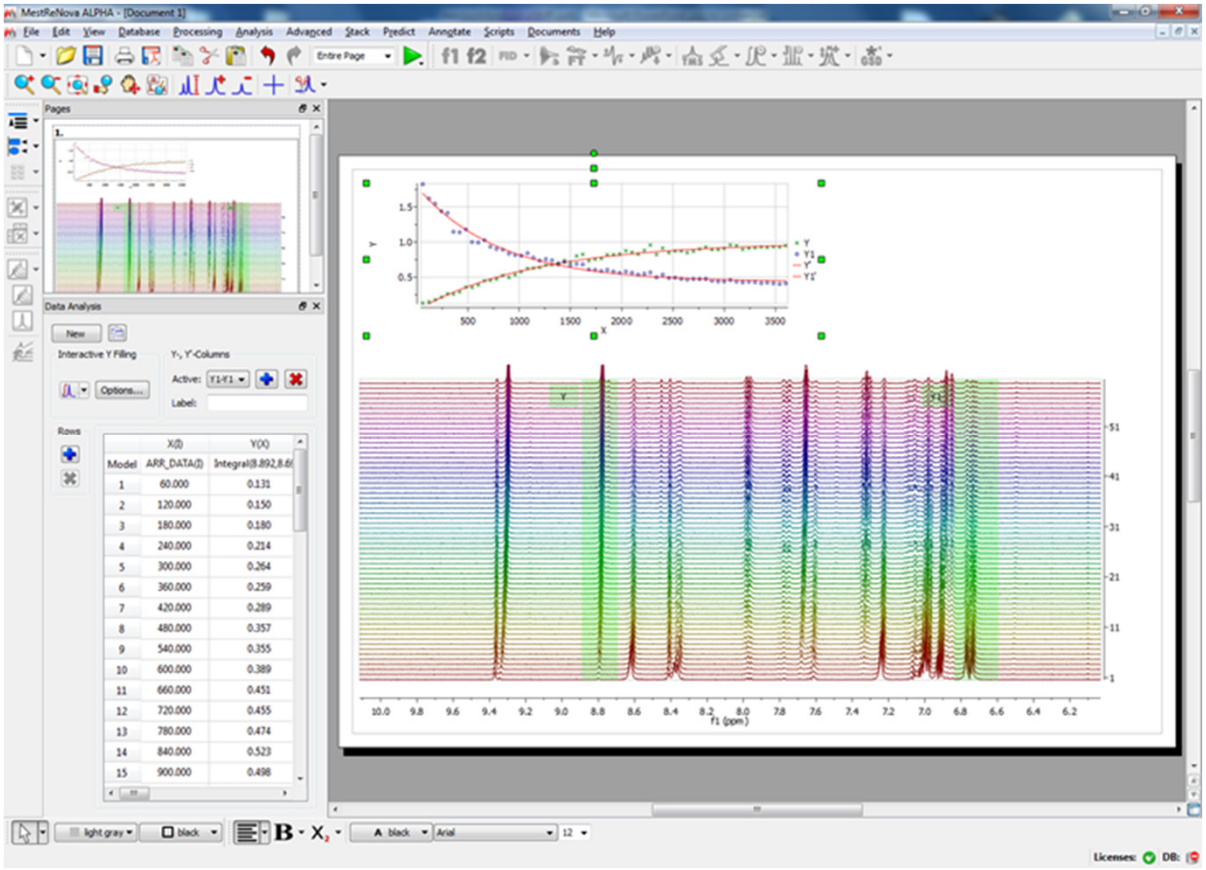
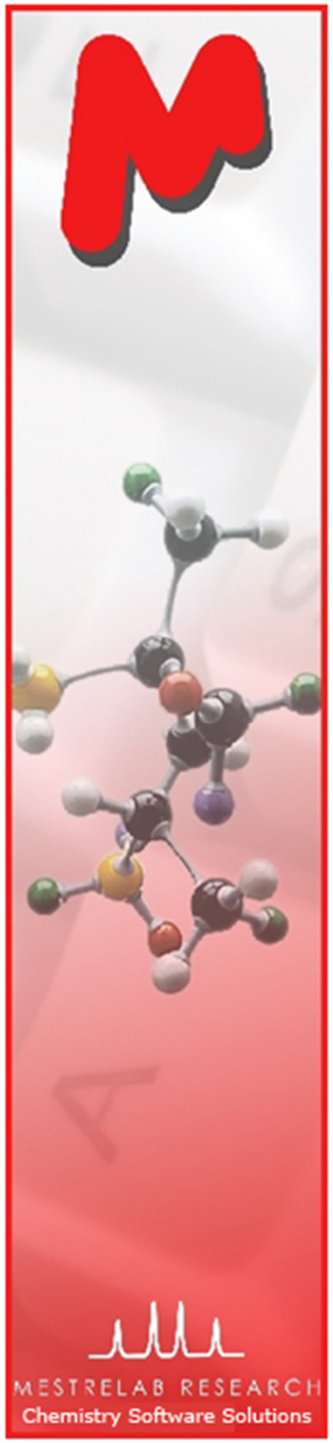
MNova for Reaction Monitoring by NMR



Version 6.2.1
Feb. 2011

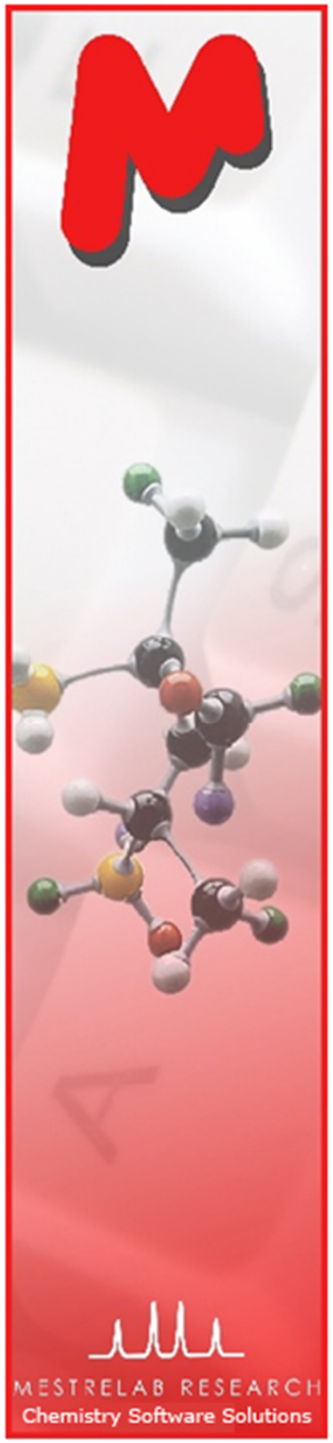
Chen Peng, PhD
Director of Business Development, US & China
Mestrelab Research SL
San Diego, CA
(858) 736-4563
chen.peng@mestrelab.com

MESTRELAB RESEARCH
Chemistry Software Solutions



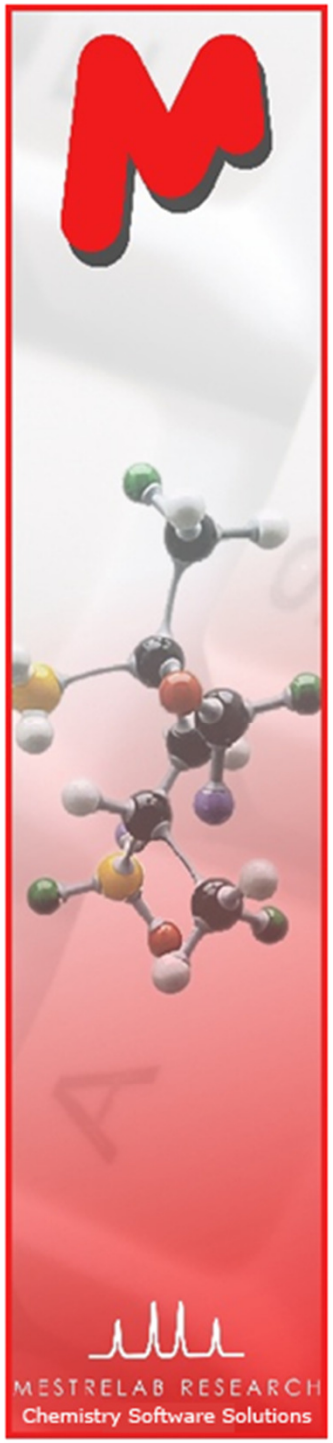
Outline

- About Mestrelab Research
- Importing and processing multiple NMR data sets
- Extracting arrayed spectral information
- Fitting spectral data to a kinetics curve
- Summary

