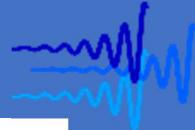


USER-GUIDE MANUAL

---

# HYSCOREAN

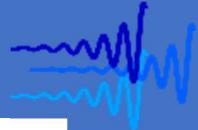
Copyright © 2018 Luis Fábregas Ibáñez



## Contents

1.	Preface .....	3
2.	Getting started.....	4
2.1	Installation .....	4
2.2	Hyscorean's GUI.....	5
2.3	Loading data into Hyscorean .....	6
2.4	Mounting of HYSCORE data .....	7
2.4.1	Mounting Bruker BES3T data .....	7
2.4.2	Mounting EPR@ETH AWG data .....	8
3.	Basic HYSCORE Processing.....	10
3.1	Background correction.....	11
3.2	Processing .....	12
3.2.1	Lorentz-to-Gauss transformation .....	12
3.2.2	Apodization .....	13
3.3	Post-processing.....	14
3.3.1	Graphical settings.....	14
3.3.2	Time-domain signal monitoring .....	17
3.3.3	Blind spots simulator .....	20
4.	NUS HYSCORE Processing .....	22
4.1	Mounting NUS HYSCORE data .....	22
4.2	NUS HYSCORE Pre-Processing.....	22
4.2.1	NUS background correction .....	22
4.3	NUS signal reconstruction.....	23
4.3.1	Iterative Soft-Thresholding (IST) reconstruction .....	25
4.3.2	Maximum Entropy (maxEnt) reconstruction .....	26
4.4	NUS HYSCORE post-processing .....	29
5.	Saving Hyscorean results .....	30
5.1	Saving/Loading settings .....	30
5.2	Saving Hyscorean's session .....	31
5.2.1	Setting the save environment .....	31
5.2.2	Save & Report .....	32
6.	Validation module.....	36
7.	EasySpin fitting module .....	37
7.1	Auxiliary lines & Field offset.....	37

7.2	Starting the fitting module.....	38
7.2.1	Loading single spectra.....	38
7.2.2	Loading multiple spectra.....	38
7.3	Fitting HYSCORE spectra .....	39
7.3.1	Defining the spin system.....	40
7.3.2	Starting/Stopping the fitting .....	42
7.3.3	Speeding-up the simulations .....	45
7.4	Saving the fit results.....	46
8.	Uninstalling Hyscorean .....	52
9.	GNU LGPL 3.0 License .....	53
10.	References .....	56



## 1. Preface

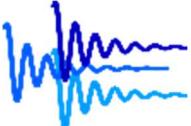
Thank you for downloading Hyscorean (i.e. HYSCORE analysis), an open-source software for the processing and manipulation of experimental HYSCORE spectra controlled via a graphical user-interface. Hyscorean enables the processing of uniform and non-uniform sampled HYSCORE experiments, background fitting, reconstruction of the signals, signal processing, validation and fitting of the spectra using EasySpin. Therefore, Hyscorean takes care of everything from the raw HYSCORE data to the fitted results ready for interpretation. This user-guide describes all elements, procedures and tricks in Hyscorean so that the user can exploit all of the features available.

This program is a free open-source software project: you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation 3.0 or any later version. This software has been written for the MATLAB environment and, therefore, is licensed under the GNU Lesser General Public License (LGPL) 3.0.

I want to acknowledge Stephan Stoll and Bradley Worley for their open-source policies on the source code of their software: EasySpin and CAMERA, respectively, which have allowed Hyscorean to reach this state.

Enjoy processing with Hyscorean,

Luis Fábregas Ibáñez



## 2. Getting started

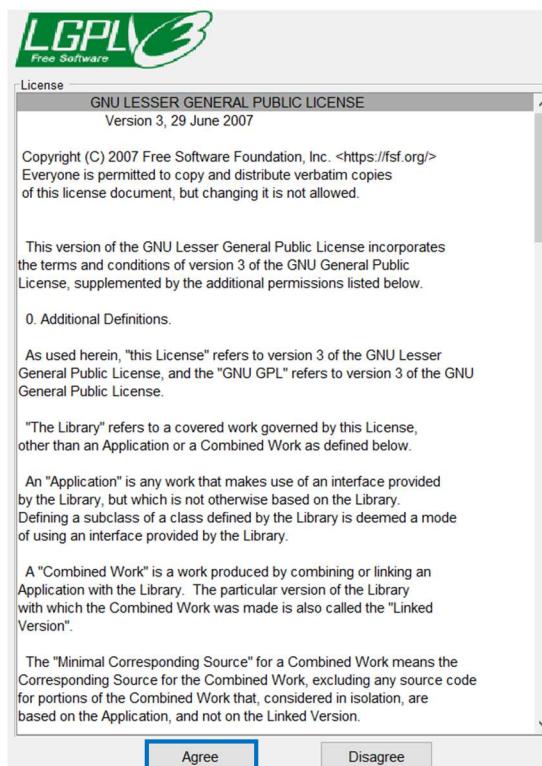
This section will cover the basic operations to be done by the user to install and prepare Hyscorean to its full use. Installation and basic default definitions will be explained step-by-step.

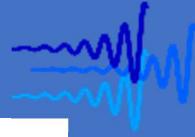
### 2.1 Installation

To install Hyscorean and set up all of the environment variables required by the software to work the following instructions need to be followed:

- I. First all files must be extracted from the ZIP file (downloaded from the EPR@ETH homepage or from the official GitHub server) and saved on a directory/folder of choice.
- II. Open MATLAB and change the current path to the Hyscorean installation folder. It is NOT necessary to add all Hyscorean files to the MATLAB path search.
- III. Execute the script `setup_hyscorean` from the MATLAB console and wait until execution finishes.  
This installation program will take care setting up everything necessary in MATLAB for Hyscorean to work properly. More precisely it takes care of the following:
  - a) All required paths by Hyscorean are saved into the path search of MATLAB for further sessions.
  - b) The installation and license of required MATLAB & Simulink packages are controlled and the functionality of Hyscorean is adapted to missing packages.
  - c) The installation and proper installation of EasySpin is controlled and again the functionality of Hyscorean is adapted if missing or not installed.
  - d) Default user-preferences are set in the MATLAB preferences to allow Hyscorean to keep user-defined defaults between sessions.

Before the all actions are taken the user has to agree to the terms of the GNU LGPL 3.0 license agreement which appears at the start of the setup.





By pressing the button [Agree](#), the license is admitted and the setup proceeds as described. Otherwise, Hyscorean cannot be used until the license agreement is accepted.

- IV. Once the setup program finishes the setup a message will be printed on the console to indicate the outcome of the installation.

A successful installation is indicated by the message:

Hyscorean was successfully and fully installed and all functionalities are operational.

Indicating that the installation proceeded smoothly without any problems and that Hyscorean is ready to be used.

In case some of the external packages is not available these will be individually notified during the installation and once finishes will be indicated by the message:

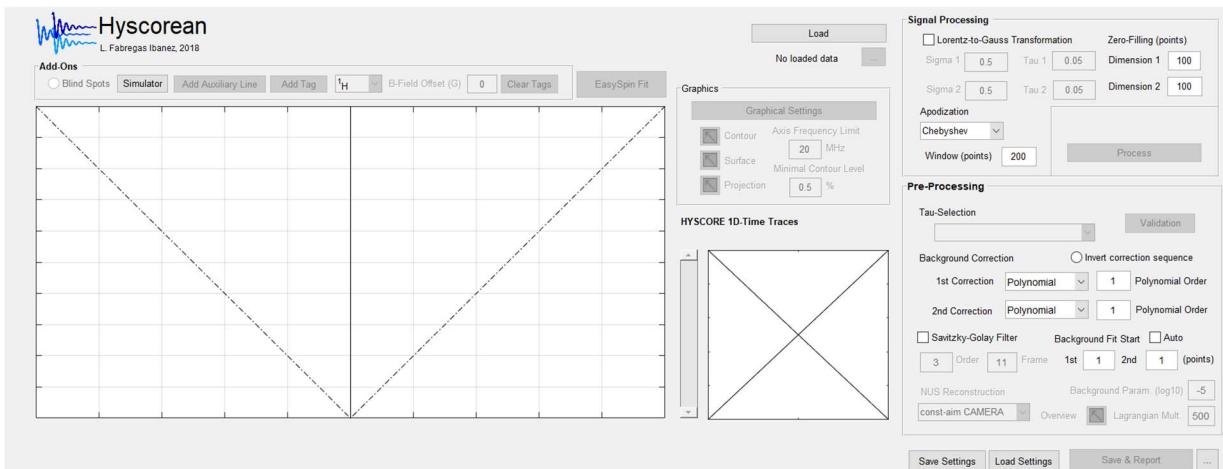
Hyscorean was successfully but incompletely installed.

#### NOTES:

- Once the user defines its own defaults and these are stored in the Hyscorean preferences, re-running [setup\\_hyscorean](#) will NOT overwrite any of these by the defaults set during the first installation.
- Missing packages which are re-installed properly will not be identified until [setup\\_hyscorean](#) is executed again.

## 2.2 Hyscorean's GUI

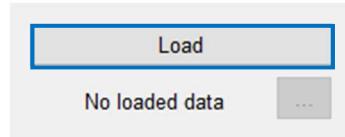
Once installation is finished, Hyscorean can be called from any path in MATLAB by executing the command [Hyscorean](#) from the console. This will prompt the Hyscorean graphical user-interface (GUI) to appear:



At this point most of the user-interface controls will be either deactivated or not visible. As a general feature of Hyscorean, the program will activate and deactivate buttons on the GUI according to what the user can and cannot do at the moment. This prevents the user from doing any action which would lead to a crash.

## 2.3 Loading data into Hyscorean

Files containing HYSCORE signal data can be loaded into the program via the [Load](#) button:



By pushing the button an OS window will appear requesting the user to select the files to be loaded. Multiple files can be loaded by selecting all of them in the window.

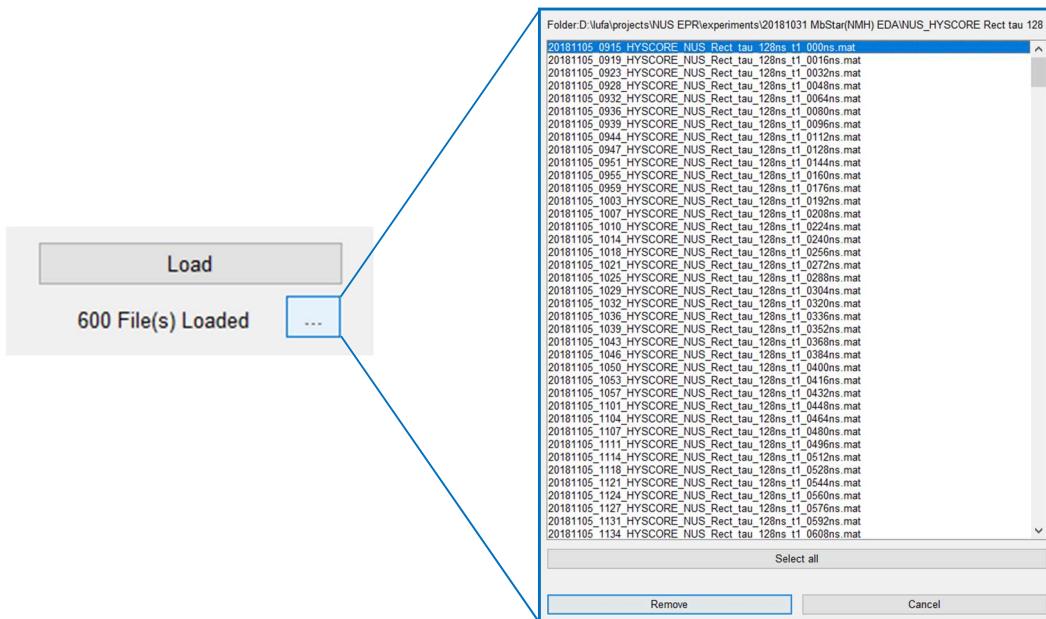
Hyscorean is compatible with the following file extensions:

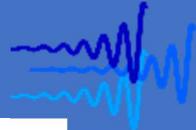
- .DSC      Bruker BEST3T data file format
- .DTA      Bruker BEST3T data file format
- .mat      MATLAB output file format

### NOTES:

Loading new data will reset Hyscorean deleting any data stored into the variables as well as all displays. Loading multiple files is required by measurement done by the EPR@ETH AWG spectrometer. For BEST3T format files a single file has to be loaded.

Once the file(s) have been loaded, the indicator below the [Load](#) button will display how many files were loaded. The user can control at any moment which files are currently loaded into the program by pressing the [\(...\)](#) button next to the display. This will open a window with a list of all loaded files where the user can remove loaded files selectively:





## 2.4 Mounting of HYSCORE data

Once the files are loaded the program automatically will start to mount the data into MATLAB variables which the program will manipulate from that point on. The protocol for mounting the data is different for all the file extensions. In the following a short description of the different mounting protocols will be presented. For a descriptions of the mounting of NUS data files see section 4.1.

**NOTE:**

Once the data has been mounted (for all file formats), the program will automatically set the zero-filling and apodization window lengths in the Processing panel to the corresponding dimensions of the mounted signal.

### 2.4.1 Mounting Bruker BES3T data

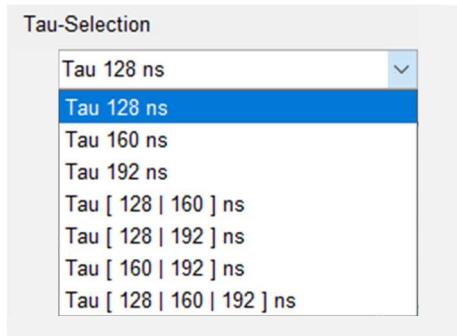
Data obtained from commercial Bruker spectrometers is mounted by loading the .DSC or .DTA files. All experimental parameters and sweep axes are extracted from the descriptors on those files.

Due to the convenience of saving all  $\tau$ -values employed in the HYSCORE experiments into one file, Hyscorean expects the an  $N \times N \cdot M$  data grid where N is the dimension of the HYSCORE signal and M is the number of  $\tau$ -values measured during the experiment. Therefore, the .DSC and .DTA files are expected to have the following structure:

$t_2$	$\tau_1$	$\tau_2$	$\tau_3$	$\tau_4$
	$t_1$	$t_1$	$t_1$	$t_1$

The  $\tau$ -values employed during the experiments are then extracted from the PulseSPEL program stored into the descriptors. It is important that the  $\tau$ -values defined in the PulseSPEL program are stored in the delay **d1**. Hyscorean also automatically detects the number of folded experiments (i.e. number of  $\tau$ -values employed) without any further input from the user.

Once the data has been mounted, all different  $\tau$ -values and their possible combinations will be updated for the user to choose from the [Tau-selection](#) list box:



```

begin exp [INTG QUAD]
for k=1 to n
totscans (n)
scansdone (k)
dy=0
d1=144
for y=1 to 512
    sweep x =1 to sx
        shot i = 1 to h
            p1[ph0]
            d1
            p1[ph0]
            d1
            dy
            p0 [ph1]
            d1
            dx
            p1 [ph2]
            d1
            d0
            acq [sg1]
        next i
        dx = dx +d30
        next x
        dx=0
        dy=dy+d31
    next y
next k
end exp

```

**NOTE:**

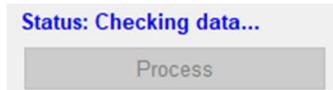
The tau-values combination determines which of the measured HYSCORE signals are added (in time-domain) together prior signal processing. Thus, choosing a different combination will restart the processing from the beginning (see later).

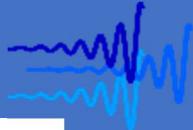
At this point the different parameters for processing of the HYSCORE signal can be selected and the processing started (see 3. Basic HYSCORE processing).

#### 2.4.2 Mounting EPR@ETH AWG data

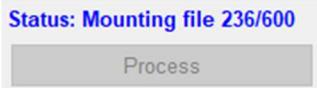
Data obtained from this home-built spectrometer is stored in multiple files, each of them containing a single sweep along one dimension. Therefore, all files have to be loaded in order to mount all of them into a single variable.

In contrast to commercial Bruker spectrometers, the data contains the raw echoes and not the integral. First the echoes are extracted from the output files and mounted. This starts with a control protocol where the consistency between all loaded files of the size of the echo arrays is checked. This procedure is reported via the status display:

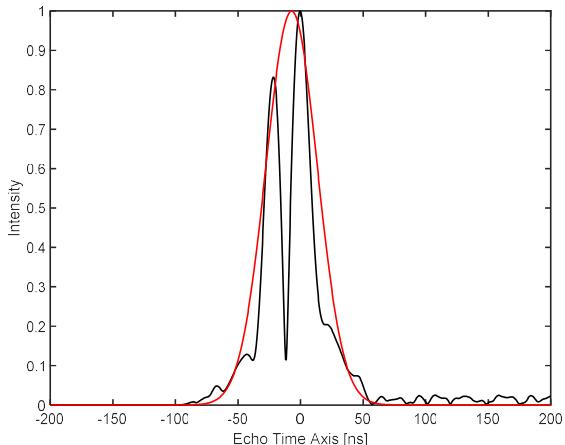




The echoes are then mounted. This procedure can be rather long due to the need to pre-process each echo prior to mounting. The user can monitor the progress again via the status display:



Next, the echoes are integrated according to the following procedure:

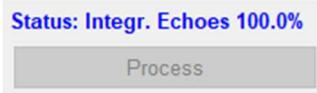


- I. A Gaussian function is fitted to the first echo of the mounted data.
- II. The fitted function is employed as a window and applied to each echo (as a matched filter)
- III. The filtered echoes are integrated

NOTE:

The Gaussian matched filtering can be changed in the function integrateEcho to boxcar integration.

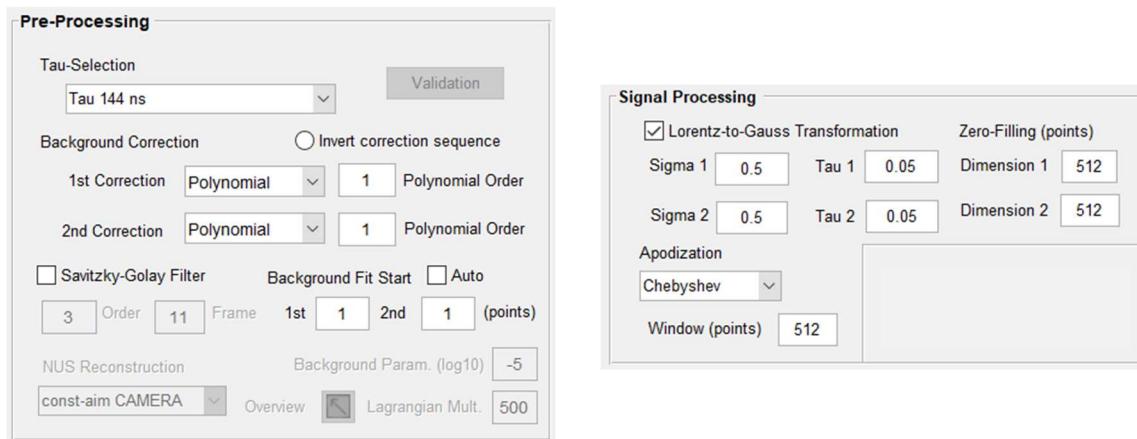
This procedure can also be monitored via the status bar:



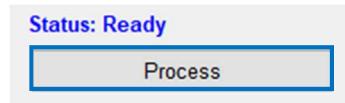
At this point the different parameters for processing of the HYSCORE signal can be selected and the processing started (see 3. Basic HYSCORE processing).

