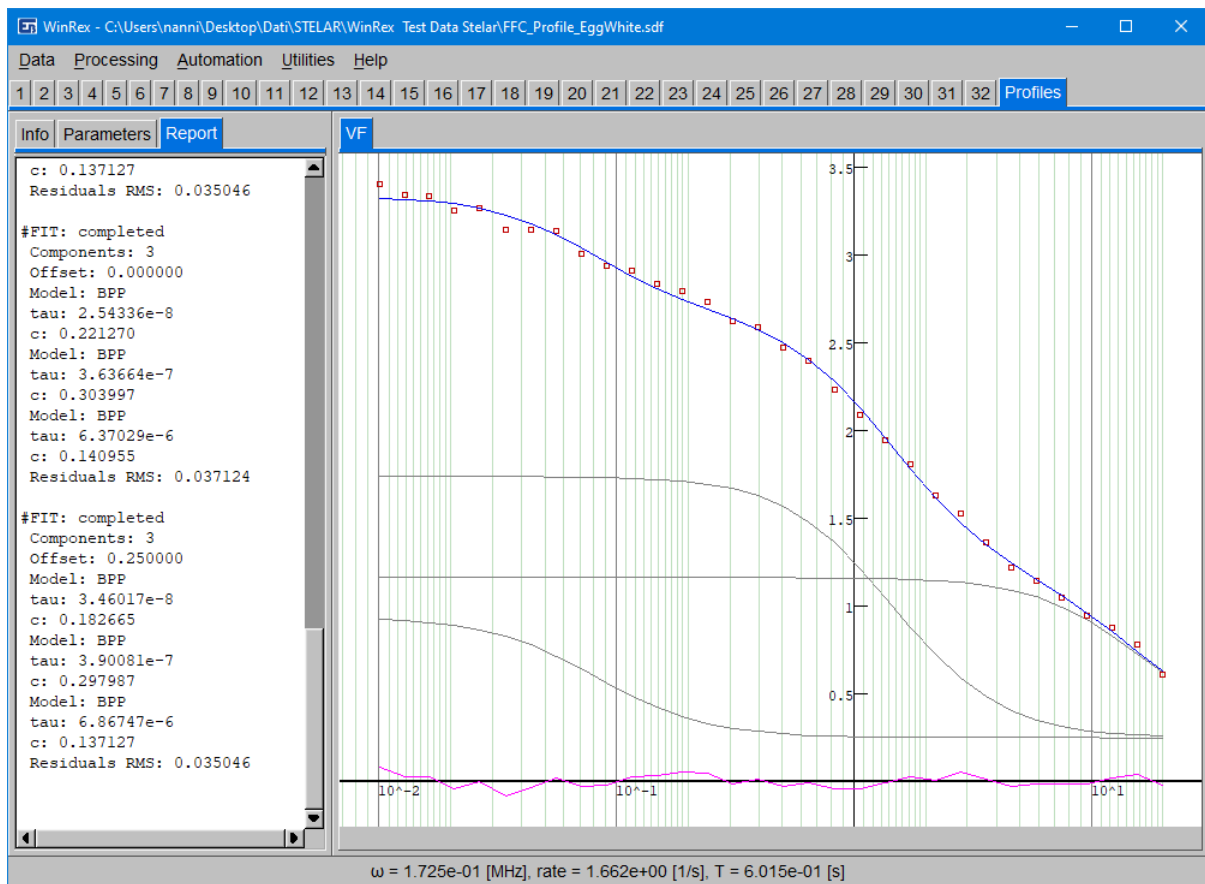


# WinRex

## 1.2 (.669) User Guide



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## What is Rex

Rex is a specialized software package for *processing NMR Relaxometry data*.

We plan to extend the list of data Rex can read to formats from instruments of any make, produced by any OEM.

Rex is intended to be an Relaxometry software Tool

- Community-oriented
- Reading data from any source
- Strongly collaborative approach
- Fast developing under community requests

It is a tool for:

- Researchers in NMR++ relaxation phenomena
- Researchers in molecular dynamics
- Developers of relaxometry applications

Its current status: covering much of what one can do with

- Fixed field TD NMR relaxometers
- FC and FFC relaxometers

Moving towards: integration with

- FD Spectroscopy
- Diffusometry
- Other relaxometry methods (dielectric, ultrasound, ...)
- Imaging

## License

### How to activate a license

Each Extra Byte Application, once installed, provides 60 days of free trial period starting from the first access. When the trial period is about to expire (<30 days left) the program sends a warning message about that at its launch.

When the trial period is over, the Application shows the Licensing interface (see [Figure 1](#)). The same can be whenever opened by the user from the program main menu bar, under the *Help* item. The License interface shows all the information about the user license: the personal data, if already registered, the *Computer ID*, the license key, if activated, the number of days left. Here user can activate a license by inserting a key. The key is provided by Extra Byte through an email exchange, after that the user has been registered and has made the payment, if required. A key can be used to activate a license on a unique computer.

The License interface allows user to register, by filling in the form with the proper entries (*Name*, *Surname*, *Institution/Organization*, *Email address*), and to send automatically an email, by clicking on the *Get key* button. This button opens the email client and prepares a new message to [licensing@ebyte.it](mailto:licensing@ebyte.it) with a formatted text. If this should not happen, user has to send the message manually, inserting all the info above, included the *Computer ID* and the number of the Application version.

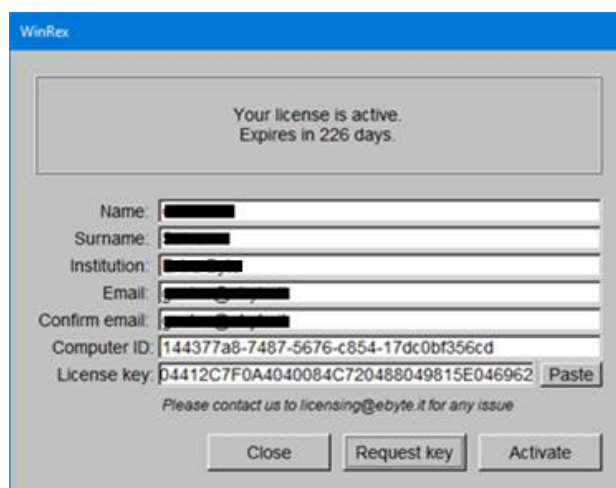


Figure 1 - Licensing interface

After sending the license key request, Extra Byte will reply to the user, providing invoice and payment details, if necessary. After payment, user will receive an email with an attached text file containing the key. He or she has to copy and paste it into the proper field in the License interface, then click the *Activate* button. The program notifies if the activation has been made successfully or not. In the next sessions, the program warns the user when the license is about to expire (<30 days left).

User is recommended to never modify the system time after the installation. This guarantees a correct execution of the program.