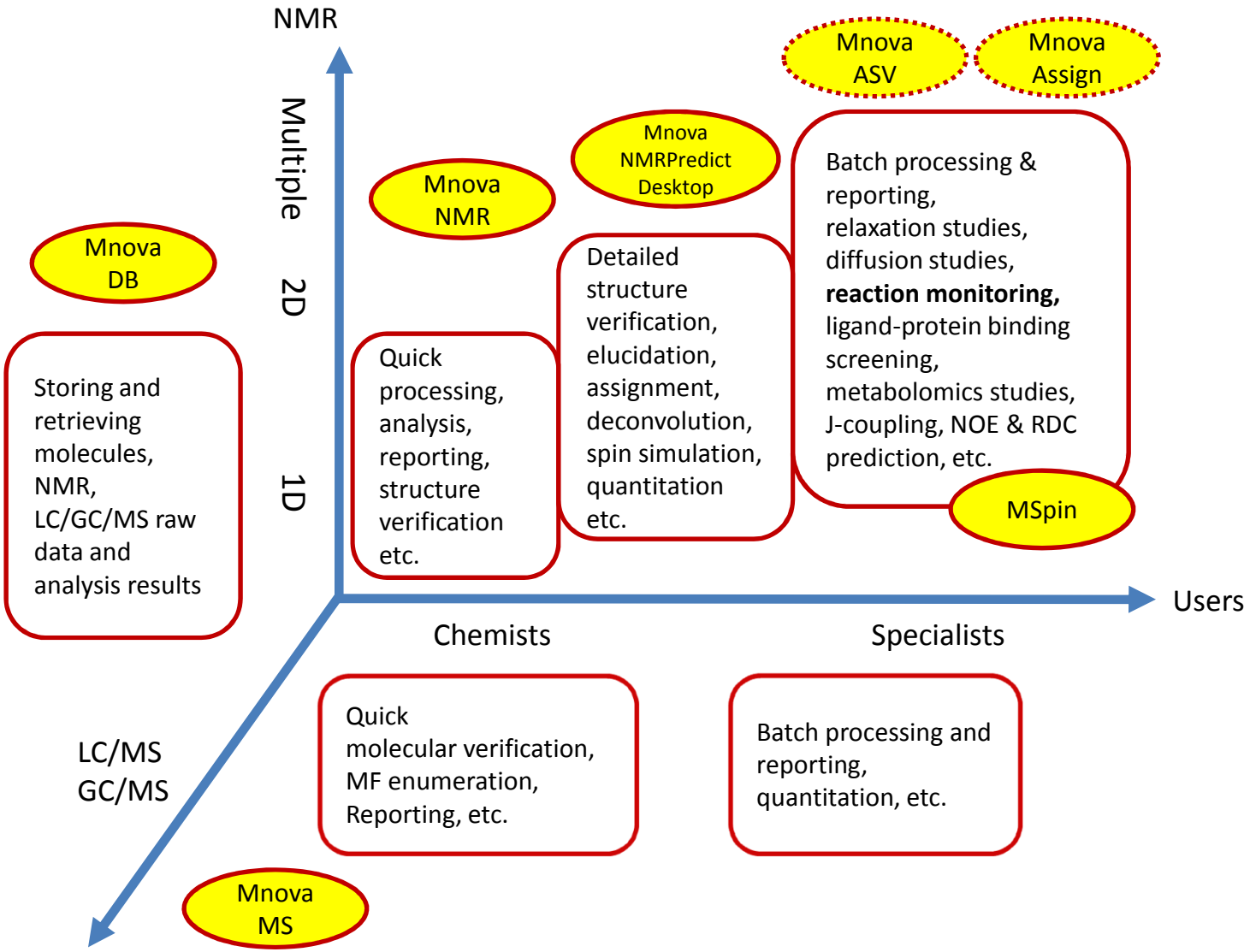


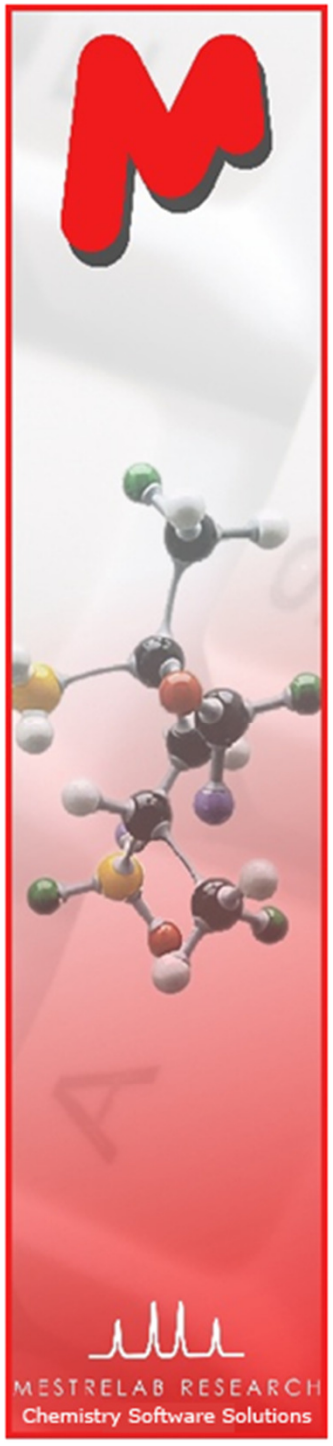
About Mestrelab Research

- 1996: A research project in University of Santiago de Compostela, Spain, developed free **MestReC** software for NMR processing
- 2004: **Mestrelab Research** incorporated in Santiago de Compostela
- 2004: New **MestreNova (Mnova)** platform and **NMR** plugin released
- 2006: **NMRPredict Desktop** plugin released with Modgraph
- 2009: **LC/GC/MS** plugin released with Sierra Analytics
- 2009: Global Spectral Deconvolution (**GSD**) algorithm released with ExtraByte
- 2010: **DB** plugin for Database Management
- 2011: **ASV** plugin for Auto. Structure Verification - to be released.
- 2011: Auto. 1D and 2D **Assignment** - to be released
- An **R&D company** with ~20 people and 70,000+ registered users



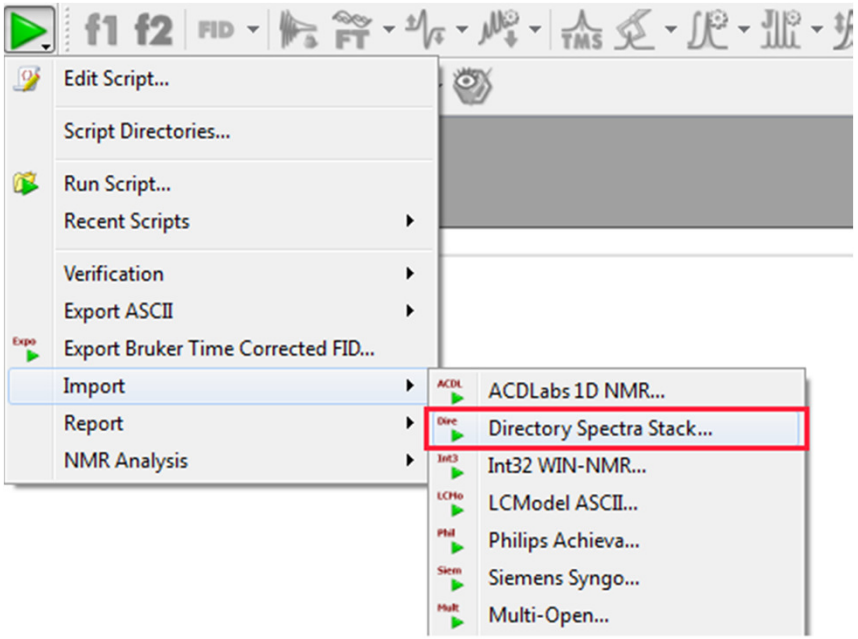
Products and Applications





To open and process reaction monitoring data

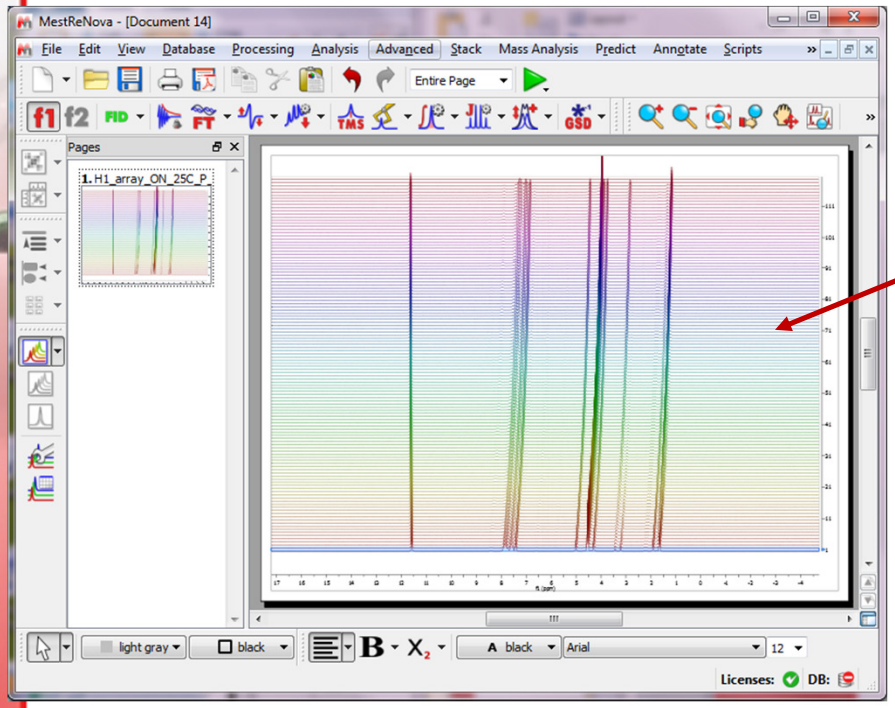
- In a typical kinetics or reaction monitoring (RM) experiment, a series of spectra are recorded at predetermined time intervals to follow the progress of the reaction:
  - Acquiring all spectra at different times and storing them as *individual spectra*
  - Acquiring all spectra into a single NMR experiment in *arrayed mode*
- Mnova supports both kinds of data
- For RM data acquired on individual basis: Run the Directory Spectra Stack script to open and stack all spectra under a base directory:

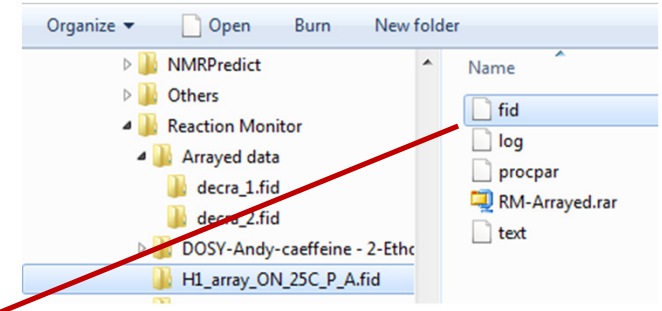


- *The user selects the directory where the spectra are located*
- *Mnova opens and process all those spectra (FIDs) automatically using the processing parameters from the instrument*
- *Mnova stacks all spectra together*

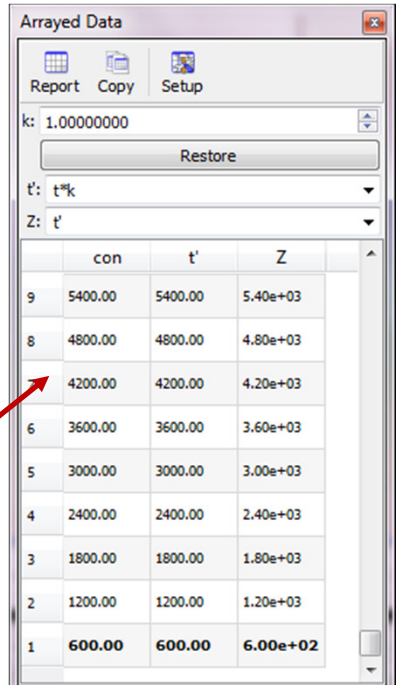
To open and process reaction monitoring data

- For RM data acquired in arrayed mode: Just open the FID (Varian) or SER file (Bruker), and the individual spectra will be processed and stacked.
  - Mnova parses the arrayed parameters automatically and displays them in the Arrayed Data Table (Choose View | Tables | Arrayed Data to open it)





