected and the effects of the baseline correction are lost. Further processing, e.g. phasing after SVD water removal, can be achieved by exporting the baseline-corrected data to file (figure 4.1, #2). If the same file is then assigned and loaded as data set 1 (figure 4.1, #1) all subsequent processing is applied to these baseline-corrected data.

## 2.4 Processing of a Pair of Spectra

All processing options can be independently applied to a second spectrum in the dual spectrum mode (#1, *2* in upper left). Individual processing options can be linked and thereby modified in synchrony if the corresponding flag at the total right end of the INSPECTOR is selected (#4).

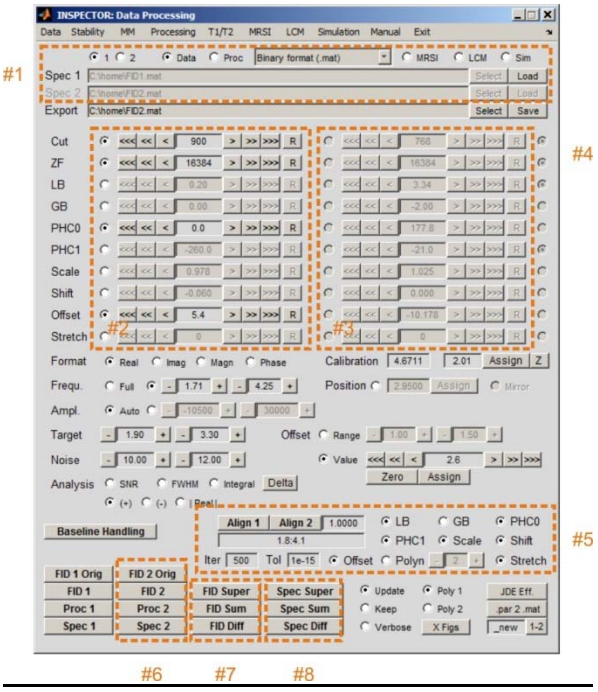



Fig. 2.5: Main *Processing* page for pair of spectra.

### *Display of Data Set 2*

Similar to data set 1, the original FID, the processed FID, and the resultant spectrum can be visualized with the corresponding buttons (#6).

### *Combination of Data Sets 1 and 2*

A variety of options are available for the combination of both data sets either in time- (#7) or in frequency (*#8*) domain. While the FID manipulations are largely for illustration purposes, the superposition, sum, and difference of spectra are regularly used for the processing of J-difference edited MRS. Remember that any of these signals can be exported to file (*Export*) or transferred to other INSPECTOR pages by ensuring that they have been visualized last.

### *Spectral Alignment*

The comparison of spectra and moreover MRS techniques relying on subtraction schemes require the spectral alignment and the minimization of secondary spectral aspects. For instance, spectra from an edited and a non-edited condition are subtracted with J-difference editing to reveal the edited metabolite. Minor differences in frequency or phase lead to subtraction artifacts that can resemble the magnitude of the metabolite signal at hand, thereby leading to suboptimal results. Parameters not essential to the individual spectra are therefore commonly matched to minimize these subtraction artifacts. While the manual adjustment is feasible for some basic parameters, it is cumbersome and becomes progressively less feasible with the inclusion of increasingly

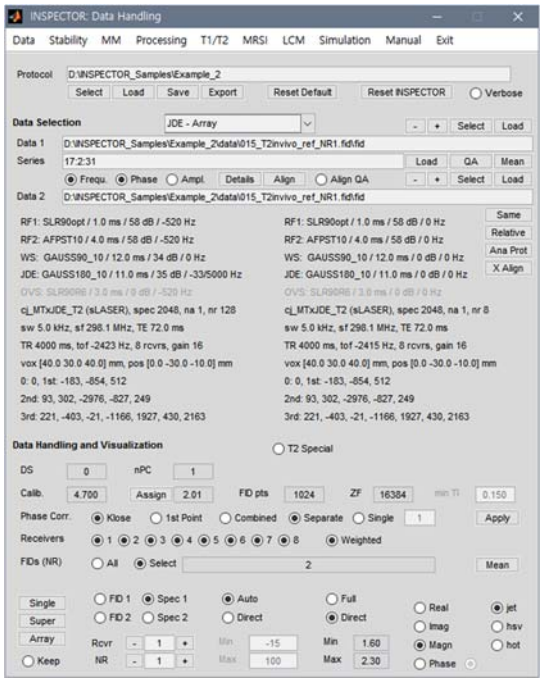
complex corrections such as a high-order polynomial baseline. The software therefore provides automated alignment algorithms to match a selection of parameters of spectrum 1 with spectrum 2 (*Align 1*) or vice-versa (*Align 2*). The alignment procedure employs a least-squares optimization based on the Levenberg-Marquardt algorithm [6,7]. The frequency alignment is based on a set of parameters that largely resemble the processing options (#5). More specifically, the supported optimization parameters include 1) exponential line broadening *LB*, 2) Gaussian line broadening *GB*, 3) zero-order phase *PHC0*, 4) first-order phase *PHC1*, 5) amplitude *scaling*, 6) frequency *shifting*, 7) a plain offset or, alternatively, 8) a variable-order polynomial baseline and 9) a stretching of the frequency axis. Please note that parameters should be selected with care to avoid the introduction of bias. The spectral bandwidth to be matched is assigned as a colon-separated pair of frequencies describing the minimum and maximum limits in ppm. The use of an arbitrary number of such frequency sectors is supported and can be assigned as a space-separated list. For instance, 2.6:2.9 3.1:3.6 3.9:4.1 considers the three frequency windows [2.6 2.9], [3.1 3.6] and [3.9 4.1] ppm for the alignment. For JDE of GABA, this selection considers only frequencies for which identical spectral signals are expected between the edited and the non-edited conditions, thereby avoiding areas affected by the editing pulse (e.g. NAA $CH_3$ at 2.01 ppm), co-edited signals (e.g. Glutamate/Glutamine around 3.75 ppm) or the edited GABA signal itself (at 3.03 ppm). Additional flags *Poly 1/2* are included to allow the application of the determined baseline to the spectra 1 and 2, e.g. for subsequent export or LCM analysis.

Additional analysis options, and routines for batch data processing and conversion are available and will be explained in more detail in a future release. Briefly, these include the determination of the achieved editing efficiency for GABA JDE (*JDE Eff.*), the import of metabolite libraries generated with Dr. de Graaf's SpinWizard software (*.par 2 .mat*), as well as batch calculation of difference signals from simulated metabolite libraries for LCM basis set generation (*1-2*).

## 2.5 Usage Example: *J*-Difference Editing of Glutathione

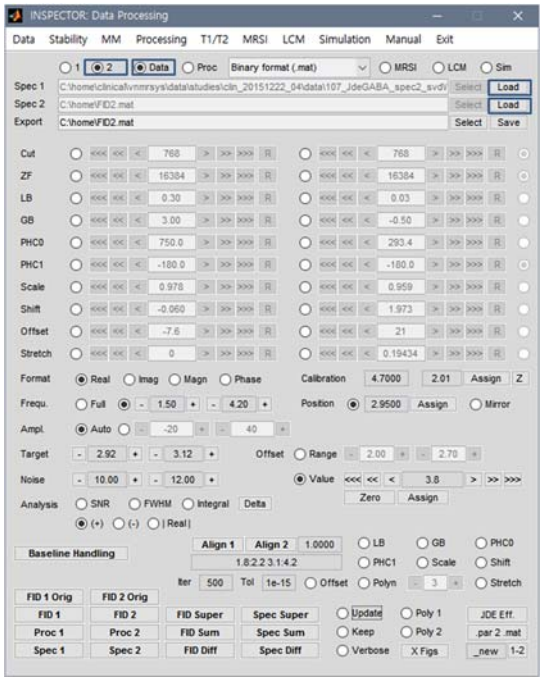
Typical processing of J-difference edited MR spectra from a single-voxel include the following steps:

1) Pre-process the experimental JDE spectra on the *Data* page and average the aligned spectra. Note that three options exist for JDE experiment data selection; it is important that the appropriate experiment type be chosen for each experiment. See *Section 1.1. General – Experiment type* for more details.

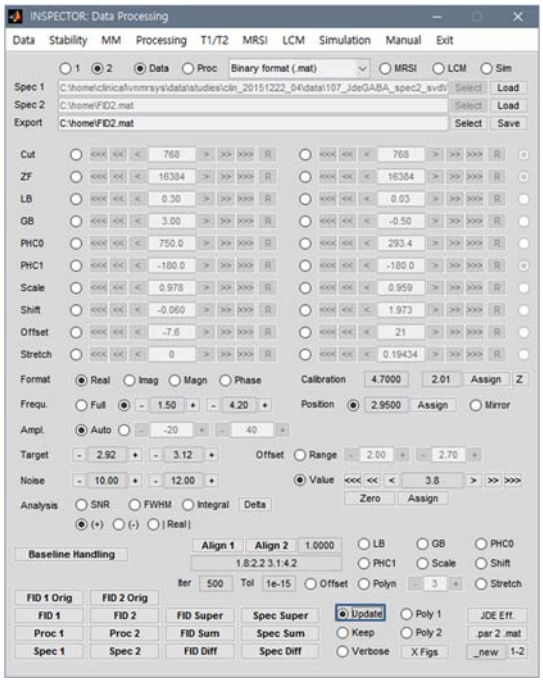



At this point, spectra have been aligned within their respective editing condition, and receivers and repetitions have been summed together in a condition-specific fashion. The alignment the editing ON with the editing OFF condition; however, has not been done yet.

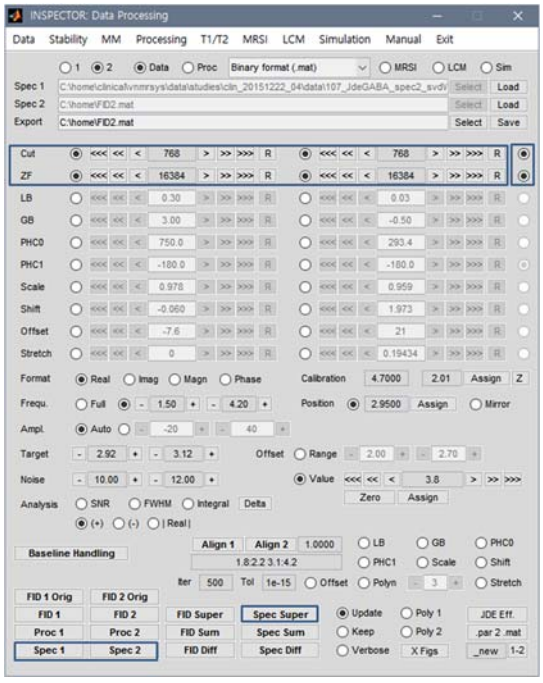
2) Select spectral processing mode *2* and *Load* the MRS of the editing ON and the editing OFF conditions as spectrum 1 and 2, respectively (note that ON is automatically assigned as *Spec 1*), from the *Data* page as selected.



