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Overview

Gannet is designed to batch-process GABA-edited MR spectroscopy data. Our aim is to manage this successfully with minimal user intervention, but it is important to understand the experiment being performed in order to judge whether this has been achieved. There is no substitute for looking at all the data at both the GannetLoad and GannetFit steps to judge the quality of the data and it is important to get a feel for what the data look like.

Difference-edited MRS for GABA

Gannet is designed to handle difference-edited spectra, usually acquired with the MEGA-PRESS experiment. More details on the experiment can be found in the original paper¹ or our recent review papers². Essentially MEGA-PRESS acquires two spectra which differ in how the GABA signal evolves, so that the GABA signal is revealed upon subtraction of the two. This subtraction is both the strength of the experiment, in that it allows a weak GABA signal to be separated from the strong overlying Creatine (Cr) signal, and its weakness, in that accurate subtraction of the Cr signals is required for accurate GABA quantification. The key pre-processing step of Gannet (frequency and phase correction) is designed to overcome some experimental instability and still achieve accurate subtraction of the Cr signal.

MEGA-PRESS harnesses the fact that coupled signals like GABA evolve during the echo time, changing the appearance of GABA spectrum at different TEs, whereas uncoupled signals like Cr do not. Furthermore, this evolution can be undone, or refocused, by applying frequency-selective inversion pulses to the GABA spins. The MEGA-PRESS experiment acquires one experiment in which editing pulses are applied to the GABA signal at 1.9 ppm (often referred to as the ON experiment) and one in which they are not (the OFF experiment). In each of these experiments, the GABA signal at 3 ppm has a different shape, and so therefore subtracting the spectra from the two experiments leaves a measureable GABA signal.

Standard MRS processing

In addition to frequency and phase correction to achieve optimal subtraction of Cr, Gannet performs standard MRS processing steps such as channel combination of phased array data (where required), fast Fourier transform (FFT) of time-domain acquired data to frequency-domain spectra, and exponential line broadening. Further details of processing steps applied to different data types are covered the section [Processing of MRS data in GannetLoad](#).

¹ Mescher et al. NMR Biomed 11:266-272 (1998).

² Puts et al. Prog NMR Spec 60:29-41 (2012); Mullins et al. Neuroimage doi: 10.1016/j.neuroimage.2012.12.004.

Getting Started with Gannet

1. Get Matlab with Optimisation and Statistics Toolboxes
2. Get the Gannet2.0 code from github (<http://github.com/cjohnnevs/Gannet2.0>) and add these files to your matlab path
Assuming familiarity with Matlab, make sure all the files downloaded from github are in your matlab path; (i.e. use “add with subfolders”). If you are not familiar with matlab, someone else in your group probably is and can assist.

3. What do I type?
Gannet is currently run from two commands – GannetLoad and GannetFit. This section will cover the load and processing of a single data file. Replace the filename with the actual name of your file.

3.1 General

In general, running GannetLoad involves typing something like:

```
MRS_struct = GannetLoad({'GE_data1.7'});
```

and running GannetFit involves typing something like:

```
MRS_struct = GannetFit(MRS_struct);
```

Gannet is designed to process a study-worth of data sequentially, so you might start like this:

```
MRS_struct = GannetLoad({'GE_data1.7' 'GE_data2.7' 'GE_data3.7' 'GE_data4.7'});
```

where the input variable to GannetLoad is a cell array of filename strings.

3.1 GE data

P files contain a water reference internally, so:

```
MRS_struct = GannetLoad({'GE_data1.7'});
```

```
MRS_struct = GannetFit(MRS_struct);
```

3.2 Philips .sdatt files

If no water reference data have been collected:

```
MRS_struct = GannetLoad({'Philips1.sdat'});
```

```
MRS_struct = GannetFit(MRS_struct);
```

If water reference data have been collected:

```
MRS_struct = GannetLoad({'Philips1.sdat'},{'Philips1_water.sdat'});
```

```
MRS_struct = GannetFit(MRS_struct);
```

In the most recent patch that includes the Spectral Correction functionality and acquires the water data within the scan:

```
MRS_struct = GannetLoad({'Philips1_act.sdat'},{'Philips1_ref.sdat'});
```

```
MRS_struct = GannetFit(MRS_struct);
```

