spant User Manual

Martin Wilson

2024-12-10

Table of contents

Welcome

This book is a user-guide for performing basic MR spectroscopy analyses with spant. The focus is on single-voxel ^{1}H MRS data acquired from the human brain - and no knowledge of R or programming is assumed. We focus on the use of a standardised analysis pipeline, developed to cover the majority of use-cases, with minimal need for adjusting the default settings.

At its core, spant is a toolkit of MRS processing and visualisation methods and is therefore capable of numerous analyses types, such as functional-MRS (fMRS), J-edited MRS, 31P MRS and MR spectroscopic imaging (MRSI). However, at the time of writing, pipelines for these less common types of analyses need to constructed by the end-user, which will most likely require a basic knowledge of R programming and MRS acquisition methodology. Help to get started with bespoke pipeline development is available in the on-line package documentation. Whilst there are long term plans to support more analysis types, the current priority is single-voxel ^{1}H MRS data acquired from the human brain, with an emphasis on commercially available sequences and from Siemens, Philips and GE.

Acknowledgements

Writing this user guide began in 2024, and it will continue to evolve, keeping up with the best practices for MRS analysis, and cover new pipelines added to the underlying package. Suggestions for changes or additions to the text and references are very welcome and useful contributions will be acknowledged below. These can be as small as pointing out a typo or a missed reference, or as large as adding a new section, but please get in touch first if you're planning to contribute anything more than a couple of sentences.

- Thanks to Paul Mullins and Chuck Gasparovic for periodically (and patiently) re-explaining the nuances of water concentration scaling. All errors are my own regarding assumptions and implementation related to water concentration scaling.
- Thanks to Jon Clayden for developing and supporting numerous R packages related to neuroimaging that spant depends on.

1 Installing spant

spant is distributed as a software package for the R programming language. Complete the following steps to install spant on your system.

1.1 Step 1 - install R

R software and installation files for Windows, macOS and Linux are made available by a generous team of contributors.

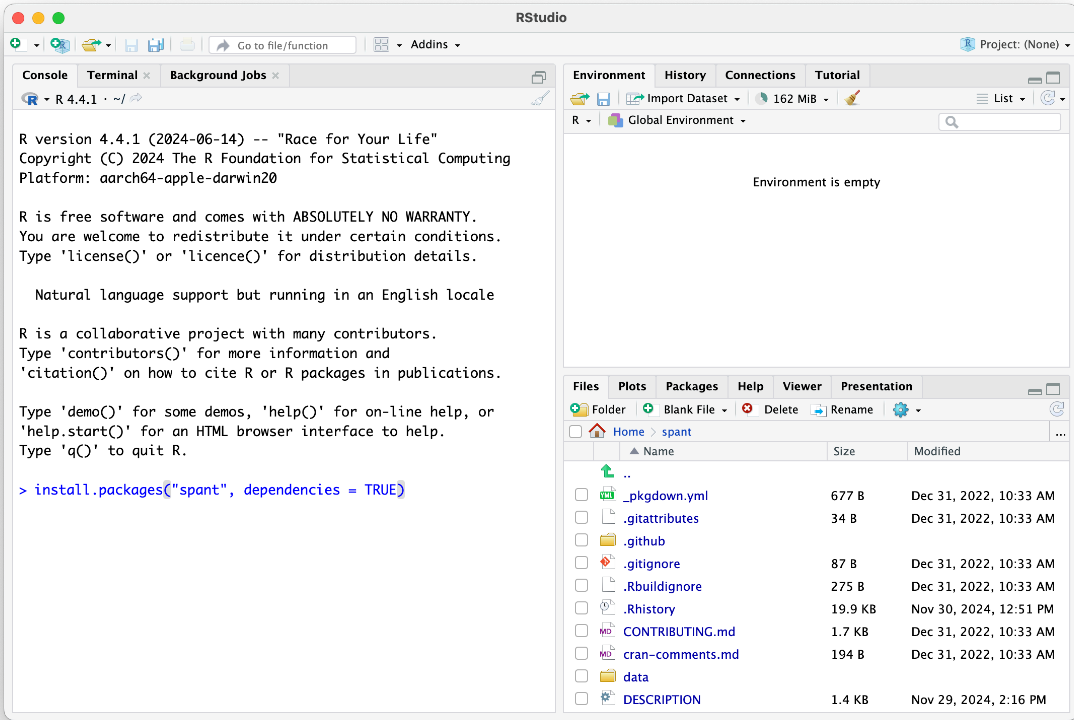
Installation instructions and files for R are available from : https://cloud.r-project.org/

1.2 Step 2 - install RStudio

RStudio is not strictly necessary to use spant, but is strongly recommended if you are a beginner to R, or want to follow along with example in this book. Installation instructions and files for RSudio are available from : https://posit.co/download/rstudio-desktop/

1.3 Step 3 - install spant

Once RStudio is installed and running you will be presented with something similar to the following window.