### 3. Basic HYSCORE Processing

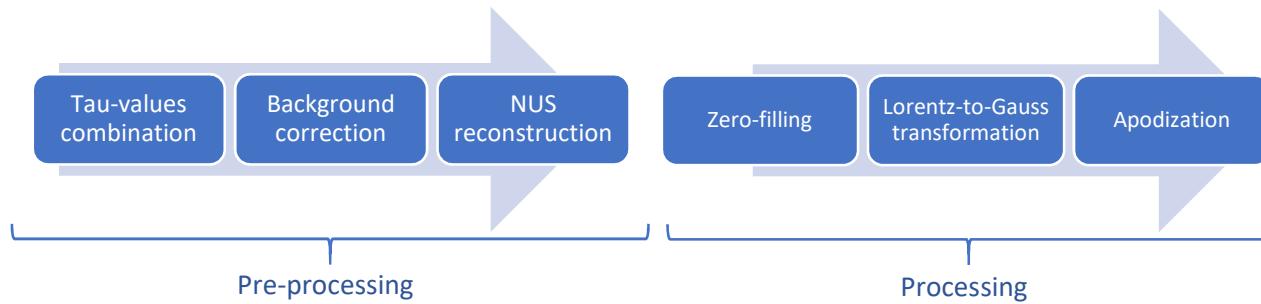
This section presents the basics of Hyscorean to get from the mounted HYSCORE signal to a spectrum. The processing is divided into two clear blocks: the pre-processing and the processing part. The parameters to be set by the user are separated into two different panels (which are activated once the mounting finished) in the Hyscorean GUI:



Hyscorean's processing protocol is started by pressing the [Process](#) button

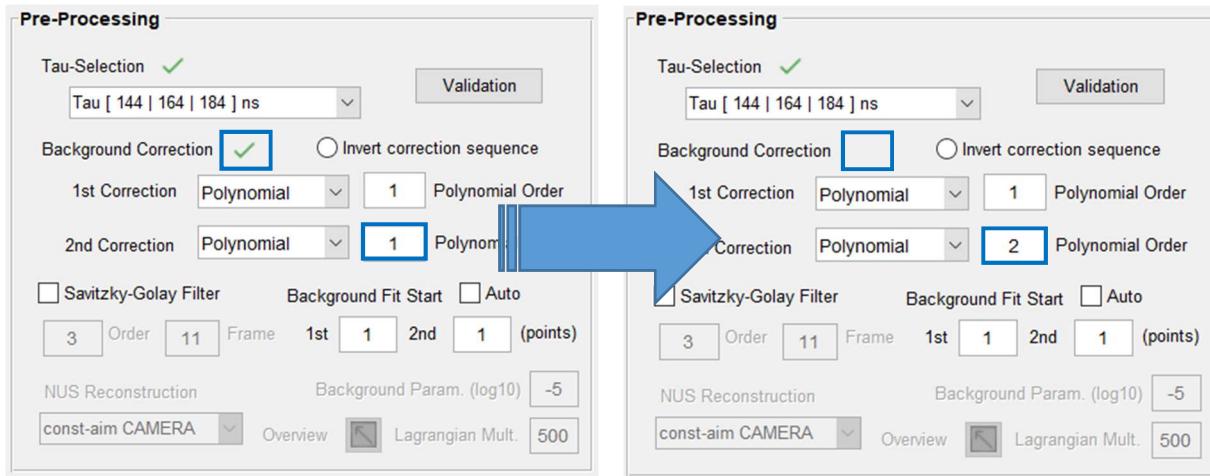
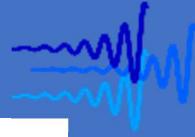


and then follows this structure:



In order to enhance the processing times of Hyscorean a series of switches are employed at each step of the pre-processing. Once the [Process](#) button is pressed and the processing finishes, all switches are activated and pressing the [Process](#) button again will result in the pre-processing being skipped. This allows to avoid performing computationally costly procedures such as background correction or NUS reconstruction (which is always skipped for non-NUS signals) when there are only changes in the processing part. If any of the parameters in the [Pre-processing](#) panel is modified the switches will be deactivated and the processing will start at that point of the pre-processing.

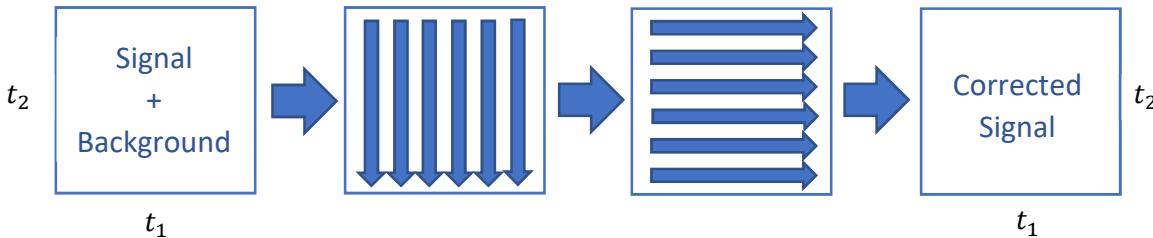
The steps of the pre-processing which have been finished and will be skipped are denoted by the marker ✓ next to the panel components.



In the following sections the different procedures in the whole processing protocol will be presented and described. The NUS reconstruction will be discussed in section 4.

### 3.1 Background correction

The background or baseline correction follows the combination of the different HYSCORE signals at different  $\tau$ -values. The correction of the 2D-background is performed sequentially. First the baselines of the single 1D-traces are corrected along one dimension and then repeated along the other.



To each trace along a first dimension, a model is fitted and then subtracted to each of them. Next, another model is fitted to the traces along the second dimension and again subtracted. The order in which the background is corrected can be inverted by enabling the [Invert correction sequence](#) option. The background models available in Hyscore are:

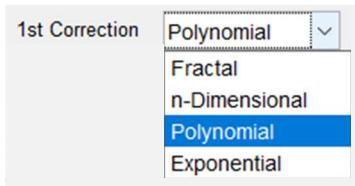
- Polynomial of n-th order
- Exponential (polynomial fitted to the logarithm)
- Stretched exponential
- Fractal

$$B(t) = \sum_{i=0}^n c_i t^i$$

$$\log(B(t)) = \sum_{i=0}^n c_i t^i$$

$$B(t) = \exp(-ct^{n/3})$$

$$B(t) = \exp(-ct^{n/3})$$



The model to fit along each dimension can be selected under the [Background correction](#) section. For each model the dimensionality parameter n can be given in the edit box next to the model list:

Polynomial Order    Exponential Order    Fractal Dimension

The background if fitted to the whole trace starting from the first point. However, the point at which the model starts to be fitted can be adjusted manually from the [Background start](#) edit boxes:

Background Fit Start			
1st	<input type="text" value="1"/>	2nd	<input type="text" value="1"/> (points)

For non-NUS data this represents the end of the pre-processing and leads to the start of the processing.

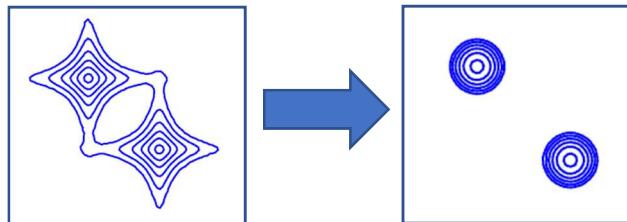
### 3.2 Processing

The processing starts by zero-filling the pre-processed signal (i.e. appending a certain number of zeros to the end of the signal). By default, the program automatically sets this number to the dimensions of the signal so that the full information of the signal is recovered later in the Fourier transform. Any further zeros can be appended by modifying the [Zero-filling](#) edit boxes in the [Processing](#) panel for aesthetic changes.

The zero-filling can be disabled by setting the values in the edit boxes to zero. However, this is not recommended due to the consequent loss information.

#### 3.2.1 Lorentz-to-Gauss transformation

Exponentially decaying time-domain signals such as in HYSCORE correspond to Lorentzian line shapes in frequency domain. The Lorentzian line shapes are characteristic for having long tails which can become very damaging in 2D-spectra such as HYSCORE's due to possible overlaps between crosspeak tails appearing as spurious crosspeaks.



The Lorentz-to-Gauss transformation allows to convert the Lorentzian line shape to the Gaussian line shape which exhibits narrower tails. Therefore, such overlaps between crosspeak tails can be avoided. This transformation is achieved by applying the following window to the signal:

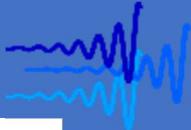
$$W(t_1, t_2) = e^{t_1/\tau_1 - (\sigma_1^2 t_1^2)/2} e^{t_2/\tau_2 - (\sigma_2^2 t_2^2)/2}$$

where the parameters in Hyscorean are computed as:

$$\hat{\sigma}_i = \frac{\hat{\sigma}_l}{\tau_i} \quad \tau_i = t_{i,max} \hat{\tau}_l$$

The factors  $\hat{\sigma}_l$  and  $\hat{\tau}_l$  can be given through Hyscorean's Processing panel. By default, Lorentz-to-Gauss transformation is disabled in Hyscorean unless the corresponding check box is enabled:

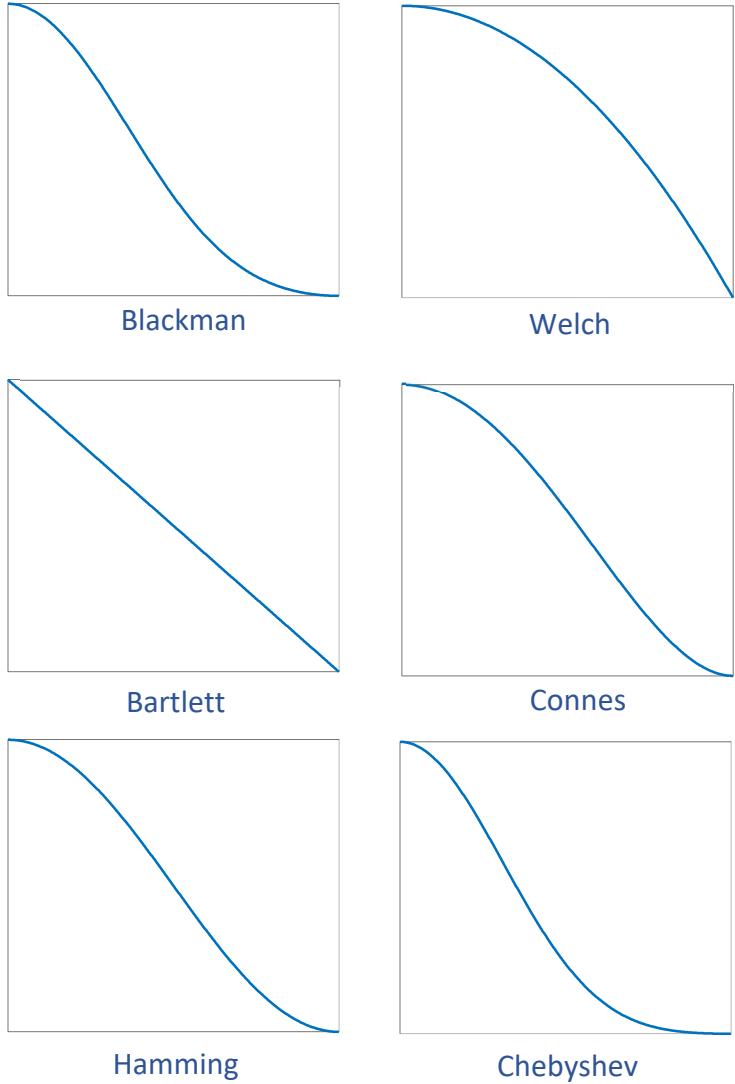
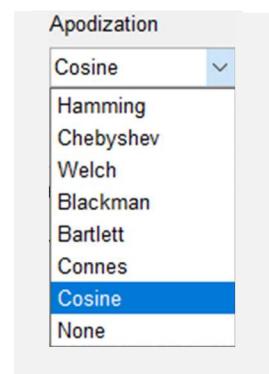
<input checked="" type="checkbox"/> Lorentz-to-Gauss Transformation			
Sigma 1	<input type="text" value="0.5"/>	Tau 1	<input type="text" value="0.05"/>
Sigma 2	<input type="text" value="0.5"/>	Tau 2	<input type="text" value="0.05"/>



### 3.2.2 Apodization

If the signal has not decayed fully to zero at the time the measurement finishes will lead to the so-called truncation artifacts in frequency-domain. This can be avoided by applying a window function which induces an artificial decay of the signal.

Hyscorean offers several different apodization windows which can be selected through the [Apodization](#) list box in the Processing panel:



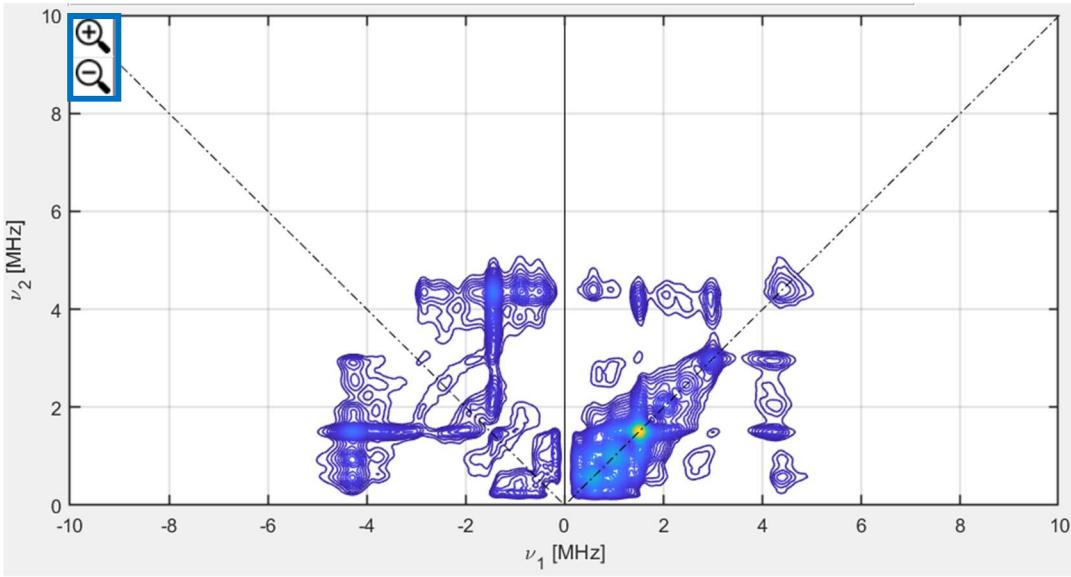
Hyscorean also allows the user to give as an input, the number of points after which the apodization window has decayed completely. This is also automatically set to the dimension of the signal after it is mounted. This value can be changed in the edit box near the window selection list box:

Window (points) 512

Apodization can be disabled and skipped by selecting the [none](#) options from the list box.

### 3.3 Post-processing

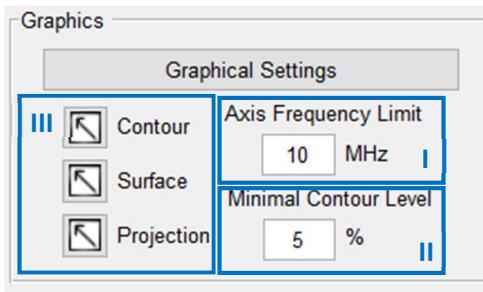
Once Hyscorean is done with the pre-processing and processing the resulting signal is Fourier transformed via Fast-Fourier transform (FFT) to the corresponding HYSCORE spectrum. This is then plotted as a magnitude contour plot (per default) on the main display. During the rendering of the spectrum, all GUI elements are disabled so that the user cannot overload the number of spectra to plot. This is denoted by the status message “Rendering...”.



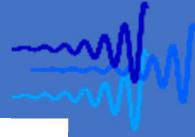
As visual guides, dashed lines are always displayed along the diagonal and anti-diagonal as well as a line along the first dimension’s zero frequency. Along the spectrum the zoom buttons are activated to allows the user to zoom in and out of the HYSCORE spectrum as in normal MATLAB plots.

#### 3.3.1 Graphical settings

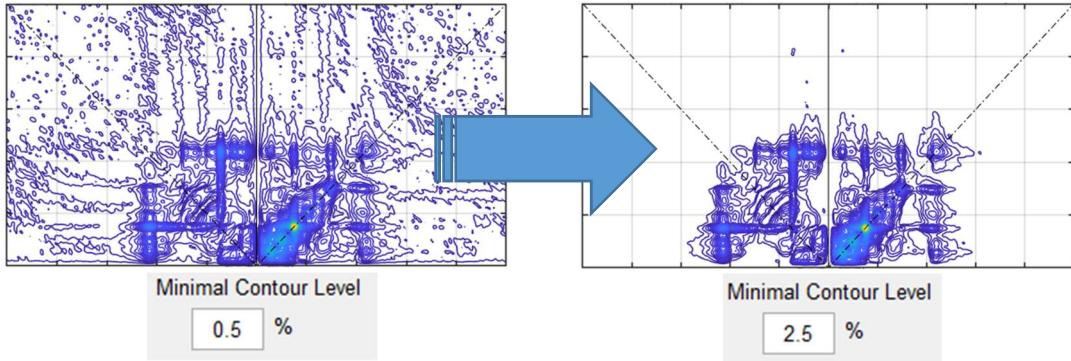
At this point the user may change the appearance of the spectrum via the now activated [Graphics](#) panel:



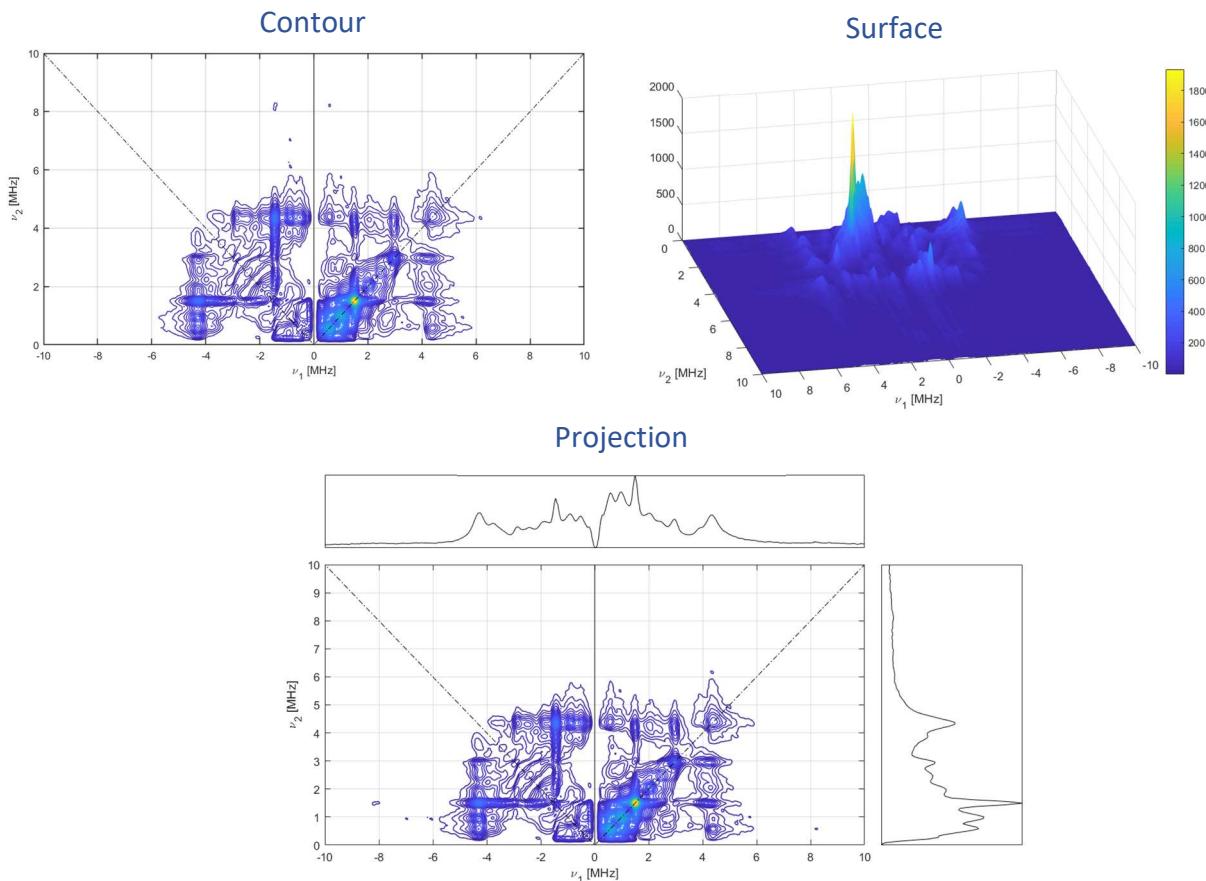
- I. The [Axis Frequency Limit](#) edit box sets the maximal absolute frequency to which the spectrum is plotted (on its zoomed out state) and given in Megahertz. This value is later passed on to any other function which plots a HYSCORE spectrum of any kind.
- II. Due to noise, contour plots can become crowded with many lines, obscure some features or just not be aesthetically appealing. This can be changed by setting the lowest contour level to a certain percentage of the maximal intensity. The contour levels are then equidistantly constructed between this lowest level and the maximal value of the spectrum. Anything below that minimal value is not

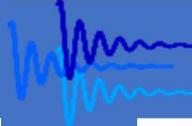


plotted as a contour. This percentage value is given by the user in the Minimal Contour Level edit box



- III. The spectrum can be detached into a separate new window by pressing one of the detach buttons on this group. Once detached the spectrum can be saved or copied just as normal MATLAB figures. Hyscorean spectra can be detached into three different formats: contour (just as in the GUI), surface (3D-surface with colorbar) and projection (contour plot with inset displaying a skyline projection along each axis).