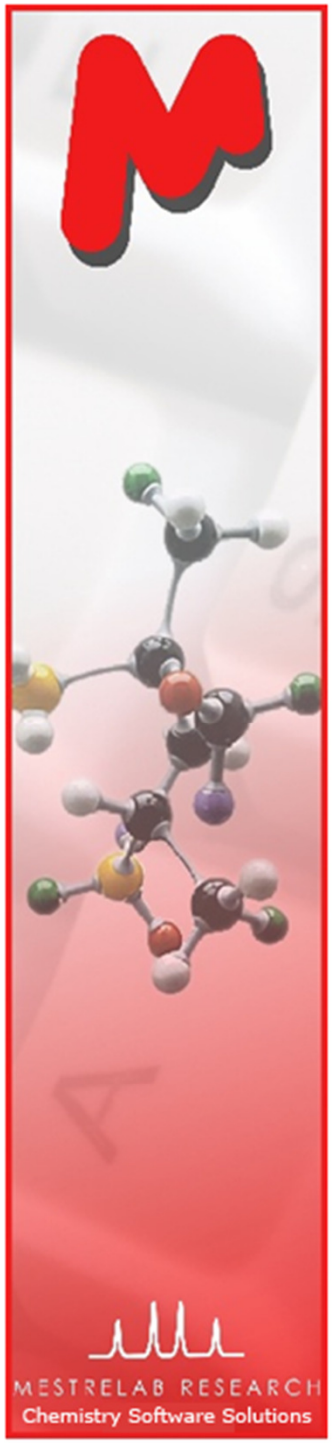
Drag & drop



	con	t'	Z
9	5400.00	5400.00	5.40e+03
8	4800.00	4800.00	4.80e+03
7	4200.00	4200.00	4.20e+03
6	3600.00	3600.00	3.60e+03
5	3000.00	3000.00	3.00e+03
4	2400.00	2400.00	2.40e+03
3	1800.00	1800.00	1.80e+03
2	1200.00	1200.00	1.20e+03
1	**600.00**	**600.00**	**6.00e+02**

t is the arrayed parameter (in column **con**). Use factor *k* and/or formulas to convert it into reaction times (*Z*) for subsequent analysis.

MESTRELAB RESEARCH
Chemistry Software Solutions

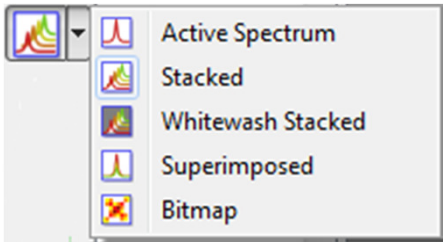


Easy handling multiple spectra in Mnova

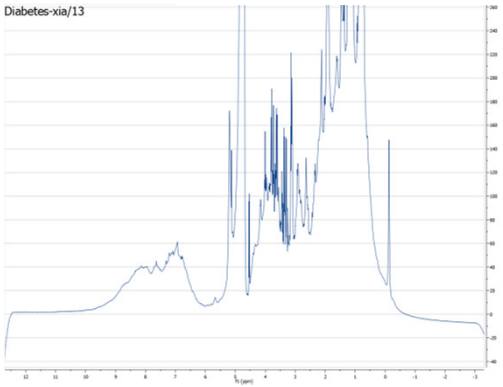
- With Mnova, it is very easy to
  - To display stacked spectra in different modes, such as Stacked, Superimposed, Active Spectrum, or Bitmap
  - To select one or several spectra and apply processing only to them
  - To hide any number of spectra for better visualization
  - To correct global or local spectral misalignment

To change the stacking mode

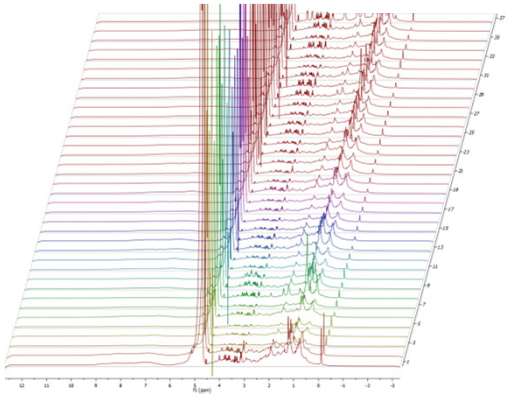
- Click  to choose the display mode for stacked spectra



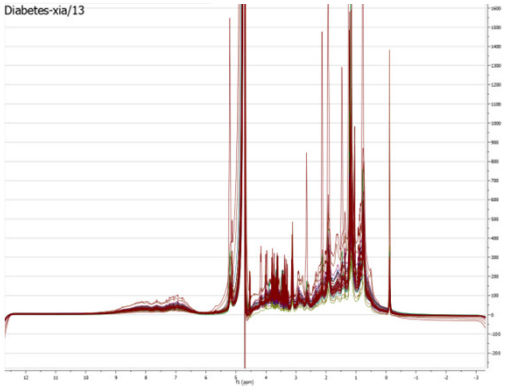
Active Spectrum



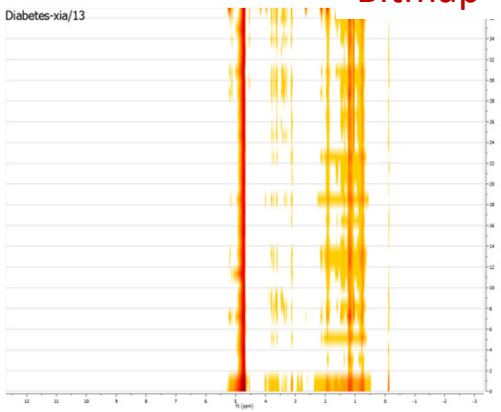
Stacked



Superimposed



Bitmap

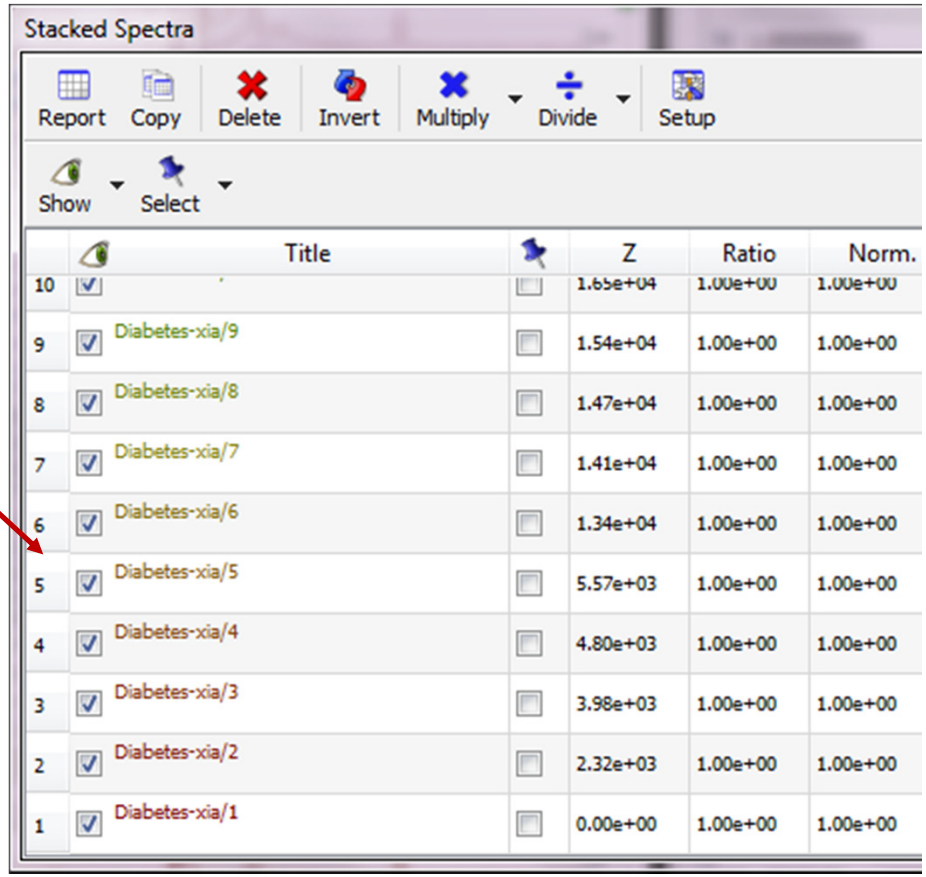


MESTRELAB RESEARCH
Chemistry Software Solutions

To re-process the stacked spectra

- Click  to toggle on the Stacked Spectra Table
- Use this table to do the following:
  - Delete spectra from the stack
  - Change order of the spectra in the stack
  - Change the Y-intensity of selected spectra
  - Change which ones to display
  - Change which ones to re-process, such as phasing, baseline correction etc.

Click and drag here to change the order of a spectrum in the stack



	Title	Z	Ratio	Norm.
10		1.65e+04	1.00e+00	1.00e+00
9	Diabetes-xia/9	1.54e+04	1.00e+00	1.00e+00
8	Diabetes-xia/8	1.47e+04	1.00e+00	1.00e+00
7	Diabetes-xia/7	1.41e+04	1.00e+00	1.00e+00
6	Diabetes-xia/6	1.34e+04	1.00e+00	1.00e+00
5	Diabetes-xia/5	5.57e+03	1.00e+00	1.00e+00
4	Diabetes-xia/4	4.80e+03	1.00e+00	1.00e+00
3	Diabetes-xia/3	3.98e+03	1.00e+00	1.00e+00
2	Diabetes-xia/2	2.32e+03	1.00e+00	1.00e+00
1	Diabetes-xia/1	0.00e+00	1.00e+00	1.00e+00