To install spant, locate the console panel (either the left or lower left panel by default) and enter the following command at the prompt:

```
install.packages("spant", dependencies = TRUE)
```

(as shown in the above image) and press return. Once this process has completed, spant is installed on your system and ready for use. If you ever want to update spant to the most recent version, simply repeat the above command.

2 Single-voxel analysis

In this section we describe the recommended way to perform analysis of single-voxel MRS data acquired from the human brain at a field strength of 1.5 or 3 Tesla with PRESS, STEAM or semi-LASER. We recommend attempting your first analysis using the provided example data to ensure the software is installed correctly.

Before starting analysis, make a note of the current working directory - as this is where the example data will be downloaded and results stored. To print the working directory enter `getwd()` on the command line. If you prefer to work in a different directory use the `setwd("/my/new/path")` command, or navigate to Session and Set Working Directory in the RStudio menu bar.

2.1 Minimal working example

Run the following code to download the example data to your working directory and unzip into a new directory called `svs_slaser_example`:

```
url <- "https://www.dropbox.com/scl/fo/ngjx9povyeroibz8cntwg/ALm-jZ_5zjygvnhXSxJyNWQ?rlkey=3
download.file(url, "svs_slaser_example.zip", mode = "wb")
unzip("svs_slaser_example.zip", exdir = "svs_slaser_example")
```

If the code fails for any reason you can use this link to manually download the data and use your system tools to copy it to your working directory and unzip.

To perform a basic analysis run the following commands:

```
library(spant)                                                        ①
ws_path <- file.path("svs_slaser_example", "sub-01_svs.nii.gz")       ②
fit_svs(metab = ws_path)                                              ③
```

① load the spant library, this only needs to be done at the start of the session
② construct a path for the metabolite (also known as "water suppressed") MRS data file, the file.path function is used to ensure the code is platform independent
③ run the analysis pipeline

Once the `fit_svs` function has completed, a new directory called `sub-01_svs_nii_gz_results` will have been created in the current working directory. The following three files will be inside the results directory:

1. `report.html` - fitting result plots and tables, online example
2. `fit_res_tCr_ratio.csv` - spreadsheet of metabolite quantities as ratios to total-creatine (tCr)
3. `fit_res_unscaled.csv` - spreadsheet of unscaled metabolite quantities

2.2 Common analysis options

2.2.1 Output directory and ratio output

By default, spant will automatically generate an output directory in the current working directory based on the input file name. However, it is good practice to provide an explicit path using the `output_dir` argument show below. The metabolite ratio denominator can be changed with the `output_ratio` argument, changed from the default of total-creatine ("tCr") to total-NAA ("tNAA") below.

```
fit_svs(metab = ws_path, output_dir = "slaser_results_tnaa",
        output_ratio = "tNAA")
```

2.2.2 Water reference data

In addition to water-suppressed metabolite data, single-voxel MRS protocols typically incorporates a short water reference scan, used to scale the metabolite levels as concentrations. The `fit_svs` function performs this automatically, according to the method described by Gasparovic [1], when water reference data is available. Note that the default behaviour assumes the voxel contains 100% white matter, however it is good practice to explicitly set the `p_vols` argument. For further details on the default water scaling method and assumptions see Section 3.1. The example below performs water reference scaling assuming the voxel contains 100% gray matter.

```
wref_path <- file.path("svs_slaser_example", "sub-01_ref.nii.gz")
fit_svs(metab = ws_path, w_ref = wref_path, output_dir = "slaser_results_wref",
        p_vols = c(WM = 0, GM = 100, CSF = 0))
```

Note that an additional file containing the water reference scaled metabolite values (`fit_res_molal_conc.csv`) will be generated in the results directory.