2.3 Philips .data files

If no water reference data have been collected (or if water reference data is 'internal' (see 4 below)):

```
MRS_struct = GannetLoad({'Philips1.data'});
```

```
MRS_struct = GannetFit(MRS_struct);
```

If water reference data have been collected in a separate acquisition:

```
MRS_struct = GannetLoad({'Philips1.data'},{'Philips1_water.data'});
```

```
MRS_struct = GannetFit(MRS_struct);
```

2.4 Siemens .rda files (saved as OFF and ON rda files separately)

```
MRS_struct = GannetLoad({'Siemens1_ON.rda' 'Siemens1_OFF.rda'});
```

```

MRS_struct = GannetFit(MRS_struct);
If water reference data have been collected:
MRS_struct = GannetLoad({'SiemensI_OFF.rda' 'SiemensI_ON.rda'
},{ 'Siemens_water.rda'});
MRS_struct = GannetFit(MRS_struct);

```

2.5. Siemens .dat TWIX files

```

If no water reference data have been collected:
MRS_struct = GannetLoad({'SiemensI.dat'});
MRS_struct = GannetFit(MRS_struct);
If water reference data have been collected:
MRS_struct = GannetLoad({'SiemensI.dat'},{'SiemensI_water.dat'});
MRS_struct = GannetFit(MRS_struct);

```

This should work for Siemens .dat data acquired either with the Siemens WIP or Jamie Near's sequence.

4. Data Formats and Assumptions

GE, Philips and Siemens data formats are supported. Vendor is chosen according to file endings, so make sure your data files end .7 .rda .sdat or .data.

For GE, data ordering according to Richard Edden's sequence is assumed. Functionality to automatically differentiate this data from Ralph Noeske's GE sequence is planned.

For Philips, we also assume the used of Richard Edden's acquisition implementation. For Philips .sdat, it is assumed that edited data are acquired as a dynamic series of alternating OFF and ON scans. In Gannet, an assumption is made as to whether data are acquired on-first, or off-first, whereas from Gannet 2.0, this is a parameter declared in GannetPreInitialise.

For Philips .data, the same assumptions apply and the data file now contains each FID rather than data time-averaged within each dynamic scan of n averages. If data are acquired with Spectral Correction on (a newer functionality for our patch), then GannetLoad extracts that water data from the same .data file as the edited data (the 'internal' case mentioned above). In Gannet, an assumption is made as to whether data are acquired on-first, or off-first, whereas from Gannet 2.0, this is a parameter declared in GannetPreInitialise.

For Siemens .rda files, it is assumed that time-domain data averaged separately for OFF and ON scans are available, as provided by the Siemens WIP. Most recently, functionality to read in raw .dat files from the TWIX server from either the Siemens WIP or Jamie Near's sequence.

If you are using this software to process your own implementation of GABA-edited MRS, please contact us gabamrs@gmail.com.

In the case where multiple files are processed in a batch by Gannet, some parameters are assumed to be conserved across the group. It is not safe to lump "different acquisitions" into a single Gannet run. While this may raise an error in Matlab, it also may continue processing with incorrect parameters.