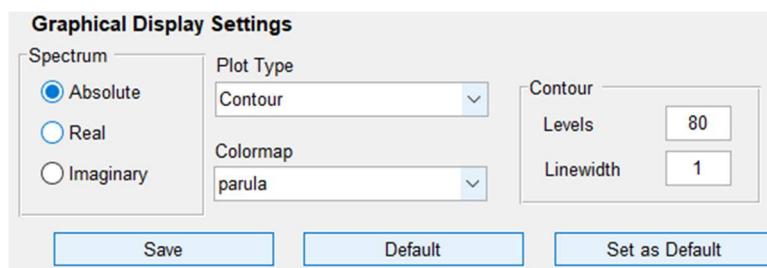




**NOTE:**

Any changes made in the [Graphics](#) panel will result in an automatic update of the spectrum with the updates settings. This is again notified by the status message: "Rendering...".

Further graphical settings can be changed by pressing the [Graphical Settings](#) button. This will prompt a new GUI window to appear as follows:



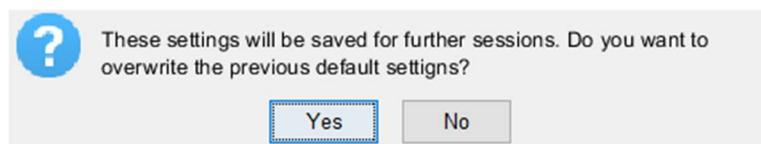
Here different changes can be made:

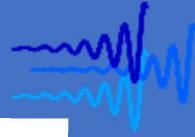
- ❖ The [Spectrum](#) panel contains buttons which determine which component of the spectrum is to be plotted: real, imaginary or absolute (default) spectrum.
- ❖ The [Plot Type](#) box list allows the user to change the way the HYSCORE spectrum is displayed in Hyscorean: contour (default), pseudocolor or filled contour (in MATLAB: contour, pcolor and contourf respectively).
- ❖ The [Colormap](#) box list allows the user to select the colormap to be used from a list of all standard MATLAB colormaps.
- ❖ In the case of the contour or filled contour display modes the [Contour](#) panel allows to set the number of contour lines and their width.

**NOTE:**

Large numbers of contour lines can have huge impacts on the rendering times of the HYSCORE spectra for computers with old graphic cards or laptops.

Once the desired settings have been selected, the [Save](#) button can be pressed to return to the Hyscorean main window and update the HYSCORE spectrum with the new settings. If the user desires to return the settings to their former default state, the [Default](#) button can be pressed. Should the user want to override the defaults with a new setting the [Set as Default](#) button can be pressed. This will prompt a confirmation window before the defaults are overridden. Selecting Yes then save the user-defined defaults for all further sessions.

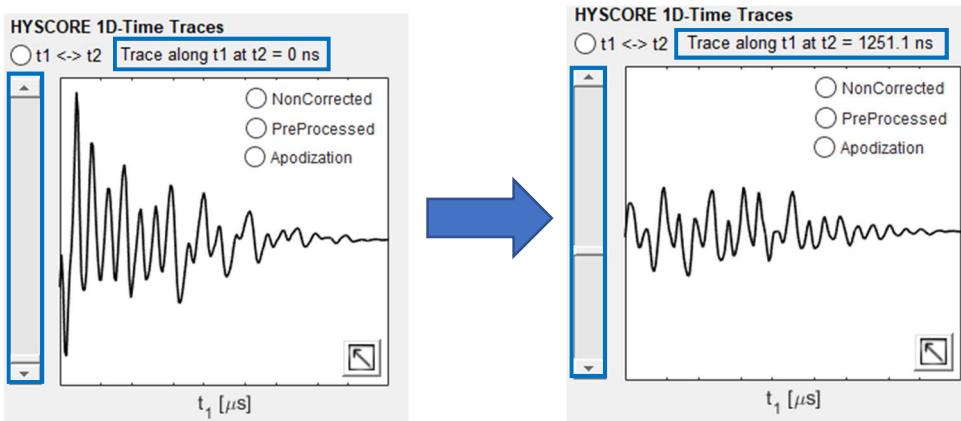




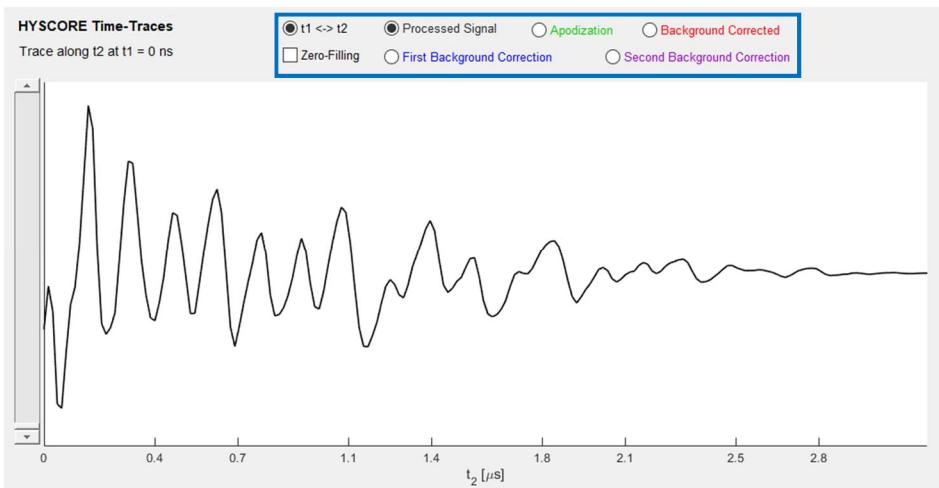
### 3.3.2 Time-domain signal monitoring

During the processing in Hyscorean, the time-domain signal undergoes several procedures which change it. Of course, ultimately the HYSCORE spectrum contains most of the information the spectroscopist is interested from signal. Nonetheless, monitoring the state of the time-signal during its processing is a good routine to adapt, since it allows a good check of whether information may be lost before obtaining the spectrum.

After the processing is finished, the processed signal is displayed as a trace in the smaller display next to the HYSCORE spectrum. By default, the traces are shown along the first dimension. However, by pressing the **t1<>t2** button will invert the display along the second dimension. In both cases the other dimension can be swept by means of the slider next to the display.



For better display and options the time-domain signal can be monitored from a detached window by pressing the **detach** button on the display. This will prompt a new GUI to open with a similar structure to the one in the main window:



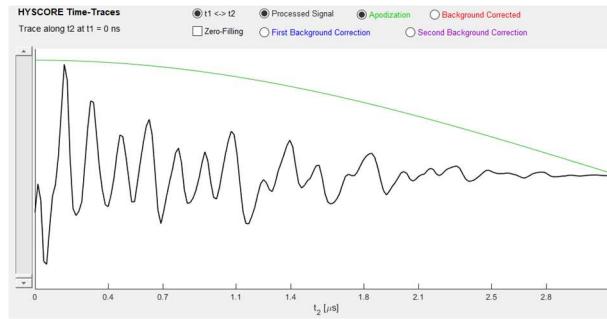
This new window works basically as described above with the addition that the signal at different states can be displayed/monitored by activating them via the highlighted buttons:

- ❖ Processed signal:

The fully processed signal from which the HYSCORE spectrum is obtained. The same signal that is automatically displayed on the main window display. The appended zeros by zero-filling can be displayed by activating the corresponding Zero-Filling check box.

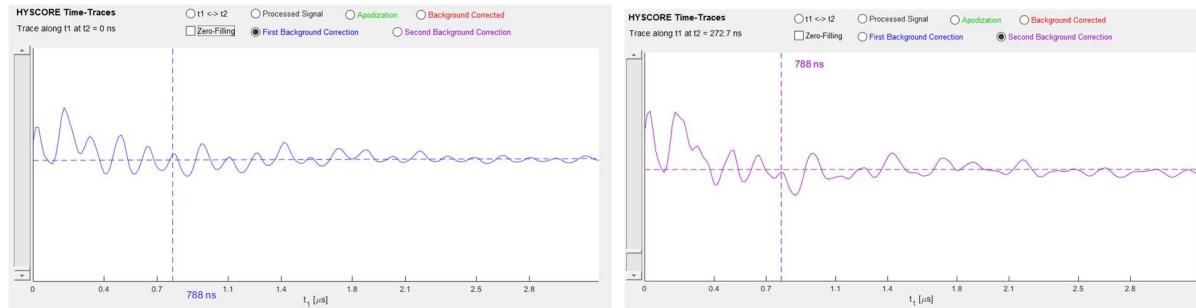
❖ Apodization:

The apodization window function employed on the pre-processed signal is displayed as a green line (can also be displayed on the main window).



❖ First/Second Background Correction:

Displays the signal before the first/second background correction overlaid with the corresponding fitted background (dashed line). A vertical dashed line marks the point at which the background starts to be fitted with and additional tag indicating the exact time.

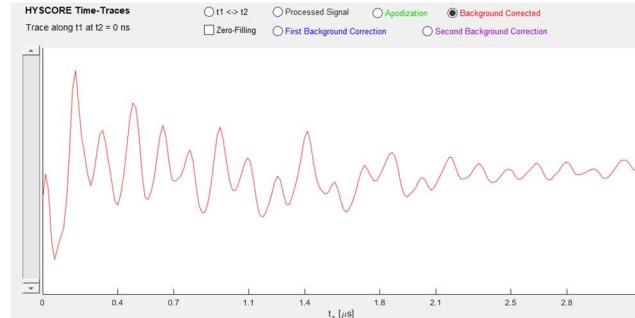


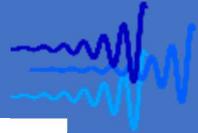
**NOTE:**

Due to the background correction being performed individually for each dimension, trying to change the dimension via the  $t_1 <-> t_2$  button will not have any effect on these displays.

❖ Background corrected:

The signal right after the pre-processing displayed as a red line (can also be displayed in the main window).





All of these displays can be enabled and disabled at the same time so that the user may compare the signal at different stages of the processing, e.g. check the fitted background during the background corrections. This detached window can be left open and should the user process the signal with new settings, Hyscorean will recognize that this window is open and update it automatically without the need to close and open it again.

**NOTE:**

This signal monitoring window sets MATLAB under a uiwait state. This means that as long as the window is open, MATLAB will not be able to open any files. To open files, the signal monitoring window needs to be closed.

### 3.3.3 Blind spots simulator

In a spectrum of a signal obtained from a HYSCORE experiment:

$$\frac{\pi}{2} - \tau - \frac{\pi}{2} - t_1 - \pi - t_2 - \frac{\pi}{2} - \tau - \text{echo}$$

The intensity of the crosspeaks appearing at  $(\pm\nu_1, \pm\nu_2)$  are given by [1]:

$$I(\nu_1, \nu_2) = \lambda z^2 \sin\left(\frac{2\pi\nu_1\tau}{2}\right) \sin\left(\frac{2\pi\nu_2\tau}{2}\right)$$

where  $\lambda$  is the modulation depth and  $z^2$  a contribution resulting from different mixing of the nuclear Zeeman states in the  $\alpha$  and  $\beta$  electron spin manifolds. From this equation it is apparent that the factor

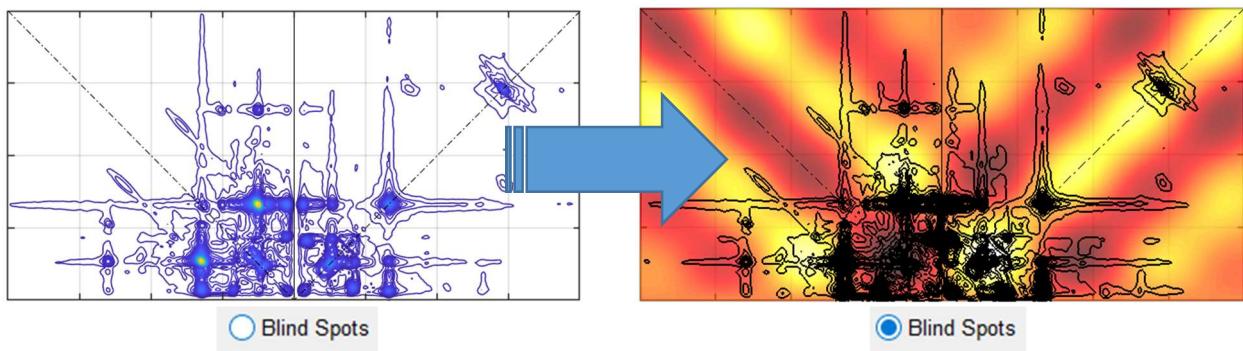
$$b(\nu_1, \nu_2, \tau) = \sin\left(\frac{2\pi\nu_1\tau}{2}\right) \sin\left(\frac{2\pi\nu_2\tau}{2}\right)$$

Introduces a  $\tau$ -dependent blind spot behavior of the HYSCORE spectrum. This blind spots can be highly damaging and dangerous for the interpretation of HYSCORE spectra. This is a reason why HYSCORE experiments should be recorded at different  $\tau$ -values.

In Hyscorean, the blind spots corresponding to the currently selected  $\tau$ -values in the Tau-Selection list box can be simulated via

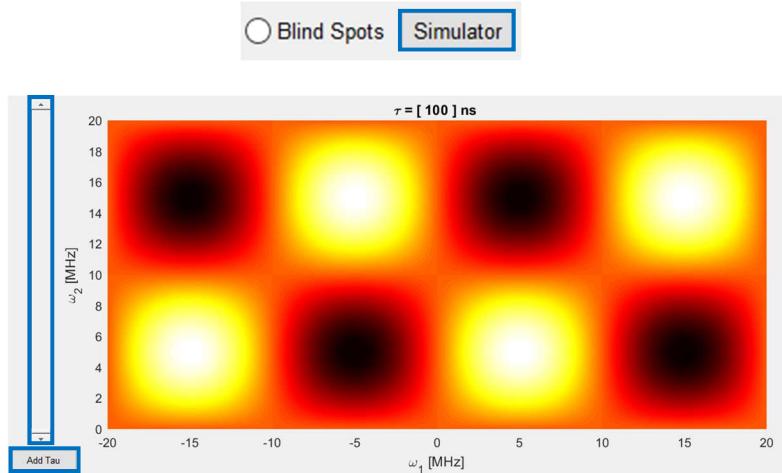
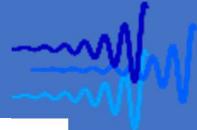
$$B(\nu_1, \nu_2) = \sum_{k=1}^N b(\nu_1, \nu_2, \tau_k)$$

where N is the number of combined  $\tau$ -values. This blind spot map is then superimposed onto the displayed spectrum in order to see which parts of the spectrum may be suppressed by such blind spots. This can be enabled and disabled from the Blind Spots button.

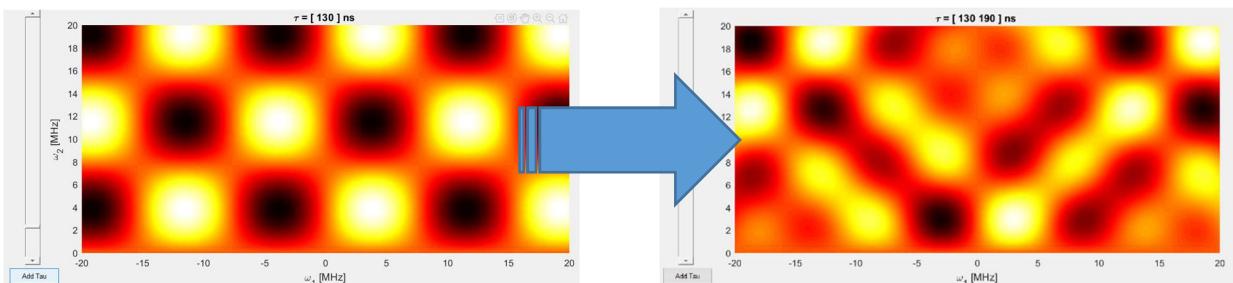


The blind spots are identified as the darker spots in the superimposed map, whereas the brighter spots represent the zones of maximal intensity.

There is also the possibility to simulate the blind spots from a constructed sequence of  $\tau$ -values. This is helpful to interactively find appropriate  $\tau$ -values for an experiment. The simulator can be opened by pressing the Simulator button.

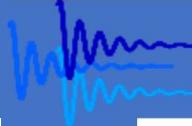


The current  $\tau$ -values are given in the top of the figure. The current  $\tau$ -value can be changed by moving the slider. Once a desired  $\tau$ -value is found it can be added to the list by pressing the Add Tau button, which will fix the  $\tau$ -value and add a next one to be changed:



NOTE:

The slider can adopt  $\tau$ -values between 100ns and 350ns. Also the response time for the simulator can be longer than expected for computers with low graphics capabilities.



## 4. NUS HYScore Processing

### 4.1 Mounting NUS HYScore data

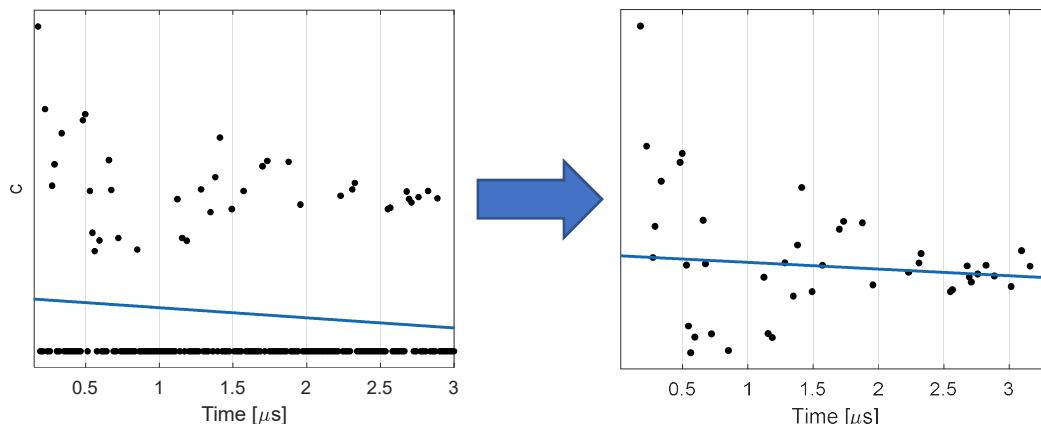
### 4.2 NUS HYScore Pre-Processing

The main difference between “standard” HYScore processing and NUS HYScore processing is the fact that an additional reconstruction step has to be incorporated. Once the NUS signal is reconstructed all remaining steps for the processing remain unchanged. Nonetheless, small differences arise during other steps of the pre-processing and these will be handled in the following sections.

#### 4.2.1 NUS background correction

Once the NUS data has been mounted, the multiple signal measured at different  $\tau$ -values are combined according to the user input. At this point the signal is zero-augmented and has to be background corrected. Due to this artificially inserted zeros, a try to fit the background from the data would be ill-posed leading to wrong backgrounds.

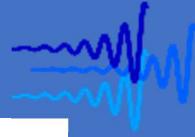
Therefore, in order to avoid this, the zeros introduced by zero-augmentation are converted to NaN values and then background corrected. MATLAB fit functions are programmed to ignore any NaN values from the data being fitted. Thus, only the measured signal points are fitted and then background corrected.



Once the background has been corrected, the NaN values in the signal are returned to zeros to give the background-corrected and zero-augmented NUS signal.

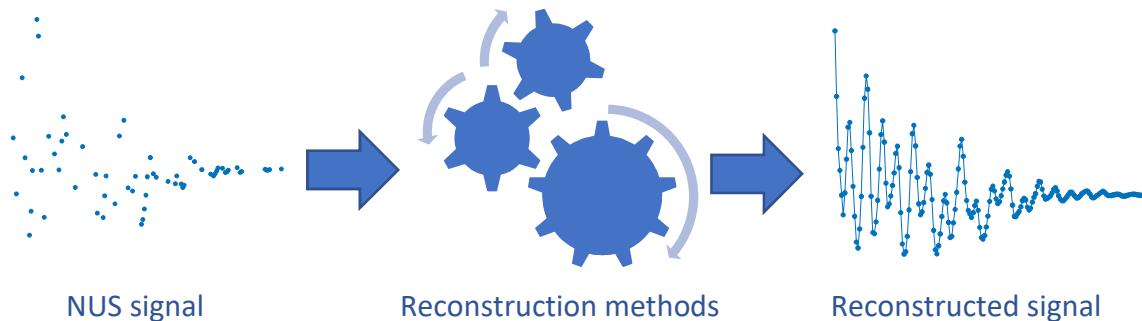
#### NOTE:

As the number of points in the traces get lower, the fitted backgrounds may start to behave strange or even look impossible. This is however not an issue since the fit between the few points is actually good. For the non-measured points it does not matter how the background is fitted since they are set to NaN and remain so even when a strange background is subtracted from them.