2.2.3 Basis signals

By default, fit_svs will automatically simulate a basis-set containing the following molecular signals:

"m_cr_ch2", "ala", "asp", "cr", "gaba", "glc", "gln", "gsh", "glu", "gpc", "ins", "lac", "lip09", "lip13a", "lip13b", "lip20", "mm09", "mm12", "mm14", "mm17", "mm20", "naa", "naag", "pch", "pcr", "sins", "tau"

The `append_basis` and `remove_basis` arguments may be used to adjust this list. Analyses may benefit from the inclusion of phosphoethanolamine and glycine for higher quality MRS acquired at 3 Tesla and above. The `append_basis` option may be specified to add these signals as follows:

```
fit_svs(metab = ws_path, w_ref = wref_path, output_dir = "slaser_results_peth_gly",
        p_vols = c(WM = 0, GM = 100, CSF = 0), append_basis = c("peth", "gly"))
```

Other common metabolites to append for brain tumour analyses include 2-hydroxyglutarate `"2hg"` and citrate `"cit"`. To see a full list of available molecular parameters run `get_mol_names()`. To print detailed output of a particular molecule, eg NAA, run `get_mol_paras("naa")` in the console.

2.2.4 HSVD water removal

HSVD water removal [2] may be applied as a preprocessing step by setting the `hsvd_width` option to a value representing the width of the filter in Hz. The following example will filter residual water signals between -30 and +30 Hz.

```
fit_svs(metab = ws_path, w_ref = wref_path, hsvd_width = 30)
```

Note that in rare cases HSVD filtering may introduce baseline distortions which are stronger than the residual water, it is recommended to check results with and without filtering to confirm the step results in an improvement.

2.2.5 Eddy current correction

Eddy current correction [3] may be applied as a preprocessing step by setting the `ecc` option to `TRUE` providing water reference data is available. Depending on how the water reference data was acquired, eddy current correction may not always yield improved spectral lineshape, it is recommended to check results with and without correction to confirm the step results in an improvement.

2.2.6 Dynamic frequency and phase correction

For data with very low single-shot SNR it may be beneficial to disable the dynamic frequency and phase correction preprocessing step by setting `dfp_corr` to `FALSE`. Always check the resultant SNR with and without to confirm the optimal setting for your data.

2.2.7 Summary measures table

Whilst around 30 signals are included in a typical analysis, and reported in the html results table, it is often the case that only a subset are of interest for clinical decision making. To aid evaluation speed, the `summary_measures` option allows a subset of signals levels and ratios to be included in a separate summary table in the html output. See below for example use:

```
biomarkers <- c("tNAA", "tNAA/tCr", "tNAA/tCho", "Lac/tNAA")
fit_svs(metab = ws_path, w_ref = wref_path, summary_measures = biomarkers)
```

2.3 Advanced analyses

For advanced users, further fitting options can be found in the on-line reference documentation: function reference.

2.4 Analysis using scripts

Interactively typing commands directly into the R console is a good way to get started, however using scripts is a far more reproducible way to perform analysis. The commands to run analyses for multiple datasets may be stored together in a single script ensuring you have a complete record of all the data files and fitting options used. See this guide for help on how to use scripts in RStudio. Copy and paste the following code into a new script and experiment with using Cmd/Ctrl + Enter to run line-by-line, and Cmd/Ctrl + Shift + S to run the full script.

```
# load the spant library
library(spant)

# define path to the MRS data files
ws_path   <- file.path("svs_slaser_example", "sub-01_svs.nii.gz")
wref_path <- file.path("svs_slaser_example", "sub-01_ref.nii.gz")

# a simple analysis
fit_svs(metab = ws_path, output_dir = "slaser_results_simple")
```

```r
# an improved analysis
fit_svs(metab = ws_path, w_ref = wref_path,
        output_dir = "slaser_results_improved",
        p_vols = c(WM = 0, GM = 100, CSF = 0),
        append_basis = c("peth", "gly"))
```

3 Water scaling details

3.1 Default assumptions

Assuming all metabolite and water signals are measured from a single tissue compartment, and neglecting T_1 and T_2 relaxation effects, we calculate the molal (moles/kg) metabolite concentration as follows:

$$[M]_{molal} = \frac{S_M}{S_{H2O}}[H_2O]_{molal} \tag{3.1}$$

where S_M and S_{H2O} are the metabolite and water signal strengths respectively, and $[H_2O]_{molal}$ is the molal concentration of pure water, 55.51 moles/kg. We also assume S_M and S_{H2O} signals are correctly scaled according to the number of contributing protons.