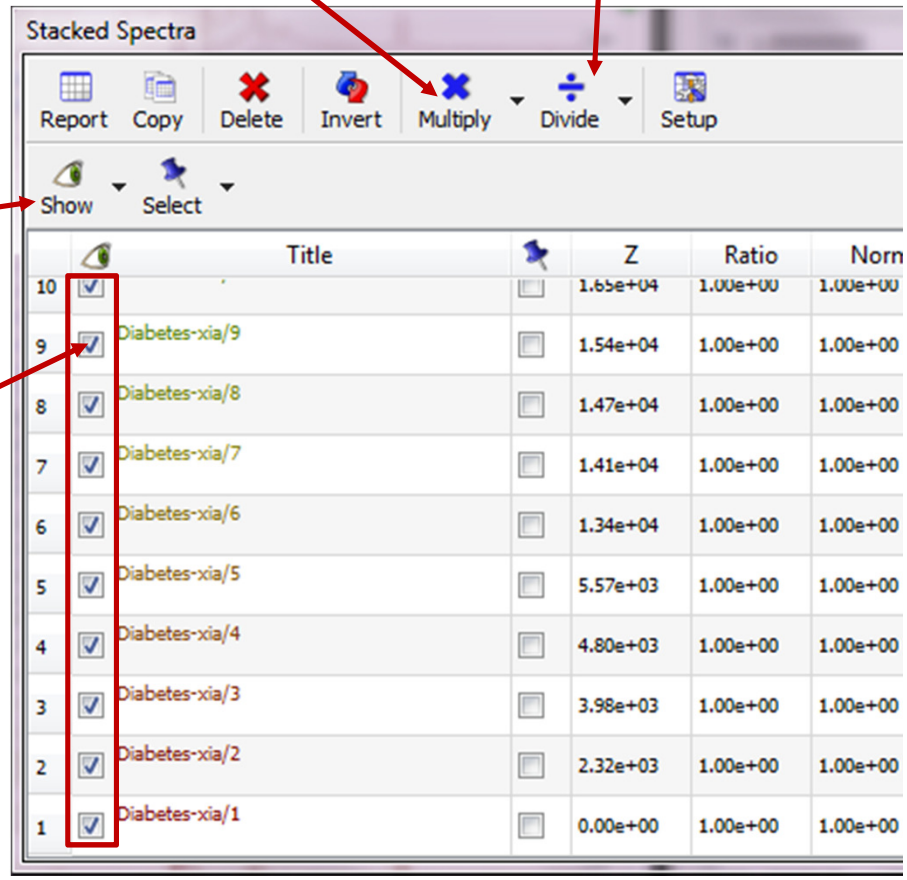
Use Stacked Spectra Table to setup the display

To increase the Y intensity of selected or all spectra *

To decrease Y intensity of selected or all spectra*

Click the arrow here for more options for spectral display

Un-check a spectrum if you don't want to show it in the stack



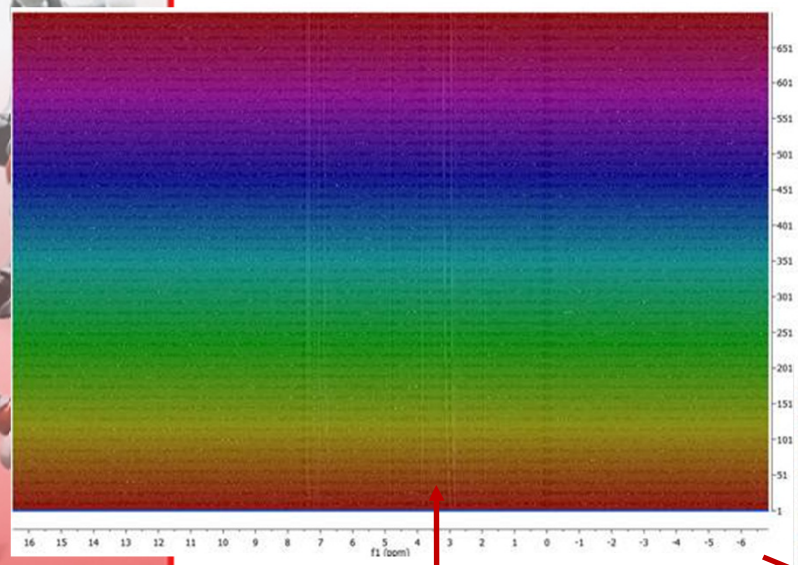
	Title	Z	Ratio	Norm
10		1.65e+04	1.00e+00	1.00e+00
9	Diabetes-xia/9	1.54e+04	1.00e+00	1.00e+00
8	Diabetes-xia/8	1.47e+04	1.00e+00	1.00e+00
7	Diabetes-xia/7	1.41e+04	1.00e+00	1.00e+00
6	Diabetes-xia/6	1.34e+04	1.00e+00	1.00e+00
5	Diabetes-xia/5	5.57e+03	1.00e+00	1.00e+00
4	Diabetes-xia/4	4.80e+03	1.00e+00	1.00e+00
3	Diabetes-xia/3	3.98e+03	1.00e+00	1.00e+00
2	Diabetes-xia/2	2.32e+03	1.00e+00	1.00e+00
1	Diabetes-xia/1	0.00e+00	1.00e+00	1.00e+00

** Note these commands change the Y intensity values of the spectrum/spectra. They are mainly used for visualization of spectra with very different intensities. Do* ***not*** *use them for quantitative analysis.*

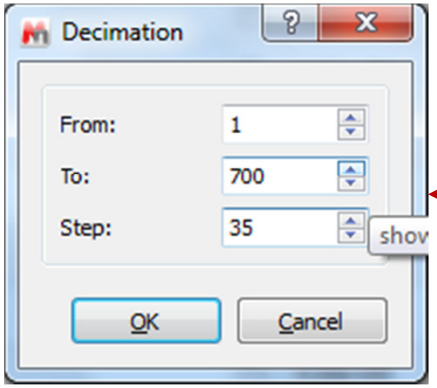
MESTRELAB RESEARCH
Chemistry Software Solutions

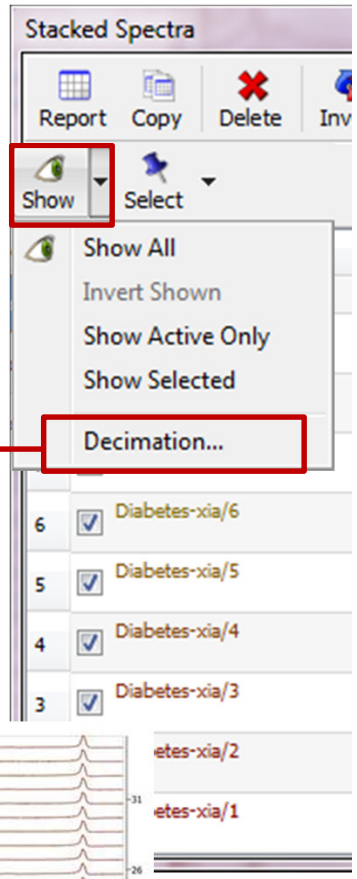
Use Stacked Spectra Table to setup the display

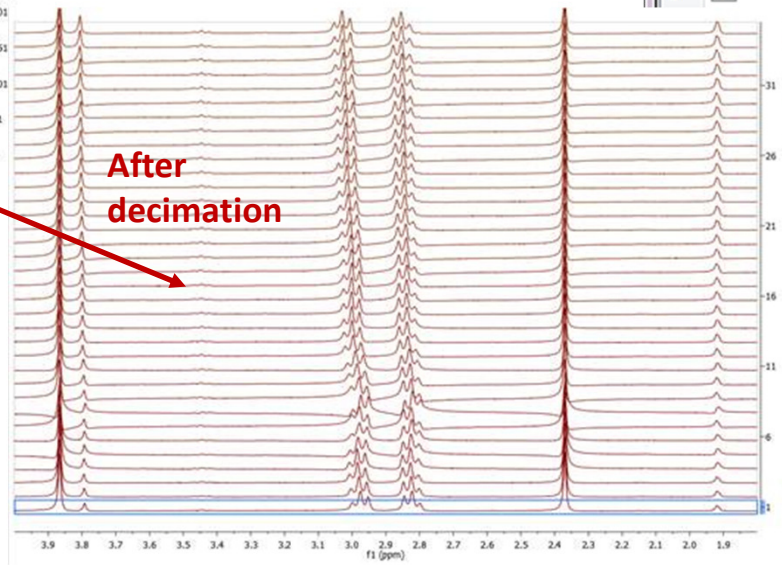
- Use the **Decimation** option when the number of stacked spectra is very large, slowing down the plotting and not showing relevant information



700 spectra



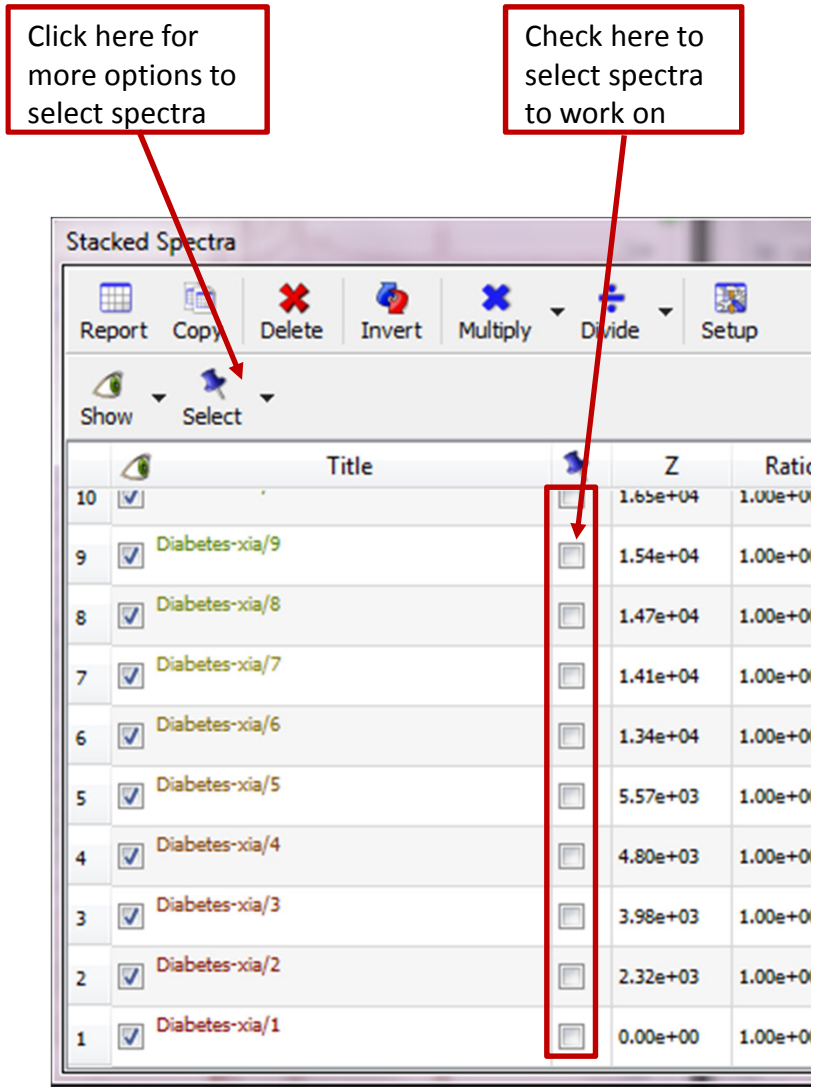




MESTRELAB RESEARCH
Chemistry Software Solutions

To re-process *all* or *selected* spectra

- Spectra are automatically processed when they are opened, but sometimes you need to manually re-process some of them
- Use the processing tools to re-process **all** or **selected** spectra:
  - If no spectrum is checked in the Select column, **all** spectra will be changed
  - If some spectra are checked in the Select column, only the **selected** ones will be changed
- Use **Undo/Redo** if you made a mistake