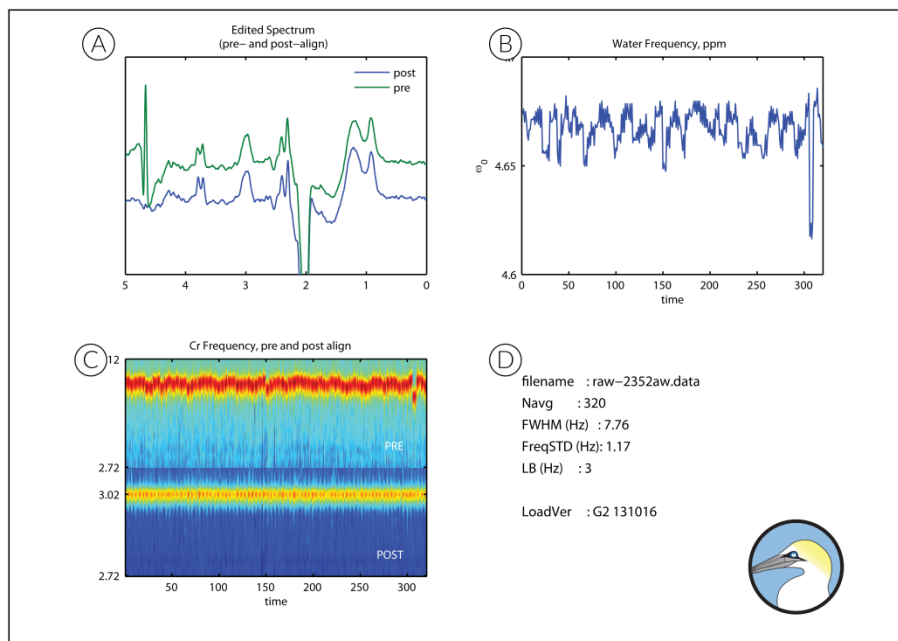
5. Batch Processing

In order to batch process data, simply add more filenames to the string arrays, e.g.:
MRS_struct=GannetLoad({'gaba1.dat' 'gaba2.dat'},{'water1.dat' 'water2.dat'});

Gannet Output

Gannet gives output in two forms: the matlab structure and the figures. The structure itself is described fully below in the section [The Gannet Output Structure](#). A separate output figure is produced for the GannetLoad and GannetFit steps of each dataset. These figures are also saved as pdf files locally.

GannetLoad Output



- A. The plot top left shows the processed GABA-edited difference spectrum, the key output of the GannetLoad module. This plot shows the spectrum before frequency and phase correction above in green and the spectrum after frequency and phase correction below in blue. Hopefully the spectrum below should look nicer than the spectrum above. If it doesn't, there is a problem; see the section [Troubleshooting](#).
- B. The plot top right shows the frequency of the maximum point in the spectrum (usually residual water signal) plotted against time. Time is measured at the resolution of time-resolved data is fed in. The y-axis is free to scale according to the data, so be sure to check the y-range. This information can be interpreted in several ways, but it gives qualitative information on the stability of the experiment whether field drift, subject motion, accuracy of prospective frequency correction etc. Field drift will appear as a non-zero slope in this trace, and movement as a discontinuity in the trace.

Some datapoints may be circled in red in this plot. These are datapoints that have been rejected – see more below.

- C. The plot bottom left presents the Cr signal over the duration of the experiment (same x-axis as B). The y-axis here represents the frequency in ppm of the Cr signal. The spectra at each timepoint are presented as a vertical stripe in the image, color-coded according to signal intensity, so the Cr signal should appear as a 'hot' stripe running through the image. In the upper half (PRE), the stripe should vary in frequency in a similar fashion to the water plot in B. In the lower half (POST), the result of

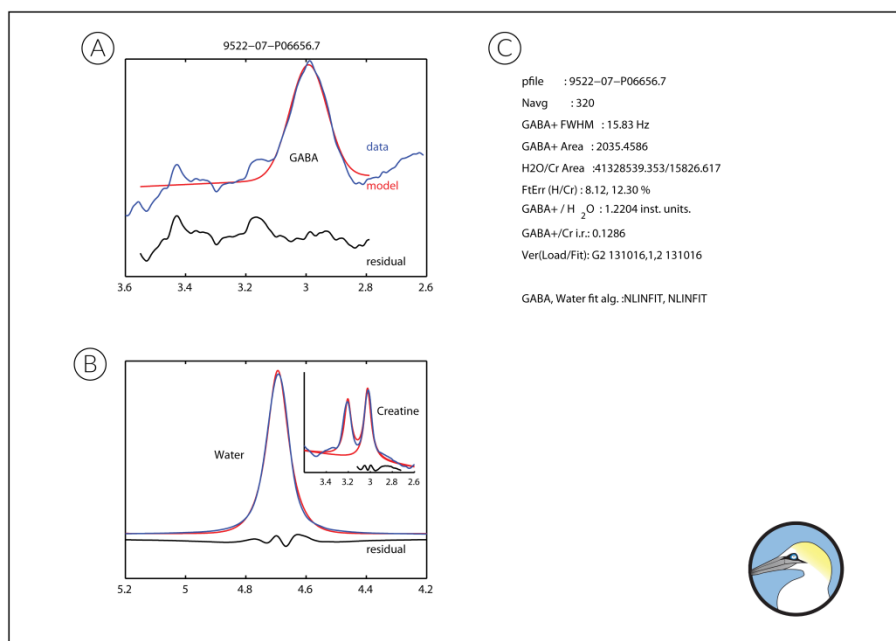
frequency and phase correction (default is spectral registration SR³) is shown and ideally a more uniform horizontal stripe should appear.