To correct for relaxation effects we define the attenuation factor, R_x, of a signal x undergoing relaxation with T_{1x} and T_{2x} time constants:

$$R_x = \exp(-TE/T_{2x})[1 - \exp(-TR/T_{1x})]$$

where TE and TR represent the MRS sequence echo time and repetition time. We modify Equation 3.1 to include relaxation effects as follows:

$$[M]_{molal} = \frac{S_M/R_M}{S_{H2O}/R_{H2O}}[H_2O]_{molal}$$

Assumed relaxation values, based on published studies of healthy adults, are given in Table 3.1.

Table 3.1: Assumed water and metabolite relaxation values. Metabolite relaxation values are taken from the average of tNAA, tCho and tCr for white and gray matter where available.

Relaxation value	1.5 T	2.9, 3.0 T	7.0 T
GM water T1 (s)	1.304 [1]	1.331 [4]	2.132 [5]
GM water T2 (s)	0.093 [1]	0.110 [4]	0.050 [6]

Relaxation value	1.5 T	2.9, 3.0 T	7.0 T
WM water T1 (s)	0.660 [1]	0.832 [4]	1.220 [5]
WM water T2 (s)	0.073 [1]	0.0796 [4]	0.055 [6]
CSF water T1 (s)	2.39 [7]	3.817 [8]	4.425 [5]
CSF water T2 (s)	0.23 [7]	0.503 [9]	1.050 [10]
Metabolite T1 (s)	1.153 [1]	1.317 [11]	1.583 [12]
Metabolite T2 (s)	0.347 [1]	0.207 [11]	0.141 [12]

When the voxel contains a mix of compartments, eg 80% white matter and 20% CSF, it is necessary to correct for water densities in each compartment. We assume the following water densities for gray matter, white matter and CSF: $d_{\mathsf{GM}} = 0.78$; $d_{\mathsf{WM}} = 0.65$; $d_{\mathsf{CSF}} = 0.97$; based on values from Ernst et al [13].

For further details on MRS water concentration scaling, including partial volume effects and tissue specific relaxation correction, please see the following papers: [1], [14], [15].

3.2 Manual scaling

Whilst the defaults described above provide reasonable values for healthy brain tissue, they are unlikely to be appropriate for all cases, and alternate assumptions and correction schemes may be required. For example, the default approach assumes all metabolites have the same relaxation properties, which could result in strong bias for certain acquisition protocols (e.g. longer echo-times) or pathology. Unscaled metabolite and water signal amplitudes are therefore provided in the `fit_res_molal_conc.csv` file in the results directory to allow custom corrections. Note that these values have already been scaled for the number of contributing protons. Example R code is listed below to calculate the molal concentrations of tNAA and tCr for a dataset acquired at 3 Tesla with an echo time of 28 ms and repetition time of 2 seconds, assuming the voxel contains 100% grey matter tissue:

```
# define constants
te       <- 0.028 # MRS sequence echo-time
tr       <- 2.0   # MRS sequence repetition-time
t1_gm    <- 1.331 # T1 of grey matter water
t2_gm    <- 0.110 # T2 of grey matter water
t1_metab <- 1.317 # T1 of metabolites
t2_metab <- 0.207 # T2 of metabolites
w_conc   <- 55510 # molal concentration of pure water (moles/kg)

# calculate water and metabolite relaxation attenuation
R_h2o <- exp(-te / t2_gm)    * (1.0 - exp(-tr / t1_gm))
```

```
R_met <- exp(-te / t2_metab) * (1.0 - exp(-tr / t1_metab))

# read the .csv of unscaled values
vals_unsc <- read.csv("fit_res_unscaled.csv")

# calculate tNAA and tCr molal concentrations
tNAA_molal_conc <- (vals_unsc$tNAA / R_met) / (vals_unsc$w_amp / R_h2o) * w_conc
tCr_molal_conc  <- (vals_unsc$tCr  / R_met) / (vals_unsc$w_amp / R_h2o) * w_conc
```

3.3 Legacy scaling

Metabolite concentration scaling using the default method for the popular LCModel MRS analysis software [16] may be added to the analysis output by setting the `legacy_ws` argument for the `fit_svs` function to `TRUE`. Concentrations are calculated as a ratio between the metabolite and water signal intensities (as in Equation 3.1) but with the additional scaling factors representing the NMR-visible water concentration (mM) in the voxel: `WCONC`; and the attenuation of the water signal due to relaxation: `ATTH2O`.