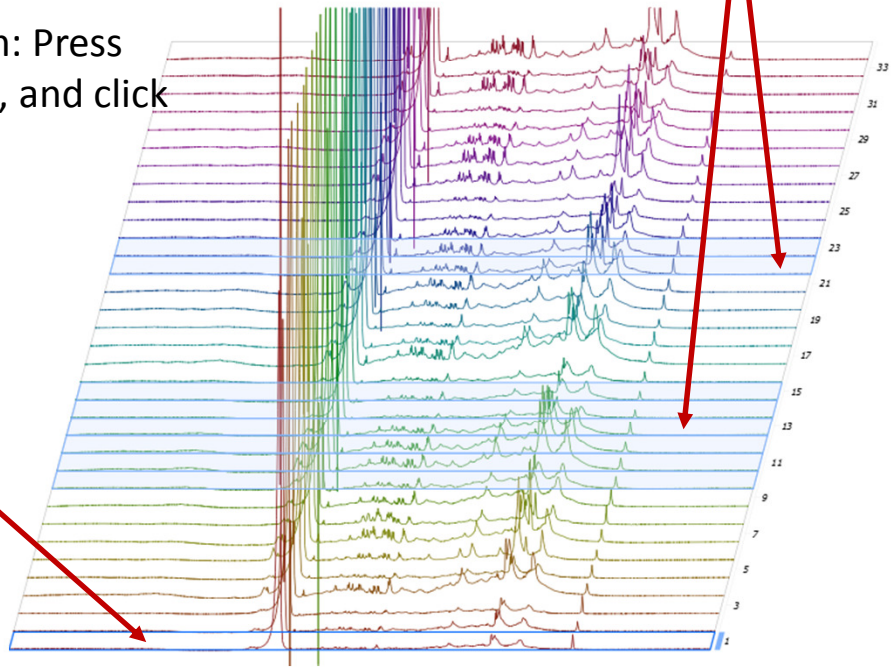
MESTRELAB RESEARCH
Chemistry Software Solutions

To select spectra from the stacked spectra

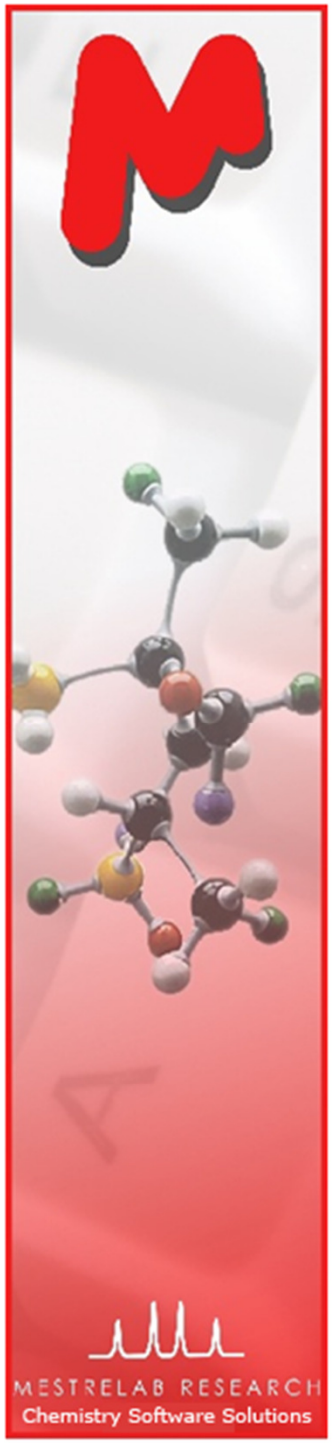
- Spectra can also be selected from the spectral stack directly.
- To select one spectrum: Press and hold **Alt** key, and click on the spectrum
- To select multiple spectra: Press and hold **Ctrl** or **Shift** key, and click on a spectrum
- To de-select one spectrum: Press and hold **Ctrl** and **Alt** keys, and click on the spectrum

Selected spectra: processing will apply on them only

The active spectrum: It will be displayed when in *Active spectrum* mode. It is also used as reference spectrum in some operations such as spectral alignment



MESTRELAB RESEARCH
Chemistry Software Solutions



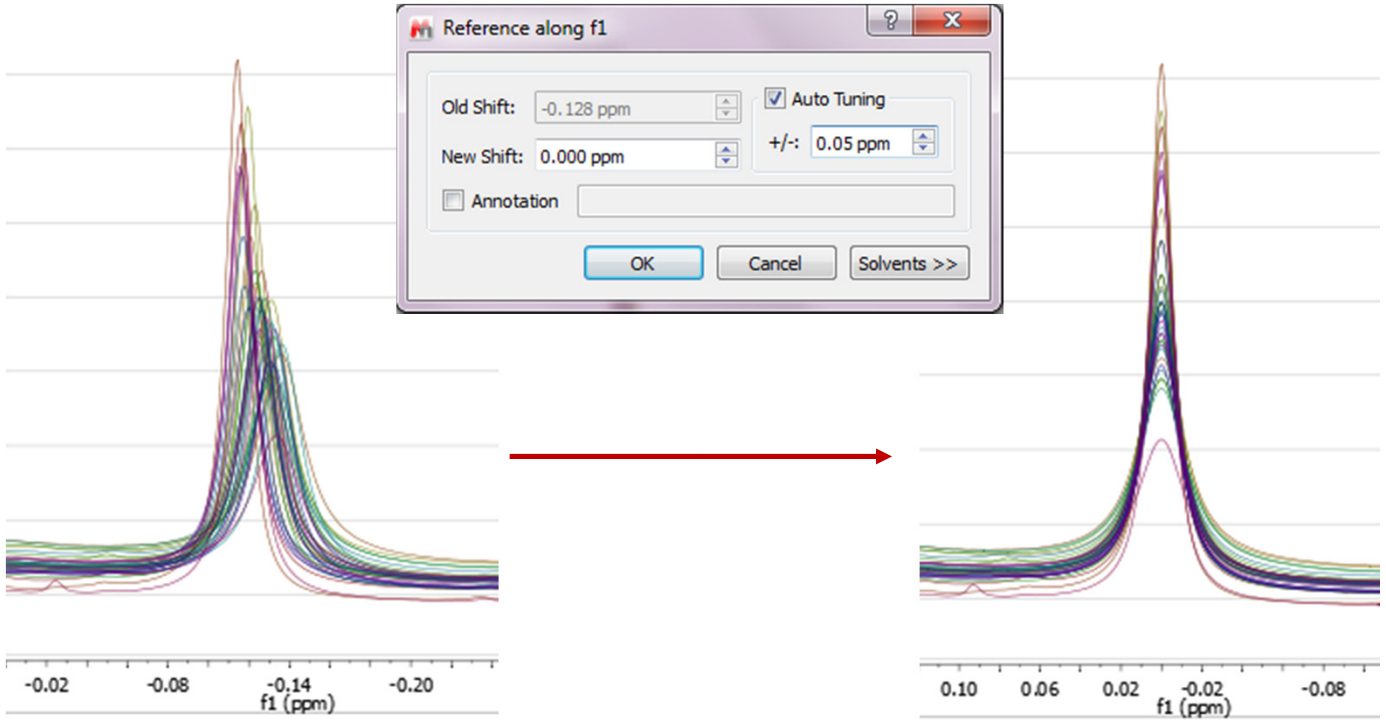
To correct phase errors and baseline

- Click  for **phase correction** if peaks are not symmetric.
  Options:
  - Global method for all positive peaks
  - Metabolomics method when there is residual solvent peaks
  - Selective method for positive/negative peaks
  - BL Optimization method using baseline optimization techniques
  - You can combine any of the methods listed above
  - Manual method if none of the above works

- Click  for **baseline correction** if baseline is not zero.
  Options:
  - Polynomial Fit
  - Bernstein Polynomial Fit
  - Whittaker Smoother
  - Manual

To align spectra by correcting reference

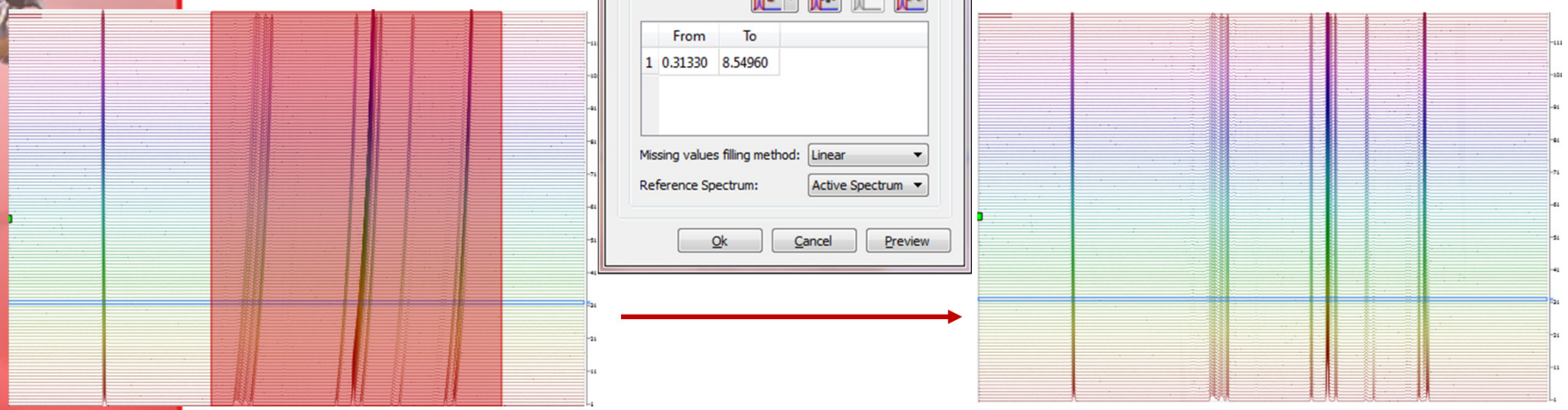
- Systematic errors of chemical shifts can be corrected if there is an internal reference peak, e.g. TSS peak.
- Click TMS and then click on the reference peak in the active spectrum
- In the following dialog, set the proper chemical shift for the reference peak, check Auto Tune, and define a tuning range (e.g. +/- 0.05 ppm):