In the lower half (POST), some rows will appear as a dark blue stripe (i.e. no Cr peak at all). These rows, corresponding to the red circled points in B, have been rejected because one of the fitting parameters used for frequency correction lie more than three standard deviations from the mean. When an outlier is identified for rejection, rejection is always performed pairwise, i.e. if an OFF scan is rejected, a neighbouring ON scan will also be rejected, so as to balance the number of OFFs and ONs for subtraction.

- D. The panel bottom right shows the filename and some descriptive variables about the data including the full-width-half-maximum linewidth of the Cr signal after SR and averaging (FWHM (Hz)), the standard deviation of the SR correction frequencies (FreqSTD (Hz)), the exponential line broadening applied to the data (3 Hz by default), and the code version used.

³ Near et al. Magn Reson Med. 2014, *In Press*.

GannetFit Output



- The plot top left shows the modeling of the GABA signal. The GABA-edited spectrum is shown in blue (across a more limited ppm range than in the GannetLoad output). Overlaid in red is the model of best fit (using a simple Gaussian model by default). Below the plot, the residual between these two is shown in black.
- The plot bottom left shows the modeling of the signal against which GABA is quantified. Again data are blue, models are red and residuals below are black. If no water data is provided, the spectrum shown will be the Cr signal from the OFF spectrum. If unsuppressed water data are supplied, then the main spectrum will be the water signal and the Cr signal will be shown in an inset. The water signal is modeled as a mixed Gaussian-Lorentzian. From Gannet 2.0 onwards, Cr and Choline (Cho) are modeled as two Lorentzians (with the same linewidth and a fixed frequency separation). In red both the full model and the Cr component of that model are shown.
- The right-hand panel contains the results of the fitting, including the file name, number of averages, the width of the fitted GABA signal, the integral area of GABA, Cr and water models, the fit error of the models (defined as the standard deviation of the residuals expressed as a percentage of the signal height), the GABA concentration expressed in institutional units relative to water and as an integral ratio relative to Cr, and the code versions of GannetFit and GannetLoad used. These numbers are also contained in the matlab output structure – for more detail, see the section [The Gannet Output Structure](#).

Acquisitions for Gannet

Gannet is designed to process difference-edited GABA spectra. In processing data, Gannet makes several assumptions as to the shape of the data, particularly with regards to how OFF and ON acquisitions are ordered within the data. More details of the files supported are given, by vendor, below.

GE implementation

Our implementation of MEGA-PRESS on the GE system (which we are happy to share with collaborators) stores alternating rows of ON and OFF data, each of which represents a time-averaged sum over NEX TRs.

Philips implementation

Our implementation of MEGA-PRESS on the Philips system (which we are happy to share with collaborators) exports .sdatt files with alternating rows of ON and OFF data, each of which represents a time-averaged dynamic scan over a certain number of averages.

It exports .data files with alternating blocks of ON and OFF data. Each block, which corresponds to one dynamic scan, consists of a number of acquisitions corresponding to the number of averages specified in the exam card. Thus the total number of averages for the MEGA-PRESS acquisition is the product of the specified number of averages and number of dynamic scans.

Siemens implementations

The Siemens-supplied WIP sequence can generate separate time-averaged rda files corresponding to the OFF and ON acquisitions. In the TWIX data export, it stores OFF and ON data separately in the NEco dimension (i.e. $NEco = 2$) with averages stored in the NSet dimension. Thus the total number of averages (as we define it) is $NSet * 2$.