### 4.3 NUS signal reconstruction

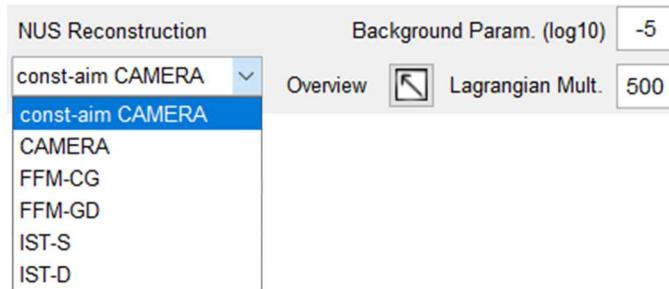
In this new step, the NUS signal has to be reconstructed:



Due to the non-uniform nature of the measurement, the Fourier transform of NUS signals no longer corresponds to an expansion in an orthonormal Fourier basis set and therefore is not a transform anymore in a strict sense [2]. However, discrete Fourier transform (DFT) of the zero-augmented NUS signal still is possible and leads to a spectrum which in Hyscorean is referred to as the nuDFT (non-uniform DFT) spectrum.

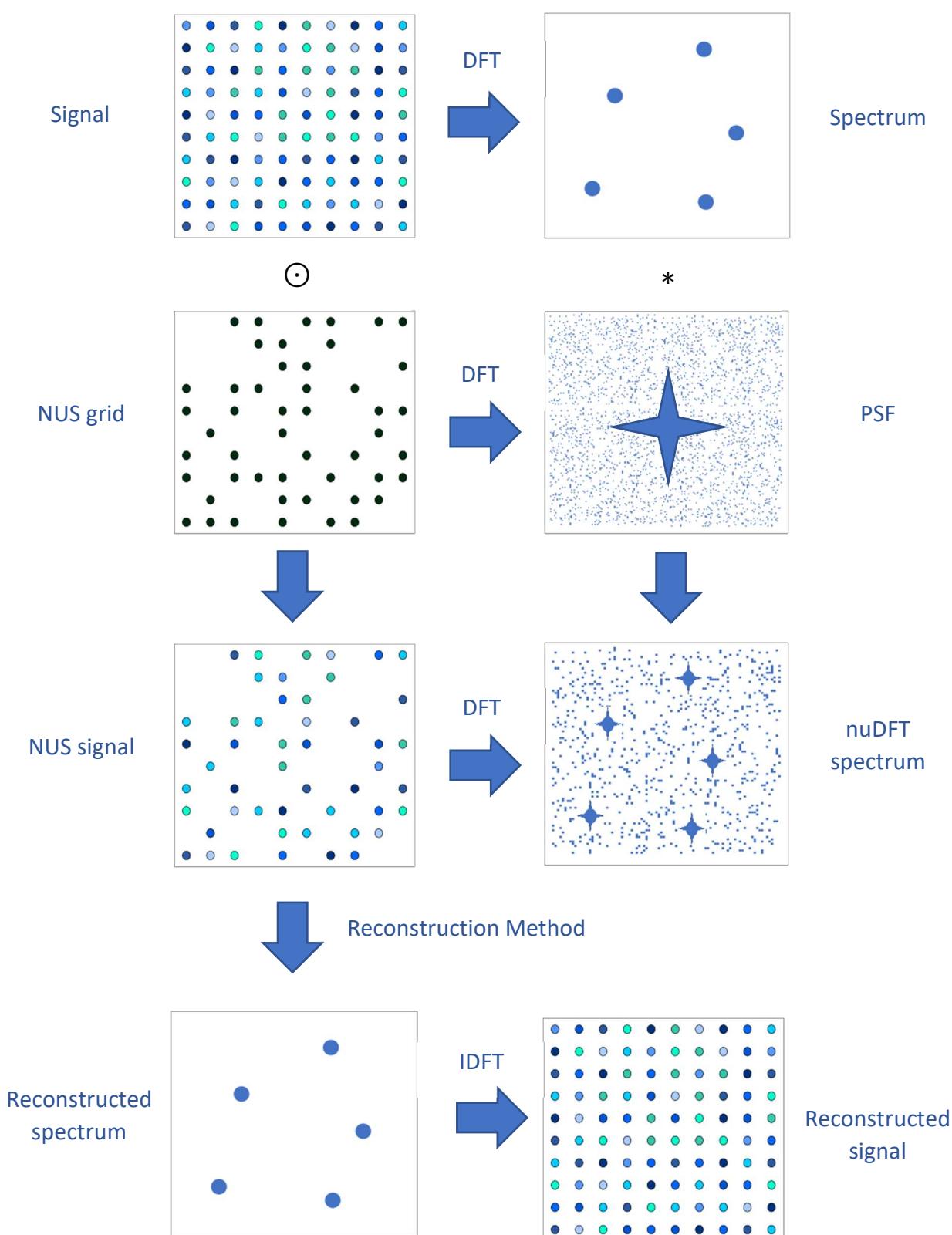
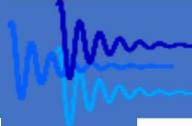
The nuDFT spectrum is the convolution of the uniformly sampled spectrum with the point-spread function (PSF) of the sampling grid, which can be obtained by Fourier transformation of the sampling grid. The reconstruction methods aim to deconvolve the PSF from the spectrum via regularization. This procedure is equivalent to reconstructing the missing points in the NUS signal.

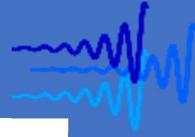
During the data mounting Hyscorean will recognize if the data are NUS and will activate the [NUS Reconstruction](#) section in the [Pre-processing](#) panel:



The user can select a reconstruction method from the list. These can be categorized into two reconstruction families: maximum entropy (maxEnt) and iterative soft thresholding (IST) reconstruction. Depending on whether the selected method requires any additional input parameters, these will be activated or deactivated.

The following sections will provide a brief description on the reconstruction methods available on Hyscorean and their way of action.





### 4.3.1 Iterative Soft-Thresholding (IST) reconstruction

An  $\ell_1$ -based regularization method based on the compressed-sensing principle, that through optimization, the sparsity of a signal can be exploited to recover it from fewer samples than required by the Nyquist theorem [3]. This method regularizes a spectrum  $\mathbf{X}$  that minimizes the functional

$$\mathbf{X}_{\text{IST}} = \underset{\mathbf{X}}{\operatorname{argmin}} \{ \|\mathbf{b} - \mathbf{DFX}\|_2 - \|\mathbf{X}\|_1 \}$$

where  $\mathbf{b}$  is the measured experimental signal,  $\mathbf{D}$  is the NUS grid and  $\mathbf{F}$  is the inverse Fourier transform kernel. This functional is solved by iterative soft-thresholding, which iteratively updates the solution  $\mathbf{X}$  with all values in  $\mathbf{F}^{-1}\mathbf{b}$  (i.e. the nuDFT spectrum) larger than the threshold value  $\eta$ .

- I. The threshold is set to  $\eta = \tau |\max(\mathbf{F}^{-1}\mathbf{b})|$ , where  $\tau$  is the damping factor.
- II. The solution  $\mathbf{X}$  is updated with all values in  $\mathbf{F}^{-1}\mathbf{b}$  larger than the threshold  $\eta$ .
- III. The input signal  $\mathbf{b}$  is updated by subtracting the term  $\mathbf{DFX}$  or  $(\mathbf{1} - \mathbf{D})\mathbf{FX}$ .
- IV. The updated spectrum  $\mathbf{F}^{-1}\mathbf{b}'$  will contain all artifacts and less meaningful peaks.
- V. The threshold is updated to  $\eta = \tau |\max(\mathbf{F}^{-1}\mathbf{b}')|$  and the next iteration is started.
- VI. After sufficient iterations the solution  $\mathbf{X}$  will contain meaningful peaks but no artifacts.

Two variants of this algorithm have been developed [3]:

❖ **IST-D**

In Drori's IST (IST-D) the term  $\mathbf{DFX}$  is subtracted from  $\mathbf{b}$  and hence the measured points in  $\mathbf{b}$  are updated and non-measured points in  $\mathbf{b}$  are left to zero. This allows for the algorithm to find a balance between data agreement and sparsity in the solution.

❖ **IST-S**

In Stern's IST (IST-S) the term  $(\mathbf{1} - \mathbf{D})\mathbf{FX}$  is subtracted from  $\mathbf{b}$  and hence non-measured points in  $\mathbf{b}$  are updated and measured points in  $\mathbf{b}$  are left unchanged. This enforces a strict accordance between the reconstruction and the measured data.

Due to the thresholding nature of the IST methods, the intensity response of the reconstructed spectrum is linear with respect to that of the experimental data. In Hyscorean both IST-D and IST-S can be chosen by the user as reconstruction methods. In both cases the damping factor  $\tau$  is set to 0.99 and updated after each iteration  $k$  according to

$$\tau_k = \tau_0 \frac{N_{\text{iter}} - k}{N_{\text{iter}}}$$

which enhances the convergence speed of the algorithm. Nonetheless, the damping factor can be later validated via Hyscorean's validation module (see section ???).

**NOTE:**

Due to the thresholding nature of these algorithms, in cases with strongly pronounced PSF's (due to e.g. low sampling densities or low randomness) the sampling artifacts may be so pronounced that these lie at the level of true peaks and be incorporated into the solution via the thresholding. In such cases, IST-S and IST-D may not be able to recover any more signal and the use of maxEnt methods are recommended.

### 4.3.2 Maximum Entropy (maxEnt) reconstruction

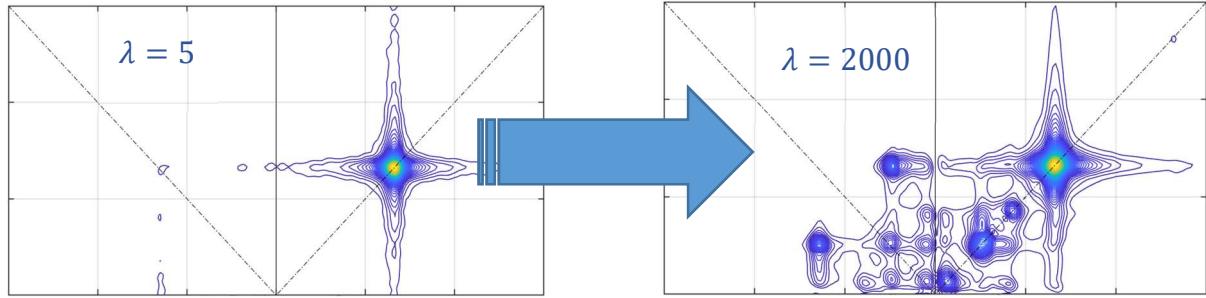
Maximum entropy (maxEnt) methods aim to find the spectrum that has minimal statistical information content (i.e. maximum entropy), while still maintaining agreement with the measured data. This is achieved by regularization of the reconstructed spectrum  $\mathbf{X}$  via minimization (by discrepancy principle) of functionals of the form

$$\mathbf{X}_{\text{maxEnt}} = \underset{\mathbf{X}}{\operatorname{argmin}} \{ \lambda \|\mathbf{b} - \mathbf{DFX}\|_2 - S(\mathbf{X}) \} \quad \text{under } \|\mathbf{b} - \mathbf{DFX}\|_2 \leq \varepsilon$$

where again  $\mathbf{b}$  is the measured experimental NUS signal,  $\mathbf{D}$  is the NUS grid,  $\mathbf{F}$  is the inverse Fourier transform kernel and  $\varepsilon$  is the noise level in the signal. The Lagrange multiplier  $\lambda$  controls the balance between the data agreement imposed by the first term in the functional and the entropy penalty. Therefore, depending on the choice of  $\lambda$  the intensity response of MaxEnt methods varies:

- ❖ Small  $\lambda$       Non-linear intensity response of the reconstructed spectrum.
- ❖ Large  $\lambda$       Linear intensity response of the reconstructed spectrum.

The effects of non-linearity can be observed in the following example:



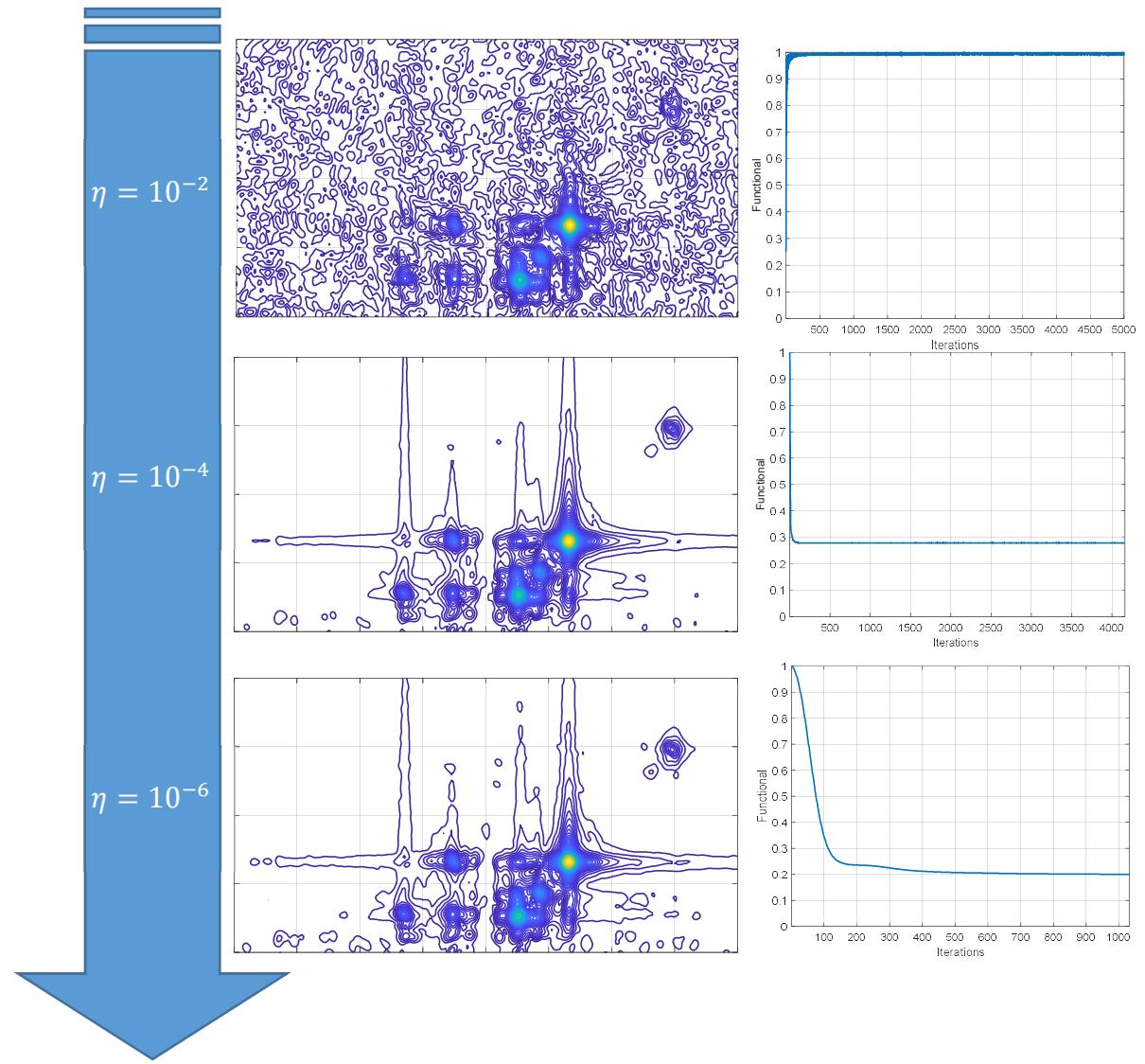
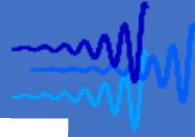
As the non-linearity increases the relative scaling of the peaks in the reconstructed spectrum becomes more drastic so that even true peaks are removed from the spectrum leaving only the most intensive ones. This does however not mean that non-linearity is to be avoided since it allows MaxEnt to recover the true spectrum from the noise and sampling artifacts (see later).

The term  $S(\mathbf{X})$  represents the spectral entropy and in Hyscorean this is implemented with the Hoch-Hore entropy [4]

$$S(\mathbf{X}) = - \sum_{i=1}^N |X_i| \log \left[ \frac{|X_i|}{\delta} + \sqrt{1 + \left( \frac{|X_i|}{\delta} \right)^2} \right] - \sqrt{|X_i|^2 + 4\delta^2}$$

which is monotonous in contrast to the Shannon and Skilling entropies as well as insensitive to the phase of the signal. The parameter  $\delta$  is the so-called background parameter, which to a large extent determines the threshold at which the non-linear effects become significant. It can also be described to control the curvature of the maxEnt functionals, leading to smoother/rougher reconstructions.

- ❖ Small  $\eta$       Smooth, slow converging functional (i.e. long computation times).  
Smoother and generally more accurate reconstructions
- ❖ Large  $\eta$       Rough functional (i.e. short computational times) but with divergence risk  
Artifacts start to populate the reconstruction.



#### 4.3.2.1 Fast-forward maxEnt (FFM) reconstruction

This maxEnt method represents the limiting case of the previous functional when  $\lambda \rightarrow \infty$ , which enforces a strict accordance with the measured data [5]. Thus, experimental measured points are directly set into the reconstruction and only the missing points are reconstructed. These are reconstructed via entropy gradient methods which in Hyscorean are called:

- ❖ **FFM-CG**

Employs the conjugate gradient method to reconstruct the missing points.

- ❖ **FFM-GD**

Employs the gradient descent method to reconstruct the missing points.

One advantage of this method is the absence of the Lagrange multiplier which reduces the parameter space to just the background parameter  $\eta$  for the entropy. In Hyscorean this can be given as a logarithmic value in the [Background Param. \(log10\)](#) edit box. However, the FFM methods are known to over-fit noise at low SNR signals.

#### 4.3.2.2 Convex Accelerated MaxEnt Reconstruction Algorithm (CAMERA)

CAMERA is a new approach to spectral reconstruction that exhibits fast, tunable convergence in both constant-aim and constant-lambda modes [4]. Hyscorean allows the use of CAMERA on both modes:

- ❖ Constant-aim CAMERA

On this mode CAMERA employs Nesterov's accelerated first-order convex optimization method where the Lagrange multiplier is iteratively updated according to

$$\lambda = \max\{0, L\varepsilon^{-1} \|\mathbf{b} - \mathbf{DFX}_{t-1} + L^{-1}\mathbf{DF}\nabla S(\mathbf{X}_{t-1})\|_2 - L\}$$

where  $L$  is a Lipschitz constant which depends on the background parameter  $\eta$ . Also the reconstruction at iteration  $t$  is updated taking into consideration the solutions in the previous iterations in order to generate a momentum which allows the algorithm to reach the optimum solution faster than other optimization methods:

$$\mathbf{X}_t = \mathbf{Y}_t + \left( \frac{t-1}{t+2} \right) (\mathbf{Y}_t - \mathbf{Y}_{t-1})$$

where  $\mathbf{Y}_{t-1}$  is the proximal gradient mapping step given by

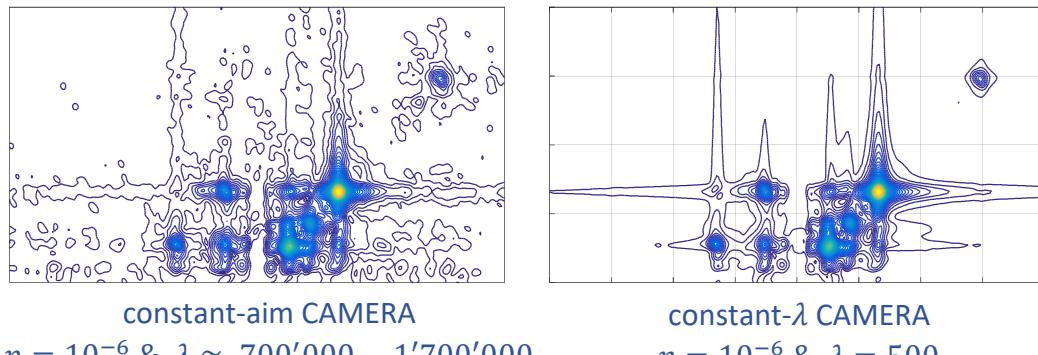
$$\mathbf{Y}_t = (\mathbf{I} + \lambda L^{-1} \mathbf{D}^T \mathbf{D})^{-1} (\mathbf{X}_{t-1} + \lambda L^{-1} \mathbf{X}_0 - L^{-1} \nabla S(\mathbf{X}_{t-1}))$$

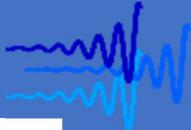
and the Lipschitz constant is also updated iteratively according to  $L \leftarrow \min\{2L, (2\eta)^{-1}\}$ .