MESTRELAB RESEARCH
Chemistry Software Solutions

To correct local peak misalignment*

- Zoom into the region of interest, select **Advanced | Align Spectra**.
- Click  , then click-and-drag to cover the peaks to align. Click Preview to see the alignment result. Adjust other parameters until satisfactory.
- Move to other regions to continue this process until done.
- Click OK to accept the results

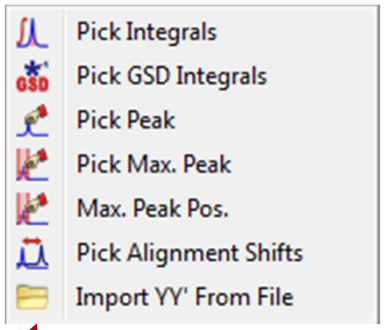


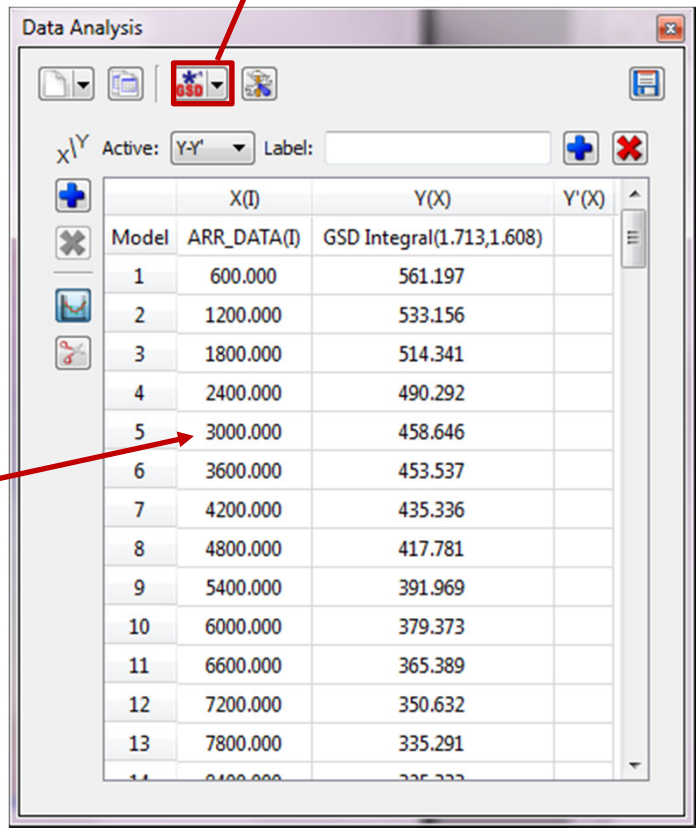
** When there is peak cross-over, it may not be good idea to use local peak alignment. Instead, use the UI feature to change the integration regions so that they follow the change of the peak locations. See later slides.*

MESTRELAB RESEARCH
Chemistry Software Solutions

To analyze stacked spectra using the Data Analysis Panel

- The Data Analysis Panel provides an intuitive way to extract and analyze multiple stacked spectral data
- Choose View | Panels | Data Analysis to open the Data Analysis Panel
- Click Create Empty Graph to create a new data series.
- Choose one of the peak picking modes (e.g. Pick GSD Integrals), click and drag in the spectra to define the range for picking GSD peaks.



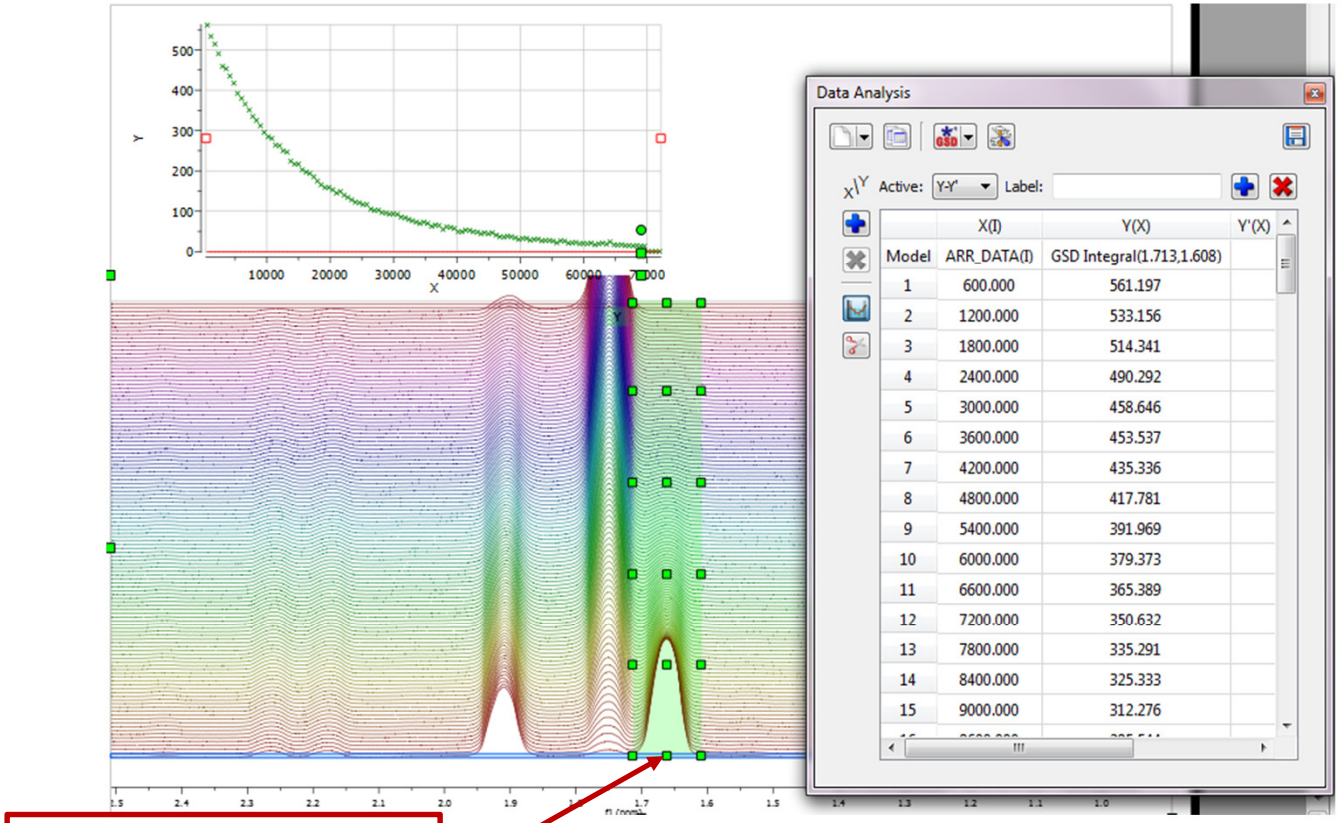


The X(I) column is automatically filled with the reaction time. Use Arrayed Data Table to preview and convert those data.
You can also manually edit these data, or copy from a .txt file.

MESTRELAB RESEARCH
Chemistry Software Solutions

To extract data using the Data Analysis Panel

- The areas of the GSD peaks in the defined region are filled in the Y(X) column, and also plotted in the X-Y graph.

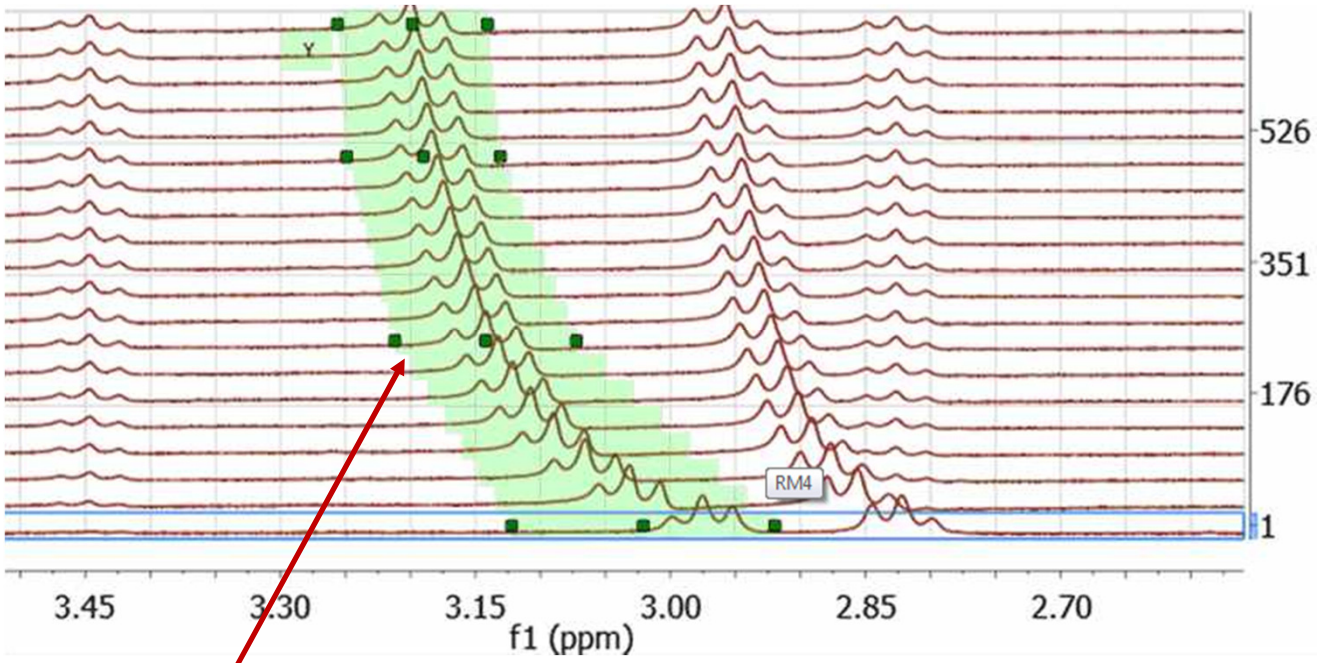


The region within which GSD peaks are integrated as Y(X) values

MESTRELAB RESEARCH
Chemistry Software Solutions

To extract data from drifting peaks

- If the peaks drift over time, you can manually change the direction of the integration regions :