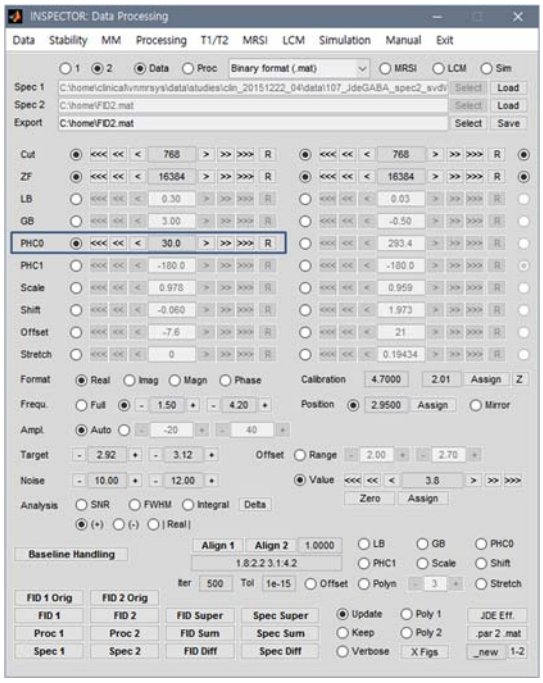
3) Select the *Update* flag to enable automated spectrum updating



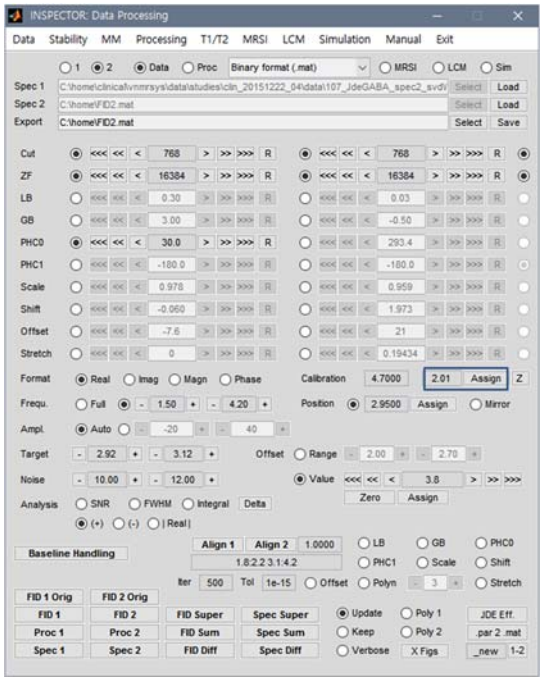

4) Select shared processing parameters for both spectra, linking them if desired using the rightmost flag for each parameter. Visualize spectrum 1 and 2 separately using the *Spec 1* and *Spec 2* buttons or superimposed using the *Spec Super* button.

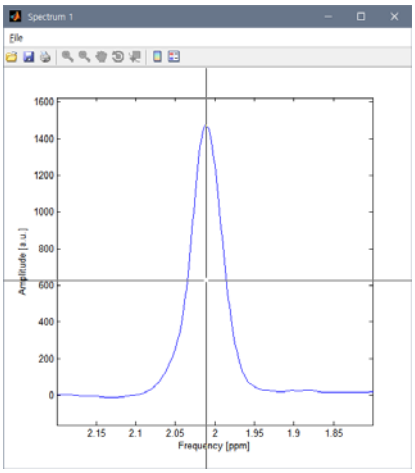


5) Apply manual zero-order phase adjustment of spectrum 1.

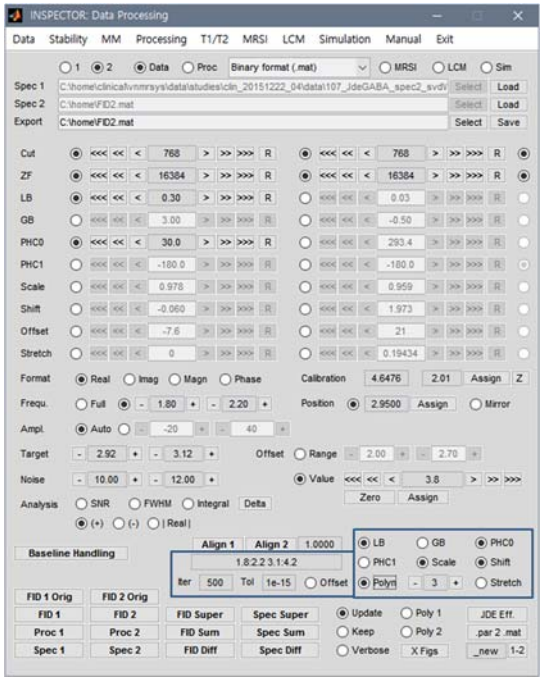



6) Apply manual frequency calibration of spectrum 1 by assigning a predefined ppm value to a known resonance (e.g., N-acetyl aspartate singlet at 2.01 ppm). Type the known ppm value into the field left of the *Assign* button. Select *Spec 1* and then click Assign to open a crosshair for manually assigning this value to the desired spectral frequency. The value of the reference frequency to the right of *Calibration* should change accordingly.

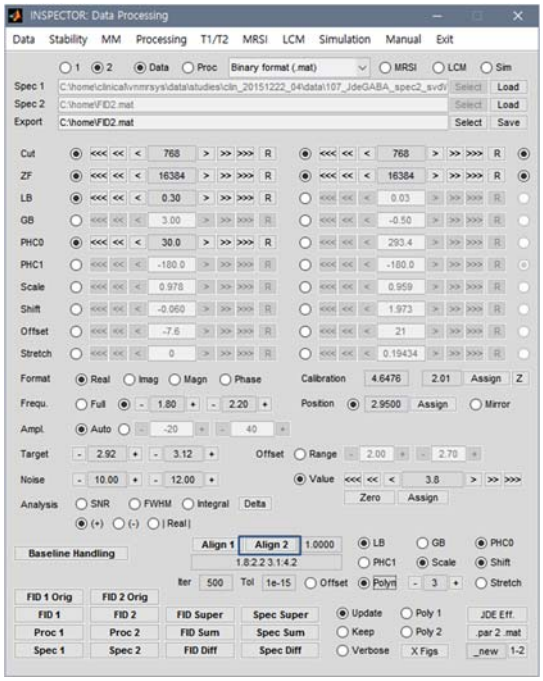


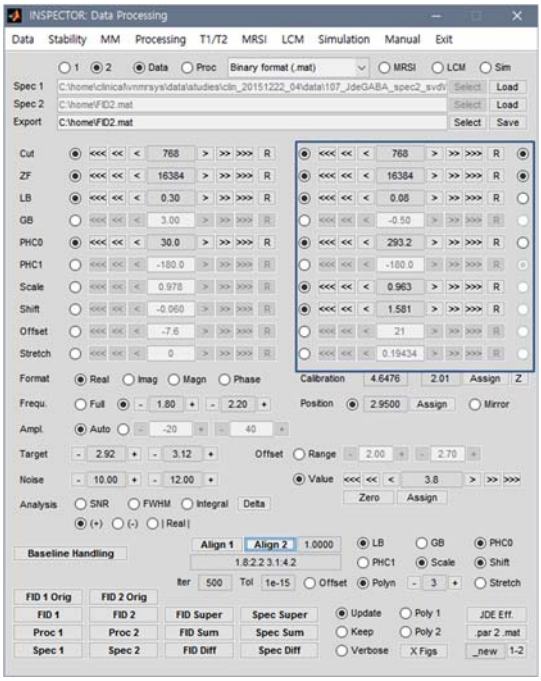



7) Specify the parameters to be aligned (PHC0, offset, LB, baseline, etc.), the frequency bands to be considered as well as desired alignment iterations and tolerance in the text field under the *Align 2* button.

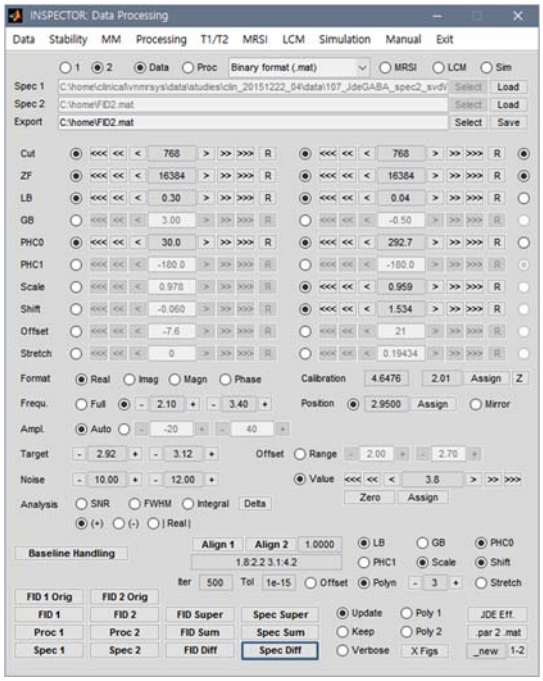


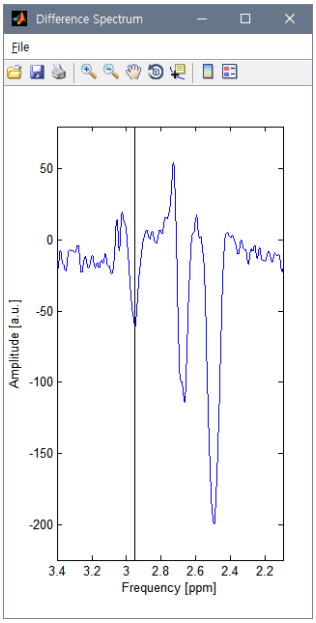

8) Perform automated alignment of spectrum 2 with spectrum 1 (via *Align 2*) to match the selected processing parameters of spectrum 2 with those of spectrum 1. Note how the specified processing parameters of spectrum 2 change as a result of the automatic alignment.



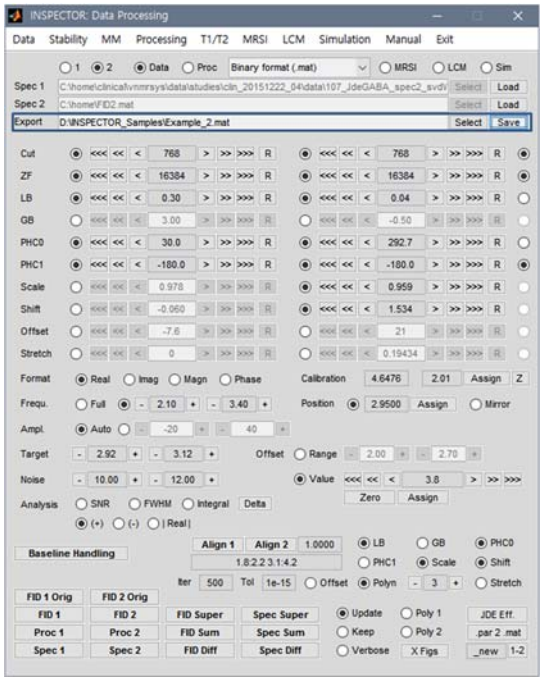



9) Display the difference spectrum (and define difference spectrum as the data set to be exported/saved) via *Spec Diff* button. Note that a ppm value of interest (in this case, 2.95 ppm for the J-difference-edited glutathione cysteinyl resonance) can be flagged using the *Assign* button to the right of the *Position* header.




10) Save difference spectrum to a specified file path via the *Export* functionality on the *Processing* page or leave in memory for transfer to the *LCM* page and quantification.



# III The Relaxation Time (*T1/T2*) Page

## 3.1 General

Quantitative MRS relies on exact knowledge of the *T1* and *T2* relaxation times. Their experimental determination is commonly applied, and the necessary processing algorithms for data visualization and processing are provided here. Note that data can also be assigned directly and the page, therefore, also serves as a general *T1* and *T2* fitting tool.