- ❖ Constant- $\lambda$  CAMERA

On this mode CAMERA does not update the Lagrange multiplier as before, but fixes it to a user-defined value. This mode allows the user to control the non-linearity of the maxEnt reconstruction manually and to impose more de-noising of the spectrum (see later).

Constant-aim CAMERA performs in a similar way as the FFM methods (as well as IST) and reconstructs the spectrum as well as in the uniform sampled case for moderate or good PSF (i.e. moderate sampling densities or good SNR). Nonetheless, constant- $\lambda$  CAMERA allows the user to impose even further regularization onto the spectrum resulting in a de-noising effect. Also, in cases when the other reconstruction methods may fail, constant- $\lambda$  CAMERA can still reconstruct the true spectrum by proper choice of the combination of background parameter and Lagrange multiplier.



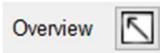


Constant-aim CAMERA has a tendency to set the Lagrange multiplier to large values setting the reconstruction in a highly linear regime (meaning that even the noise level is reconstructed). However, constant- $\lambda$  CAMERA can set the algorithm into a highly non-linear regime which is necessary if the artifacts in the nuDFT spectrum are too large.

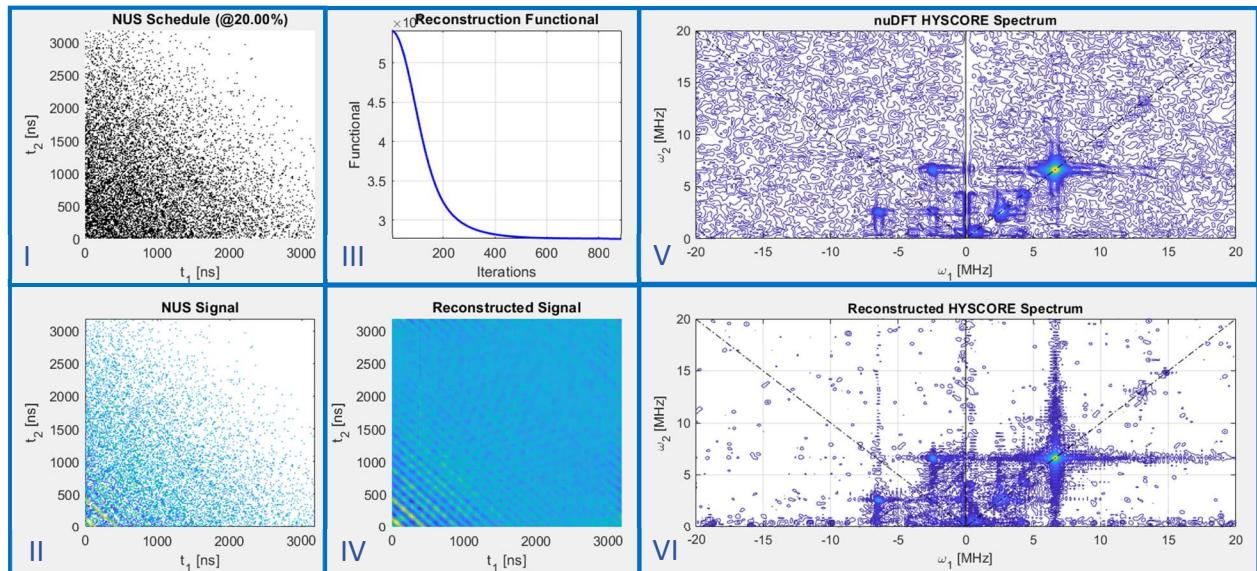
#### 4.4 NUS HYSCORE post-processing

Once the NUS signal has been reconstructed, it undergoes all of the processing steps remaining as normal. As in the rest of the steps of the pre-processing, pressing the Process button again without changing any of the NUS reconstruction settings will result in the reconstruction being skipped employing the pre-processed reconstructed signal already available.

An overview of the results of the reconstruction can be displayed by pressing the [Overview](#) detach button



which opens a new MATLAB figure with the following displays:



- I. The NUS grid employed for the measurement. The black points indicate measured points.
- II. The experimental NUS HYSCORE signal after background correction.
- III. The functional values of the reconstruction methods at each iteration of the reconstruction.
- IV. The reconstructed HYSCORE signal.
- V. The nuDFT HYSCORE spectrum of the signal prior to reconstruction.
- VI. The reconstructed HYSCORE spectrum right before the rest of the processing.

A comparison of the nuDFT and reconstructed HYSCORE spectra is already a good control point to check the quality of the reconstruction, since most of the true peaks are usually already visible from the nuDFT spectrum and may have been removed, e.g. due to strong non-linearity of maxEnt reconstruction.

## 5. Saving Hyscorean results

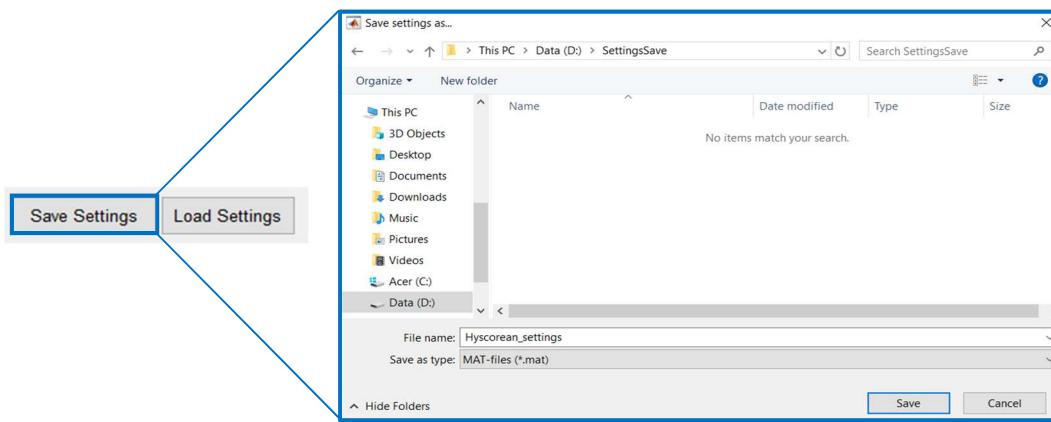
From the help provided in the previous sections the user can process its HYSCORE data into a well-processed spectrum. Now the question comes how to save/export the data outside of Hyscorean. A straightforward way would be to detach the current spectrum (as described in 3.3.1) and save it via the MATLAB save interface.

Nonetheless, the processing of spectra is a process which requires many parameters and quickly generates many different spectra of similar interest. It is rather easy to get lost in similarly looking spectra and settings. Therefore, Hyscorean offers a saving system which not only allows for the save export of data but an organized book-keeping system of all processed spectra as well.

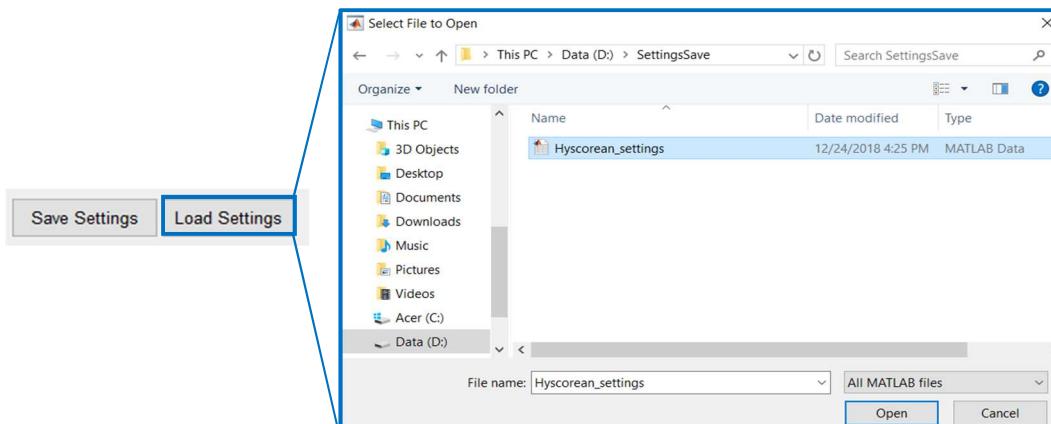
This section will describe the setup, description and operation of this saving system.

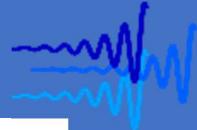
### 5.1 Saving/Loading settings

Hyscorean features a wide range of processing opportunities. This comes, however, at the cost of a large quantity of parameters and settings to be set. The user can save the current settings (no graphical settings) manually via the [Save Settings](#) button. The button will prompt a new OS window which will ask the user to select a folder and filename to save the settings to.



All settings (edit and list boxes in the GUI) will then be saved into a .mat file of the given name. These settings can then be loaded back by the [Load Settings](#) button, which will override all current settings by those in the loaded file.



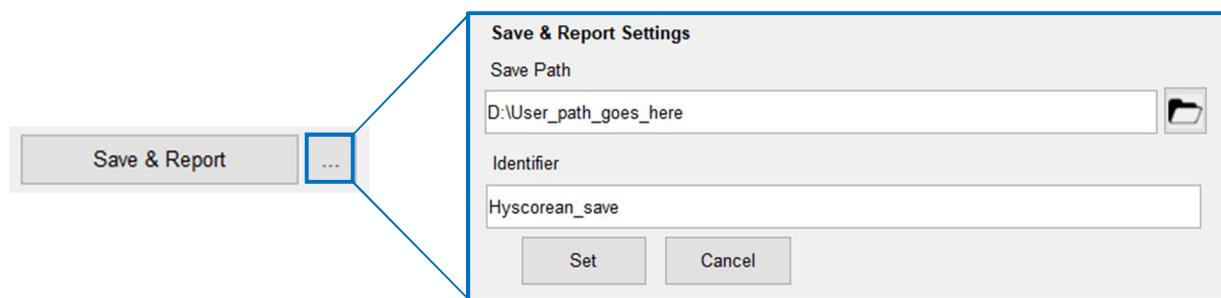


## 5.2 Saving Hyscorean's session

As mentioned in the introduction of this section Hyscorean offers a saving system which allows the user to maintain an automated book-keeping of all saved results. The user only has to press the [Save&Report](#) button to prompt the automatic saving system. However, the saving path and name can be defined first by the user.

### 5.2.1 Setting the save environment

Prior to saving Hyscorean's session the user can change the save setting by pressing the (...) button on next to the [Save & Report](#) one, which will prompt a new window to appear:



This new window has two fields: [Save Path](#) and [Identifier](#). The file management system works as follows:

- ❖ All saved files will be saved on the folder given by [Save Path](#) within a subfolder with the following automated name which includes the date of generation and identifier:

20181224\_Hyscorean\_save

- ❖ The saved files will have a name constructed from the date, identifier and file type:

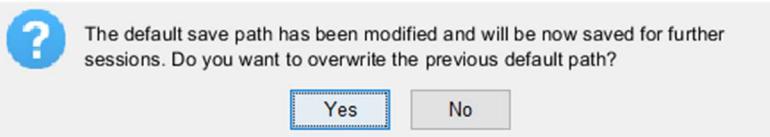
20181224\_Hyscorean\_save\_OutputData      20181224\_Hyscorean\_save\_spectrum

- ❖ Files saved with the same identifier will have the same name with an appended number to indicate the order of creation:

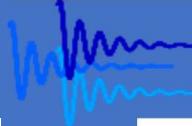
20181224\_Hyscorean\_save\_spectrum      20181224\_Hyscorean\_save\_spectrum\_2

Therefore, for every different date and identifier a different subfolder will be generated in the folder given by [Save Path](#).

The user can enter the save path manually or select it via a OS window by pressing the  button. The [Save Path](#) field is stored between Hyscorean sessions and does not need to be specified again unless the user wants to change it. The [Identifier](#) field, however, is reset at each session to its default Hyscorean\_save. The user-defined settings can then be stored by means of the [Set](#) button. If the save path was added the program will recognize it and ask the user for permission to overwrite the old path.

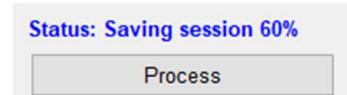


Answering Yes then closes the [Save & Report Settings](#) and return to Hyscorean's main window.



## 5.2.2 Save & Report

The [Save & Report](#) button will generate a set of files consisting the following. The progress of the saving is reported via the status bar with an indication of the percentage of files saved.



Filename: [Date\\_Identifier\\_](#)

- ❖ [settings.mat](#)

A MATLAB data file containing Hyscorean settings as the ones generated via the [Save Settings](#) button, which can also be loaded back via the [Load Settings](#) button.

- ❖ [spectrum.fig](#) & [spectrum.pdf](#)

A MATLAB figure and PDF files containing an exact copy of the spectrum as in the Hyscorean main display.

- ❖ [OutputData.mat](#)

A MATLAB data file containing a structure with the following fields:

```
.RawSignal           % Signal mounted from raw experimental data  
.ProcessedSignal    % Processed signal used for spectrum  
.FrequencyAxis1     % X-axis of spectrum  
.FrequencyAxis2     % Y-axis of spectrum  
.Spectrum           % Spectrum (real, imag or abs depending of choice)  
.TimeAxis1           % X-axis of time-domain signal  
.TimeAxis2           % X-axis of time-domain signal
```

- ❖ [DataForFitting.mat](#)

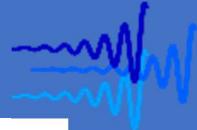
A MATLAB data file containing a set of data necessary by the EasySpin Fitting module as input (see section 7.2).

- ❖ [Report.pdf](#)

A PDF obtained via the report generator (requires the Report Generator MATLAB license). This creates a report containing all details (experimental conditions, processing parameters, NUS reconstruction details, ...) about the signal and spectrum. This enables the user to have a detailed description of the processing in a compact and automated way enabling book-keeping of the measurements, signals and spectra in a comfortable way.

NOTE:

Any NaN in the report indicates that the corresponding parameter is not available from the loaded experimental file or does not apply to that kind of spectrometer at all.



# Hyscorean - Processing Report

24-Dec-2018 17:35:37

**File:**

600 files

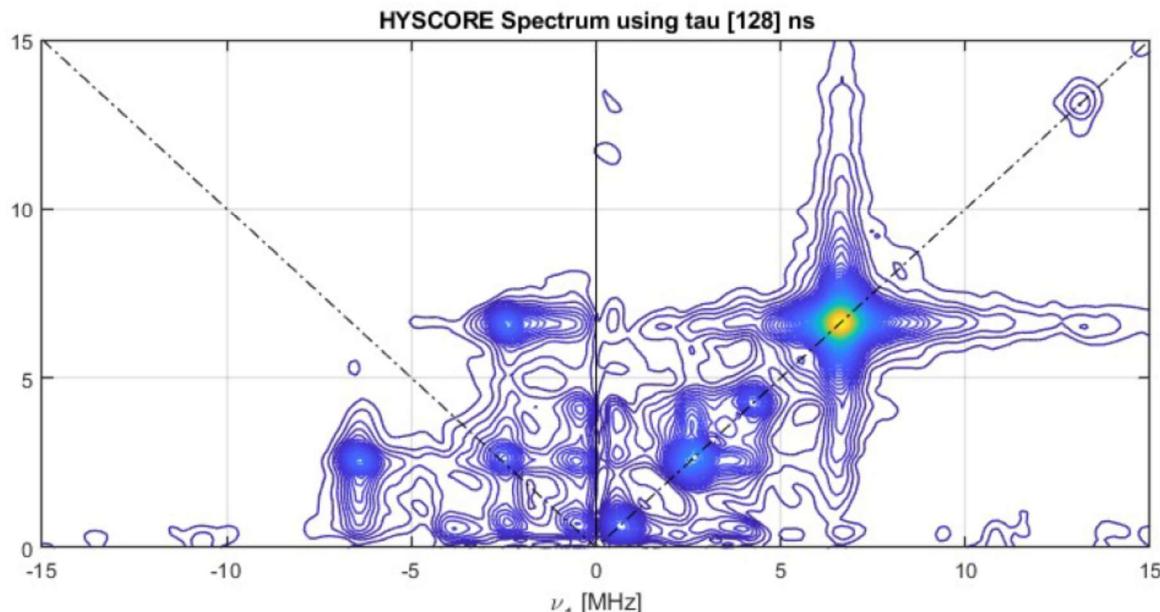
**Path:**

**Table 1. Experimental Settings**

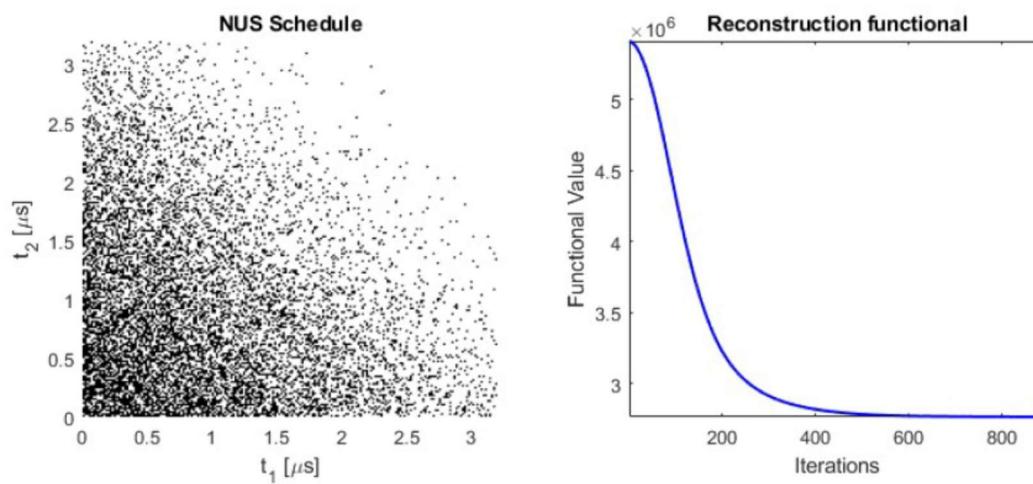
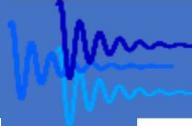
<b>MW Freq</b>	9.4500 GHz	<b>SRT/SPP/Scans</b>	2 us / 160 / 1
<b>B-Field</b>	3460 G	<b>B-Field Offset</b>	0 G
<b>X-Points/TimeStep</b>	200 / 16 ns	<b>Y-Points/TimeStep</b>	200 / 16 ns
<b>Video Gain</b>	Nan dB	<b>Video Bandwidth</b>	Nan MHz
<b>Pulse Lengths 90°/180°</b>	16 ns / 16 ns	<b>Tau Values</b>	[128 160 192] ns

**Table 2. Processing Settings**

<b>1st Corrected Dimension</b> 1	<b>Fitting Model</b> Polynomial	<b>Polynomial Order</b> 1	<b>Background Start</b> 0 ns
<b>2nd Corrected Dimension</b> 2	<b>Fitting Model</b> Polynomial	<b>Polynomial Order</b> 1	<b>Background Start</b> 0 ns
<b>Lorentz-to-Gauss TF</b> deactivated	<b>Savitzky-Golay Filter</b> deactivated	<b>Polynomial order</b> 3	<b>Frame length</b> 11
<b>Zero-filling in t1</b> 200 points	<b>Zero-filling in t2</b> 200 points	<b>Apodization Window</b> chebyshev	<b>Window decay</b> 200 points
<b>Contour Levels</b> 80	<b>Minimal Contour Level</b> 2 %	<b>Spectrum</b> Absolute	