## Overview

### Main menu

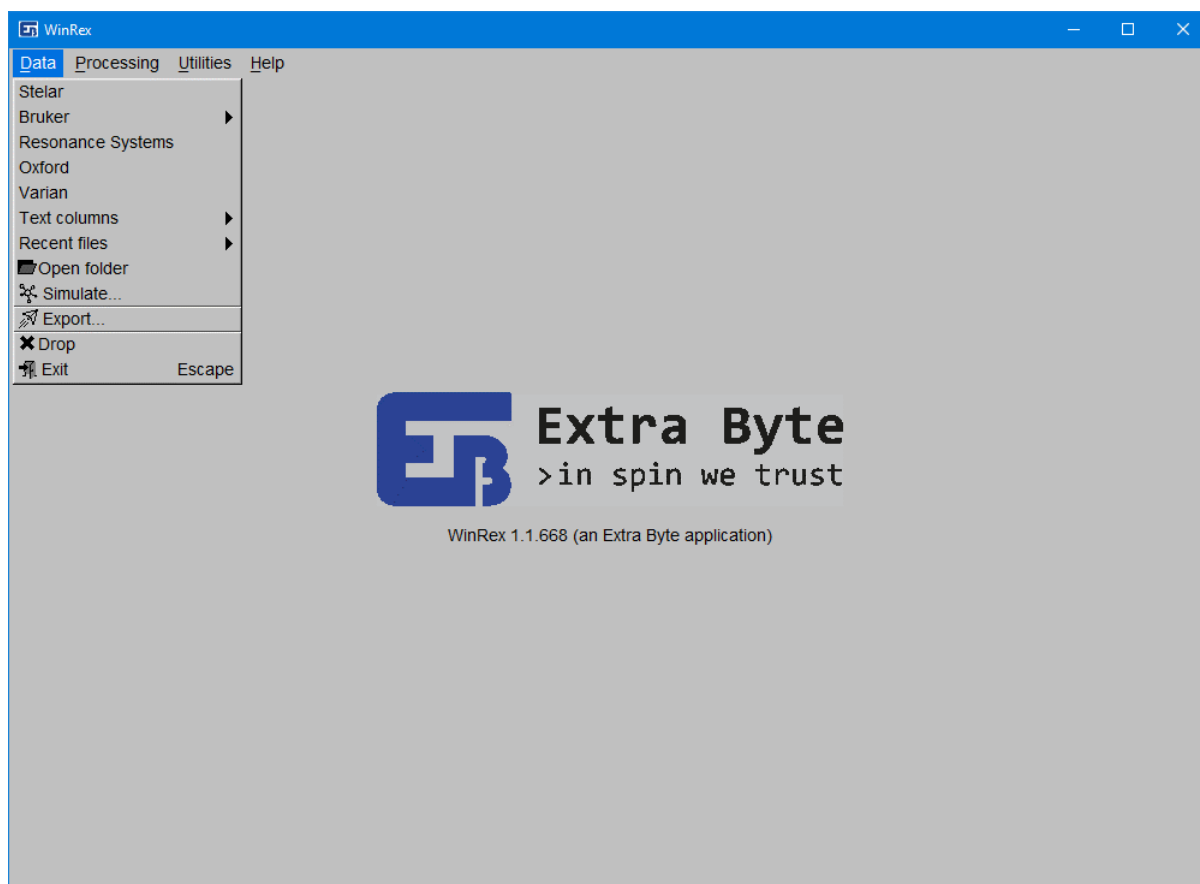


Figure 2. Main window appearance at program launch

WinRex main window (see [Figure 2](#)) presents a menu bar with the following commands:

#### Data

- "Stelar"  
Opens a Stelar Data File (choosing between *.sdf* or *.hdf5* file extension).
- "Resonance Systems"  
Opens Resonance Systems files (extension *.TD-Rel*).
- "Bruker"  
Chooses to open Bruker data files coming from MiniSpec instrument (*.dps*) or in TopSpin format (select *fid* or *ser*).  
Note: MiniSpec time-domain data are exported into two different files, containing the real and the imaginary part respectively. It is sufficient to select one of them and they both will be loaded (the file names must include the words "real" and "imag" respectively). Also, it is possible to load many files together and they will be treated in an analogous way of Stelar concept of "zones".

- "Oxford"  
Opens Oxford Instruments data files (.RiDat).  
Note: only 1D experiments are supported up to this version.
- "Varian"  
Opens Varian data files (*fid*).
- "Text columns"  
Imports data from text files (.txt, .csv, ...).  
Note: data must be encoded as text columns with ASCII characters, separated by blank spaces or commas. They may be preceded by blank lines or comments (that means lines starting with "//"). It can be chosen to import as:
  - ♦ time-domain signal (FID or CPMG).  
Columns correspond to time [s], real part [au] and, optionally, the imaginary part
  - ♦ multiblock data.  
Columns: tau (block parameter) [s], time [s], real and, optionally, imaginary part [au]
  - ♦ relaxation curve (two columns: tau [s] and signal intensity [au])
  - ♦ VF dispersion profile: relaxation rate [1/s] vs frequency [MHz]
  - ♦ VT profile: relaxation rate [1/s] vs temperature [K]

Note: Rex assumed the data to be expressed in the specified units. There is no possibility to change it once the files are loaded.

- "Recent files"  
Opens a recent file from a list. It's not possible to open multiple files from here.
- "Simulation..."  
Opens the interface for simulating relaxometry experiments (see Paragraph [Simulation](#)).
- "Export..."  
Opens a simple interface where user can decide what to export (any data, plot, report or acquisition parameters) and the destination file format (Scalable Vector Graphic image (.svg), Comma-Separated Values text (.csv), or Stellar Data File (.sdf)). Data may be exported directly from the canvas popup menu, as well.
- "Open file location"  
Opens the directory of the loaded files in the file explorer window.
- "Drop"  
Closes current data files.
- "Exit"  
Closes current data files and exits the application.

## Processing

- "Setup"  
Opens the Setup interface (see Paragraph [Processing Setup and Commands](#)) where user can set all processing parameters.
- "Automation":
  - ♦ "Plain"  
Runs the plain list of commands selected in the *Automation* section of the processing Setup window for all the selected zones. Attention: evaluation starts

from the current state of the data. So, if user needs to repeat it, commands “Undo” or “Restart” should be launched before.

- ◆ "Plain + Profile"

Like "Plain", then builds the NMRD Profiles page. The profile is built according to the options set in the *Profiles* section of the Setup.

Important: it is necessary to have already built the relaxation curves or have selected the command *Build Relax curve* in the automation list in order to create the profile. Also, it is necessary that the parameters include the acquisition frequency for building variable frequency profiles, or the temperature for building variable temperature profiles.

- "Zones->New multiblock..."

Extract a single block data (FIDs) from any zones and create a new multiblock zone.

- "Zones->Stack"

It will creates a new page, labelled “Stack”, where the relaxation curves from all the zones are plotted together in stack. It is necessary that all the pages contain relaxation curves (canvas labelled “Relax”) with the same time values. From “Stack” plot, user can execute the multiexponential fitting on all the curves and then execute PCA (Principal Component Analysis) on either the relaxation curves or the fitting parameters (intensity and relaxation rate). Read section [PCA](#) for more information.

- "Undo"

Undoes last operation.

- "Restart"

Resets all the processing done and restart from the original data.

## Utilities

- "NMR Calculator"

Runs some useful NMR tools (see Paragraph [NMR Calculator](#))

## Help

- "Manual"

A link to a copy of this user Manual, which is installed in the application folder.

- "Website"

Opens Rex webpage ([www.extrabyte.eu/products/rex/](http://www.extrabyte.eu/products/rex/)) on Extra Byte website with user’s default browser.

- "Contact us"

Opens user’s default email client and prepares a draft message addressed to Extra Byte ([info@extrabyte.eu](mailto:info@extrabyte.eu)).

- "License"

Shows information about user’s license. Here a license can be activated, providing a proper key. For information about licensing se Paragraph [License](#).

- "About"

Shows information about WinRex current version and Extra Byte contacts.