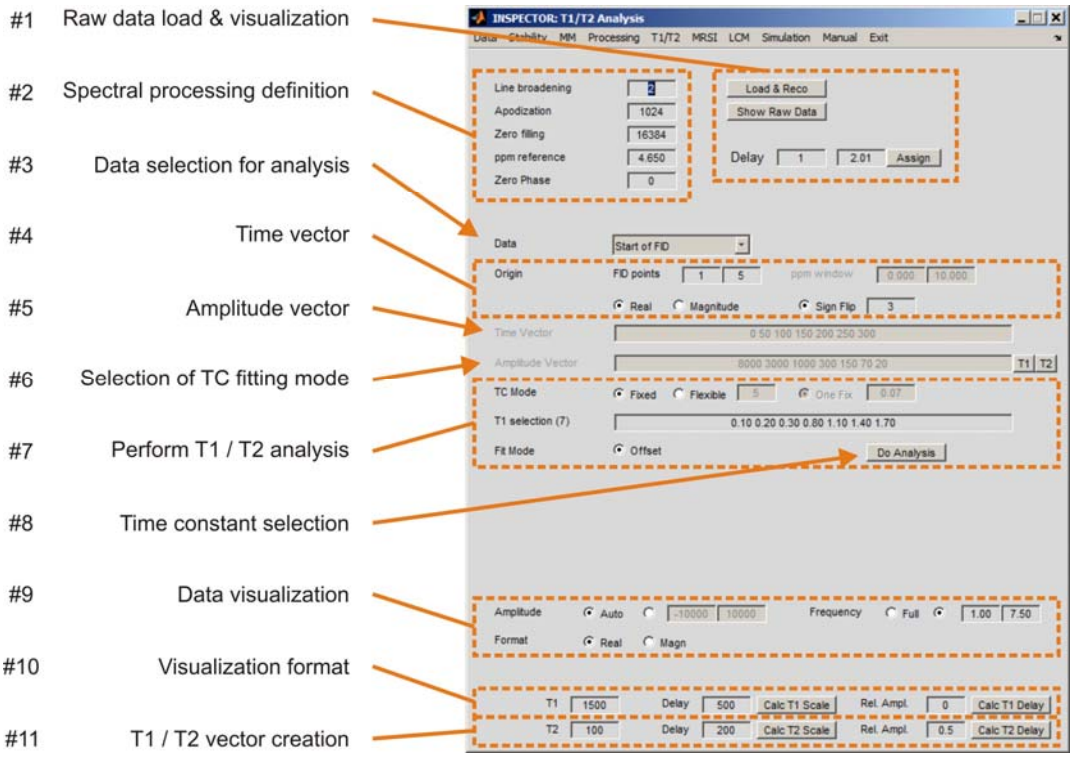



Fig. 3.1: Main *T1/T2* page.

The determination of T1 and T2 time constants of a variety of data types and conditions is supported on this page (#3, pulldown menu). These include 1) the first point(s) of an array of FIDs, 2) spectral peaks and 3) the integral of spectral peaks. In addition, vectors can be assigned directly and decomposed into their 4) T1 and 5) T2 components. If experimental data is to be analyzed, this data is expected to be loaded and preprocessed on the *Data* page before it is loaded and reconstructed (#1, Load & Reco) on the *T1/T2* page for the basic processing parameter assigned on the left (#2). A visualization option is available (#1, *Show Raw Data*) for basic data assessment.

Exponential fitting is then applied based on the parameter selection in #6 to derive the contributing T1 or T2 components. Note that time constants can be set or optimized, and an offset can be included in the optimization.

The fitting result is visualized given the data *Format*, *Amplitude* range and *Frequency* window assigned in section #10.

In addition to the derivation of time constants from data vectors, data vector can also be generated for a given time constant and amplitude behavior for both T1 and T2 (#11).

The *T1/T2* page enables the quantification of relaxation time constants and has been developed primarily for the analysis of spectroscopic data obtained by customized T1- and T2-encoded MRS methods on a Varian MR scanner. We are in the process of establishing equivalent methods on MR systems of other vendors and will make them available as soon as they are complete, thereby allowing the measurement of T1s and T2s of biochemical compound based on the full functionality of this page.

## 3.2. Usage Example: $T_2$ Fitting

As a basic example the analysis of water T2 at 7 Tesla measured with a STEAM sequence (TE 10 ms, TM 50 ms) is shown.

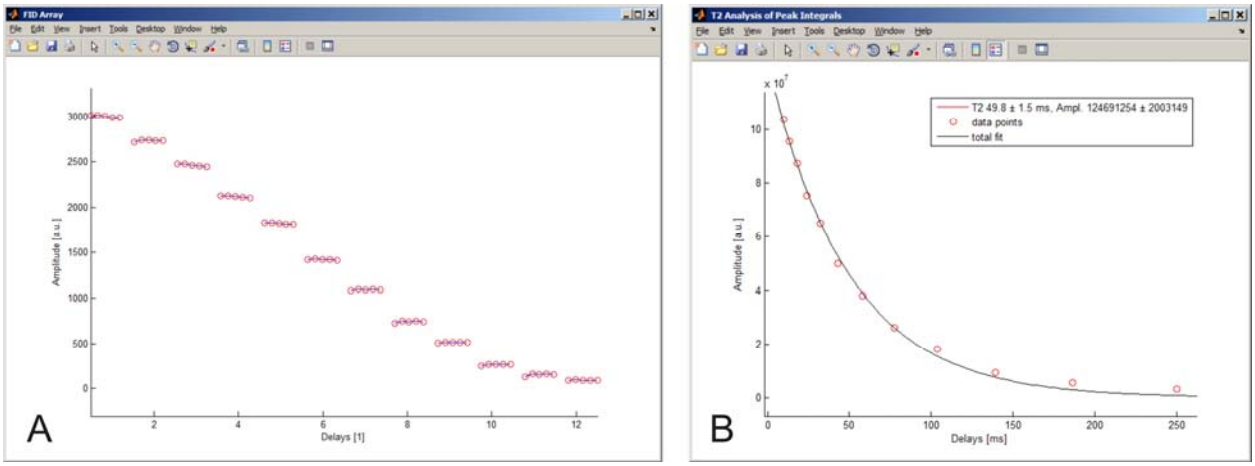



Fig. 3.2: Example T2 analysis.

Fitting of points 1 through 5 of the magnitude FID (A) with a single exponential with a flexible time constant without offset leads to a water T2 of 50 ms (B).

# IV The Quantification (*LCM*) Page

## 4.1. General

Quantification of MR spectra is achieved by modeling them as a linear combination of model spectra. While the basic concept resembles the work by S. Provencher [5,8] and others, many characteristics differ. These distinct features include the way basis functions are handled and selected, the details of the quantification algorithm, and the reporting of confidence levels. In addition, INSPECTOR provides extended options for result visualization, documentation and data export.

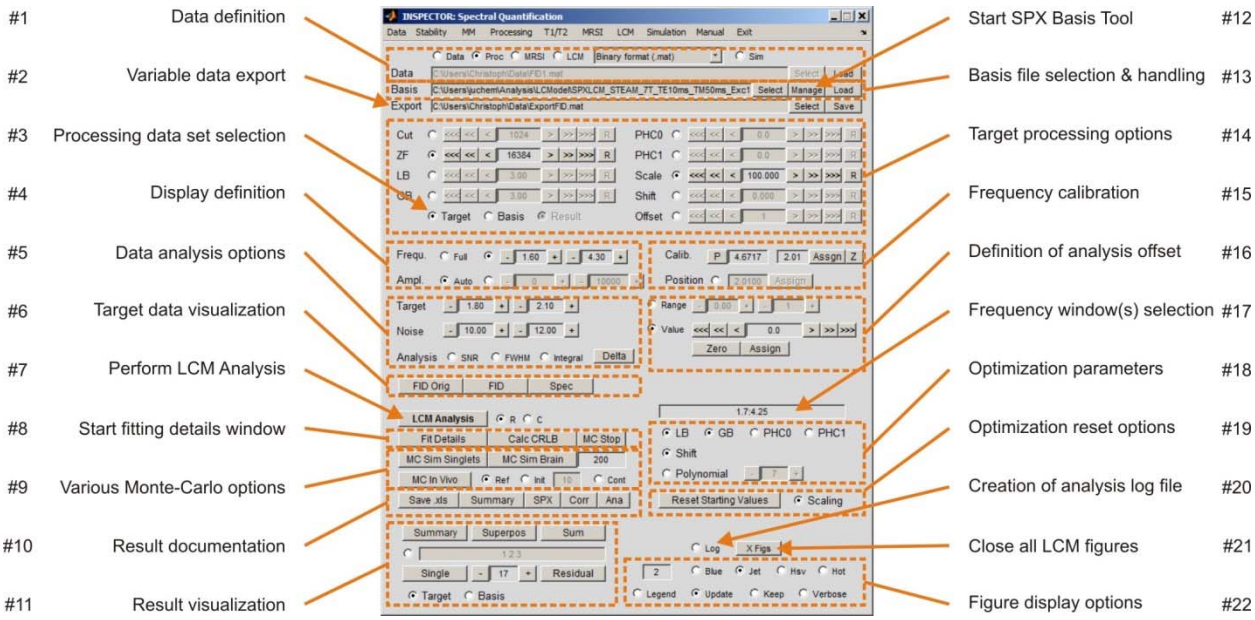



Fig. 4.1: Main *LCM* page.

## 4.2. Data Preparation

The spectrum to be quantified can be loaded directly from a file (#1, *LCM*) or transferred from other INSPECTOR pages such as (experimental) *Data*, e.g. for single-voxel MRS, or from the *Processing* page, e.g., after the difference calculation of *J*-Difference Editing (JDE) data. Basic processing similar to the *Processing* page is necessary for data preparation and definition of the target spectrum to be analyzed (#14). Its application to the target spectrum is enabled with the Target flag (#3). The target data can be visualized without any processing applied (#6, *FID orig*), with the selected processing applied (*FID*) or as resultant spectrum (*Spec*). Again, similar to the *Proc*essing page, options defining the display (#4) and the frequency calibration (#15), as well as the details for the analysis of *SNR*, *FWHM*, signal *integral*s and frequency differences (*Delta*) are available (#5, #16).

An arbitrary number of frequency windows can be analyzed. Assignment of the corresponding frequency ranges is equivalent to that of the spectral alignment procedure on the *Proc*essing page (#17). A number pair 1:4.2 separated by a colon, for instance, results in the analysis of a single frequency window covering the 1.0 to 4.2 ppm range. Multiple frequency windows are assigned as a space-separated list of such number pairs (with intermediate colon). A basic parameter selection for LCM quantification is available on the LCM main page (#18) along with reset options for the selected parameters (#19). More detailed selection options of the employed optimization parameters are available on the *Fit Details* window (#8) that is described in detail in section 4.3.

## 4.3. Basis Tool

The decomposition of the target spectrum at hand into a scaled superposition of basis spectra requires the latter input. The *Manage* button (#12 on *LCM* page) opens a separate window for the generation and management of such basis sets. Basis functions can be established one-by-one by loading individual spectra to the *LCM* page and subsequently assigning to a specific position of the basis set (#6 of *Basis Tool*). Note that the target spectrum is used as source data for this purpose and, consequently, the same options for transfer from other INSPECTOR pages or for loading from file are available. Alternatively, the *Import* function of the *Basis Tool* can be used to create a new basis from all spectra of a given directory in a fully automated fashion. This functionality supports INSPECTOR's own data format (.mat), S. Provencher's .raw data format and jMRUI's .mrui format [9]. The *Proc*essing page furthermore includes a dedicated formatting function for the conversion of NMRWizards (.par) files to the INSPECTOR format (.mat). As such, full metabolite libraries can be reformatted to establish LCM basis sets. Note that the data format is chosen by selecting an input format on the main LCM page (#1, center). Additional processing (e.g. line broadening) can be applied to the basis functions by selecting the *Target* flag on the main LCM page (#3) as well as any combination of processing parameters. If the individual metabolite signals are to be compiled to form a basis without further processing, be sure to disable the *Target* flag, thereby preventing automated processing. The metabolite names are initiated with the file names when libraries are imported but can be modified at any time. Once a file name and path have been chosen for the basis set on the main *LCM* page (#13), the new basis set can be saved to file for future use by clicking the *Save* button. Existing basis files can be loaded, modified, and re-saved at any time.