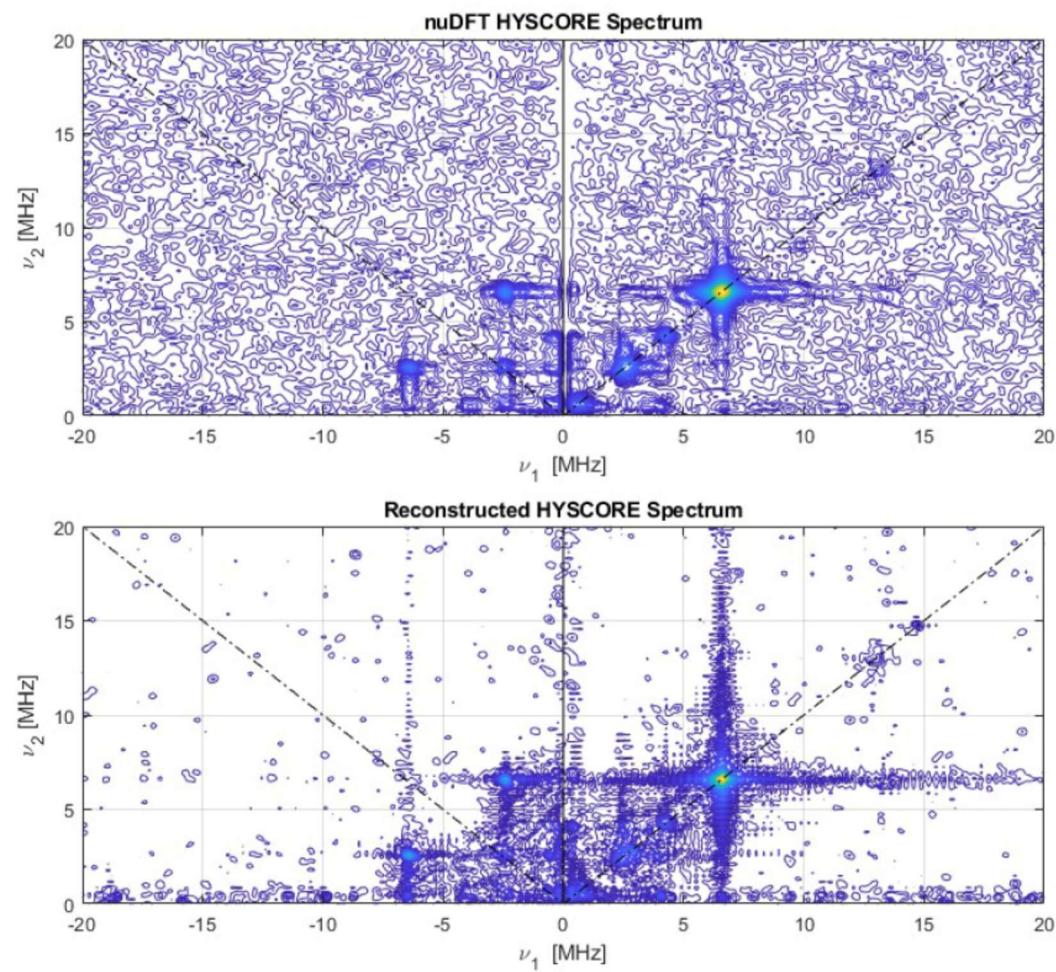


- This report has been automatically generated by Hyscorean -



**Table 3. Non-Uniform Sampling & Reconstruction Settings**

Sampling Density	Reconstruction Method	Lagrange multiplier	Background parameter ( $\log_{10}$ )
20.00%	CAMERA	500	-6



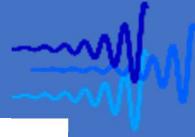


Figure 1. HYSCORE Time Trace - First Background Correction

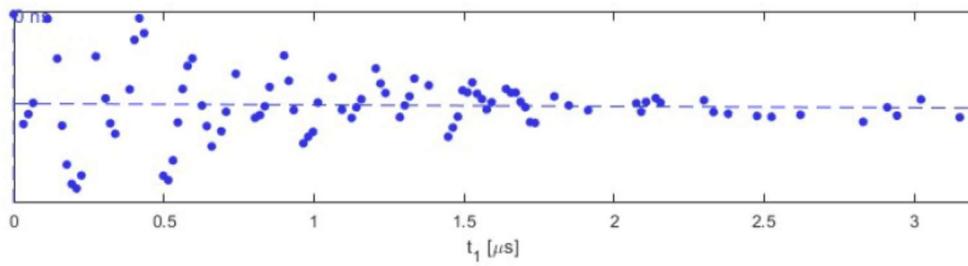


Figure 2. HYSCORE Time Trace - Second Background Correction

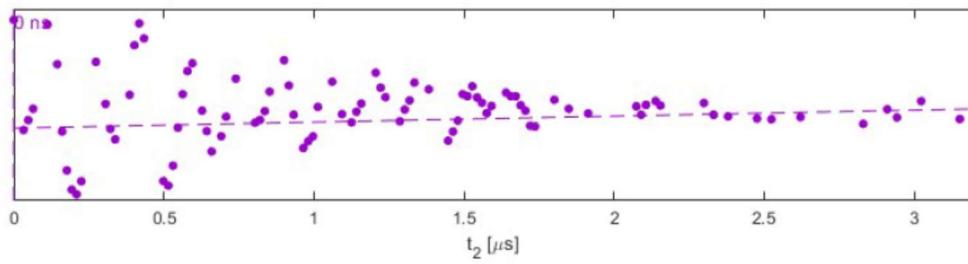


Figure 3. HYSCORE Time Trace - Processed Signal (First Dimension w/o Zero-Filling)

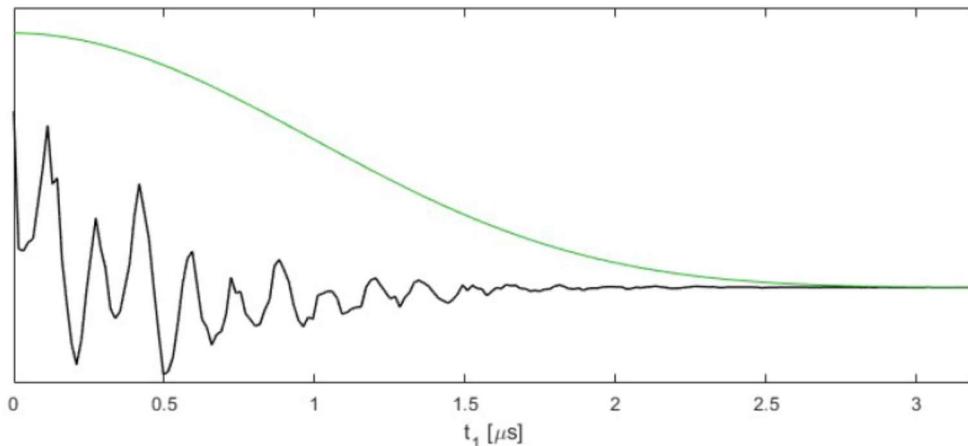
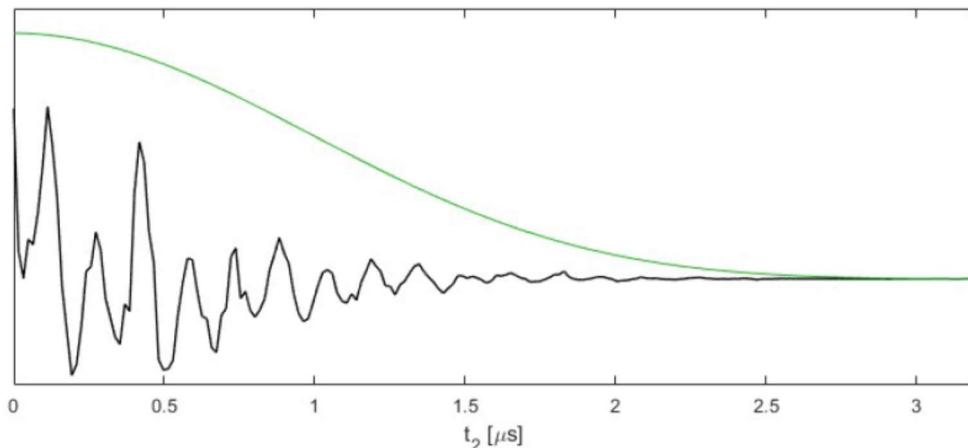
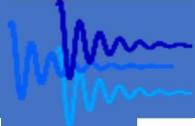


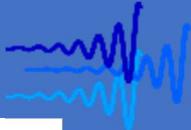
Figure 4. HYSCORE Time Trace - Processed Signal (Second Dimension w/o Zero-Filling)





## 6. Validation module

(to be added)



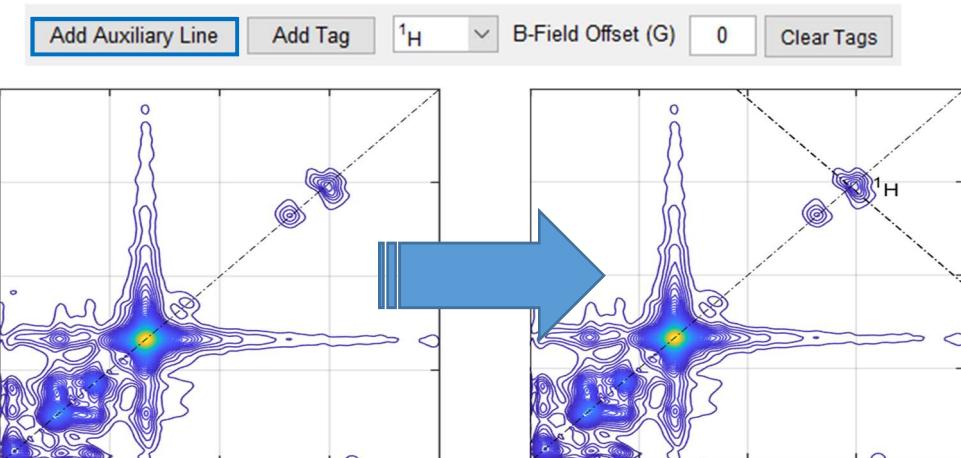
## 7. EasySpin fitting module

Hyscorean creates the link between processing and fitting by including a fitting module based on EasySpin [6] simulation routines.

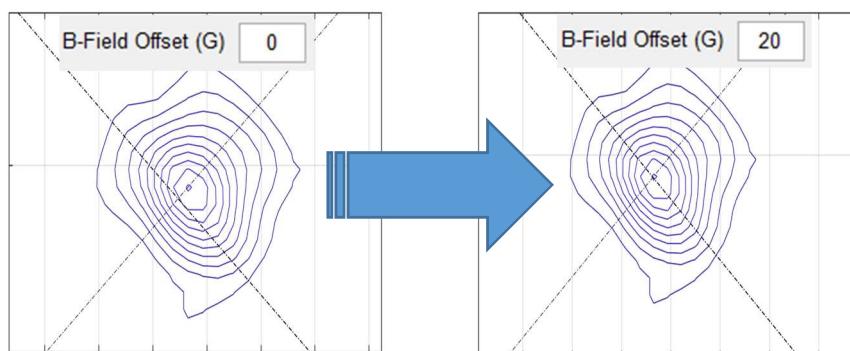
### 7.1 Auxiliary lines & Field offset

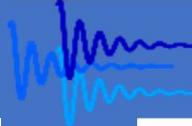
One of the first steps to prepare for the fitting is to determine the magnetic field offset of the current measurement. This offset can be determined from a matrix peak on the HYSCORE spectrum from a known nuclear Larmor frequency.

Hyscorean facilitates this procedure by including auxiliary lines which can be used for calibrating the field offset. These lines can be added from the [Add-Ons](#) panel on top of the main display, by pressing the [Add Auxiliary Line](#) button which will appear at the resonance frequency of the nuclei currently selected on the list box of the same panel. The resonance frequency is computed by means of the gyromagnetic ratio of that nuclei, the magnetic field employed in the measurement and the magnetic field offset given by the [B-Field Offset](#) edit box in the same panel. A tag with the isotope name next to the matrix peak at the corresponding frequency can be added via the [Add Tag](#) button. All tags and lines can be removed from the spectrum by means of the [Clear Tags](#) button.



Then in order to calibrate the magnetic field offset one can adjust its value via the [B-Field Offset](#) edit box and add a new lin. The offset value corresponds to that at which the line crosses the center of the matrix peak. The value can be fine-tuned by zooming in the corresponding matrix peak and adding the line:





## 7.2 Starting the fitting module

Hyscorean's EasySpin fitting module is a revamped version of the well-known EasySpin esfit function adapted for HYSCORE spectra. However, a big difference to EasySpin (besides the display) is the fact that each simulation performed by EasySpin to fit the HYSCORE spectra is processed exactly the same as the experimental spectrum in Hyscorean (with exception of the background correction which is performed differently). This can make big differences in the RMSD-based fit of EasySpin when using processing techniques such as the Lorentz-Gauss transformation or some apodization windows.

Hyscorean makes this connection between processing and simulation automatically and without the need for the user to give any input. There exist two routes to start the fitting module of Hyscorean depending on the needs of the user.

### 7.2.1 Loading single spectra

The most straightforward way to start the fitting module is directly from the Hyscorean main window by pressing the [EasySpin Fit](#) button.

EasySpin Fit

This will open the fitting module GUI and load all required data for the fit: spectrum and axis, processing settings, experimental values (e.g. magnetic field and offset,  $\tau$ -values, time-sweeps steps, ...) and construct all corresponding EasySpin structures.

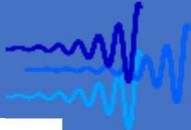
### 7.2.2 Loading multiple spectra

It may be of interest for the user to fit several HYSCORE spectra of a same system measured at different magnetic field positions. Hyscorean's fitting module allows the simultaneous fitting of several spectra at the same time via the same interface as for single spectra. This, however, is started in a different way although with minimal effort for the user:

- I. Process all HYSCORE spectra in Hyscorean
- II. Save them individually via the [Save&Report](#) button. As described in 5.2.1. this will create an output file with the suffix [DataForFitting.mat](#) containing all necessary input for the fitting module.
- III. Create a MATLAB script which defines a variable containing the paths and filenames of those files and call the validation module as a standalone GUI via the [launch\\_Hyscorean\\_fit](#) function. The script should look as follows:

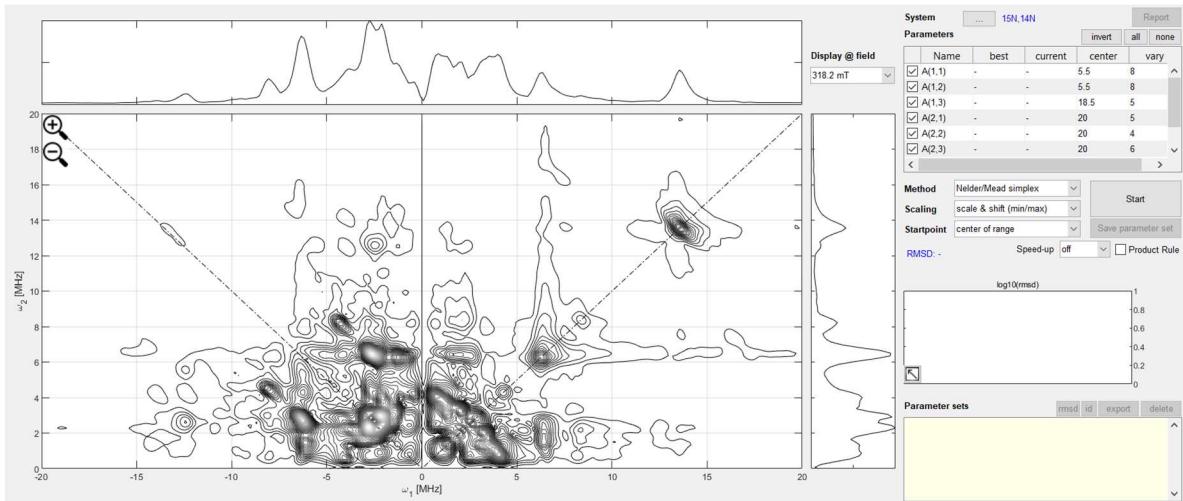
```
clear Files  
  
Path = 'D:\This\Is\An\Example\Path';  
Files{1} = '20181206_FieldPosition1_DataForFitting';  
Files{2} = '20181206_FieldPosition2_DataForFitting';  
Files{2} = '20181206_FieldPosition3_DataForFitting_2';  
  
launch_Hyscorean_fit(Files, Path)
```

- IV. By running the script, the fitting module will be started and all of the spectra will be loaded along their individual experimental and processing settings.

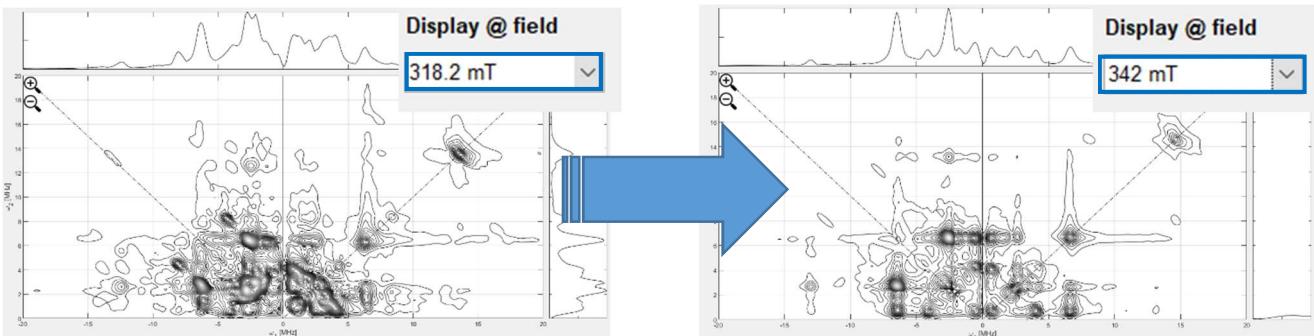


### 7.3 Fitting HYSCORE spectra

Once the Hyscorean fit module is started as described in the previous section the following window will open. Users familiar with the EasySpin fitting tool GUI will recognize familiar elements on this version:



The loaded HYSCORE spectra will be automatically displayed as a monochromatic contour plot in the main display with skyline-projection plots along each dimension in the insets. The spectra can be switched at any time (even during fitting) by means of the **Display@field** list box. This box contains the field positions of the spectra loaded into the fit module. By selecting a different field position, the program will switch the main display to the corresponding spectrum.



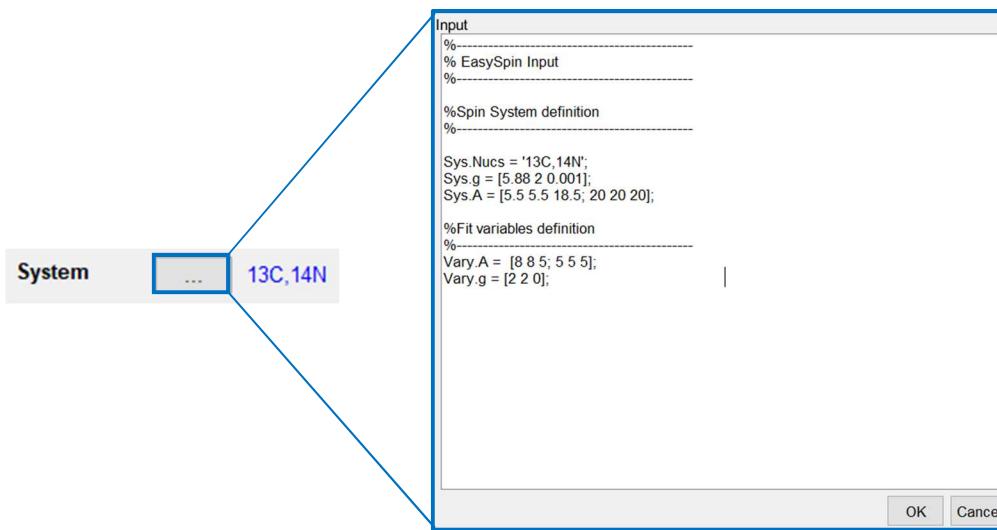
#### NOTE:

If the user changes the field position to be displayed while the fitting algorithm is running, the spectrum will not be changed until the current iteration is finished and the program updates the spectrum. Therefore, should simulations take long computation times the change of display will also require the same time.

As in the main Hyscorean window, the spectra can be zoomed-in and out via the same  $\oplus \ominus$  buttons. The insets adjust automatically to the zoom status of the main display. The limits to which the zoom-out button resets are given by the [Axis Frequency Limit](#) in Hyscorean set during the processing.

### 7.3.1 Defining the spin system

In contrast to EasySpin in Hyscorean the spin system is defined via the GUI and allows the user to modify it without the need to start the module anew. This can be done via the (...) button next to the **System** tag. The current system's nuclei considered are displayed next to this button as a blue text.



By pressing the button, a new window is opened which allows the user to modify a text box where the EasySpin Sys and Vary structures can be constructed using MATLAB notation. The spin system is defined by the Sys structure and the fitting parameters are given in Vary. For further details, please refer to the EasySpin documentation.

**NOTE:**

The Exp and Opt variables usually required by EasySpin for simulations are automatically constructed from the experimental parameters in the measurement files and do not need to be constructed by the user. The user can, however, define further fields on those structures from the same window.