## Graphic interface

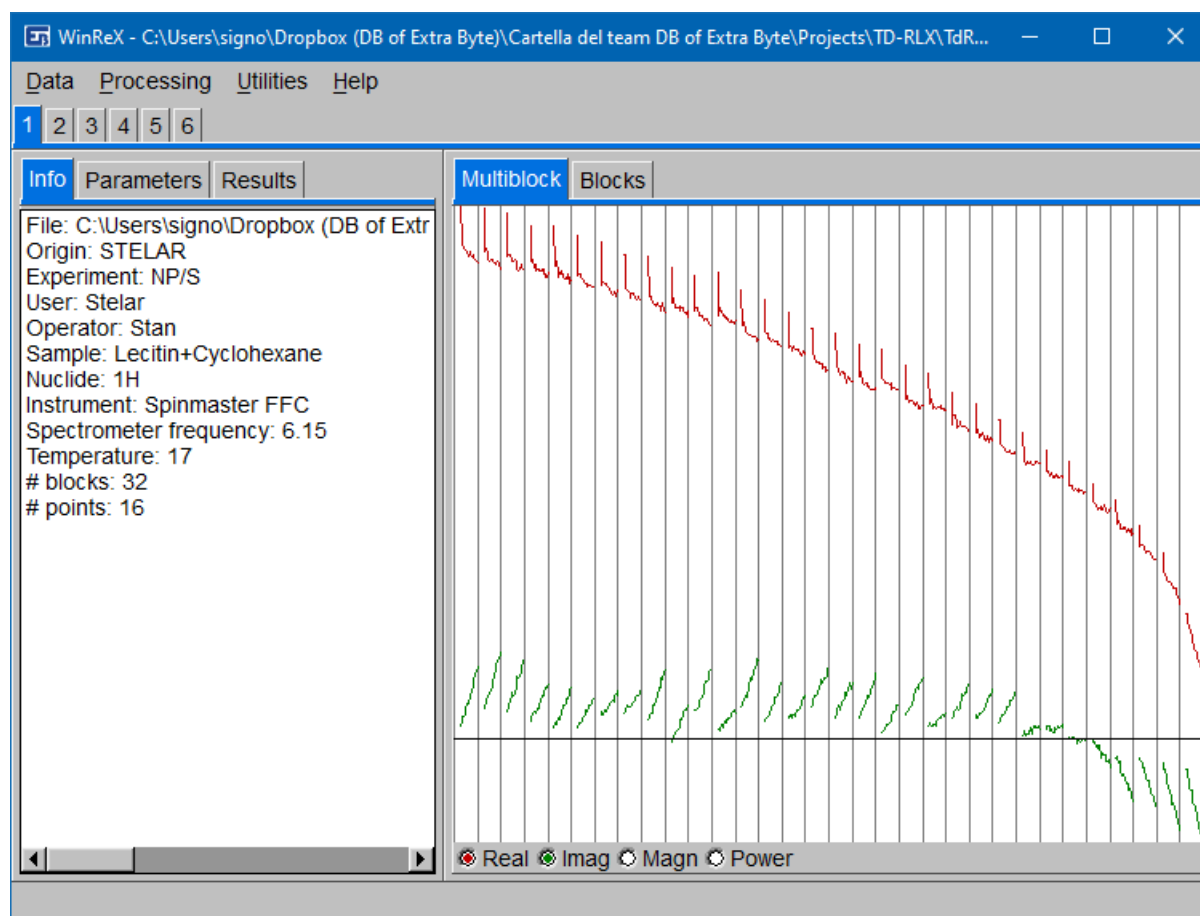


Figure 3. Main window appearance when data are loaded

When data are loaded successfully the file name is shown in the window title bar. A multi-tab panel, which is useful to navigate through different numbered pages, appears just under the menu bar (see [Figure 3](#)). When pages are too many, the multi-tab panel is substituted with arrow buttons.

Each page is divided in two sections. At the left side, a multi-tab panel includes some quick information about the currently loaded data, a list of the acquisition/experimental parameters and a space where processing results are output. At the right side, there is a multi-tab panel for the graphical display of data. All possible canvas types that may appear, depending on the data and the evaluation, are labelled as *Multiblock*, *Blocks* or *Signal* for time domain data, *Spectrum* for frequency domain, *Relax* for relaxation curves, *ILT* and for the Inverse Laplace Transform (1D and 2D), *VF* and *VT* for variable frequency and variable temperature NMR dispersion profiles, *PCA* for Principal Component Analysis score plot. Canvases appear dynamically during the evaluation.

After loading any file, raw data are plotted. Multiblock canvas displays vertically stacked blocks showing stacked experiments data (eg. Inversion Recovery), while Blocks and Signal canvas displays singular blocks data, which can be browsed with the arrows in the tool bar, at the bottom of the canvas.

Other canvases will appear after some specific commands. When the relaxation curve of a 2D experiment is created (or imported from a text file), new panel Relax appear. Freq. dom. canvas appears when Fourier transform is executed and it shows frequency domain spectra. When the



Inverse Laplace Transform is performed, either discrete or continuous, ILT canvas appear. VT and VF Profiles canvas appears when profiles are built or imported from a text file. Dispersion profiles are plotted as the relaxation rate [1/s] versus the frequency [MHz] (VF) or versus the temperature [1000/K] (VT).

Under all the canvases a tool bar is present, which includes some useful buttons. For example toggle buttons to view displayed arrays of data, like the real and the imaginary parts of a signal, or the fit function and residuals. In some canvases, residuals arrays may be expanded by a factor 10, 100 or 1000, and plot coordinates axes (x- or y-axis) may be switched between linear and logarithmic scale with the appropriate button. In the , some buttons allow user to select which principal components to plot.

All plots can be zoomed in by selecting a rectangle area with the mouse and can be also zoomed out (horizontally) with 'Ctrl' button pressed down. Plots can be moved in any directions by dragging the mouse and pressing 'x' on the keyboard. They can be expanded vertically (changing the intensity) by using the mouse wheel, and horizontally in the same way while pressing 'Ctrl' button. All zoom states can be reset with a mouse double click.

Wherever the mouse is, its coordinates are shown in the status bar, along with the closest point index (from 0 to n-1) and the block number (in case of Multiblock canvases).

A right click on any canvas opens a pop-up menu with the processing commands list. Commands are intended to run on the visible canvas of the current page. Each canvas type has a particular list of commands. The parameters related to any commands are found in the Setup (see next paragraph).

## Processing Setup and Commands

"Setup" menu item opens a window, shown in [Figure 4](#), where user can set the parameters related to any commands.