Further information defining the basis function at hand such as the metabolite name (#1) or a potential compound-specific comment (#4) can be entered directly. Future extensions will consider the metabolite's T1 and T2 constants. To date, however, this functionality is not yet supported and the corresponding fields act solely as place holders. Individual basis spectra can be visualized via the Show buttons (#5). Both experimentally measured and simulated basis functions should possess linewidths well below those obtained *in vivo*. The appearance of spectra, however, largely depends on their line width, and the spectral shapes known from *in vivo* MRS might not be immediately recognizable in their corresponding basis functions. All processing options on the main LCM page are therefore also available for the visualization of

basis functions and can be enabled (or disabled) via selection of the *Target* flag on the main *LCM* page (#14 box, right of *Target* flag #3).

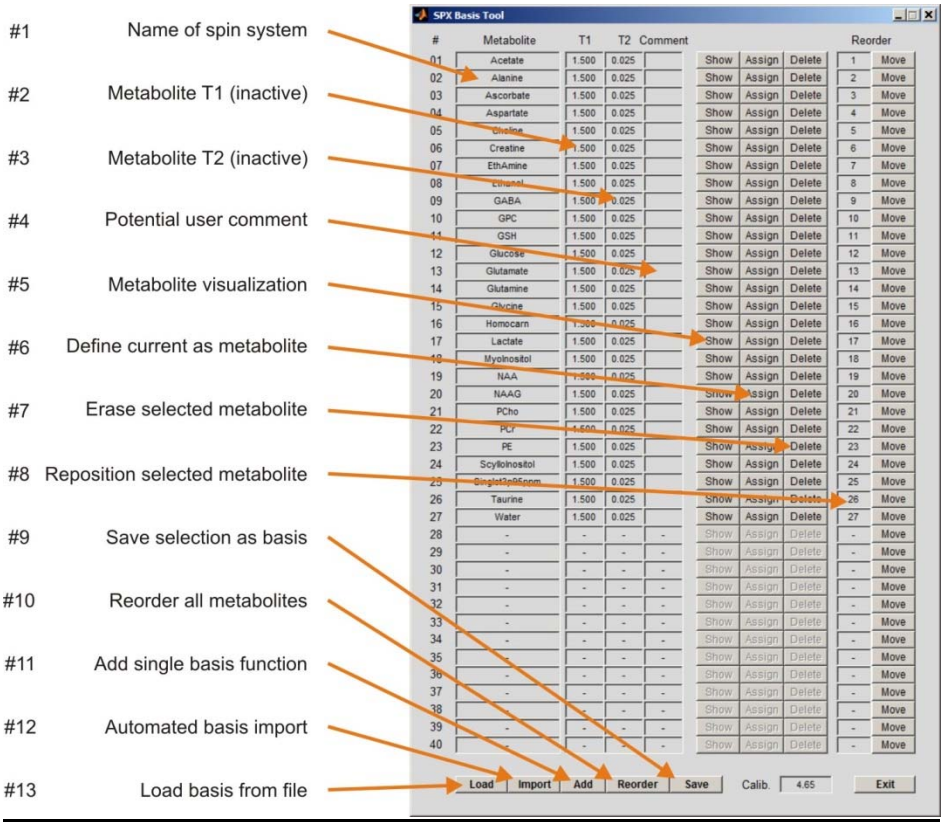



Fig. 7.2: Basis Tool.

## 4.4. Fit Details Tool

The *Fit Details* button opens a new window for the selection of all parameters defining the LCM analysis. The available basis functions are listed on the left (#3). An ensemble of basis functions to be applied for a given analysis can then be flexibly chosen by the appropriate flag selection (#11). Note that all text fields and functionalities are disabled for compounds that are not part of the current analysis selection.

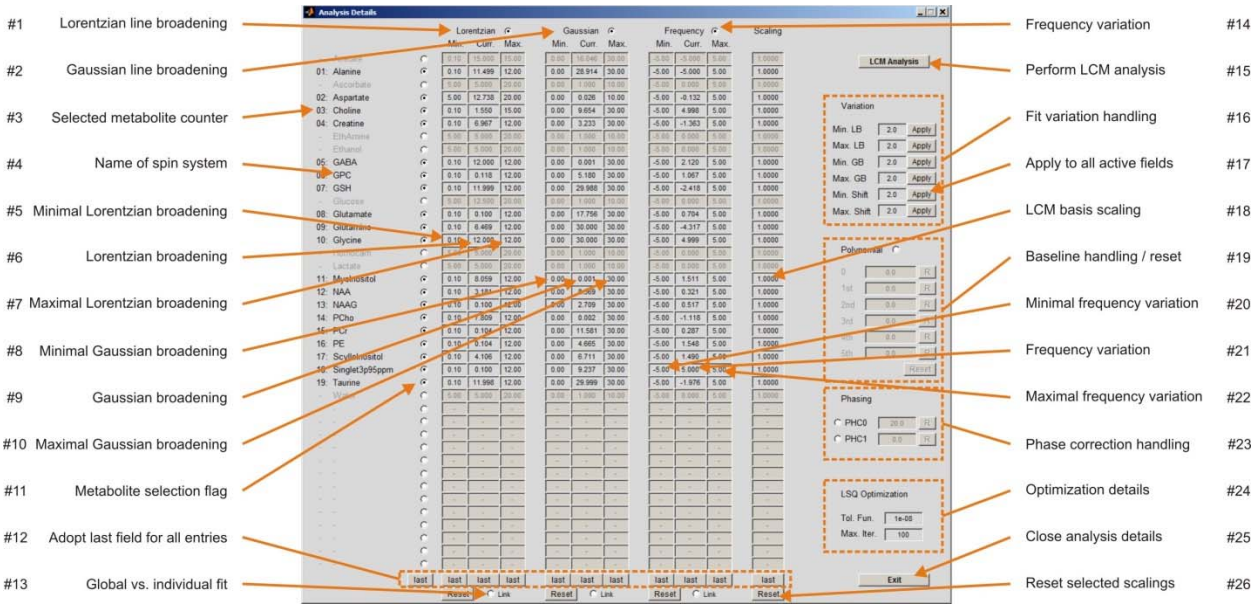



Fig. 7.3: Fitting Details Tool.

Potential optimization parameters beyond amplitude scaling (#18) include 1) Lorentzian line broadening (#1), 2) Gaussian line broadening (#2), 3) frequency shifting (#14), 4) a polynomial baseline (#19), 5) zero-order phasing, and 6) first-order phasing (#23). The three columns for Lorentzian line broadening, Gaussian line broadening and frequency shifting correspond to the metabolite-specific minimum boundaries (#5, #8, #20), their maximum boundaries (#7, #10, #22) and their respective parameter values (#6, #9, #21). All parameter values, including the scalings, are updated with every fit. As such, they correspond to both the starting values of an optimization as well as their results. This functionality allows the employment of arbitrary starting conditions, including the results from previous or similar optimizations, or zero values, thereby starting from scratch. Note that Lorentzian line broadening, Gaussian line broadening, and frequency determination can be applied in either a global or a metabolite-specific fashion by linking or unlinking the underlying parameters (#13). When parameter optimizations are linked, the corresponding boundary conditions are synchronized automatically for consistency. Zero- and first-order phase corrections are always applied in a global fashion. For convenience, a variety of basic functionalities have been included for easier parameter handling. For instance, changing all conditions of a certain type one by one might be cumbersome. This can be achieved more easily by assigning a given value to the lowest activated entry field of that type, e.g. minimum value for Lorentzian line broadening. Subsequently pressing the *Last* button (#12) will then apply this value to all other active entry fields of the same kind. Additionally, all active parameters of a given type can be reset at once (#26 and equivalent). It is sometimes desirable to the optimization that some variation or wiggle room be enabled around a given value, e.g. and extra +/-2 Hz frequency offset. This can be achieved by the corresponding parameter selection (#16) and subsequent application to all active fields (#17). In addition, the break-off conditions of the least-square optimization can be assigned directly, thereby providing a handle on the balance between fit accuracy and duration (#24). As with other menus in the software, the fitting tool is properly closed via the *Exit* button (#25) of the graphical interface instead of the close button of the window.

The LCM optimization can be started directly from the *Fit Details Tool* (#15) or via the equivalent button on the main *LCM* page (#7).

## 4.5. Result Visualization and Documentation

Various display and analysis options are provided for efficient, in-depth access to the results (#11). These include the visualization of 1) a fit summary, 2) the superposition, 3) the summation and 4) the individual display of selected compounds. Superposition and summation can involve all compounds (flag off) or any given combination of metabolites (flag on). The numbering of the selection resembles that of the *Basis Tool*. Individual compounds are also accessible with the *Single* button and scrolling through the results is possible via the (+) and (-) functionalities next to it. Note that the spectral baseline – if fitted – is accessible as an additional, last compound. The inclusion of the *Target* spectrum and the *Baseline* (if fitted) can be chosen separately by the appropriate flags. The selection of the *Baseline* option not only displays the baseline itself but also underlies the baseline to all metabolite signals. Such consideration of the baseline resembles the appearance of the target data and the original LCM fit. In addition, the residual, ie., the difference between the target spectrum and the best fit, can be visualized separately.

Similar to any other INSPECTOR page, visualization of a spectrum, such as an individual metabolite or a sum of a given selection (e.g. NAA, glutamate and glutamine), allows saving that signal to file or its transfer to other INSPECTOR pages. This is a distinct feature not only for further processing, but also for display and demonstration purposes.

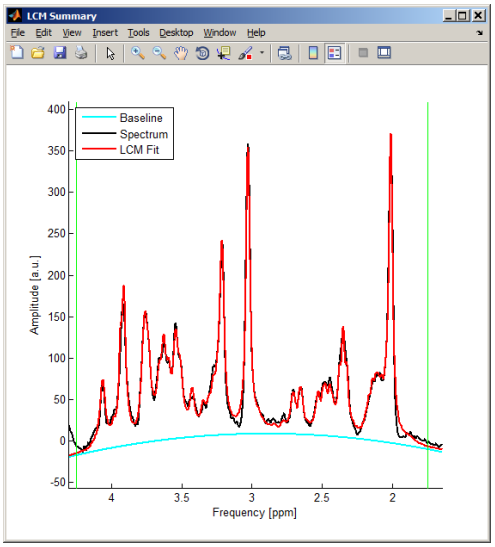



Fig. 7.4: LCM result summary figure.

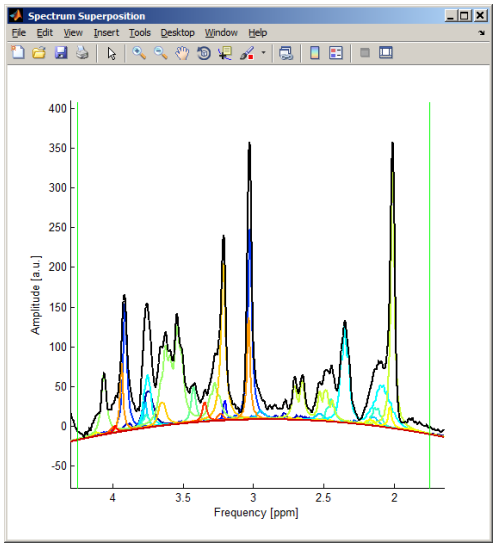



Fig. 7.5: LCM metabolite superposition.

Several dedicated export and documentation options are available (#10), including the writing of all fitting details to file in Microsoft Excel format (*Save .xls*), saving of the *Summary* figure, saving of screenshots of all three *LCM*-related figures (main *LCM* page, *Basis Tool*, *Fit Details Tool*),

saving of the correlation information between metabolites and direct export of the FID analysis result of a single metabolite or a combination thereof (*Ana*).