We have additionally programmed the TWIX data read to accommodate Jamie Near's implementation of the sequence. For this experiment, OFF and ON data are stored separately in the NIda dimension (i.e. $NIda = 2$) with averages stored in the NSet dimension. Thus the total number of averages (as we define it) is still $NSet * 2$.

Other implementations

We are keen to accommodate further implementations of MEGA-PRESS in order to widen the applicability of Gannet. If you are acquiring data from a currently unsupported data format and would like to use Gannet, contact us at gabamrs@gmail.com.

Other experiments

In addition to GABA editing, we are in the process of developing a Gannet variant that handles glutathione-edited MEGA-PRESS data. Please get in touch if this is of interest, at gabamrs@gmail.com.

Processing of MRS data in GannetLoad

Depending on the degree of pre-processing applied before data export from the scanner, a different number of steps are applied to each data type.

GE p-file data

1. Combination of phased array coil data
2. Time-domain frequency-and-phase correction using spectral correction
3. Exponential apodization function (line broadening)
4. FFT
5. Time averaging
6. Frequency and phase correction based upon fitting of the Cho and Cr signals
7. Pairwise rejection of data for which fitting parameters are greater than 3 s.d. from the mean
8. Subtraction to generate the edited difference spectrum (and extraction of OFF spectrum)

The distinction between step 2 and step 6 is that spectral correction ensures that the frequency and phase of time-domain data are internally consistent, but does not ensure either are 'correct'. In step 6, the frequency of the Cr signal is set to 3.02 ppm and the phase of the spectrum is chosen so as to maximize the match between the data and a simple absorption lineshape model. Prior to Gannet 2.0, frequency and phase correction was performed in the frequency domain, prior to time averaging, according to Evans et al. 2012⁴.

Philips .sdatt data

1. Time-domain frequency-and-phase correction using spectral correction
2. Exponential apodization function (line broadening)
3. FFT
4. Time averaging
5. Frequency and phase correction based upon fitting of the Cho and Cr signals
6. Pairwise rejection of data for which fitting parameters are greater than 3 s.d. from the mean
7. Subtraction to generate the edited difference spectrum (and extraction of OFF spectrum)

The distinction between step 1 and step 5 is that spectral correction ensures that the frequency and phase of time-domain data are internally consistent, but does not ensure either are 'correct'. In step 5, the frequency of the Cr signal is set to 3.02 ppm and the phase of the spectrum is chosen so as to maximize the match between the data and a simple absorption lineshape model. Prior to Gannet 2.0, frequency and phase correction was performed in the frequency domain, prior to time averaging, according to Evans et al. 2012³.

Philips .data data

Processing of Philips .data files proceeds similarly to .sdatt (as above). The only difference between the two is the reduced time averaging of the .data format – one row is exported per TR, rather than one per dynamic scan for .sdatt. The only additional complication this adds is that pairwise rejection for .data will result in rejection of a matching (ON/OFF) scan that comes from the next dynamic scan.

Siemens .rda files

1. Time-domain frequency-and-phase correction using spectral correction

⁴ Evans et al. J Magn Reson Imag 38(4):970-975 (2013).

2. Exponential apodization function (line broadening)
3. FFT
4. Frequency and phase correction (of OFF.rda to ON.rda) based upon fitting of the Cho and Cr signals
5. Subtraction to generate the edited difference spectrum (and extraction of OFF spectrum)

The Siemens rda files are already time-averaged – Gannet still performs a registration of the OFF scan to the ON scan to minimize subtraction artefacts, but there is a limit to what can be done!

Siemens .dat files

1. Combination of phased array coil data
2. Time-domain frequency-and-phase correction using spectral correction
3. Exponential apodization function (line broadening)
4. FFT
5. Time averaging
6. Frequency and phase correction based upon fitting of the Cho and Cr signals
7. Pairwise rejection of data for which fitting parameters are greater than 3 s.d. from the mean
8. Subtraction to generate the edited difference spectrum (and extraction of OFF spectrum)

The distinction between step 2 and step 6 is that spectral correction ensures that the frequency and phase of time-domain data are internally consistent, but does not ensure either are 'correct'. In step 6, the frequency of the Cr signal is set to 3.02 ppm and the phase of the spectrum is chosen so as to maximize the match between the data and a simple absorption lineshape model. Prior to Gannet 2.0, frequency and phase correction was performed in the frequency domain, prior to time averaging, according to Evans et al. 2012⁵. While this frequency and phase correction function still exists, the default and our general recommendation is to use spectral registration³.