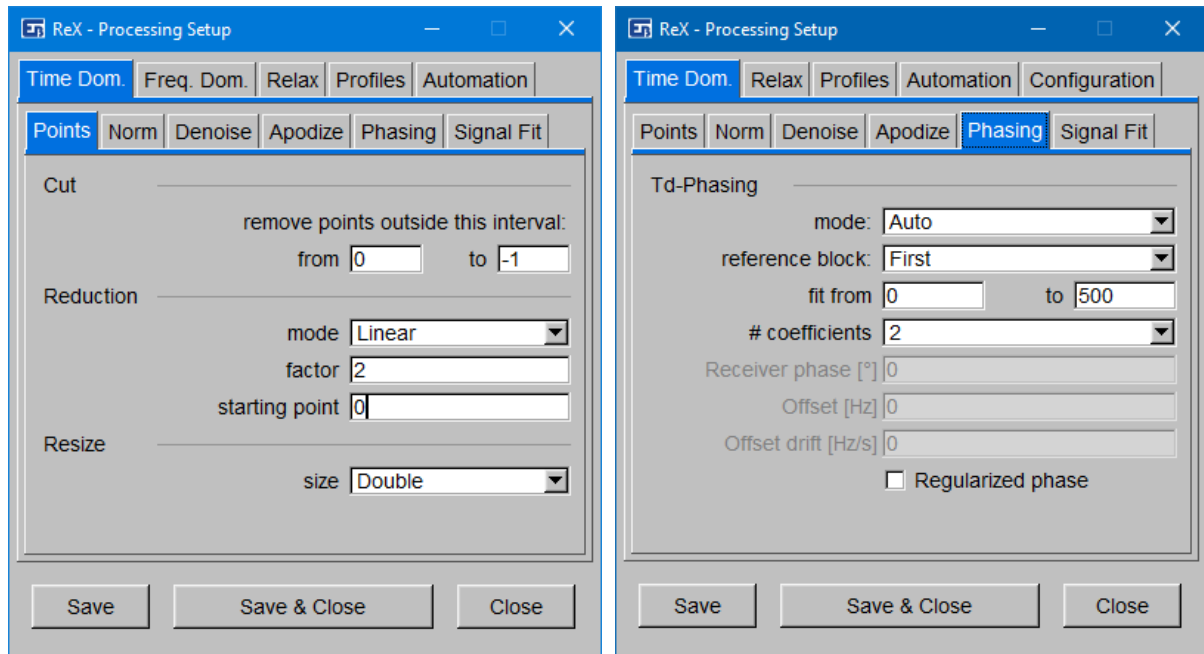


Figure 4 - Processing setup interface: points and phasing sections for time-domain commands.

## General description of the Setup

The Setup interface presents a multi-tab, each panel containing sections of commands parameters. Generally, inputs fields have explanatory tooltip windows. If any field remains empty, or a bad number (like a negative index) is input, then a default value is used for the related command. Whenever points indexes are required, they vary from 0 to n-1.

Commands launch happens through pop-up menus directly opened from a canvas through the mouse right click. Commands are addressed to data visible in the current canvas. During the commands execution, the setup interface may remain opened (it is not necessary to close it). Only commands related to *Automation* section are launched from the menu bar in the main window, as they (may) refer to many zones and data sets.

Commands are launched with the last input parameters, even if not saved. In fact, "Save" button (at the bottom of the Setup) saves all the current input parameters for the next WinRex session. "Close" button just closes the Setup window.

As mentioned above, commands are generally launched from a pop-up menu, which is opened by right-clicking on a canvas. Commands are executed on data related to that canvas, with the parameters currently set in the related section in the Setup. When each evaluation has terminated, a concise report is displayed in the Report panel, even if an error occurred.

A command which is common for all the canvas is "**Report data**". It reports the data plotted in the canvas as tabulated values in the Report panel at the left side of the GUI. Also, "**Export**" is a common command, which allows to export the data or the plot to different file formats or to clipboard. In the following sections all the other commands and related parameters are described.

## Time Domain commands

The first panel present in the Setup contains sections of parameters for time domain data (canvases *Multiblock*, *Blocks* and *Signal*). Commands launched from a Multiblock canvas are addressed to all the blocks.

"Points" section is intended to include parameters for commands that change the blocks size (number of points). Command "**Cut**" removes points outside a certain range, e.g. with a range from 1 to -1 it just removes the first points (index 0). "**Reduction**" reduces blocks size by a factor, according to a grid type (either linear or logarithmic), starting from a certain point. This may be useful to reduce the points number before fitting a decay. The effect of "**Resize/Zero Filling**" command depends on the blocks size: if the set size is greater than the current blocks size, then the blocks are zero filled, that means expanded and the new points are set zero; on the other hand, if the size is smaller then blocks are just truncated. Also, commands "**Remove first point**" and "**Remove this point**" are present and they don't need any parameters. The latter refers to the point nearest to the mouse pointer and its index is specified in the status bar. After the execution of each of these commands the new number of points is reported.

"**Group Delay correction**" correct group delay artifact from Bruker FIDs. This avoids to obtain typical smileys in frequency domain spectrum.

"**Normalization**" section refers to just one command which apply a change in the intensity of the vertical scale. The input parameters are the rescaling reference, that is what is to be rescaled (only "Maximum" available for now), and the scale value (default is 1.0).

"**Denoising**" applies a denoising filter to the selected data. Two methods are implemented so far: the Savitzky-Golay filter (Savitzky & Golay, 1964) and the P-Spline (Eilers & Marx, 1996). The noise estimation is reported.

Four **Apodization** functions are available:

- Stanning 
$$A(t) = \begin{cases} \cos^2(\alpha t) & \text{for } \alpha t < \pi/2 \\ 0 & \text{for } \alpha t \geq \pi/2 \end{cases}$$

Input parameter: number of times the mean decay time at which is  $\alpha t = \pi/2$ .
- Exponential 
$$A(t) = e^{-\alpha t}$$

This apodization corresponds to a convolution product in the frequency domain between the spectrum and a Lorentz function, whose width is the input parameter.
- Gaussian 
$$A(t) = e^{-\alpha t^2}$$

This apodization corresponds to a convolution product in the frequency domain between the spectrum and a Gauss function, whose width is the input parameter.
- Sinc (Cardinal Sine) 
$$A(t) = \sin(\alpha t) / \alpha t$$

This apodization mode corresponds to a convolution product in the frequency domain between the spectrum and a rectangle function, whose width is the input parameter.

Command "**Td-Phasing**" executes a phase correction which depends on the mode set in the related section (see [Figure 4b](#)). "Magnitude and Phase" and "Power and Phase" modes just substitute the real and imaginary part of the signal into the magnitude (or power) and the phase array, respectively. Flag "Regularized phase" can be selected to obtained a phase which drifts throughout the signal, if this occurs, otherwise it will always be calculated within the interval  $(-\pi; \pi]$ .

"Manual" mode, instead, allows to manually adjust the receiver phase, the frequency offset and/or the offset drift (the latter conceived for unstable magnets). "Auto" mode does the same automatically, by calculating the parameters so that the imaginary part of the signal starts from zero (one coefficient), with null first derivative (two coefficients) and/or with null second derivative (three coefficients). The phase correction is calculated through a polynomial fit of the signal phase, in a range of points, with  $n$  input coefficients (from 1 to 3). The signal phase can be regularized or not through the relative flag. Unlike a point by point calculation of the phase, the fitting procedure does not distort the signal and allows to eliminate noise effects. After the execution of the command the correction of the phasing parameters is reported.

In case of multiblock data, a reference block (the first or last block, the strongest one in magnitude) can be chosen to fit the phase. This fitted phase is then used to correct all the blocks. Also, the blocks can be phased individually, with no reference block, or individually with maintaining the sign (so that negative real signal are not inverted).

Time domain data can be **Fourier Transformed**. A new graphic panel opens to plot frequency domain spectra.

Also, time-domain signals can be fitted through the command "**Signal fit**". In the appropriate section of the Setup (see [Figure 5a](#)) one can set the point window, choose the channels to fit, between the real one only and complex (real + imaginary), and select the minimization function.

The available models are: exponential, gaussian and Abragamian function (that is a Gaussian function times a cardinal sine). They can be combined together as adding functions and, for each of them, the number of components can be chosen.

The parameters can be automatically initialized by setting the appropriate flag, otherwise one can manually do it in the new interface window which can be opened by clicking on the button "Advanced...". In the advanced fitting interface (see [Figure 5b](#)) is present a section for each available model, which shows the list of all the parameters relative to that model (signal intensity, decay rate e frequency offset) along with a brief description. Also the global parameters (baseline offset and starting phase), which don't depend on the single components, are placed at the bottom. User can set the flag on any parameter he wants to fit, or remove that flag if he wants to keep that parameter fixed. Also, he can set the starting values in the appropriate fields (the limiting values, minimum and maximum, are not yet handled). If left empty, these will be set automatically. A parameter to be fixed with an empty starting value will be fitted. In any case, after the fit has run, the starting value fields will be filled with the effective starting values. One could even fix all the parameters, just to calculate and plot the function along with the data.