## 4.6. Error Analysis

The INSPECTOR software provides error estimates not only for the derived scaling factors used for metabolite quantification, but also for every parameter of the fitting routine, including Lorentzian/Gaussian line broadenings, frequency shifts, and zero- and first-order phasing. Three different types of error estimates are supported:

- Cramer-Rao Lower Bonds (CRLB)
- Monte-Carlo simulations of the LCM fit at identical noise floor
- Hessian error of the employed least-squares fit

CRLBs represent the lower limit of the confidence interval. For unbiased modeling, the CRLBs approximate the standard deviation of the parameter estimates. Monte-Carlo simulations are helpful to assess the reproducibility of the fit for a given noise floor. The Hessian error provides a confidence measure of the least-square optimization itself. CRLBs and Hessian errors are automatically calculated and reported, whereas additional computations are required for estimation of confidence levels via Monte-Carlo simulation.

CRLBs are automatically calculated following the work by Cavassila et al.[10,11] for all optimization parameters when the LCM analysis is performed. This not only includes the scaling parameters leading to metabolite concentrations, but also the derived frequency shifts, line shape characteristics, and spectral phases. In addition to individual CRLBs, INSPECTOR also supports the computation of up to three metabolite combinations (compare upper right corner of Fitting Details window). This feature can be used to derive the confidence levels e.g. of total NAA as the sum of NAA and NAAG, or total Creatine as sum of Creatine and Phosphocreatine. Metabolites to be combined are assigned as absolute numbers of the basis functions (number in parenthesis on the left). An arbitrary number and selection of basis functions can be assigned in each of the field as long as no basis function is used in more than one combination. CRLBs can also be explicitly (re)calculated, e.g. for a new combination of metabolites via the 'Calc CLRB' button on the main *LCM* page. This explicit call furthermore displays 1) the Fisher information matrix, 2) the correlation matrices of the individual fitting parameters such as amplitudes or frequency shifts as well as 3) the overall correlation matrix describing the parameter dependencies of all optimization parameters with respect to the optimization at hand. The 'Corr' button below in the line of saving options saves all these plots to Matlab figure files (.fig) for documentation and further analysis.

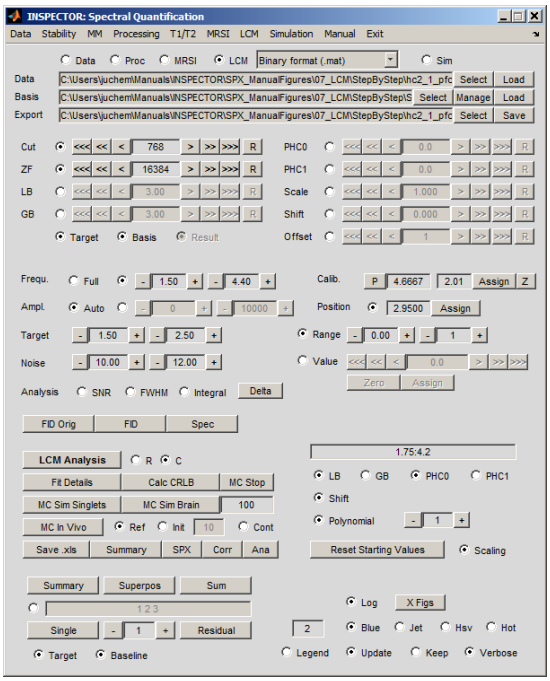
Monte-Carlo simulations can be used to investigate the reproducibility of the metabolite quantification, especially under the influence of noise. *In vivo* MRS is inherently SNR limited and extensive signal averaging is required for quantifiable signals. The multifold repetition, e.g. 1000 times, of entire MRS scans including all averaging to derive meaningful standard variations is therefore unrealistic. The simulation of this process is furthermore complicated by the apparent mingling of metabolite and noise contributions in the target spectrum at hand. The superposition

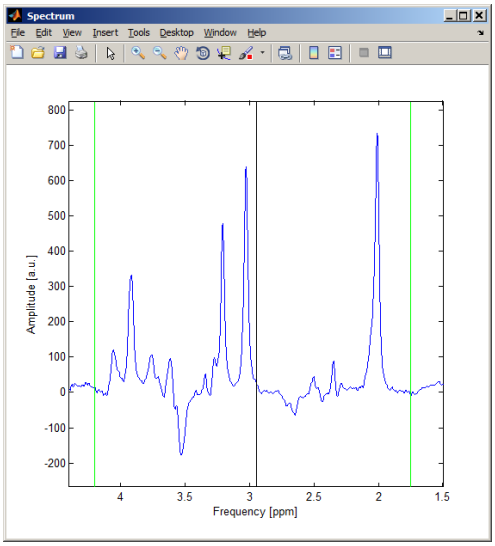
of the correct metabolite signals with varying noise floors of identical power is therefore not possible. This scenario is therefore approximated by considering the fitting result of a first analysis. In the current implementation (#9), a dedicated first analysis can be enforced (*Ref*erence flag); alternatively, the existing outcome of the last analysis is used. After the total number of simulations has been selected, it is performed by the *MC In Vivo* button. The results are shown as figures and written outcome files in the *Export* (or result) directory. The number of iterations necessary for convergence is sometimes difficult to predict. To this end, an option has been included (*Cont*inue flag) for the extension of a previous analysis. If selected, an additional number of simulations is added when the *MC In Vivo* button is pushed again. This allows the assessment of the results from a smaller number of iterations, before more iterations are added. The *Init* flag allows to initialize the fitting parameters with values spread around the result of the first analysis. The corresponding percentage value describes the standard deviation of the amplitudes as well as frequency and phase ranges relative to assumed 5 Hz and 10 degree ranges, respectively. Note, however, that this option should be used with care. While some parameter initialization might be suitable to reduce the processing time, the results must not be 'guided' and thereby affected. The initialization range should therefore be reasonably large, e.g. 20% or more. If the *Init* flag is deselected, no starting conditions are assumed and every analysis is performed from scratch. Note that while CRLBs are calculated automatically, Monte-Carlo simulations need to be started explicitly due to their computational burden.

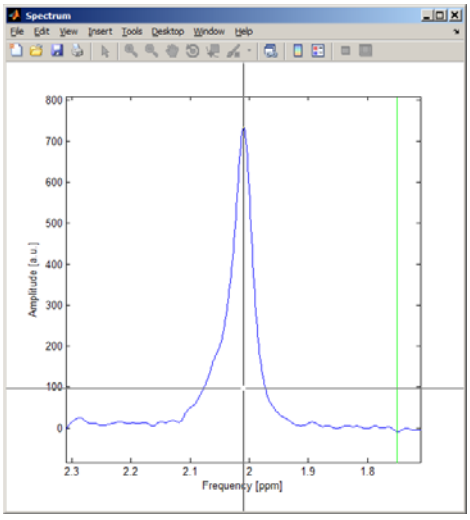
## 4.7. Usage Example: Quantification of semi-LASER MRS

Typical processing of a single-voxel MR spectrum (e.g. of the non-edited condition of a MEGA sLASER scan for JDE of GSH) includes the following steps:

1) Transfer or direct import of target FID to be analyzed, via *Load* button in upper right corner of the *Data* row. Then select processing parameters of target FID, process and visualize.

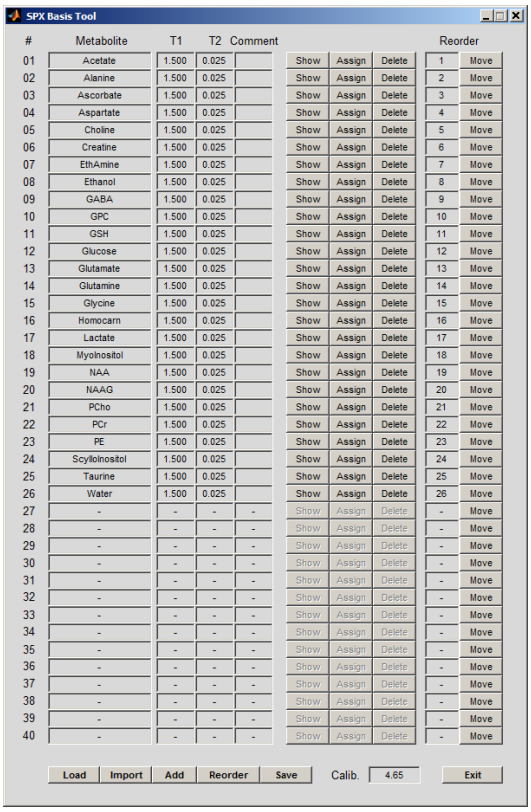


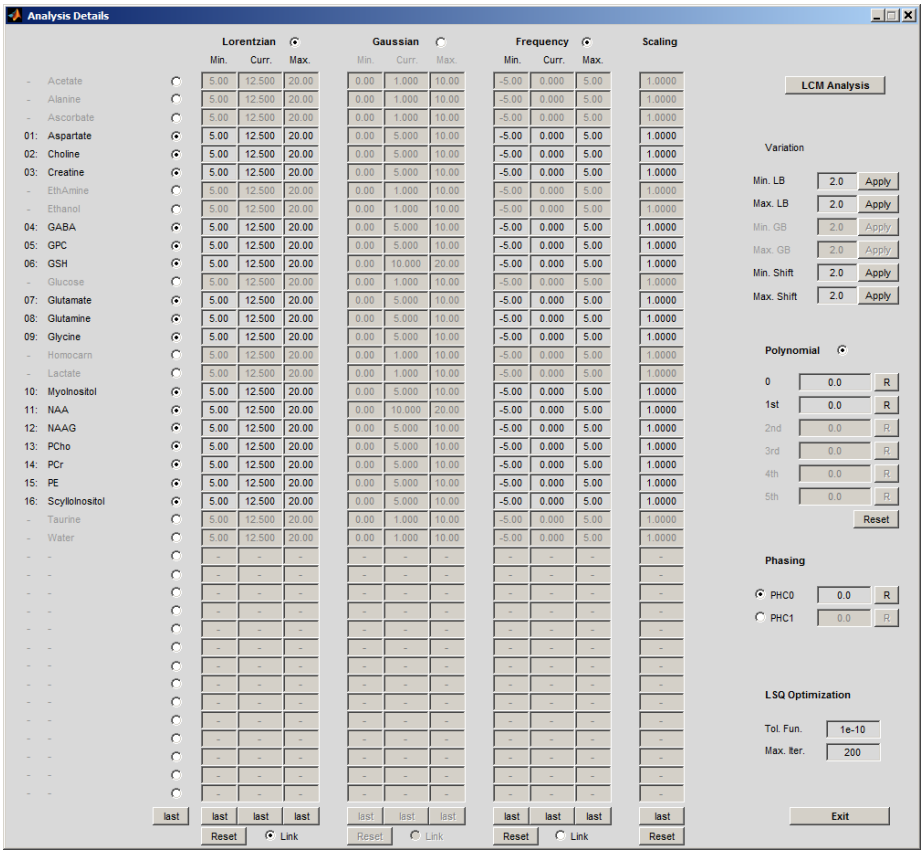




Calibrate the spectral frequency relative to the 2.01 ppm NAA resonance by pushing one of the *Assign/Z* buttons and subsequent peak selection.

2) Selection and loading of metabolite basis set by the functionalities in the *Basis* row. Opening the *Basis Tool* by pushing the *Manage* button allows to visualize and potentially manipulate the basis set. Note that the overall basis set represents a library of potential signals including *Water* and those not necessarily relevant for every experiment (e.g. *Ethanol*).