⁵ Evans et al. J Magn Reson Imag 38(4):970-975 (2013).

Troubleshooting

In this section, we will try to identify some “things that can go wrong” and “what to do about it”.

1. Difference spectra appear negative

If the GABA signals in GannetLoad both look negative, it is likely that the `MRS_struct.onofforder` parameter is set wrong (the options are the strings ‘onfirst’ or ‘offfirst’). This is set in the GannetPreInitialise script in the Gannet code folder, and determines whether the data are treated as if the on data or the off data are acquired first. Since the correct edited spectrum is “ON subtract OFF”, incorrect assignment of this parameter results in a negative GABA peak.

However, a negative spectrum may also occur if the ON and OFF spectra are correctly assigned, but phased negatively. If that is the case, follow 2.

2. Creatine stripes are negative

If, as seen in the figure above, the creatine signal in the GannetLoad output is negative i.e. it appears as a blue (cold) stripe on a red (hot) background, rather than a red (hot) stripe on a cold (blue) background, then it is likely that the parameter `MRS_struct.WaterPositive` is set incorrectly. In loading the data, a preliminary phasing is performed based on the first point of the time-domain data, and if the residual signal has negative phase relative to the rest of the spectrum (as can happen with some water suppression schemes) then the creatine signals may become phased negatively. The parameter is set to 1 (yes) by default and 0 (no) as appropriate.

Note that the Difference spectrum may appear negative in this case also.

3. Matlab can't find GannetLoad

Make sure the gannet code folder including subdirectories is listed in your matlab path.

4. GannetLoad can't find my files

Either the filenames has been mistyped, the syntax of the call array is incorrect, or the current working directory (type `pwd`) is a mismatch to the filenames used.

5. “Subscripted assignment dimension mismatch”

This is one of the errors that may appear during batch processing if acquisition parameters are not consistent across scans. A similar message appears with improper syntax in calling GannetLoad.

The Gannet Output Structure

After running GannetLoad, e.g.

```
MRS_struct = GannetLoad({'gabafile1.typ' 'gabfile2.typ'}, {'waterfile1.typ' 'waterfile2.typ'}); ,
```

the outputted structure, MRS_struct in this case, will have some of the following attributes. Not all attributes are filled for all calls, for example e.g. water data is not filled where none is supplied).

The structure has been organized to include fields for acquisition parameters (MRS_struct.p), frequency domain data (MRS_struct.fids), spectra (spec) and output results (MRS_struct.out).

MRS_struct.

versionload	String corresponding to the version and date of GannetLoad
ii	During analysis, the current file being loaded. After analysis, the number of files loaded
gabafile	String array containing the inputted GABA filenames {'gabafile1.typ' 'gabfile2.typ'}
waterfile	String array containing the inputted water filenames {'waterfile1.typ' 'waterfile2.typ'}

MRS_struct.p

sw	Spectral width (Hz)
npoints	Number of time-domain complex datapoints per FID loaded
TR	Repetition time (ms)
TE	Echo time (ms)
LarmorFreq	Larmor Frequency, where possible, this is determined from data headers but in some cases (e.g., Philips .data), is taken from GannetPreInitialize
target	Metabolite of interest. By default this is GABA but implementations for GSH are currently being tested
spar_string	Philips-specific string to track capitalization of file endings
ONOFForder	Defined in GannetPreInitialize, this states which of the "ON" and "OFF" editing pulse was applied first in the acquisition
Water_Positive	The polarity of the residual water spectrum.
LB	Exponential line broadening applied to data in Hz
ZeroFillTo	Number of points for zero-filled data
AlignTo	Frequency and phase correction scheme applied. By default and recommended is 'SpecReg'
Reference_compound	Concentrations will be calculated relative to this – 'H2O' is data provided, otherwise 'Cr'
vendor	Scanner vendor (Siemens, GE or Philips) and data type if relevant
npoint_water	Number of time-domain complex datapoints per FID of the water data
nrows	Number of interleaved ON/OFF dynamics
nrows_water	
Navg	Total number of averages
Navg_water	Number of water averages
Nwateravg	Total number of averages of water data
zf	Zero-filling multiple