The estimation of the error of the fitted parameters is not implemented yet.

A brief report shows the parameters of each components obtained with the fit, along with some statistical information about the quality of the fit results: the residuals root mean square and a model-data match compatibility index based on the residuals  $\chi^2$  test.

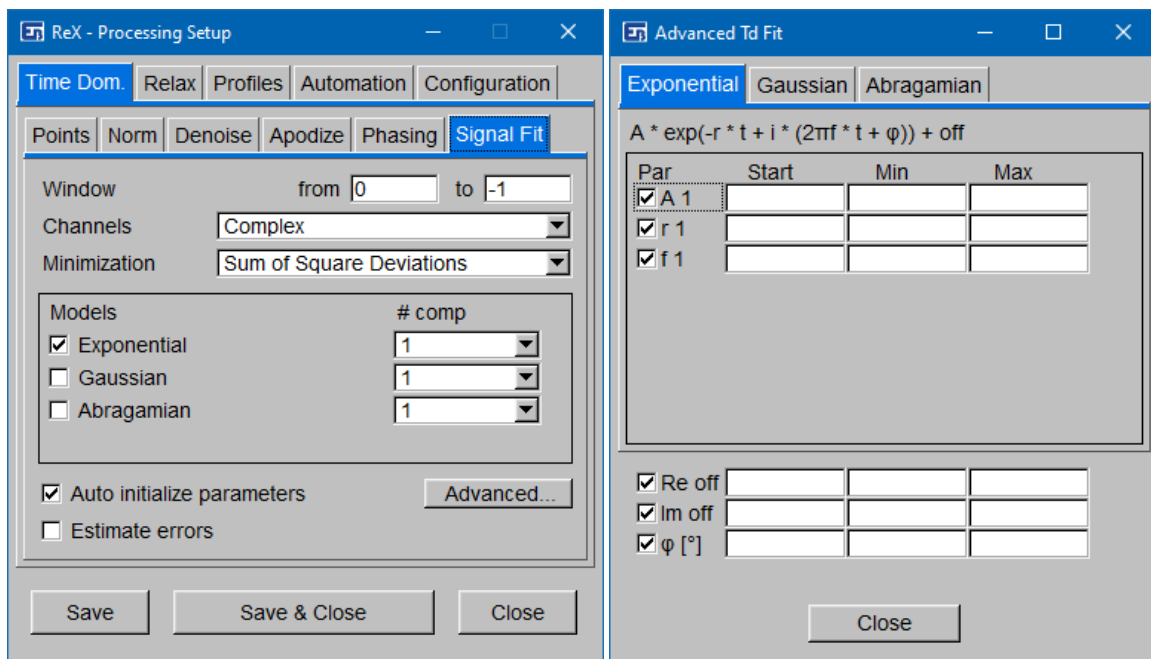


Figure 5 - Setup section for Td-signal fit and relative Advanced interface

### Relax commands

"**Build Relax curve**" command quantifies the blocks of a staggered (2D) experiment and shows the quantification values in a new panel labelled "Relax". Real or magnitude channel can be quantified and the available modes are: arithmetic averaging of a window and extrapolation to zero with Savitzky-Golay algorithm. For 1D experiment, instead, the command "**Promote to Relax curve**"

builds the relaxation curve by copying the signal as it is. This is thought for single FIDs or CPMG, where user wants to run the inverse Laplace transform, which is available only for Relax curves.

Commands available in "Relax" panel includes those for (re)building and handling the relaxation curve, with the same parameters for operating on points, normalization and denoising as those in Time-Domain.

A command which is present in the menu of Relax canvas only for ANGLE experiment, is **Calibrate pulse**. This calculates and reports the time width for a 90 and 180 degrees pulse, by executing a fitting with the sine function.

Relaxation curves can be fitted as with a discrete number of components or with a continuous distribution. "Fit" section contains parameters for the **DILT** (Discrete Inverse Laplace Transform), that is a finite number of exponential functions (up to 8 components). An automatic determination of the best number of exponentials is available (see [Figure 6a](#)). The DILT fits either the relaxation rates and the weights of each components, in addition to the baseline offset.

A second available model is implemented and can be used: the stretched exponential function  $f(x) = A \exp(-(rt)^b)$  where  $b$  is a parameters to fit in the interval (0; 1). Again, one can choose the up to 8 components, but no automatic determination is available. Unlike for time-domain fitting, different models cannot be combined together.

Here again, as for time-domain signal fitting, parameters can be initialized either automatically or manually through an analogous interface. In the advanced fitting interface, user can initialize the global parameters and those relative to the selected models and decide which one is to be fitted and which is to be kept fixed.

One has the possibility to execute simultaneous fitting of relaxation curves by selecting the appropriate flag and select the curves to fit. In the advanced interface, one can select which parameters are to fit simultaneously, that means which parameters must be identical for different curves and which not. The parameters that are not selected will be fitted separately for different curves.

A brief report shows the parameters of each components obtained with the fit, along with some statistical information about the quality of the fit results: the residuals root mean square and a model-data match compatibility index based on the residuals  $\chi^2$  test.

In the continuous version (**CILT**), the rates values are got from a fixed grid whose number of components can be set in the Setup (see [Figure 6b](#)), so that only the weights and the offset remain to be calculated. The grid is logarithmic and the range is found automatically. The problem reduces to the solution of the linear system  $Mx = r$  where  $r$  is the signal and  $M$  is the Laplace kernel. But given the huge number of variables, the problem is ill-posed and an explicit solution cannot be found numerically through matrix inversion, and this determines the intrinsic instability of the Inverse Laplace Transform. Thus, some regularization terms have to be introduced.

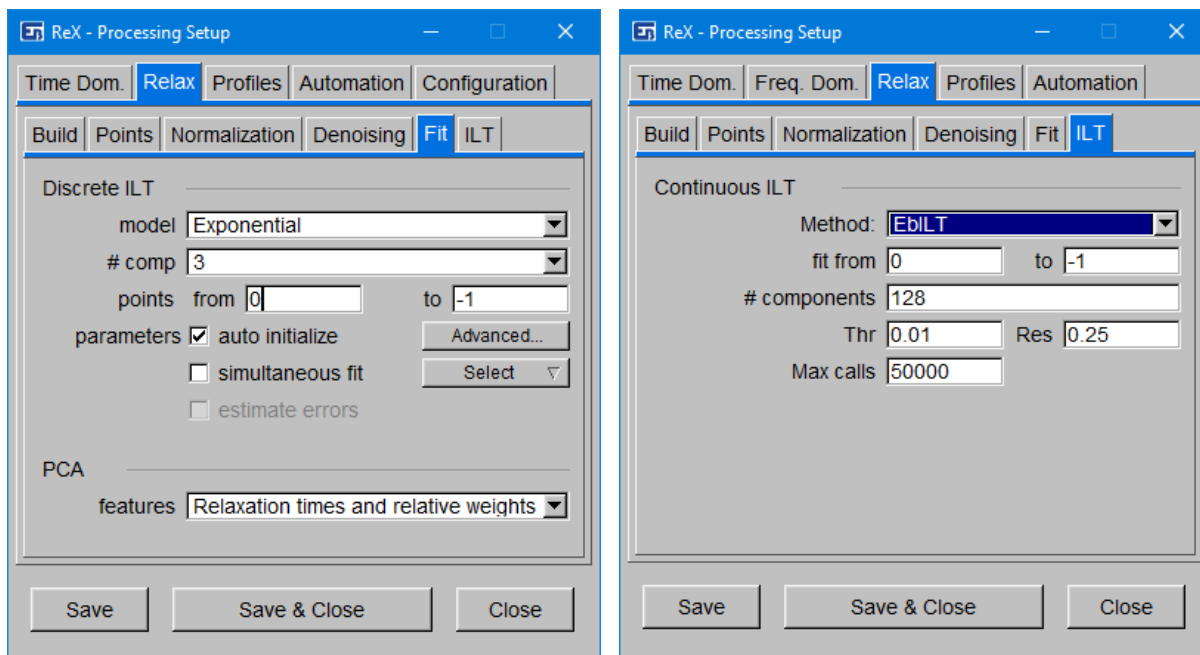


Figure 6 - Discrete (a) and Continuous (b) ILT setup

Three methods are implemented so far:

- **Truncated Singular Value Decomposition (SVD)**  
This one solves the linear system through a low-rank approximation. Matrix  $M$  is decomposed as  $M = USV^+$  where  $U$  and  $V^+$  are unitary matrices and  $S$  is the diagonal matrix containing the singular values. The algorithm truncates  $S$  to include only values above a threshold, which can be set from the GUI (parameter "Thr"). This drastically reduces the dimension of  $M$  and the system can be solved. This method is fast but its main drawback is that it is not guaranteed to obtain a positive distribution everywhere, as no constraint can be set on the weights sign.
- **EbILT**  
This iterative algorithm, based on UPEN (Borgia, Brown, & Fantazzini, 1998), performs a fit that minimizes the residuals, but also it includes constraints on the first and the second derivative. This allows to obtain a relatively smooth distribution of the relaxation rates. Parameter "Res" can be used to adjust the initial resolution of the distribution peaks, expressed in Neper, and "Max calls" to set the maximum number of calls of the objective function. Unlike SVD, this method guarantees an always positive distribution.
- **Multi-Gaussian**  
This algorithm models the ILT distribution as a sum of Gaussian distributions. User has to set just the number of gaussian components and the starting resolution.

Due to the instability of the ILT, the solution depends heavily on the initial condition and on the parameters used. User can set the range of points of the relaxation curve on which to perform the ILT, along with the number of components, while the limit values of the relaxation times are set automatically.

Both DILT and CILT commands create a new canvas labelled as "ILT", if not already present, where either DILT and CILT relaxation times distributions are plotted together. They are rescaled to their maximum value, to be compared.



## Profiles

NMRD profiles can be **built** from raw data by selecting the zones from a list in the setup (see [Figure 7a](#)). From the main window menu, command "**Plain + build Profile**" executes, in addition to the commands selected in the *Automation* panel, the discrete ILT for all the selected zones according to the parameters set in *Relax->Fit* section in the setup. Then, it plots the profiles in a new dedicated canvas. The relaxation curves must be necessarily built before for all the selected zones.

The **fit** section ([Figure 7b](#)) includes the point windows to fit, the relaxation type (longitudinal or transversal) and the minimization function. Also, user can specify the temperature at which the variable frequency profile has been acquired, and, viceversa, the frequency for variable temperature profiles. The available fitting models are:

- Rotational BPP (Abragam, 1961)
- Diffusional translation (Abragam, 1961)
- Lorentz. An empirical model based on Lorentz (Cauchy) distribution  $r(\omega, \tau) = c/(1 + (\omega\tau)^2)$
- Stretched Lorentz. Like a Lorentz distribution, with a different slope  $r(\omega, \tau) = c/(1 + (\omega\tau)^\beta)$
- Cole-Cole dispersion (Hallenga & Koenig, 1976)
- Solomon equation for dipolar coupling (Solomon, 1955)
- Gd(III)-based paramagnetic contrast agents solutions (Chapter 2: Gadolinium-based Contrast Agents, 2018)
- Quadrupole Relaxation Enhancement peaks (Fries & Belorizky, 2015)
- Havriliak-Negami dispersion relation, which is a generalization of Cole-Cole

Models can be selected and combined together. Button "Advanced" opens a new window with a section dedicated to each model, where user can choose to fit or fix any parameters and to set the starting values and the minimum and maximum (these are not handled yet), in the same way for time-domain signals fitting (see paragraph Time Domain). The baseline offset can be fitted too. The parameters in the right side of each section are considered only for variable temperature profiles only. They all are activation energies, involved in the Arrhenius (or sometimes Eyring)

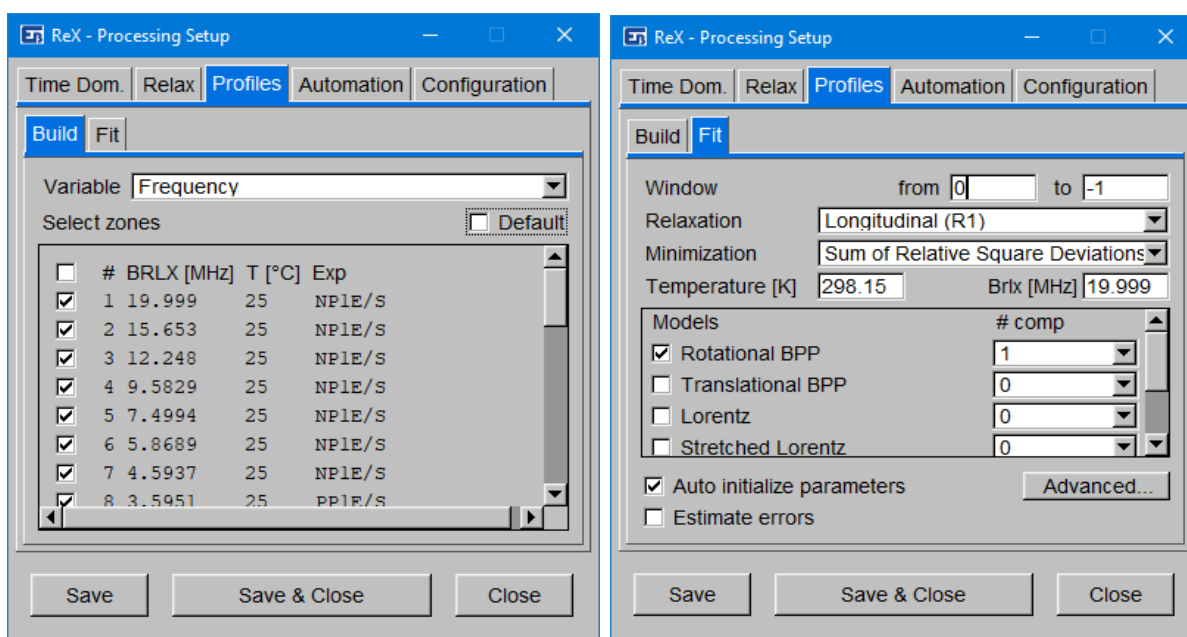


Figure 7 - Setup section for NMR dispersion profiles building and fitting

dependency of some variables, like the correlation times, on the temperature (eg.  $\tau(T) = \tau_0 \exp(E_a/k_B T)$ ). For frequency profiles, these activation energies are ignored, in order to avoid to fit redundant parameters. Then the dependency on the temperature is not considered and the fitted parameter is the entire function  $\tau$ .  
 At last, a flag for the estimation of parameters error can be set (not implemented yet).

### Automation

Each command can be launched individually from the canvas pop-up menu. This section allows to accelerate the processing, by creating a list of commands to execute in a row on any zones.