3) Selection of fitting parameters in *Fit Details Tool*



Here, a set of metabolites has been chosen for the analysis and a global linewidth is fitted since the individual Lorentzian line broadening parameters are linked (*Link* flag selected). Gaussian line broadening is disabled (*Gaussian* flag deselected), but a 5 Hertz frequency variation is allowed in a metabolite-specific fashion (*Frequency* flag selected, *-5..5* Hz range, *Link* flag deselected). A first-order polynomial baseline is included in the analysis (*0, 1st*) as well as zero-order phasing (*PHC0*).

5) Performing of LCM analysis by pushing the *LCM Analysis* button either on *INSPECTOR*'s main *LCM* window or directly in the *Fit Details Tool* starts the analysis and yields:

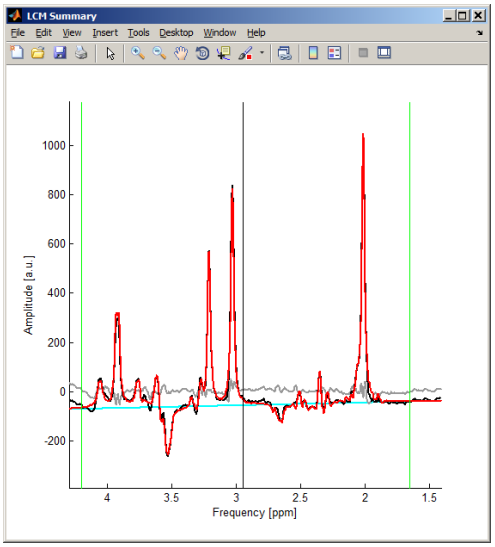
```
Spectral range for LCModel analysis:
1: 1.650..4.200 ppm (2491 pts)
Total: 2491 pts
LCModel analysis of REAL & IMAGINARY part applied

LCModel starting values (16 metabolites):
Scaling:   [1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00]
LB (L):    [12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5] Hz
PHC0:       0.00 deg
Shift:     [1.00 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000] Hz
Real poly (1st..0 order):   [0.00000 0.00000]
Imag. poly (1st..0 order):  [0.00000 0.00000]
Real poly (complete):  [0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000
0.00000]
Imag. poly (complete): [0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000
0.00000]

…

LCModel result:
Scaling:   [0.000 0.778 7.95 3.62 0.000 2.12 16.7 2.79 0.336 12.8 19.1 1.61 3.17 7.96 1.83 0.372]
LB:        [6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39 6.39] Hz
PHC0:       0.65 deg
Shift:     [-5.00 5.00 -0.539 0.639 -4.99 0.254 1.43 -0.247 5.00 1.48 0.698 2.73 0.507 0.741 4.05 2.40] Hz
Real poly (fit, 1st..0 order):  [-11.782 -21.032]
Imag. poly (fit, 1st..0 order): [3.5537 -13.733]
Real poly (complete):  [0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 -11.782 -
21.032]
Imag. poly (complete): [0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 3.5537 -13.733]


LCMODEL ANALYSIS SUMMARY:
(errors as CRLB / Hessian of LSQ)
Aspartate:       0.00 mM (0.000, 100+/100+%) / Shift -5.00 Hz (50+/NaN Hz)
Choline:         0.41 mM (0.778, 0.1/17.9%) / Shift 5.00 Hz (<0.1/0.5 Hz)
Creatine:        4.16 mM (7.953, <0.1/1.1%) / Shift -0.54 Hz (<0.1/<0.1 Hz)
GABA:            1.89 mM (3.617, 0.2/5.0%) / Shift 0.64 Hz (<0.1/0.1 Hz)
GPC:             0.00 mM (0.000, 100+/100+%) / Shift -4.99 Hz (50+/NaN Hz)
GSH:             1.11 mM (2.121, 0.1/2.9%) / Shift 0.25 Hz (<0.1/0.1 Hz)
Glutamate:       8.71 mM (16.665, <0.1/1.8%) / Shift 1.43 Hz (<0.1/<0.1 Hz)
Glutamine:       1.46 mM (2.786, 0.4/11.5%) / Shift -0.25 Hz (<0.1/0.3 Hz)
Glycine:         0.18 mM (0.336, 1.0/20.1%) / Shift 5.00 Hz (<0.1/1.0 Hz)
MyoInositol:     6.69 mM (12.790, <0.1/0.7%) / Shift 1.48 Hz (<0.1/<0.1 Hz)
NAA:             10.00 mM (19.132, <0.1/0.3%) / Shift 0.70 Hz (<0.1/<0.1 Hz)
NAAG:            0.84 mM (1.609, 0.1/3.1%) / Shift 2.73 Hz (<0.1/0.1 Hz)
PCho:            1.66 mM (3.169, <0.1/5.1%) / Shift 0.51 Hz (<0.1/<0.1 Hz)
PCr:             4.16 mM (7.958, <0.1/1.1%) / Shift 0.74 Hz (<0.1/<0.1 Hz)
PE:              0.96 mM (1.832, 0.6/28.1%) / Shift 4.05 Hz (<0.1/1.1 Hz)
ScylloInositol:  0.19 mM (0.372, 0.3/5.8%) / Shift 2.40 Hz (<0.1/0.3 Hz)
LB:              6.4 Hz (<0.1/<0.1 Hz)
PHC0:            0.6 deg (<0.1/0.1 deg)
```

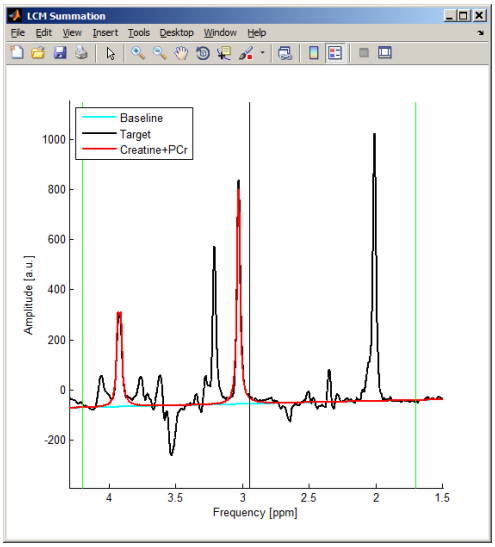
If NAA is included in a basis set, a 10 mM relative scaling is applied to the printout for context. The original numerical outcome of the LCM analysis is returned as the following number. Here,

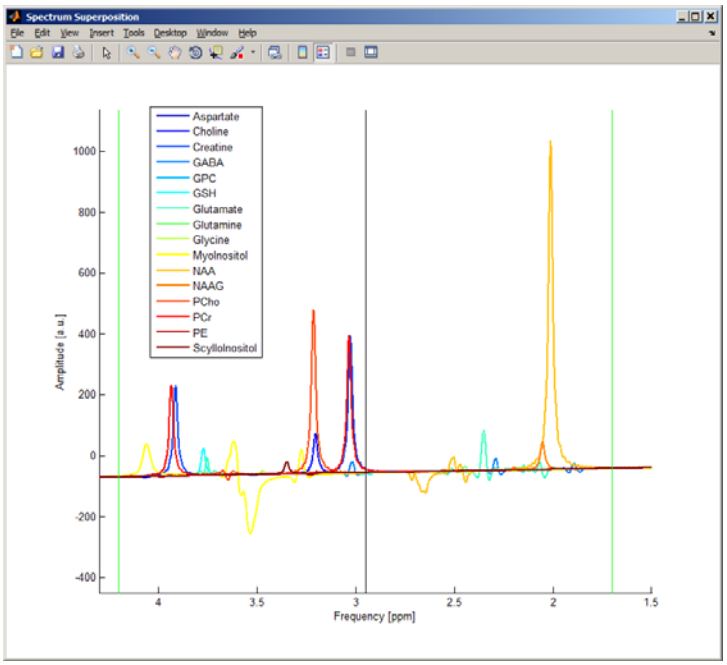
every metabolite has its own CRLB for amplitude and frequency shift as these were fitted individually. Instead, the global parameter fits are associated with single CRLB values for exponential line broadening (LB) and zero-order phase (PHC0). The basis functions in this example have been simulated at a line width of 1 Hz and this offset has to be added to fitted line broadening to derive to the apparent line width of the spectrum. Note that parameters between *LCM* windows is automatically maintained and parameters are updated by the fitting results, thereby allowing a refinement if desired.

## 6) Result visualization and documentation

The summary window is opened automatically. Additional printouts and visualization options are available on the main *LCM* page, including the extraction of single metabolites, the superposition of an arbitrary selection of metabolites or their summation, e.g. for further processing or export.






7) Monte-Carlo simulation

A third error value approximating the susceptibility of the LCM analysis to the influence of noise can be obtained by multiple execution of the LCM analysis at varying noise patterns of the same noise power. The ground truth, i.e. the pure metabolite signals without noise, is not available and a selected metabolite fit is therefore used and noise is added to it.

# V The *Simulation* Page

## 5.1. General

The *Simulation* page provides synthesized spectra ranging from a singlet or an array of singlets to full $^{1}$H MRS of the human brain. In addition, well-defined noise components and a polynomial baseline can be added to resemble typical experimental problems and to systematically explore their impact on quantification. Both noise and baseline effects are added to the fully processed spectrum.

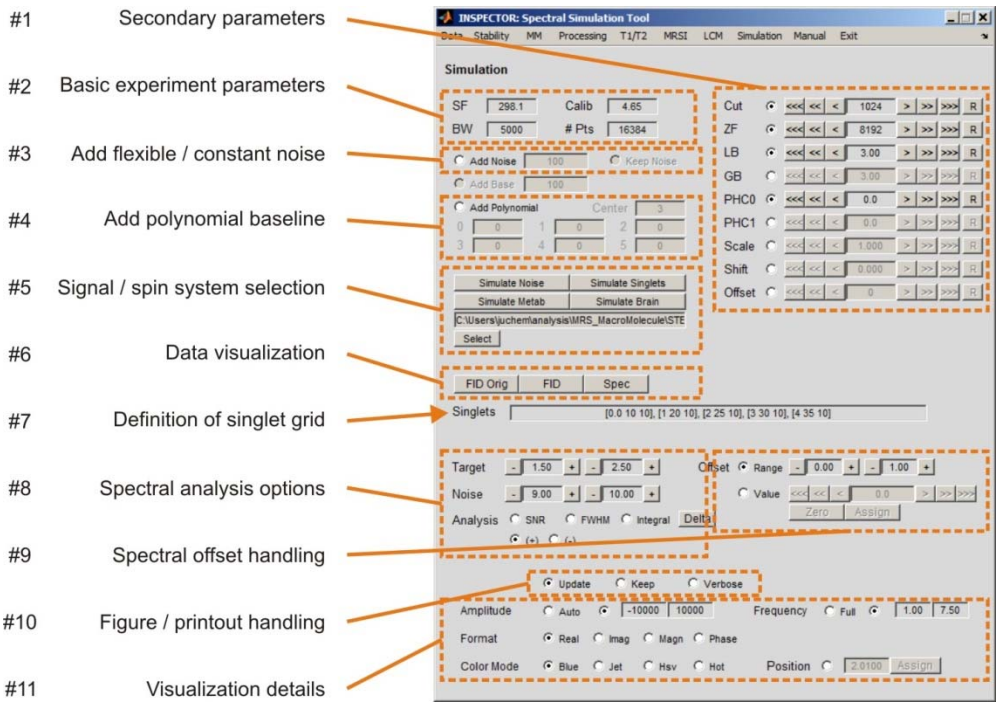



Fig. 8.1: Main *Simulation* page.