By pressing the **OK** button, the window is closed and the input is compiled. Should there be any errors in the spin system definition, the program will recognize this and prompt an error window and request the user to check the input again. Once the input has been compiled, the new Vary structure parameters will be updated on the **Parameters** table immediately.

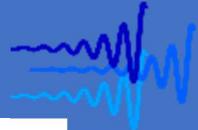
The diagram illustrates the update of a parameter table after defining a spin system. It consists of two side-by-side tables with a large blue arrow pointing from the left to the right.

**Left Table (Initial State):**

System		13C			Report		
Parameters					invert	all	none
	Name	best	current	center	vary		
<input checked="" type="checkbox"/>	A(1,1)	-	-	5.5	8		
<input checked="" type="checkbox"/>	A(1,2)	-	-	5.5	8		
<input checked="" type="checkbox"/>	A(1,3)	-	-	18.5	5		

**Right Table (Final State):**

System		13C,14N			Report		
Parameters					invert	all	none
	Name	best	current	center	vary		
<input checked="" type="checkbox"/>	A(1,1)	-	-	5.5	8		
<input checked="" type="checkbox"/>	A(1,2)	-	-	5.5	8		
<input checked="" type="checkbox"/>	A(1,3)	-	-	18.5	5		
<input checked="" type="checkbox"/>	A(2,1)	-	-	20	5		
<input checked="" type="checkbox"/>	A(2,2)	-	-	20	4		
<input checked="" type="checkbox"/>	A(2,3)	-	-	20	6		



The table rows indicate the different parameters of the spin system which are to be fitted and the columns indicate the following properties:

- ❖ Name      Name of the parameter to be fitted as defined in the input
- ❖ Best      Value of the parameter in the best fit spectrum
- ❖ Current    Value of the parameter for the current iteration spectrum
- ❖ Center     Value given to the parameter in the Sys variable
- ❖ Vary      Variation range of values which the parameter can adopt

Next to this table a set of buttons can be pressed to [Invert](#) the check boxes in the table, select them [All](#) or [None](#).

The user has also the option to pass the spin system programmatically from MATLAB scripts as an input to the [launch\\_Hyscorean\\_fit](#) function, which then will pass the Sys and Vary as variables to Hyscorean's fit module. This can be done by passing the Sys and Vary structures as fields of the structure which is given as input:

```
Sys.Nucs = '15N,14N';
Sys.g = [5.88 2 0.001];
Sys.A = [5.5 5.5 18.5; 20 20 20];

Vary.A = [8 8 5; 5 4 6];
Vary.g = [2 2 0; 5 2 1];

Input.Sys = Sys;
Input.Vary = Vary;

launch_Hyscorean_fit(Files, Path, Input)
```



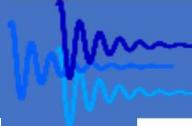
```
Input
%-----
% EasySpin Input
%-----

% Spin System definition
%-----
Sys.Nucs = '15N,14N';
Sys.g = [5.88 , 2.00, 0.00; 6.00 , 1.00, 0.00];
Sys.A = [5.50 , 5.50, 18.50; 20.00 , 20.00, 20.00];

% Fit variables definition
%-----
Vary.A = [8.00 , 8.00, 5.00; 5.00 , 4.00, 6.00];
Vary.g = [2.00 , 2.00, 0.00; 5.00 , 2.00, 1.00];
```

**NOTE:**

Hyscorean stores the text used for the spin system definition as a MATLAB preference. This means that once the program is closed, the next time it is opened the text in the window (and thus the spin system definition) will be exactly the same as when it was closed.



### 7.3.2 Starting/Stopping the fitting

With the spin system defined, the fit settings can be selected in order to start the fitting. All the options available for the fitting can be chosen from the list boxes next to the **Start** button.

Method	Nelder/Mead simplex	▼	Start
Scaling	scale & shift (min/max)	▼	
Startpoint	center of range	▼	Save parameter set

#### ❖ Method

The optimization algorithm to be employed to find the best fit spectrum corresponding to the minimum of some optimization functional. The details can be found on the EasySpin online documentation [7]:



Beyond a good starting parameter set or search range, the performance of the fitting depends crucially on two things: the choice of the optimization algorithm, and the choice of the target function. Let's have a look at each of them in turn.

#### Optimization algorithms

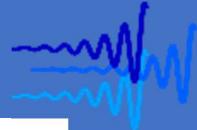
EasySpin provides several optimization algorithms that are in widespread use: (1) the [Nelder/Mead downhill simplex](#) method, (2) the [Levenberg/Marquardt](#) algorithm, (3) [Monte Carlo](#) random search, (4) a [genetic](#) algorithm, (5) a [systematic grid search](#), as well as others.

The first two are local search algorithms, which start from a given starting set of parameter values and try to work their way down a nearby valley of the parameter space to find the minimum. Both methods are quite fast, although there are some differences in general performance between them: The downhill simplex is somewhat slower than Levenberg/Marquardt, but it is more robust in the sense that it does not get stuck in a local minimum as easily as Levenberg/Marquardt.

The latter three are global search methods: they do not have a single starting parameter set, but use many, distributed over the entire parameter search space. The Monte Carlo method simply performs a series of random trial simulations and picks the best one. It is very inefficient. The systematic grid search is better: It covers the parameter space with a grid and then does simulations for each knot of the grid, in random order. Thus, no point is simulated twice, and the method is more efficient than the Monte Carlo search. However, if the minimum is between two grid points, it will never be found.

The third global method is a genetic algorithm: It makes simulations for several, let's say  $N$ , parameter sets (called a population), computes the fitting error (called the fitness) for all of them and then proceeds to generate  $N$  new parameter sets from the old ones using mechanisms like mutation, cross-over and reproduction. This way, a new generation of parameter sets is (pro-) created, just like in biological evolution. The benefit of this algorithm is that if a good parameter is encountered, it is likely to propagate down the generations and across the population.

*Fitting EPR spectra, EasySpin Documentation, Stoll et al.*



## ❖ Scaling

The different ways the spectrum can be scaled once it has been simulated in order to compare it with the experimental spectrum. The options are:

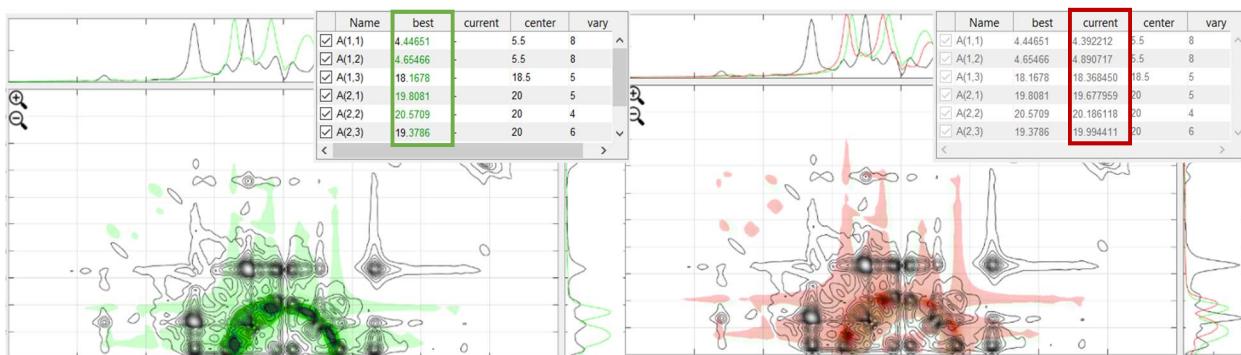
- scale & shift  
The spectra are scaled to the absolute maxima and their absolute minima are shifted to match
- scale only  
The spectra are scaled only to the absolute maxima.
- no scaling  
The spectra are used as simulated without scaling

## ❖ Startpoint

The way the initial values of the fitting parameters are selected at the start of the optimization routine. The options are:

- center of range  
Starts with the values given in the center column of the [Parameters](#) table.
- random within range  
Starts at a random value within the range given by the values given in the [center](#) and [vary](#) columns of the [Parameters](#) table.
- selected parameter range  
Starts at the values saved in the currently selected parameter set in the [Parameter Sets](#) panel (see later).

Once the user has chosen the settings for the fitting, this can be started via the [Start](#) button. Hyscorean employs the following color coding to show the state of the fitting in the main display as well as in both insets:



❖ Black

Experimental spectrum processed in Hyscorean.

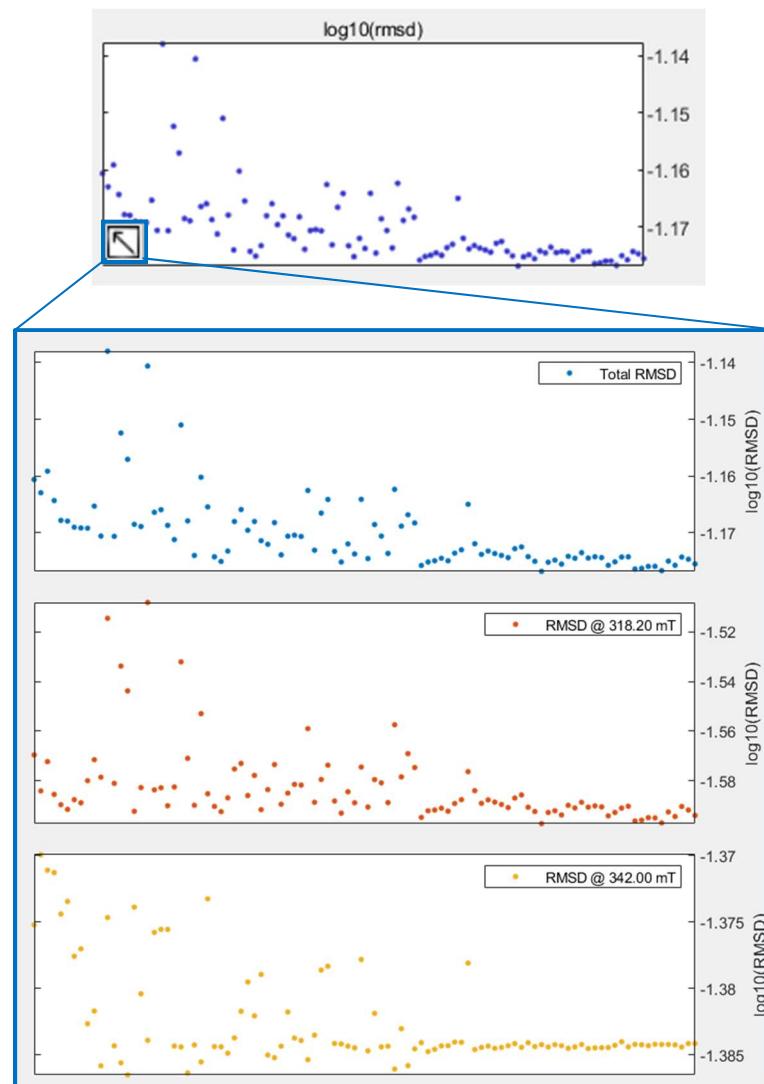
❖ Green

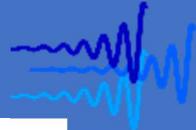
Best fit spectrum given by the parameters in the [best](#) column of the [Parameters](#) table. Displayed during and after the fitting.

❖ Red

Spectrum corresponding to current iteration with the parameters in the current [column](#) of the [Parameters](#) table. Displayed only during the fitting.

The fitting can be also monitored via the evolution of the RMSD values of the fitted spectra in the display below the [Start](#) button. The RMSD displayed in here is the total RMSD computed from the sum over the individual RMSD values of the different spectra being fitted (e.g. if several field positions are being fitted). Nonetheless, in case the user wants to monitor the RMSD evolution of the individual fitted spectra the user can press the detach button to prompt a new window which will contain an individual display for each of the spectra RMSD as well as one for the total RMSD.





Once the fitting has started, all UI control elements are disabled and the [Start](#) button is changed into the [Stop](#) button. This [Stop](#) button can be used to stop the fitting prematurely even if the optimization algorithm has not found the minimum yet. Otherwise, the fitting will continue until a minimum is found, point at which the fit ends and all UI control elements are enabled again.

NOTE:

By pressing the [Stop](#) button, a trigger is sent to the program to stop the optimization at the next iterate. Therefore, pressing the button will not result in an immediate abortion of the fitting. If the simulations are computationally costly then the program may require a prolonged lapse of time until it is fully stopped. Nonetheless, the user can abruptly kill the fitting by pressing the [Ctrl+C](#) combination in the MATLAB console. This will result in a complete loss of the fit data.

In either case, once the fitting routine has stopped, the parameters of the best fitted spectrum are updated at the [Parameters](#) table under the [best](#) column:



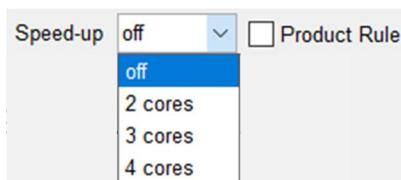
	Name	best	current	center	vary	^
<input checked="" type="checkbox"/>	A(1,1)	-	-	5.5	8	^
<input checked="" type="checkbox"/>	A(1,2)	-	-	5.5	8	^
<input checked="" type="checkbox"/>	A(1,3)	-	-	18.5	5	^
<input checked="" type="checkbox"/>	A(2,1)	-	-	20	5	^
<input checked="" type="checkbox"/>	A(2,2)	-	-	20	4	^
<input checked="" type="checkbox"/>	A(2,3)	-	-	20	6	^

	Name	best	current	center	vary	^
<input checked="" type="checkbox"/>	A(1,1)	4.31031	-	5.5	8	^
<input checked="" type="checkbox"/>	A(1,2)	4.7469	-	5.5	8	^
<input checked="" type="checkbox"/>	A(1,3)	18.4586	-	18.5	5	^
<input checked="" type="checkbox"/>	A(2,1)	20.2431	-	20	5	^
<input checked="" type="checkbox"/>	A(2,2)	19.9268	-	20	4	^
<input checked="" type="checkbox"/>	A(2,3)	20.4277	-	20	6	^

### 7.3.3 Speeding-up the simulations

HYSCORE spectra often require large spin systems with multiple nuclear spins and the fitting of several magnetic field positions. These facts combined contribute to potentially very long simulation times for EasySpin which overall slowdown the fitting and lead to long computational times. Hyscorean offers a couple of options to potentially speed-up those fitting times.



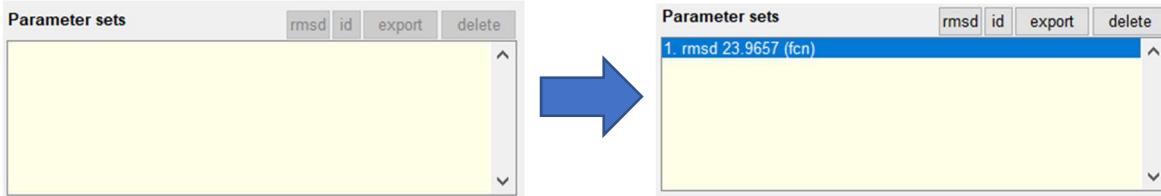
If the spin system contains more than two nuclear spins it may be beneficial to use the [Product Rule](#) options of the saffron simulation function since it may enhance simulation times. By default it is disabled until the check box is clicked.

Another feature available in Hyscorean is to distribute the load of simulating different spectra in different workers of the computer. This allows the different field positions to be simulated at the same time in parallel largely enhancing the computation times. Hyscorean automatically detects the available workers to be set in parallel and these can be selected from the [Speed-up](#) list box. This requires the Parallel Computing Toolbox of MATLAB to be properly installed and with a valid license.

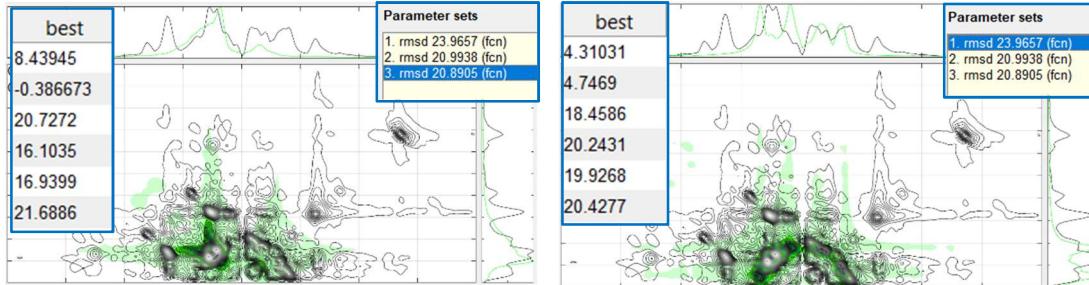
Further speed-up options integrated in EasySpin can be added to the Opt structure via the [System](#) input window. For details, see the EasySpin documentation for the saffron function.

## 7.4 Saving the fit results

If the user finds the current fit of the spectrum satisfactory, wants to save the current parameters for later or use them as starting point for another fitting round, the current parameter set under the best column can be stored for later use via the [Save parameter set](#) under the Start/Stop button. Doing this will update the [Parameter sets](#) panel with the newly saved parameter set. The sets are named according to their total RMSD value.



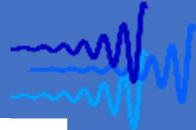
Once several sets have been saved, these can be displayed in the [Parameter](#) table and their corresponding spectrum by just clicking their names in the [Parameter sets](#) panel.



The [Parameter sets](#) panel also possesses a set of buttons with the same functionalities as in EasySpin:

- ❖ **rmsd**  
Sorts the saved parameter sets by in the [Parameter sets](#) panel by increasing RMSD value.
- ❖ **id**  
Sorts the saved parameter sets by in the [Parameter sets](#) panel by order of creation.
- ❖ **delete**  
Removes the selected parameter set from the panel definitely.
- ❖ **export**  
Saves the selected parameter set to the MATLAB workspace as a structure variable with name `fit#` and the following fields:

```
.rmsd      % Total RMSD of the parameter set  
.fitSpec   % Cell array of fitted spectra  
.expSpec   % Cell array of experimental spectra  
.residuals % Cell array of residuals  
.bestValues % Array of the parameter values in the best column  
.Sys        % Structure with spin system definition  
.ID         % ID of the parameter set
```



As for the processing, Hyscorean's fitting module includes a report generator to keep an organized record of the fits and their results. The report can be generated by pressing the [Report](#) button next to the [Parameters](#) table in the upper-right corner of the window.

The report contains the following:

- ❖ An exact copy of the input given to EasySpin with the definitions of the Sys and Vary variables.
- ❖ A summary of the fitting options employed and a copy of the [Parameters](#) table containing the [best](#) values of the fit.
- ❖ For each fitted spectrum the report includes a copy of the experimental spectrum overlaid with the best fit as in the GUI. Additionally, both the experimental spectrum as the fitted spectrum are displayed separately for a better visualization.
- ❖ A summary of the evolution of the total RMSD as well as the RMSDs of the individual fitted spectra.