As show in the **Figure 8**, at the left user can select the zones to process and, on the right, the list of commands. It can be run through the main menu bar, with "**Automation->Plain**" button. Instead, "**Automation->Plain + build Profile**" executes the same and creates Fast Field Cycling Profile chart at the end.

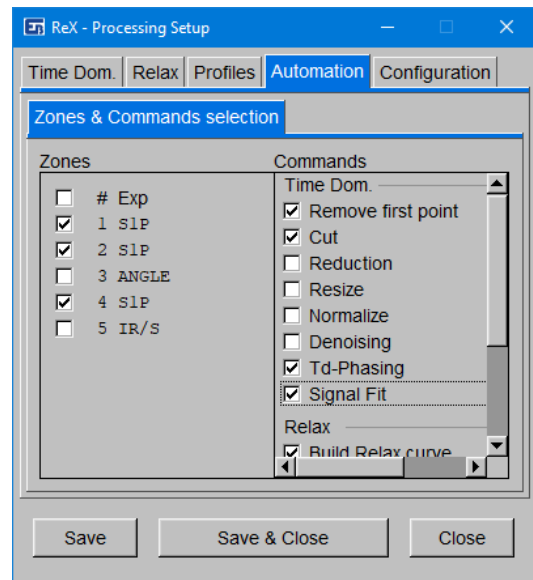


Figure 8 - Automation section of the Setup

### Configuration

Last panel in the Setup include a list of configuration parameters for different evaluation procedures. Among them, the threshold used to stop the automatic determination of the components (should be a few smaller than 1), or the significance level used for statistical analysis of the fitting residuals (usually 0.05).



## PCA

Rex offers the possibility to execute the **Principal Component Analysis** on either the relaxation curves points or the resulting parameters of a multiexponential fitting, which are the relaxation times (or rates) and weights.

Once some data files have been loaded, and a set of relaxation curves are present in different pages of Rex interface, the software is ready to prepare data to execute the PCA. The relaxation curves may be loaded directly from text files or built from raw data; it is just necessary that they have the same time values.

Through the command **"Stack Relax"** from the menu **"Data->Zones"**, a new page labelled **"Stack"** will be opened, and a canvas labelled **"Relax"** will show all the relaxation curves in a stack plot (see **Figure 9a**). Here, user can execute PCA, using the curves points as features. A new canvas labelled **"PCA"** appears showing the 2D score plot (see **Figure 9b**). Here, user can use the arrows present in the toolbar to choose different Principal Components. The mouse cursor coordinates in the status bar tells the point number too, which refers to the page number of the associated relaxation curve.

It is also possible to execute PCA by using the resulting parameters of a multi-exponential fitting as features, that means the relaxation times (or rates) and the components weights. From the **"Stack"** canvas, user can launch command **"ILT: Discrete"** in order to fit all the curves. From the Setup (Relax->Fit section), user can set the number of components (auto mode not available here, it just turns into one component). A new canvas labelled **"ILT"** appears which plots the relaxation times distributions. Hence, user can launch PCA directly from this canvas. In the Setup user can choose between to use the relaxation times or rates and between absolute or relative (percent) component weights (**Figure 6a**). They are used in the following order: firstly all the rates, in increasing order (or viceversa the times in decreasing order) then the associated weights.

In any case, on the left side are reported the first eigenvalues and their explained variance (also cumulative) in percentage. From the canvas pop-up menu it is possible to export the points coordinates (PCA scores), the eigenvectors (loading coefficients) and the eigenvalues, into a new file or to clipboard.

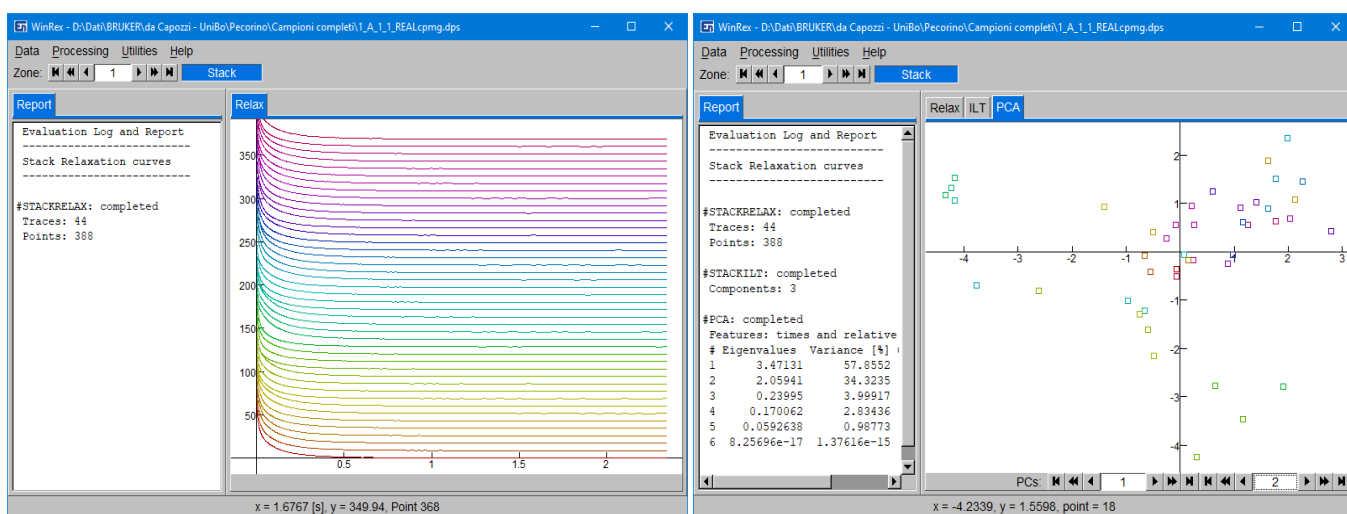


Figure 9 - Stack relaxation curves and Principal Component Analysis plot

## Simulation

WinRex allows to simulate simple relaxometry experiments data. Button "Simulation" in the menu bar opens the simulation interface, as it is shown in [Figure 10](#).

In the General tab user can choose the experiment (or kind of synthetic data) and other general option, like the noise root mean square. Parameters present in 1D tab refers to *free induction decays* (FIDs) settings and those in 2D tab to other, like 2D experiments or CPMG. NMRD tab is only for FFC profiles. The available experiments are, according to Stellar names: S1P, IR/S, SR/S, CPMG, ANGLE.

- S1P (Single 1 Pulse) simulates an FID, whose pulse sequence consists of  $90^\circ_x$
- IR/S (Inversion Recovery-Staggered) is a 2D (staggered) experiment whose sequence is  $180^\circ_x - \tau - 90^\circ_x$  where  $\tau$  is the varying parameter.
- SR/S (Saturation Recovery-Staggered) sequence is  $90^\circ_x - \tau - 90^\circ_x$ . IR/S and SR/S 2D curves relax with  $T_1$ .
- CPMG (Carr-Purcell-Meiboom-Gill) is a 1D experiment composed of  $90^\circ_x$  and then a train of  $\tau/2 - 180^\circ_x$  (spin-echos sequence). CPMG signal relaxes with  $T_2$ .
- ANGLE is a 2D series of FIDs where the varying parameter is the B1 pulse width.

Other special synthetic data can be generated:

- Phantom Decay is a stack of S1P blocks, with noise root mean square as array parameter.
- Relax curve is a simple relaxation curve, directly simulated (not generated from raw data).
- NMRD profile is a profile directly simulated (not generated from raw data) by combining components described by many models. Can choose between variable frequency or temperature. The models available are the same used for fitting, described in paragraph [Profiles](#).