## 5.2. Spectral Simulation and Processing

The parameters describing the overall spectral simulation (#1) include the Larmor frequency (*SF*), the synthesizer or *Calib*ration frequency, the acquisition bandwidth (*BW*), and the length of the FID assigned as number of points *# Pts*.

The parameters describing the spectral processing are outlined in a column similar to those of previous INSPECTOR pages (#1). Note that all these modifications are applied ***before*** noise is added to enable studying the impact of noise on the spectral appearance and quantification. Further processing be achieved after noise or a spectral baseline has been added can be achieved by transferring it to other INSPECTOR pages, e.g. the processing page.

Various types of simulated signals can be provided by the functionalities associated with the buttons in box #5:

#### *1) Simulate Noise*

A pure noise spectrum is simulated at the noise power defined next to the *Add Noise* flag.

#### *2) Simulate Singlets*

An array of singlet peaks is simulated at frequencies, amplitudes and linewidths defined in the text field labeled *Singlets* (#7).

#### *3) Simulate Metab*

This function loads the FID of a selected metabolite from the file defined in the text field below the simulation buttons or via the Select button below it. Note that overall processing parameters have to be chosen consistent with the underlying simulation.

#### *4) Simulate Brain*

This functionality provides a $^{1}$H MR spectrum resembling those obtained from the human brain and includes all relevant metabolite signals at (average) concentrations taken from the literature. Note that the files containing the individual metabolite signals are expected to be located in the directory of the metabolite path assigned below. Some examples and libraries will be provided with the next release.

## 5.3. Spectral Noise

Well defined levels of noise can be added to any spectrum using the *Add Noise* field (#3). The assignment of the same noise spectrum at different noise power or a different noise spectrum at identical noise power allows studying the effects of noise on spectral appearance and quantification. For instance, spectral simulations along with added noise can be used to confirm the CRLB calculations on the *LCM* quantification page. By default, new noise vectors are generated with every update. The modification of the noise contribution can be suppressed, e.g. for assessment of the identical noise pattern at varying amplitude, with the selection of the *Keep Noise* flag.

## 5.4. Spectral Baseline

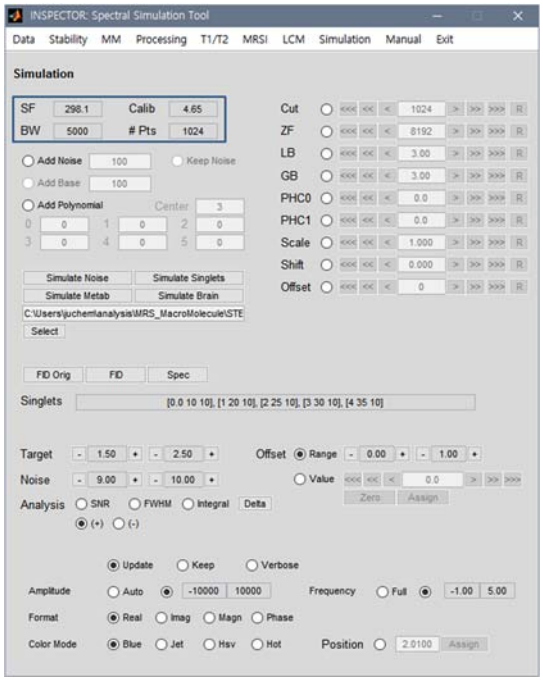
Similarly, well-defined polynomial baselines can be added to any given spectral simulation to mimic the challenges encountered *in vivo* (#4). Zero- through fifth-order polynomials are

supported, and parameters are defined per ppm (instead of per point) to make them independent of the employed spectral resolution. An explicit *Center* frequency parameter (in [ppm]) has been added for easy placement of the polynomial center to a specific frequency position. Note that this parameter is redundant as all aspects of a polynomial are already described by its coefficients.

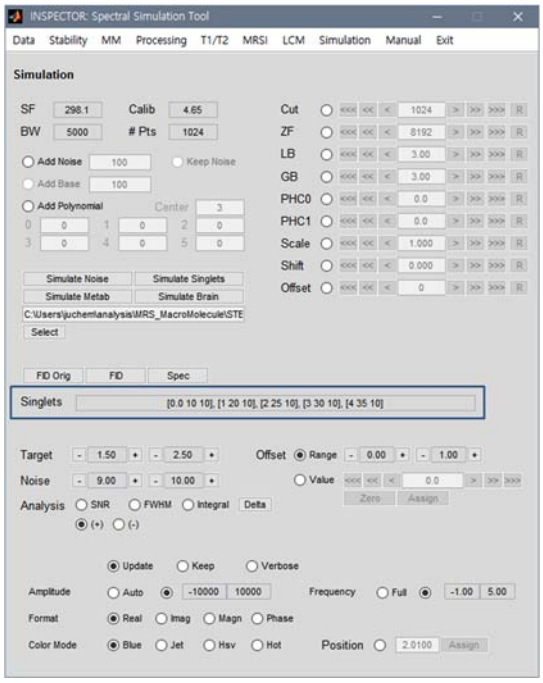
## 5.5. Usage Example: Simulation of an Array of Singlets

Here we generate an array of five singlets between 0 and 4 ppm with 1-ppm spacing and increasing relative amplitudes. The full width at half maximum (FWHM) of all peaks is chosen to be 10 Hz.

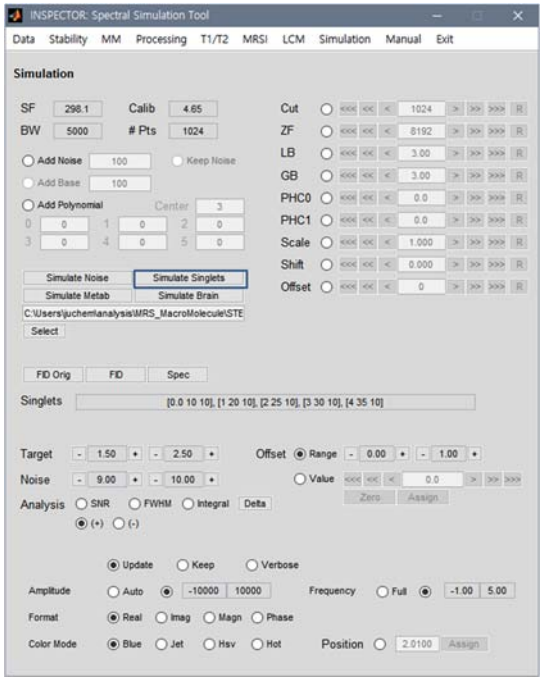
1) Select the general parameters for the simulation at hand (*SF*, *Calib*, *BW*, *# Pts*).



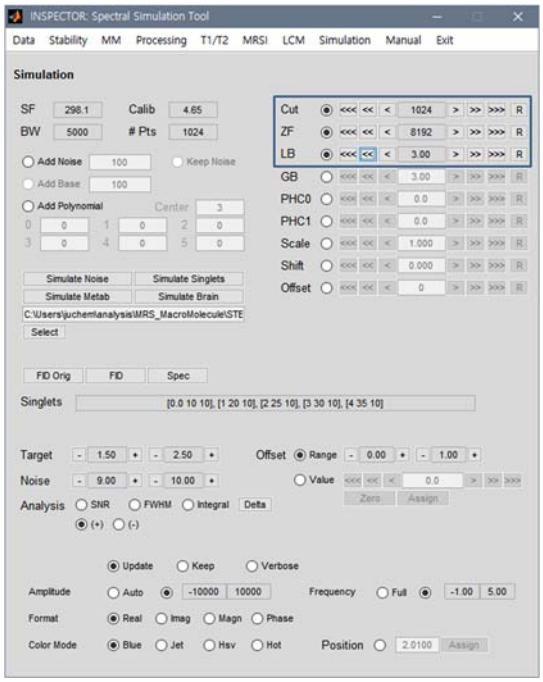

2) Define the singlet array: [0.0 10 10], [1 20 10], [2 25 10], [3 30 10], [4 35 10]



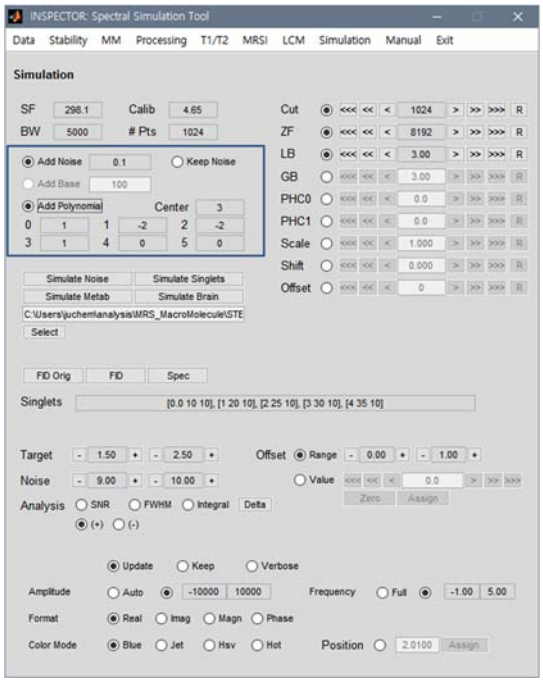

3) Perform the simulation via the *Simulate Singlets* button



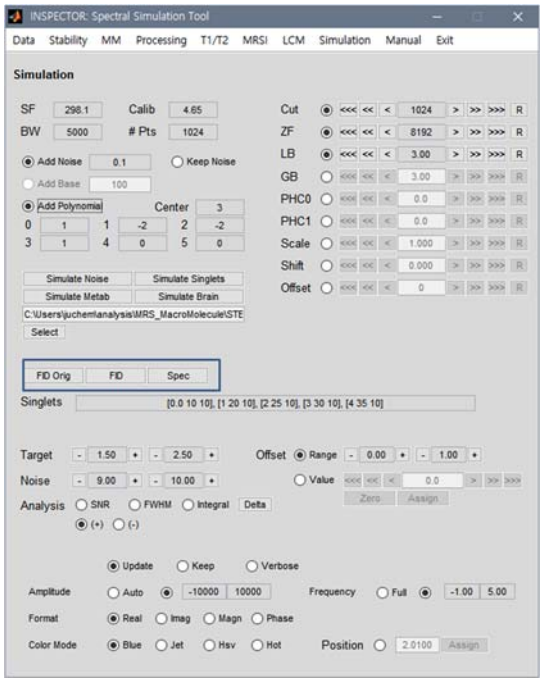

4) If desired, further manipulations can be applied to the simulated FID as defined by the parameters in the upper right part of the *Simulation* page.

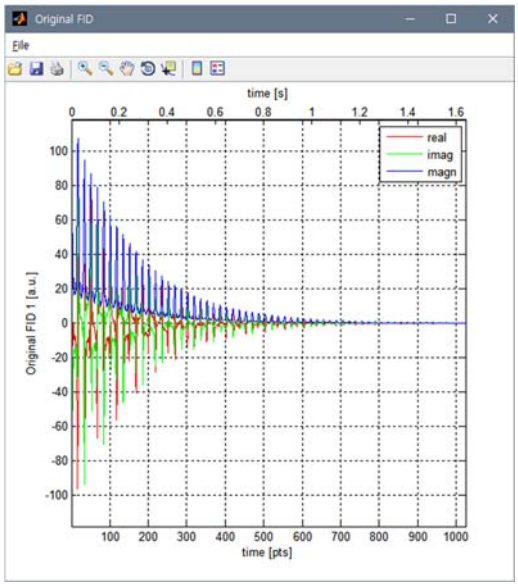


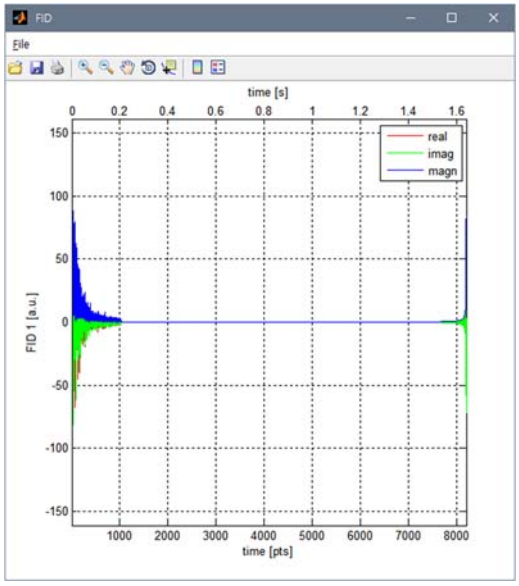

5) Noise and a polynomial baseline can also be added as defined by parameters in the upper left part of the *Simulation* page.