$$[\mathsf{M}]_{\mathsf{lcm}} = \frac{\mathsf{S_M}}{\mathsf{S_{H2O}}} \times \texttt{WCONC} \times \texttt{ATTH2O}$$

The default values for `WCONC` and `ATTH2O` are 35880 and 0.7 respectively. The LCModel manual [17] states that concentrations derived from this method:

"should be labelled 'mmol per Kg wet weight'. We use the shorter (incorrect) abbreviation mM. The actual mM is the mmol per Kg wet weight multiplied by the specific gravity of the tissue, typically 1.04 in brain."

Note that we don't recommend this method of water scaling, but have implemented it to simplify comparison with legacy analysis packages such as LCModel [16] and TARQUIN [18].

3.4 Legacy relaxation assumptions

The following relaxation values were assumed for spant versions older than 3.1.0.

Relaxation value	1.5 T	2.9, 3.0 T
GM water T1 (s)	1.304	1.331
GM water T2 (s)	0.093	0.110
WM water T1 (s)	0.660	0.832
WM water T2 (s)	0.073	0.0792

Relaxation value	1.5 T	2.9, 3.0 T
CSF water T1 (s)	2.93	3.817
CSF water T2 (s)	0.23	0.503
Metabolite T1 (s)	1.15	1.15
Metabolite T2 (s)	0.3	0.3

4 Analysis citations

If you're using the `fit_svs` function in your research please cite [19], [20].

The following methods are used in `fit_svs` by default:

- rats dynamic frequency and phase correction [21]
- water reference concentration scaling [1]

When separate coil information is available the GLS method of combination is used [22].

The following methods are used in `fit_svs` depending on the input options:

- Klose method for eddy current correction [3]
- HSVD residual water removal [2]

References

[1] C. Gasparovic *et al.*, "Use of tissue water as a concentration reference for proton spectroscopic imaging," *Magnetic Resonance in Medicine*, vol. 55, no. 6, pp. 1219–1226, Jun. 2006, doi: 10.1002/mrm.20901.

[2] H. Barkhuijsen, R. de Beer, and D. van Ormondt, "Improved algorithm for noniterative time-domain model fitting to exponentially damped magnetic resonance signals," *Journal of Magnetic Resonance (1969)*, vol. 73, no. 3, pp. 553–557, Jul. 1987, doi: 10.1016/0022-2364(87)90023-0.

[3] U. Klose, "In vivo proton spectroscopy in presence of eddy currents," *Magnetic Resonance in Medicine*, vol. 14, no. 1, pp. 26–30, Apr. 1990, doi: 10.1002/mrm.1910140104.

[4] J. P. Wansapura, S. K. Holland, R. S. Dunn, and W. S. Ball Jr., "NMR relaxation times in the human brain at 3.0 Tesla," *Journal of Magnetic Resonance Imaging*, vol. 9, no. 4, pp. 531–538, 1999, doi: 10.1002/(SICI)1522-2586(199904)9:4<531::AID-JMRI4>3.0.CO;2-L.

[5] W. D. Rooney *et al.*, "Magnetic field and tissue dependencies of human brain longitudinal 1H2O relaxation in vivo," *Magnetic Resonance in Medicine*, vol. 57, no. 2, pp. 308–318, 2007, doi: 10.1002/mrm.21122.

[6] R. Bartha *et al.*, "In vivo 1H2O T2+ measurement in the human occipital lobe at 4T and 7T by Carr-Purcell MRI: Detection of microscopic susceptibility contrast," *Magnetic Resonance in Medicine*, vol. 47, no. 4, pp. 742–750, 2002, doi: 10.1002/mrm.10112.

[7] M. A. Ibrahim, J. F. Emerson, and C. W. Cotman, "Magnetic resonance imaging relaxation times and gadolinium-DTPA relaxivity values in human cerebrospinal fluid," *Investigative Radiology*, vol. 33, no. 3, pp. 153–162, Mar. 1998, doi: 10.1097/00004424-199803000-00005.