Each parameter is self-explanatory, with the help of tooltip windows, but here we give some more

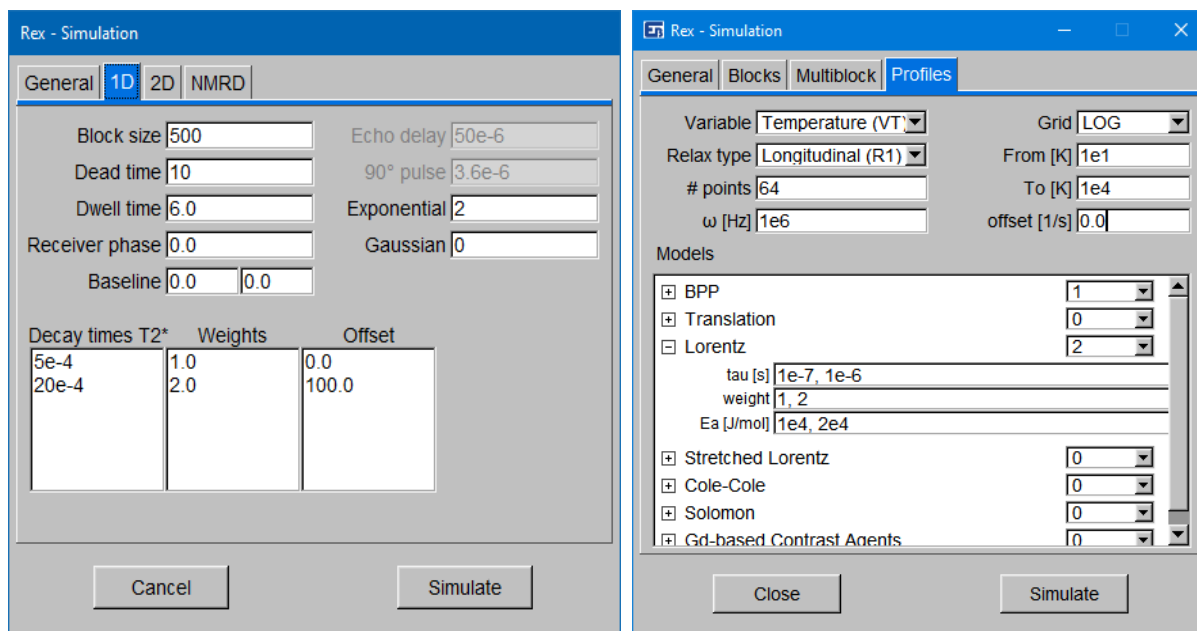


Figure 10 - Simulation interface

explanation about relaxation times input field. These are input as a list of elements (see Figure 10a), along with their weights and frequency offset. Decay times  $T_2^*$  are intended as discrete (delta-shaped) components and they must be at least equal to the sum of "Exponential" and "Gaussian" parameters. Instead, relaxation times (either  $T_1$  or  $T_2$ , depending on the selected experiment) must be equal to "N components" and may have finite width. In this case they are log-normally distributed and their width is expressed in Neper (1 Neper = 1 order of magnitude). Instead, parameters for dispersion profiles components must be separated by commas (see Figure 10b).

Data are simulated and displayed on the canvases and then they can be treated exactly as they were loaded from a file.

## NMR Calculator

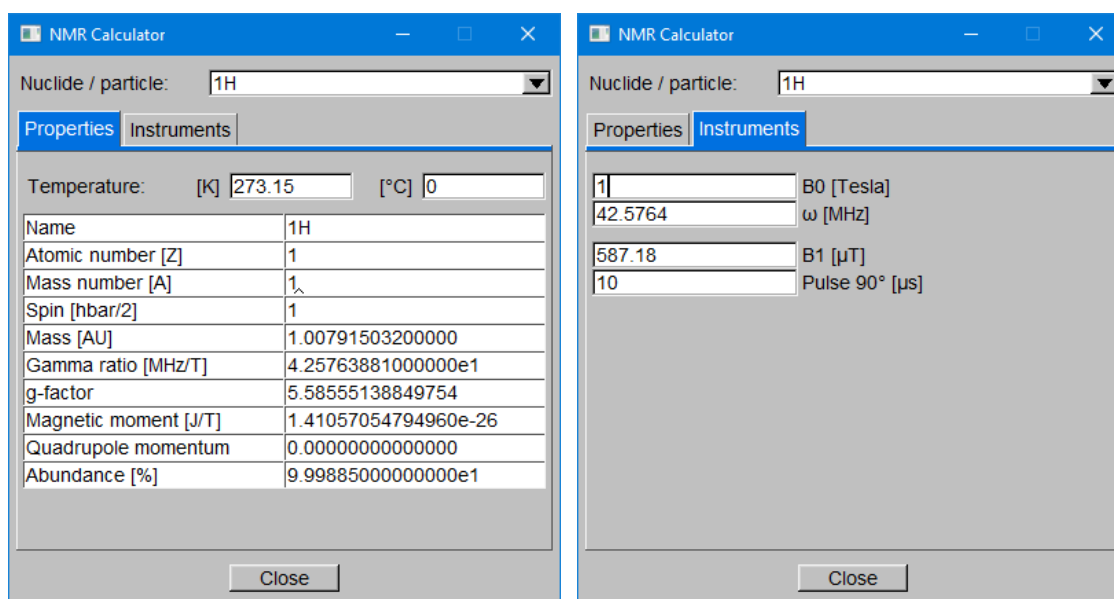


Figure 11. NMR Calculator

NMR Calculator (see Figure 11) is a simple utility for any NMR-ists. In the current version there are two sections. One section shows information about the nuclides properties. More than 100 nuclides are available. In the second section user can convert the Larmor frequency of the selected nuclide from MHz to Tesla and vice versa. Also, it calculates the B1 field intensity starting from the time duration radio frequency pulse and vice versa, for the selected nuclide. The nuclide (or particle) can be chosen from a menu or can be input with the keyboard.

## Tutorial

A presentation/tutorial that shows how to use WinRex is present in a separate document, along with some example data. It can be downloaded from Rex webpage ([www.extrabyte.eu/products/rex/](http://www.extrabyte.eu/products/rex/)).

## Bibliography

- Abraham, A. (1961). *The principles of Nuclear Magnetism*. University Press, Oxford.
- Borgia, G. C., Brown, R. J., & Fantazzini, P. (1998). Uniform-Penalty Inversion of Multiexponential Decay. *Journal of Magnetic Resonance*, 65-77.

Chapter 2: Gadolinium-based Contrast Agents. (2018). In J. R.-P.-F.-G.-I. Lothar Helm, *Contrast Agents for MRI: Experimental Methods*. doi:10.1039/9781788010146-00121

Eilers, P., & Marx, B. (1996). Flexible Smoothing with B-splines and Penalties. *Statistical Science*, 11(2), 89-102.

Fries, P. H., & Belorizky, E. (2015). Simple expressions of the nuclear relaxation rate enhancement due to quadrupole nuclei in slowly tumbling molecules. *J. Chem. Phys.*

Hallenga, K., & Koenig, S. H. (1976). Protein Rotational Relaxation as Studied by Solvent 1H and 2H Magnetic Relaxation. *Biochemistry*, 15(19), 4255-4264.

Savitzky, A., & Golay, M. J. (1964). *Smoothing and Differentiation of Data by Simplified Least Squares Procedures*. doi:10.1021/AC60319A045

Solomon, I. (1955). Relaxation Processes in a System of Two Spins. *Physical Review*.