MRS_struct.fids	
gabadata	Raw time-domain GABA data (dimensions: npoints, nrows) of the last loaded dataset
data_water	Time-domain water data (dimensions: 1, npoints) of the last loaded dataset
flattarget	Real representation of complex data for 'SpecReg'
tempwater	Time-domain water data prior to time averaging
ON_OFF	Vector representing each transient as an ON or OFF acquisition.
waterfreq	ppm value of residual water maximum in time-resolved gaba spectra as plotted top right in GannetLoad output (dimensions: ii, nrows)
data_align	Frequency and phase corrected data in time-domain following SpecReg

MRS_struct.spec

water	Frequency-domain water spectra (dimensions: ii, npoints*zf)
freq	ppm x-axis corresponding to waterspec and gabaspec
off	Frequency-domain OFF subspectra (dimensions: ii, npoints*zf)
on	Frequency-domain ON subspectra (dimensions: ii, npoints*zf)
diff	Frequency-domain difference spectra after frequency/phase correction (dimensions: ii, npoints*zf)
diff_noalign	Frequency-domain difference spectra prior to frequency/phase correction (dimensions: ii, npoints*zf)
diff_scaled	Frequency-domain difference spectra scaled for plotting output

MRS_struct.out

ChoCrMeanSpecFit	Fitting parameters of the Cho and Cr peaks
FreqStdevHz	Standard deviation of waterfreq in Hz
CrFWHMHZ	Width of Cr peak fit in Hz (dimensions: ii, nrows)
rejects	Number of time-resolved spectra rejected due to stability thresholding

After running GannetFit, e.g. MRS_struct=GannetFit(MRS_struct); the outputted structure will have some of the following additional attributes.

MRS_struct.

versionfit	String corresponding to the date of most recent changes to GannetFit
------------	--

MRS_struct.out

fitparams_iter	Record of output parameters from GABA fitting, which is looped 100 times (dimensions: 100, 5, ii)
GABAFitError	Standard deviation of the residual of the GABA peak fit expressed as a percentage of the GABA peak height (dimension: ii)
GABAArea	Integral of Gaussian model fit to GABA peak (dimension: ii)
GABAFWHM	Width (FWHM) of fitted GABA peak in Hz (dimension: ii)
GABAModelFit	Fitting parameters of the model of best GABA fit (dimension: ii,5)
WaterModelParam	Fitting parameters of the model of best waterfit (dimension: ii,6)
WaterFitError	Standard deviation of the residual of the Water peak fit expressed as a percentage of the water peak height (dimension: ii)
GABAIU_Error_w	Error combination of GABAFitError and WaterFitError (dimension: ii)
WaterArea	Integral of Gaussian-Lorentzian model fit to water peak (dimension: ii)
GABAconciu	Main GABA concentration output (dimension: ii)

CrFitError	Standard deviation of the residual of the Cr peak fit expressed as a percentage of the Cr peak height (dimension: ii)
GABAIU_Error_cr	Error combination of GABAFitError and CrFitError (dimension: ii)
CrArea	Integral of Lorentzian model fit to Cr peak (dimension: ii)
ChoArea	Integral of Lorentzian model fit to Cho peak (dimension: ii)
GABAconcCr	GABA concentration output calculated relative to Cr (dimension: ii)
GABAconcCr	GABA concentration output calculated relative to Cho (dimension: ii)

Additional Resources

Jiscmail mailing list

GABAMRS@jiscmail.ac.uk is a mailing list to which any questions about MRS of GABA can be asked. The list also has a searchable archive.

Direct email - GABAMRS@gmail.com

More specific enquiries about Gannet can be made to gabamrs@gmail.com.

Blog

The blog gabamrs.blogspot.com has occasional updates and quite a lot of useful information – the difficulty is finding the useful information.

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