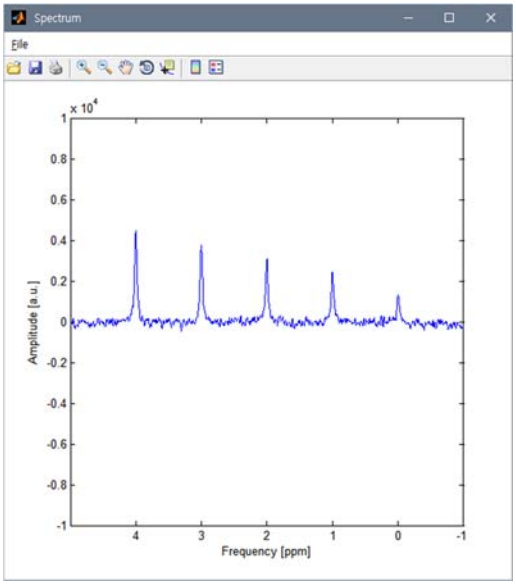



6) Visualize the result as the FID as simulated (*FID Orig*), the FID including potential additional processing (*FID*), or the resultant spectrum (*Spec*).




Notably, if the macromolecule baseline of short echo time MRS is not measured, but approximated by Gaussian or Lorentzian functions in the LCM quantification, this functionality can be used to synthesize them, before they are transferred to the *LCM* page and added to a given *LCM* basis set.

# VI The *Manual* Page

The *Manual* tab of the INSPECTOR window does not switch to other pages but opens the software manual that you are currently reading in Adobe Acrobat© if available. You can place the PDF file of the manual that comes with the software anywhere on your computer. The first time you press the *Manual* tab, you are prompted to select the file location. This location is remembered by the software and the manual will be opened immediately with subsequent use.

# VII The *Exit* Page

The *Exit* tab saves all current parameters to a default protocol file and subsequently closes the program. This procedure allows INSPECTOR to reopen in the page that has been used last with all parameter settings as selected previously. Closing INSPECTOR through the operating system's Close button in the upper right corner of the window is not advised as parameter settings are thereby lost. Note that explicitly loading a specific protocol on the *Data* page is always possible.

# VIII Supported Data Formats

The INSPECTOR software supports the processing of standard single-voxel MRS (e.g. STEAM, PRESS or LASER) as well as J-difference edited MRS from a variety of vendors and in various formats:

1) Varian: fid files

2) Bruker: old/new fid files

3) Bruker: rawdata.job0 files

4) Siemens: .rda files

5) Siemens: .dat files (VB, VD/VE)

6) General Electric: P-files (.7, revision 24)

7) Philips: .raw

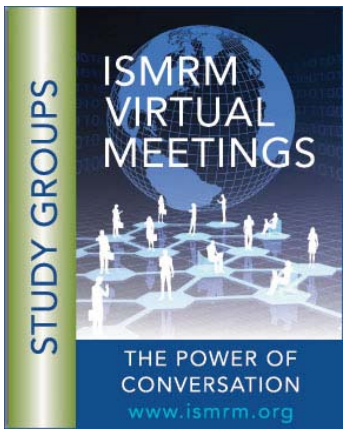
8) Philips: .SDAT

The software automatically retrieves the parameters describing the experimental data set, e.g. number of FID points, number of receivers, or number of acquisitions, in order to set up the processing environment as well as a variety of additional information relevant for the understanding of the data at hand including experiment type, sequence timings, RF pulse shapes or employed $B_0$ shim settings. The corresponding parameter files therefore have to exist and expected to be located in their usual file location with the data.

INSPECTOR reliable works for the data sets of above formats that have come my way. However, it is unlikely that all potential constellations are covered, especially if customized MRS sequences are used. The software is work-in-progress and I am eager to extend the number of data formats it supports. Therefore, feel free to contact me for further details. I am more than happy to get additional data formats to work!

# IX Further Reading, Training and User Community

### *ISMRM Virtual Meeting, 06/15/2017*




MR Spectroscopy Study Group

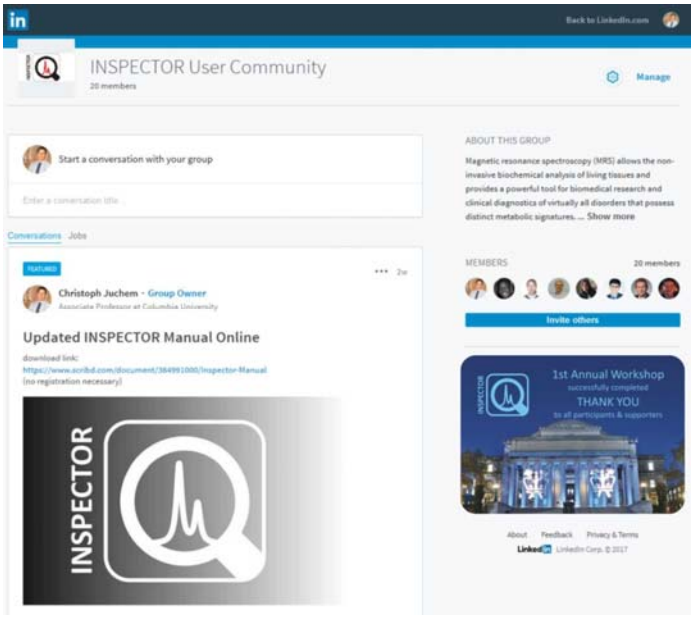
INSPECTOR: The New Kid on the Block (video)

(ISMRM member login required)

### *First Annual INSPECTOR Workshop, 12/08/2017*


1st Annual INSPECTOR Workshop a success. Thank you to all speakers, participants and supporters for this wonderful day. Hope to see you again next year!

### *INSPECTOR User Community*




Join the [INSPECTOR User Community] as an informal platform for communication, support and exchange. Everybody is welcome.

# X Glossary

| | |
|---|---|
| GABA | γ-aminobutyric acid |
| GSH | glutathione |
| JDE | J-difference editing |
| NAA | N-acetyl aspartate |
| STEAM | Stimulated echo acquisition mode [12] |
| T1 | spin-lattice or longitudinal relaxation time constant |
| T2 | spin-spin or transversal relaxation time constant |

# XI Terms of Use and Additional Remarks

### *Purpose and Liability*

The INSPECTOR software is meant for research purposes only. Neither the creator, Christoph Juchem, nor Columbia University assumes any responsibility of any kind. Please refer to the license agreement for further details.

### *Legal and Academic Concerns*

INSPECTOR software is made publically available under a term-limited license. The software can be used free of charge in a non-commercial academic setting with the understanding that it is referenced properly in any publication for which it has been used [13]:

Prinsen H, de Graaf RA, Mason GF, Pelletier D, Juchem C. *Reproducibility measurement of glutathione, GABA, and glutamate: Towards in vivo neurochemical profiling of multiple sclerosis with MR spectroscopy at 7T*. J Magn Reson Imaging 45:187-198 (2017).

If the software has been instrumental to your research, please consider offering the creator of the software, Christoph Juchem, an authorship on the first publication for which INSPECTOR has been used.

### *Join Us in the Fight against Multiple Sclerosis*

The National Multiple Sclerosis Society (NMSS) and patient advocate groups such as the Nancy Davis Foundation (*Erase MS*) provide instrumental support for MS patients and researchers aiming to end this devastating disease. While we do not ask for monetary contributions for ourselves, we encourage you to consider a donation to these organizations. If you chose to support their invaluable efforts based on the free use of the INSPECTOR software, please assign *Juchem Laboratory* as the occasion of the donation.

http://www.nationalmssociety.org/Donate

http://www.erasems.org/donate/

### *Questions and Feedback*

The development of the INSPECTOR software is still a work in progress. Questions and feedback are always welcome to help us to further tailor it to the needs of clinical research:

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Associate Professor
Departments of Biomedical Engineering and Radiology
Columbia University in the City of New York
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w: juchem.bme.columbia.edu

*Acknowledgements*

I am very grateful for the many fruitful discussions I had with colleagues over the years and the wealth of constructive comments I received. Special thanks go to the members of my laboratory for being the first true users of this software. Their feedback and suggestions have been instrumental for the transition of INSPECTOR from personal software to a general tool usable by and useful for others. In addition, the help from Vincent Boer, Ph.D., and Jannie Wijnen, Ph.D., with the reading of Philips data is acknowledged.

# XI References

1 Juchem, C. *INSPECTOR - Magnetic Resonance Spectroscopy Software*, http://innovation.columbia.edu/technologies/cu17130_inspector (2016)

2 Klose, U. In vivo proton spectroscopy in presence of eddy currents. *Magn Reson Med* **14**, 26-30 (1990).

3 Wald, L. L., Moyher, S. E., Day, M. R., Nelson, S. J. & Vigneron, D. B. Proton spectroscopic imaging of the human brain using phased array detectors. *Magn Reson Med* **34**, 440-445 (1995).

4 van Eijsden, P., Behar, K. L., Mason, G. F., Braun, K. P. & de Graaf, R. A. In vivo neurochemical profiling of rat brain by 1H-[13C] NMR spectroscopy: cerebral energetics and glutamatergic/GABAergic neurotransmission. *J Neurochem* **112**, 24-33 (2010).

5 Provencher, S. W. Automatic quantitation of localized in vivo 1H spectra with LCModel. *NMR Biomed* **14**, 260-264 (2001).

6 Levenberg, K. A Method for the Solution of Certain Problems in Least Squares. *Quart Appl Math* **2**, 164-168 (1944).

7 Marquardt, D. W. An Algorithm for Least-Squares Estimation of Nonlinear Parameters. *SIAM J Appl Math* **11**, 431-441 (1963).

8 Provencher, S. W. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* **30**, 672-679 (1993).

9 *jMRUI - Software for clinical and biomedical MRS*, http://www.jmrui.eu/

10 Cavassila, S., Deval, S., Huegen, C., Van Ormondt, D. & Graveron-Demilly, D. Cramer-Rao Bound Expressions for Parametric Estimation of Overlapping Peaks: Influence of Prior Knowledge. *J Magn Reson* **143**, 311-320 (2000).

11 Cavassila, S., Deval, S., Huegen, C., van Ormondt, D. & Graveron-Demilly, D. Cramer-Rao bounds: an evaluation tool for quantitation. *NMR Biomed* **14**, 278-283 (2001).

12 Frahm, J., Merboldt, K. D. & Hanicke, W. Localized Proton Spectroscopy Using Stimulated Echos. *J Magn Reson* **72**, 502-508 (1987).

13 Prinsen, H., de Graaf, R. A., Mason, G. F., Pelletier, D. & Juchem, C. Reproducibility measurement of glutathione, GABA, and glutamate: Towards in vivo neurochemical profiling of multiple sclerosis with MR spectroscopy at 7T. *J Magn Reson Imaging*, doi:10.1002/jmri.25356 (2016).