(to be added)

# Hyscorean - EasySpin Fit Report

29-Dec-2018 19:04:33

File(s):

Path(s):

---

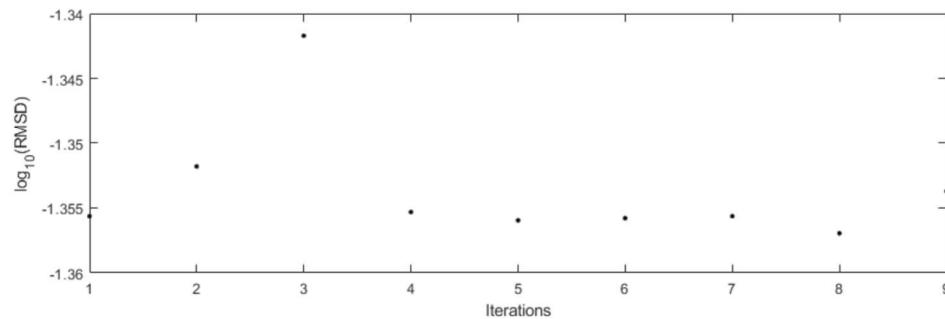
**Spin-System Input Definition:**

```
%-----  
% EasySpin Input  
%-----  
% Spin System definition  
%-----  
Sys.Nucs = '15N,14N';  
Sys.g = [5.50 , 3.00, 0.01];  
Sys.A = [5.50 , 5.50, 18.50; 20.00 , 20.00, 20.00];  
% Fit variables definition  
%-----  
Vary.A = [8.00 , 8.00, 5.00; 5.00 , 4.00, 6.00];  
Vary.g = [2.00 , 2.00, 0.00];
```

---

Method	Scaling	Startpoint	
Nelder/Mead simplex	scale & shift (min/max)	center of range	
Fit Variable Name	Best Fit	Initial guess	Vary
A(1,1)	5.500	5.500	8.000
A(1,2)	5.500	5.500	8.000
A(1,3)	18.500	18.500	5.000
A(2,1)	20.000	20.000	5.000
A(2,2)	20.000	20.000	4.000
A(2,3)	20.000	20.000	6.000
g(1)	5.700	5.500	2.000
g(2)	3.000	3.000	2.000

**Figure 1. Total RMSD (Best fit: 26.5368)**



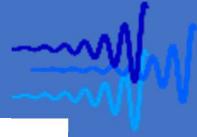
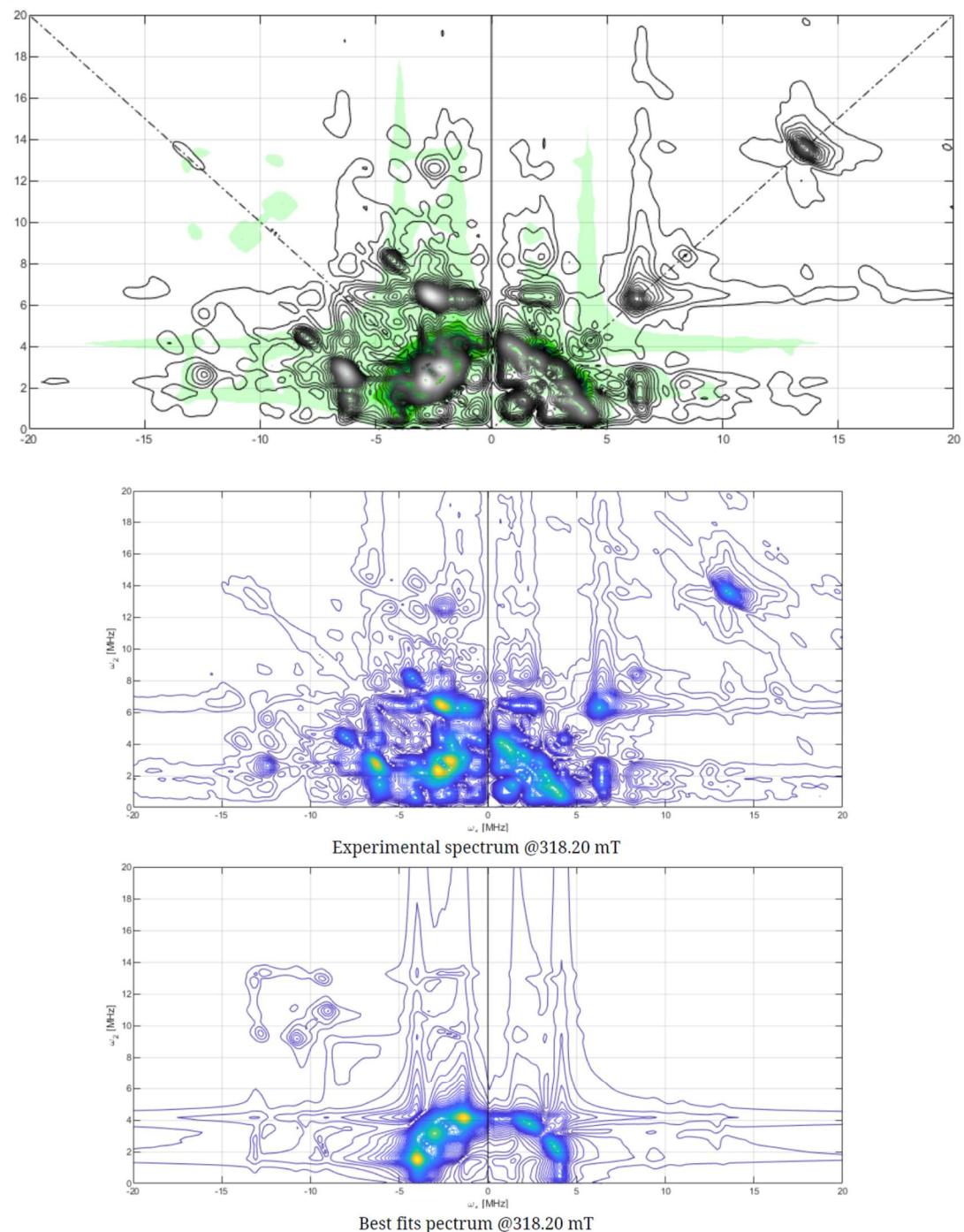


Figure 2. Spectrum @318.20 mT (Best Fit RMSD: 11.6138)



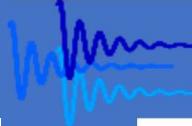
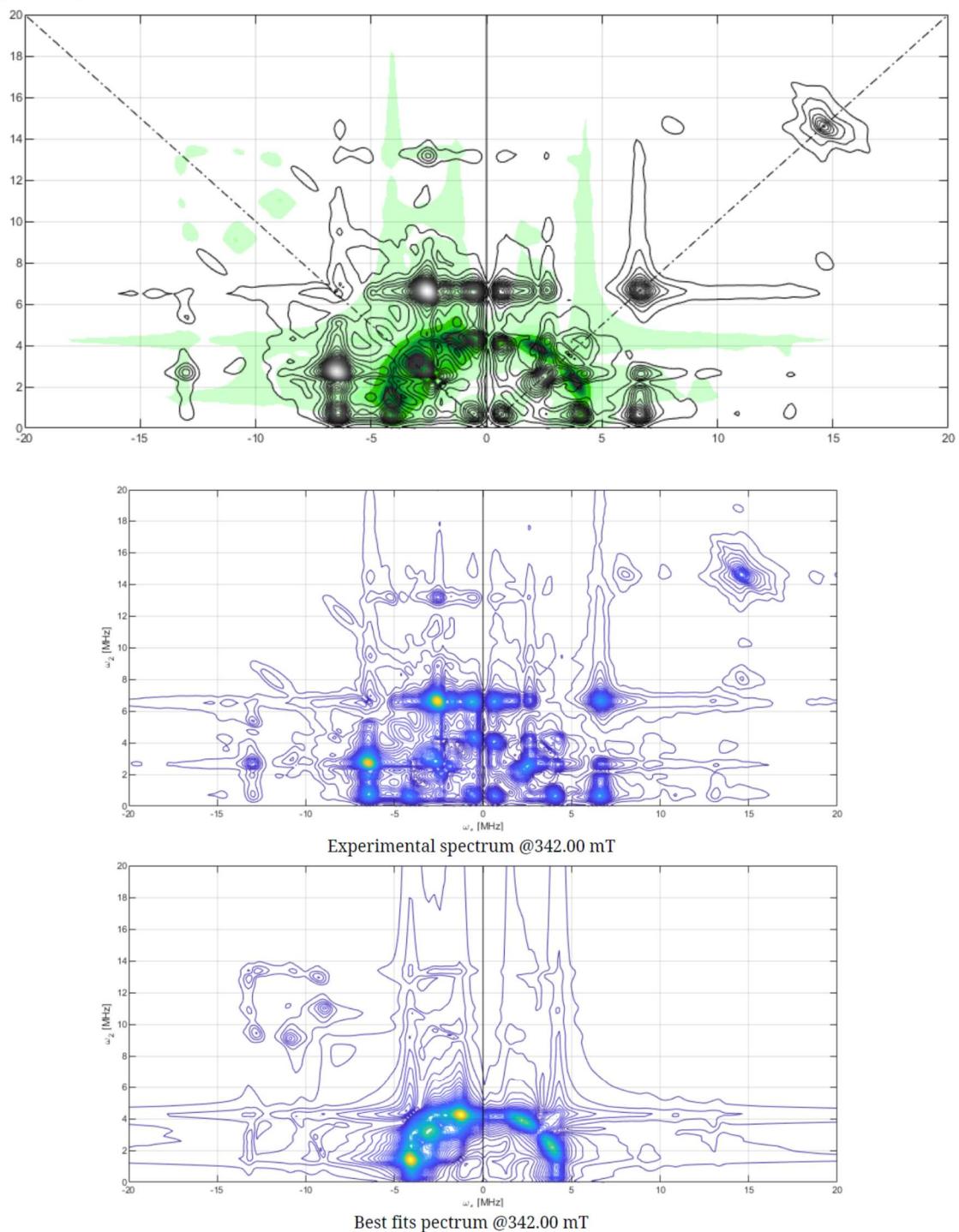


Figure 3. Spectrum @342.00 mT (Best Fit RMSD: 14.9231)



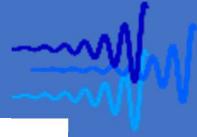


Figure 4. RMSD Evolution of Spectrum @318.20 mT (Best Fit RMSD: 14.9231)

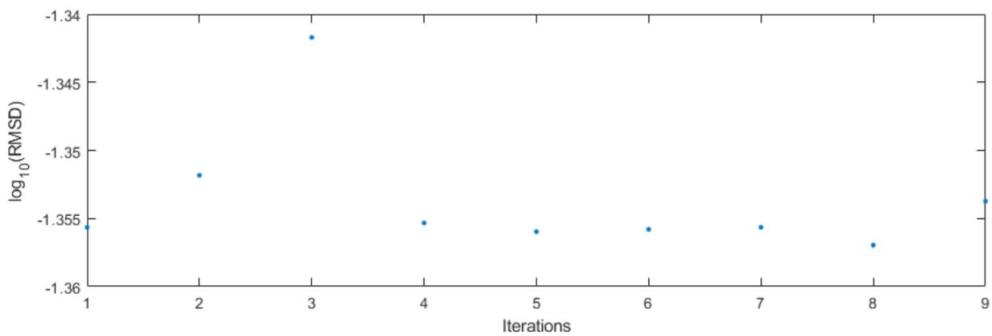
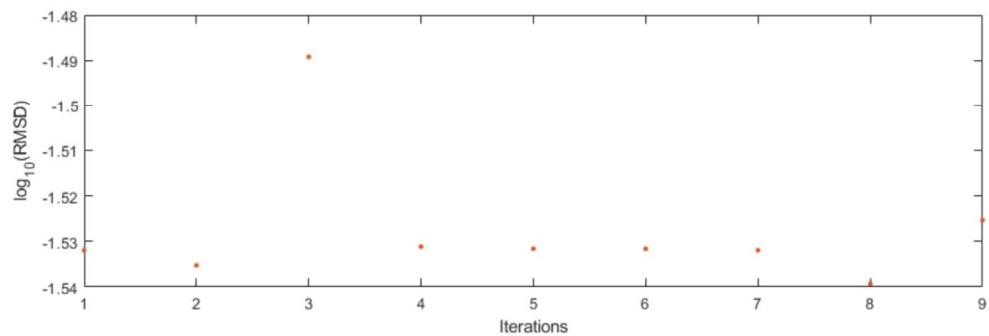


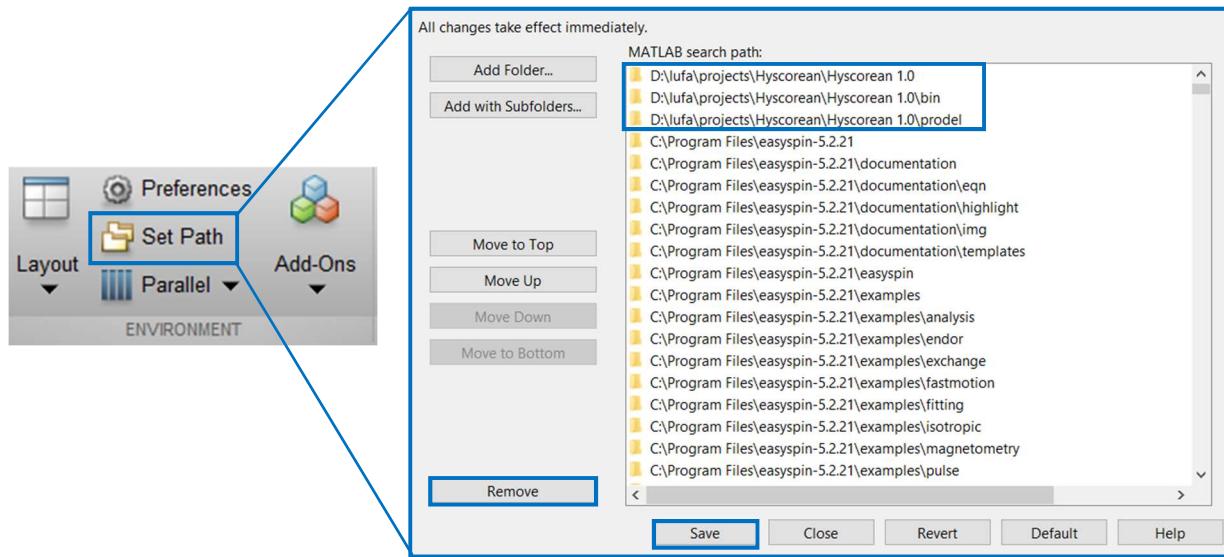
Figure 5. RMSD Evolution of Spectrum @342.00 mT (Best Fit RMSD: 14.9231)



## 8. Uninstalling Hyscorean

In order to remove Hyscorean from the computer and from MATLAB the user has to follow these instructions:

- I. Remove Hyscorean from the MATLAB search path. This can be done via the MATLAB GUI selecting the [Home>Environment>Set Path](#) option and then in the new window selecting all Hyscorean paths and removing them via the [Remove](#) button. Make sure to exist the window via the [Save](#) button to ensure the paths are removed in further session.

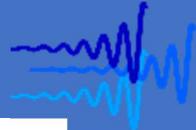


- II. Remove the MATLAB preferences set by Hyscorean by executing the following command in the MATLAB console.

```
rmpref('hyscorean')
```

- III. Remove all files from the computer by deleting them from the location chosen by the user to save them.

At this point Hyscorean will be completely removed from the system as prior to installation. Should the user want to re-install Hyscorean it will have to be done as described in 2.1. at the start of this manual.



## 9. GNU LGPL 3.0 License



GNU LESSER GENERAL PUBLIC LICENSE  
Version 3, 29 June 2007

Copyright (C) 2007 Free Software Foundation, Inc. <<https://fsf.org/>>  
Everyone is permitted to copy and distribute verbatim copies of this license  
document, but changing it is not allowed.

This version of the GNU Lesser General Public License incorporates the terms  
and conditions of version 3 of the GNU General Public License, supplemented by  
the additional permissions listed below.

### 0. Additional Definitions.

As used herein, "this License" refers to version 3 of the GNU Lesser General  
Public License, and the "GNU GPL" refers to version 3 of the GNU General Public  
License.

"The Library" refers to a covered work governed by this License, other than  
an Application or a Combined Work as defined below.

An "Application" is any work that makes use of an interface provided by the  
Library, but which is not otherwise based on the Library. Defining a subclass  
of a class defined by the Library is deemed a mode of using an interface provided  
by the Library.

A "Combined Work" is a work produced by combining or linking an Application  
with the Library. The particular version of the Library with which the Combined  
Work was made is also called the "Linked Version".

The "Minimal Corresponding Source" for a Combined Work means the Corresponding  
Source for the Combined Work, excluding any source code for portions of the  
Combined Work that, considered in isolation, are based on the Application, and  
not on the Linked Version.

The "Corresponding Application Code" for a Combined Work means the object  
code and/or source code for the Application, including any data and utility  
programs needed for reproducing the Combined Work from the Application, but  
excluding the System Libraries of the Combined Work.

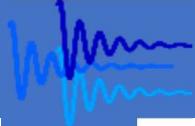
### 1. Exception to Section 3 of the GNU GPL.

You may convey a covered work under sections 3 and 4 of this License without  
being bound by section 3 of the GNU GPL.

### 2. Conveying Modified Versions.

If you modify a copy of the Library, and, in your modifications, a facility  
refers to a function or data to be supplied by an Application that uses the  
facility (other than as an argument passed when the facility is invoked), then  
you may convey a copy of the modified version:

- a) under this License, provided that you make a good faith effort to ensure  
that, in the event an Application does not supply the function or data,



the facility still operates, and performs whatever part of its purpose remains meaningful, or

- b) under the GNU GPL, with none of the additional permissions of this License applicable to that copy.

### 3. Object Code Incorporating Material from Library Header Files.

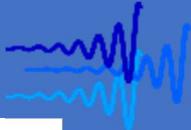
The object code form of an Application may incorporate material from a header file that is part of the Library. You may convey such object code under terms of your choice, provided that, if the incorporated material is not limited to numerical parameters, data structure layouts and accessors, or small macros, inline functions and templates (ten or fewer lines in length), you do both of the following:

- a) Give prominent notice with each copy of the object code that the Library is used in it and that the Library and its use are covered by this License.
- b) Accompany the object code with a copy of the GNU GPL and this license document.

### 4. Combined Works.

You may convey a Combined Work under terms of your choice that, taken together, effectively do not restrict modification of the portions of the Library contained in the Combined Work and reverse engineering for debugging such modifications, if you also do each of the following:

- a) Give prominent notice with each copy of the Combined Work that the Library is used in it and that the Library and its use are covered by this License.
- b) Accompany the Combined Work with a copy of the GNU GPL and this license document.
- c) For a Combined Work that displays copyright notices during execution, include the copyright notice for the Library among these notices, as well as a reference directing the user to the copies of the GNU GPL and this license document.
- d) Do one of the following:
  - 0) Convey the Minimal Corresponding Source under the terms of this License, and the Corresponding Application Code in a form suitable for, and under terms that permit, the user to recombine or relink the Application with a modified version of the Linked Version to produce a modified Combined Work, in the manner specified by section 6 of the GNU GPL for conveying Corresponding Source.
  - 1) Use a suitable shared library mechanism for linking with the Library. A suitable mechanism is one that (a) uses at run time a copy of the Library already present on the user's computer system, and (b) will operate properly with a modified version of the Library that is interface-compatible with the Linked Version.
  - e) Provide Installation Information, but only if you would otherwise be required to provide such information under section 6 of the GNU GPL, and only to the extent that such information is necessary to install and execute a modified version of the Combined Work produced by recombining or relinking the Application with a modified version of the Linked



Version. (If you use option 4d0, the Installation Information must accompany the Minimal Corresponding Source and Corresponding Application Code. If you use option 4d1, you must provide the Installation Information in the manner specified by section 6 of the GNU GPL for conveying Corresponding Source.)

## 5. Combined Libraries.

You may place library facilities that are a work based on the Library side by side in a single library together with other library facilities that are not Applications and are not covered by this License, and convey such a combined library under terms of your choice, if you do both of the following:

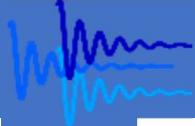
- a) Accompany the combined library with a copy of the same work based on the Library, uncombined with any other library facilities, conveyed under the terms of this License.
- b) Give prominent notice with the combined library that part of it is a work based on the Library, and explaining where to find the accompanying uncombined form of the same work.

## 6. Revised Versions of the GNU Lesser General Public License.

The Free Software Foundation may publish revised and/or new versions of the GNU Lesser General Public License from time to time. Such new versions will be similar in spirit to the present version, but may differ in detail to address new problems or concerns.

Each version is given a distinguishing version number. If the Library as you received it specifies that a certain numbered version of the GNU Lesser General Public License "or any later version" applies to it, you have the option of following the terms and conditions either of that published version or of any later version published by the Free Software Foundation. If the Library as you received it does not specify a version number of the GNU Lesser General Public License, you may choose any version of the GNU Lesser General Public License ever published by the Free Software Foundation.

If the Library as you received it specifies that a proxy can decide whether future versions of the GNU Lesser General Public License shall apply, that proxy's public statement of acceptance of any version is permanent authorization for you to choose that version for the Library.



## 10. References

- [1] Stoll et al., *Journal Magnetic Resonance*, vol. 177, pp. 91-101, 2009.
- [2] Maciejewski et al., "Nonuniform Sampling and Spectral Aliasing," *Journal of Magnetic Resonance*, vol. 199, no. 1, pp. 88-93, 2009.
- [3] Shchukina et al., "Pitfalls in compressed sensing reconstruction and how to avoid them," *J. Biomol. NMR*, vol. 68, pp. 79-98, 2017.
- [4] B. Worley, "Convex accelerated maximum entropy reconstruction," *Journal of Magnetic Resonance*, vol. 265, pp. 90-98, 2016.
- [5] Balsgart et al., "Fast Forward Maximum entropy reconstruction of sparsely sampled data," *Journal of Magnetic Resonance*, vol. 223, pp. 164-169, 2012.
- [6] Stefan Stoll, A. Schweiger, "EasySpin, a comprehensive software package for spectral simulation and analysis in EPR," *Journal of Magnetic Resonance* 178 (2006) 42–55, vol. 178, pp. 42-55, 2006.
- [7] Stoll et al., " EasySpin Documentation: Fitting EPR spectra," [Online]. [Accessed 12 2018].