[8] H. Lu, L. M. Nagae-Poetscher, X. Golay, D. Lin, M. Pomper, and P. C. M. van Zijl, "Routine clinical brain MRI sequences for use at 3.0 Tesla," *Journal of Magnetic Resonance Imaging*, vol. 22, no. 1, pp. 13–22, 2005, doi: 10.1002/jmri.20356.

[9] S. K. Piechnik, J. Evans, L. H. Bary, R. G. Wise, and P. Jezzard, "Functional changes in CSF volume estimated using measurement of water T2 relaxation," *Magnetic Resonance in Medicine*, vol. 61, no. 3, pp. 579–586, Mar. 2009, doi: 10.1002/mrm.21897.

[10] J. M. Spijkerman, E. T. Petersen, J. Hendrikse, P. Luijten, and J. J. M. Zwanenburg, "T2 mapping of cerebrospinal fluid: 3 T versus 7 T," *Magnetic Resonance Materials in Physics, Biology and Medicine*, vol. 31, no. 3, pp. 415–424, Jun. 2018, doi: 10.1007/s10334-017-0659-3.

[11] V. Mlynárik, S. Gruber, and E. Moser, "Proton T(1) and T(2) relaxation times of human brain metabolites at 3 Tesla," *NMR in biomedicine*, vol. 14, no. 5, pp. 325–331, Aug. 2001, doi: 10.1002/nbm.713.

[12] Y. Li *et al.*, "T1 and T2 Metabolite Relaxation Times in Normal Brain at 3T and 7T," *Journal of Molecular Imaging & Dynamics*, vol. 2, no. 2, pp. 1–5, 2012, doi: 10.4172/2155-9937.S1-002.

[13] T. Ernst, R. Kreis, and B. D. Ross, "Absolute Quantitation of Water and Metabolites in the Human Brain. I. Compartments and Water," *Journal of Magnetic Resonance, Series B*, vol. 102, no. 1, pp. 1–8, Aug. 1993, doi: 10.1006/jmrb.1993.1055.

[14] J. Near *et al.*, "Preprocessing, analysis and quantification in single-voxel magnetic resonance spectroscopy: Experts' consensus recommendations," *NMR in biomedicine*, vol. 34, no. 5, p. e4257, May 2021, doi: 10.1002/nbm.4257.

[15] C. Gasparovic, H. Chen, and P. G. Mullins, "Errors in 1H-MRS estimates of brain metabolite concentrations caused by failing to take into account tissue-specific signal relaxation," *NMR in biomedicine*, vol. 31, no. 6, p. e3914, Jun. 2018, doi: 10.1002/nbm.3914.

[16] S. W. Provencher, "Estimation of metabolite concentrations from localized in vivo proton NMR spectra," *Magnetic Resonance in Medicine*, vol. 30, no. 6, pp. 672–679, Dec. 1993, doi: 10.1002/mrm.1910300604.

[17] S. Provencher, *LCModel & LCMgui User's Manual.* 2021. Available: http://s-provencher.com/lcm-manual.shtml

[18] M. Wilson, G. Reynolds, R. A. Kauppinen, T. N. Arvanitis, and A. C. Peet, "A constrained least-squares approach to the automated quantitation of in vivo ^{1}H magnetic resonance spectroscopy data," *Magnetic Resonance in Medicine*, vol. 65, no. 1, pp. 1–12, Jan. 2011, doi: 10.1002/mrm.22579.

[19] M. Wilson, "Adaptive baseline fitting for 1H MR spectroscopy analysis," *Magnetic Resonance in Medicine*, vol. 85, no. 1, pp. 13–29, Jan. 2021, doi: 10.1002/mrm.28385.

[20] M. Wilson, "Spant: An R package for magnetic resonance spectroscopy analysis," *Journal of Open Source Software*, vol. 6, no. 67, p. 3646, Nov. 2021, doi: 10.21105/joss.03646.

[21] M. Wilson, "Robust retrospective frequency and phase correction for single-voxel MR spectroscopy," *Magnetic Resonance in Medicine*, vol. 81, no. 5, pp. 2878–2886, May 2019, doi: 10.1002/mrm.27605.

[22] L. An, J. W. van der Veen, S. Li, D. M. Thomasson, and J. Shen, "Combination of Multichannel Single-Voxel MRS Signals Using Generalized Least Squares," *Journal of magnetic resonance imaging : JMRI*, vol. 37, no. 6, pp. 1445–1450, Jun. 2013, doi: 10.1002/jmri.23941.