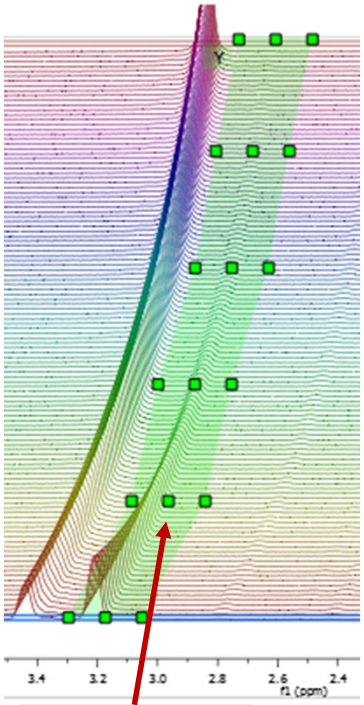
Click & drag the handles to change the shape of the selection region.
Press Shift to move all points simultaneously

Tip: you can change the number of handles by clicking the Options button on the Data Analysis Panel: 

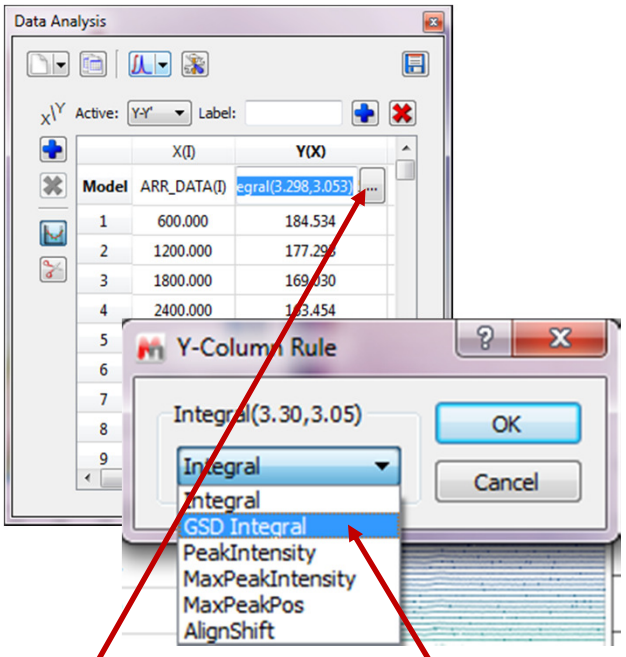
MESTRELAB RESEARCH
Chemistry Software Solutions

What if it is too slow?

- When in the Pick GSD Integrals mode, changing the integration regions can be very slow, as it does local GSD across all spectra every time you change the regions.
- You can first choose the Pick Integrals mode (which is fast), correct the integration regions, and then switch to Pick GSD Integrals mode:

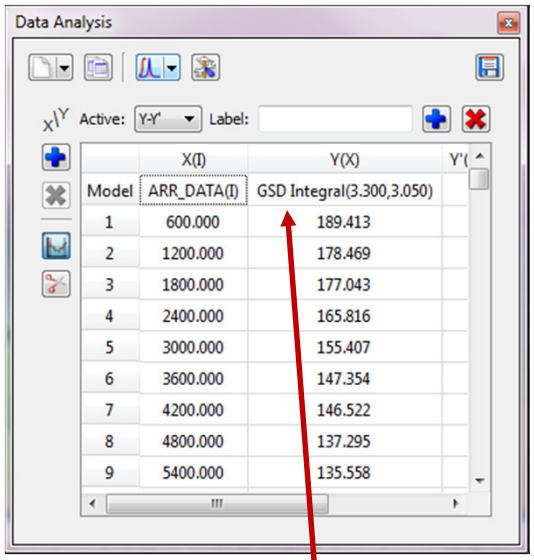


Define the integration region in Pick Integral mode, which is fast



Double click here to pop up the Y-Column Rule dialog

Change the rule to GSD Integral

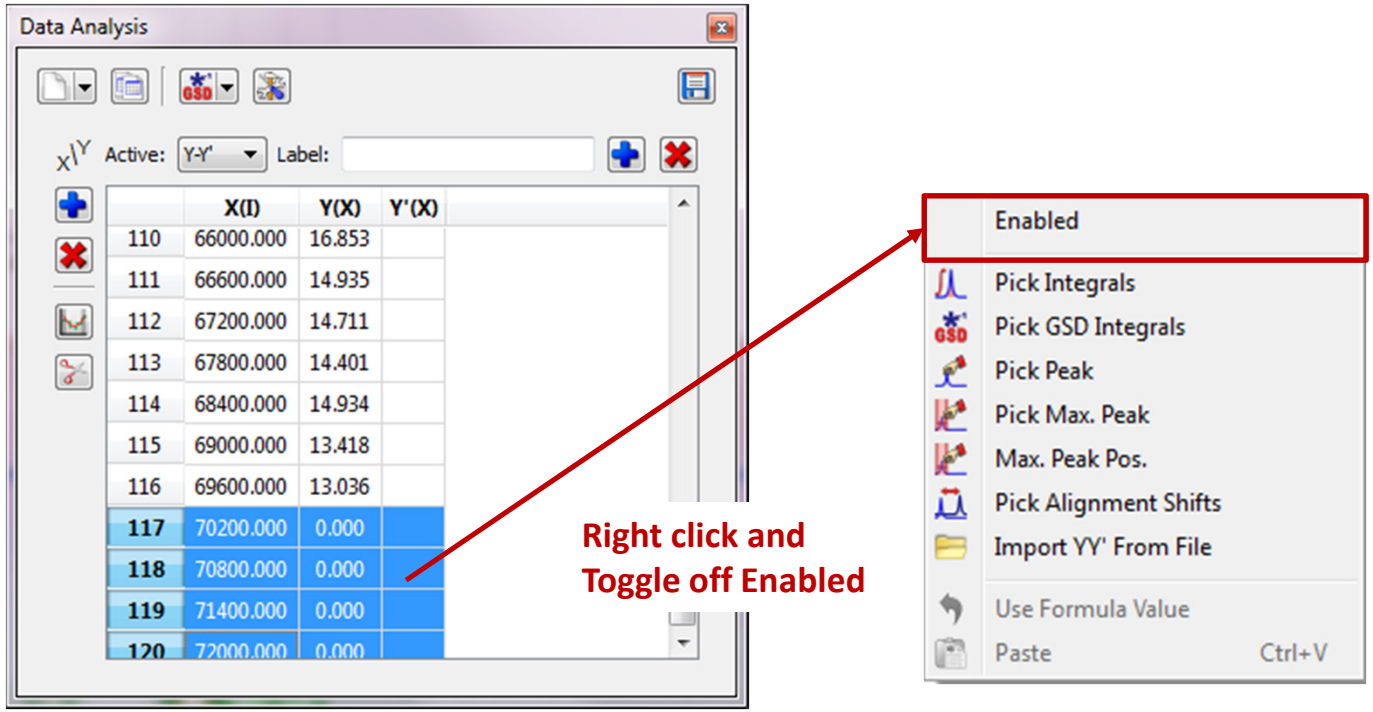


The GSD integrals are calculated and listed in the column

MESTRELAB RESEARCH
Chemistry Software Solutions

What to do with bad points?

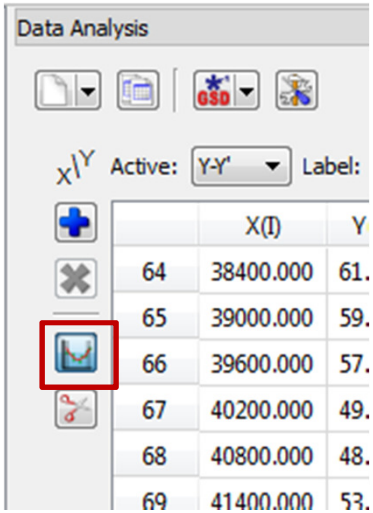
- To exclude some data points, highlight them in the table, and right click and turn off Enabled:

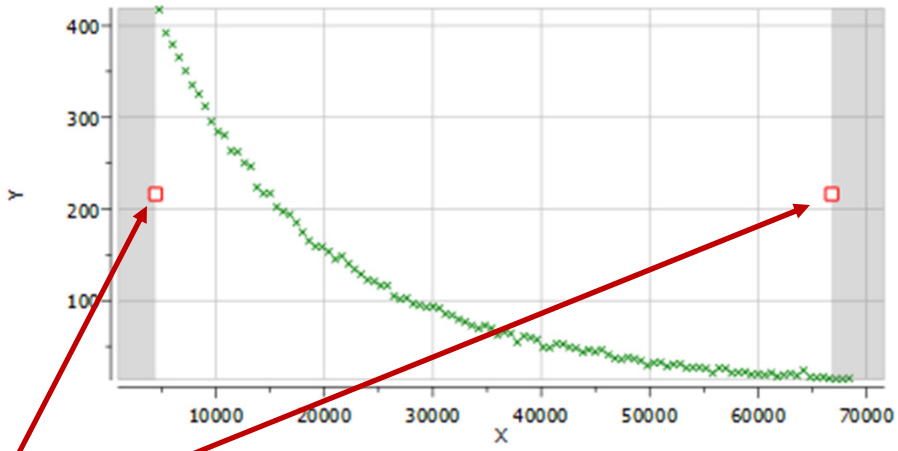


MESTRELAB RESEARCH
Chemistry Software Solutions

What to do with bad points?

- To exclude some data points at the beginning or at the end of the reaction, you can toggle on the Use Fitting Limit button, and exclude the data points on the XY Graph:



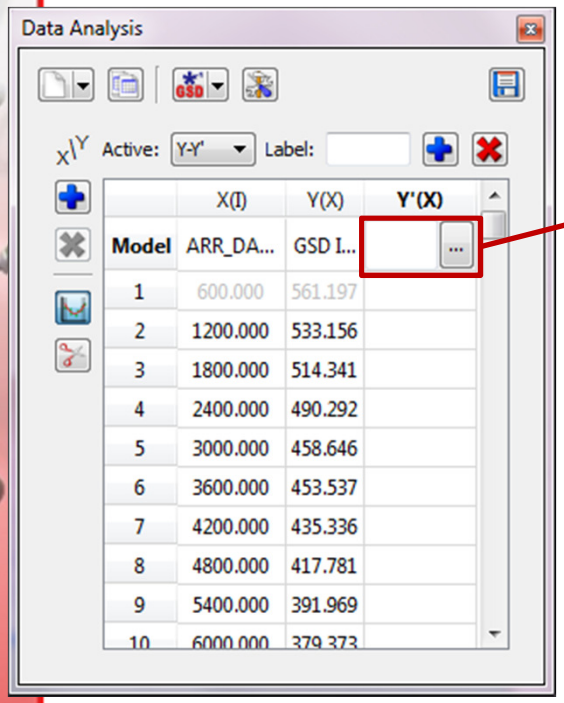


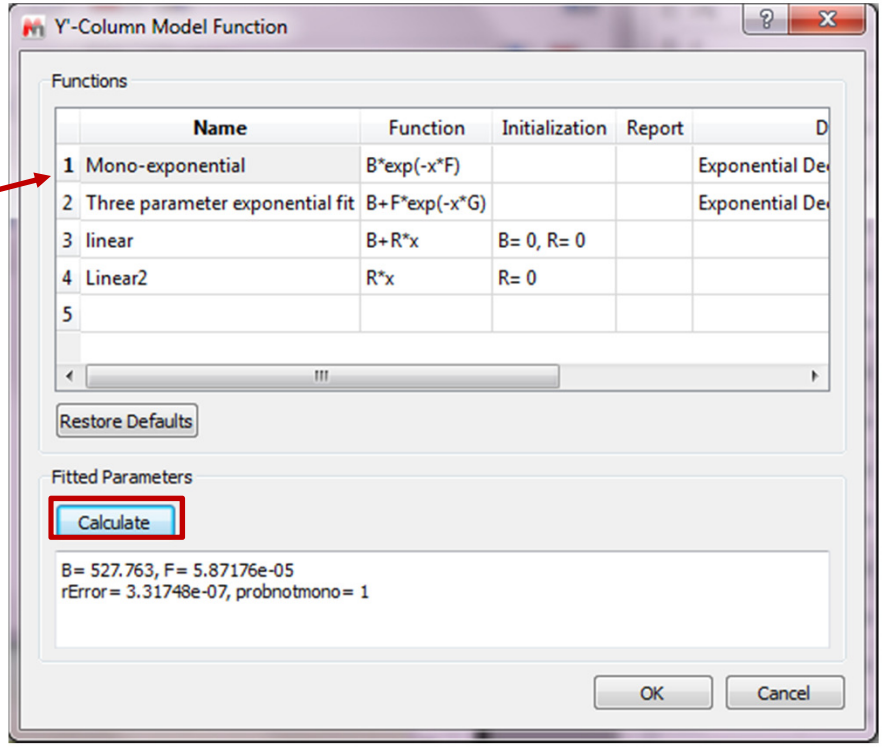
Click & drag the handles to exclude data points at either end of the data series

MESTRELAB RESEARCH
Chemistry Software Solutions

To fit the data to a function

- To fit the XY points to a function, double click the first cell in the Y'(X) column, and choose (or define) a function, and click Calculate to do the fitting. Click OK to accept the results:



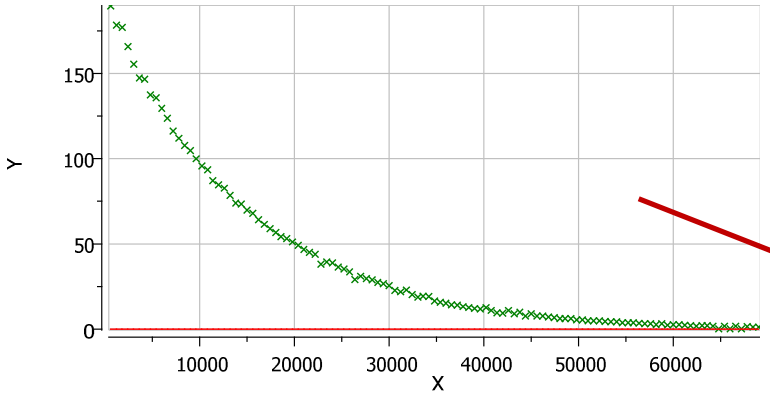


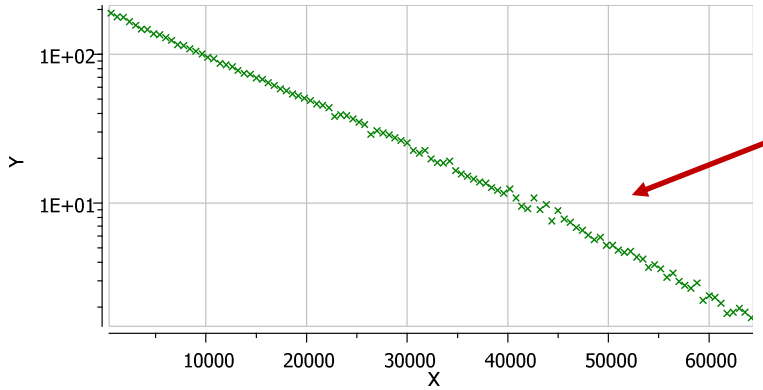
This example shows a first order reaction. F is the rate constant (k). The half-life $t_{1/2}$ = 0.693/F

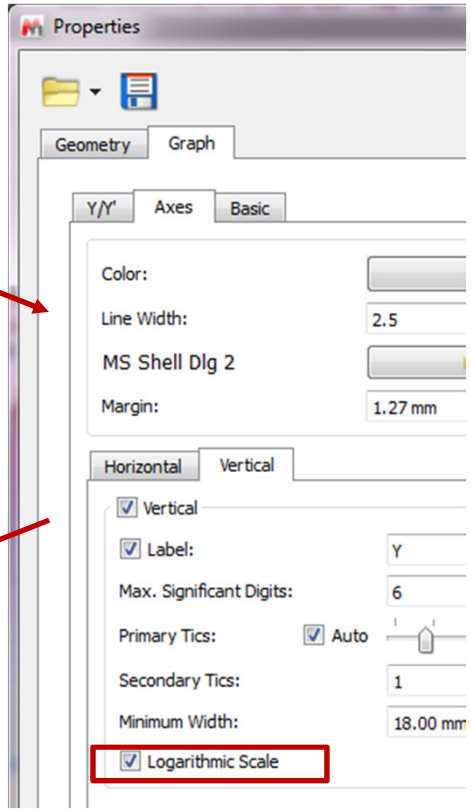
MESTRELAB RESEARCH
Chemistry Software Solutions

To fit the data to a function

- To change the Y scale of the XY graph to logarithmic, right click on the graph and choose Properties, and toggle on the Logarithmic Scale option:

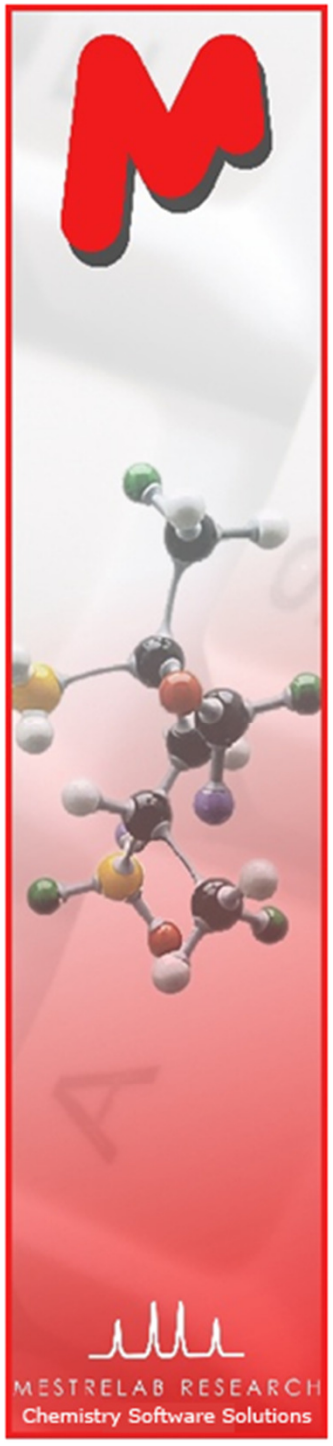






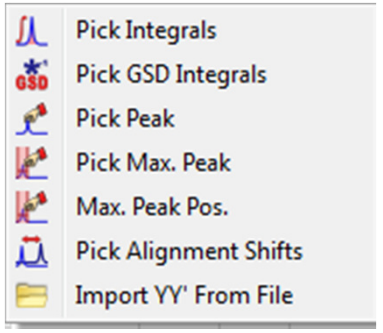
Tip: We recommend you to fit the original values to exponential function directly to avoid bigger numeric fitting errors. Also: Make sure you exclude zero or negative values before converting them to logarithmic scale.

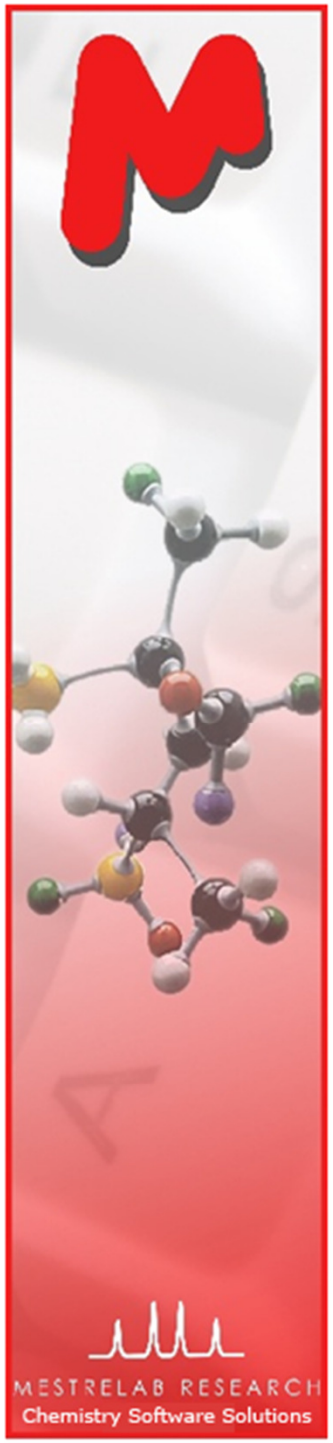
MESTRELAB RESEARCH
Chemistry Software Solutions



More about the Data Analysis Panel

- You can click the "+" button to add another Y(X) column to the current table/plot, or you can start with a new table/plot by clicking the Create New Plot button.
- You can use other types of spectral properties as Y(X) values:
  - Integrals: analog peak areas
  - GSD Integrals: areas of deconvoluted peaks
  - Peaks: intensities of the peaks near a defined location
  - Maximum Peaks: intensities of the highest peaks in a defined region
  - Max. Peak Positions: positions of the highest peaks in a defined region.
  - Pick Alignment Shifts: the shifts of peaks relative to the peak in the first spectrum





Summary

- Mnova NMR provides powerful and easy-to-use tools for processing and analysis of multiple NMR spectra for reaction monitoring
- Such tools can be used for many other types of studies, such as relaxation, diffusion, binding studies, etc.
- See Mnova > Help > Contents > Advanced Menu > Data Analysis for more info.
- For 45 day free trial of Mnova, go to http://mestrelab.com/software/mnova-suite/download/ or email us at sales@mestrelab.com