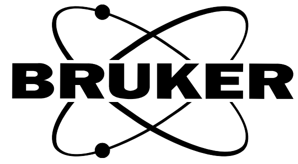

XENON DATA PROCESSING REFERENCE

Xenon Data Processing Reference

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Bruker BioSpin Corporation
Billerica, MA USA

Manual Version 1.0
Part Number 863????

Software Version 1.1b60
June, 2012

Xenon Data Processing Reference
Manual Version 1.0
Software Version 1.1b60

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This manual is part of the original documentation for the Bruker Xenon spectrometer.

Preface 0

Bruker strives to supply you with instructional and accurate documentation. We encourage you to tell us how we are doing. Please send us your suggestions for improvements, corrections, or bug reports. If there is anything you particularly liked, tell us as well. With your input and assistance, Bruker can continually improve its products and documentation.

You can send your messages and correspondence via e-mail, FAX, telephone, or mail. It is important to include the document name, product name, version number, and page number in your response. Here are the addresses and numbers to which you can send your messages.

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Thank you for your help.

Electrical Safety 0.1

Do not remove any of the protective covers or panels of the instrument. They are fitted to protect you and should be opened by qualified service personnel only.

Power off the instrument and disconnect the line cord before starting any cleaning work in the spectrometer. Never operate the instrument with the grounding cord disconnected or by passed. Facility wiring must include a properly grounded power receptacle.

Chemical Safety 0.2

Individuals working with hazardous chemicals, toxic substances, or enclosed liquid samples must take every precaution possible to avoid exposure to these agents. As a general rule, THINK OF THE CHEMICAL LABORATORY AS A HAZARDOUS ENVIRONMENT IN WHICH YOU MUST CONTINUALLY MAINTAIN A HIGH STANDARD OF VIGILANCE. Do not assume a cavalier attitude -- the substances with which you work present very real, and very serious threats to your health and safety.

Adhere to all currently recommended guidelines for standard laboratory safety as promulgated by governmental codes and contemporary laboratory practice. Inform yourself about the specific risks that are present when you handle actual or potential carcinogens (cancer-causing agents), explosive materials, strong acids, or any liquids that are sealed in glass containers.

Specifically:

- Be extremely careful when you handle sealed glass samples that are rapidly heated or cooled. The rapid cooling of some samples may result in the formation of a solid bolus in the sample tube that may make the tube prone to explosive rupture.
- Educate yourself about the temperature at which chemicals evaporate. When a sample gets close to the temperature at which it evaporates, it may quickly become volatile.
- In general, the safety threat posed by flying glass and violently escaping gases and liquids should not be underestimated.
- Wear safety glasses, face masks, and other protective clothing whenever there is any risk of spillage, breakage, or explosion. Protective shields should also be employed when there is any risk of explosion.
- Be sure that both storage and working areas are properly ventilated. They should be equipped with powerful blowers and fume heads.
- Store chemicals safely. Avoid integrating containers of chemicals that may result in dangerous combinations.
- Practice good housekeeping in work and storage areas. Clean up spills and refuse promptly. Do not leave volatile, combustible, or acidic liquids exposed on counters, benches, or other work areas.
- Make certain all chemical containers are properly labeled and classified, and that especially hazardous materials are appropriately designated with clearly understood decals or warnings.
- Never taste or inhale unmarked chemicals.
- All laboratories should be equipped with fire doors, fire extinguishers, fire smothering materials, and sprinkler systems or showers, as well as a detailed fire safety plan.

Microwave Safety 0.3

As long as the microwaves are contained in metal structures, microwaves can be very safe. Here are some precautions which, if followed, will eliminate the possibility of injury due to the microwaves.

- Do not have an open waveguide when the microwave power is on.
- Switch the bridge to standby when you remove or change EPR cavities.
- Never look down an open waveguide when there is microwave power. The eyes are very susceptible to damage from microwaves.

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Introduction 1

This document describes the data processing functions of the Bruker Xenon EPR software. It is assumed that you have already read and mastered the material in the Xenon User's Guide and that you are familiar with CW (Continuous Wave) EPR.

Using this Manual 1.1

How to Find Things 1.1.1

Preface First, you should read the safety guide in the preface of the manual. Microwaves can be dangerous, particularly to your eyes. With normal precautions, the risk for injury can be minimized.

Chapter 2 This section introduces the reader to the essential concepts and components of the Bruker Xenon software.

Chapter 3 This chapter describes the Processing taskbar.

Chapter 4 A description of the Baseline Correction task.

Chapter 5 This section describes Peak Picking.

Chapter 6 An explanation of the Integration and Derivative tasks.

Chapter 7 A description of Fitting.

Chapter 8 This section describes Filtering.

Chapter 9 An explanation of the Algebra tasks.

Chapter 10 This section describes operations on Complex data.

Chapter 11 This chapter describes the many Transformations operations available in Xenon.

Chapter 12 An explanation of Structure operations.

Chapter 13 A description of Quantitative EPR.

Chapter 14 This section explains SpinFit.

Typographical Conventions 1.1.2

Special fonts are used in the text to differentiate between normal manual text and text displayed in the program.

Times This is the font used for the normal text in the manual.

Helvetica This is the font used for text that is displayed by the program or must be entered into the program by you.

`Courier` This is the font used for text in examples of PulseSPEL pulse programs.

Special notes 1.1.3

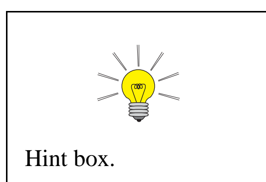
Some special notation is employed in this manual to simplify the descriptions.

< ... > The content between the brackets needs to be substituted with proper entries by the user.

> The right bracket indicates sequential selection of the menu entries. For example, Processing > Filtering > Smoothing means clicking the Processing button in the menu bar, followed by clicking Filtering in the sub-menu, and then clicking Smoothing.

Warning box.

You will see a warning box sometimes in the lefthand margin. These are meant to point out critical information. In particular, it warns you about any procedures or operations that may be dangerous to the spectrometer or you. Always read and follow this advice.


Hint box.

In addition, there are also hint boxes in the lefthand margin These are meant to be helpful hints and point out important information.

Essential Concepts of Xenon 2

We shall explore some of the most fundamental Xenon operations in this chapter. You will need these operations for processing data. Therefore, we highly recommend reading this chapter before exploring the remaining sections.

Basic Components of an Xenon Window 2.1

There are two operational modes for Xenon, processing and acquisition. Figure 2-1 shows the basic components of a Xenon processing window. It has the following basic components:

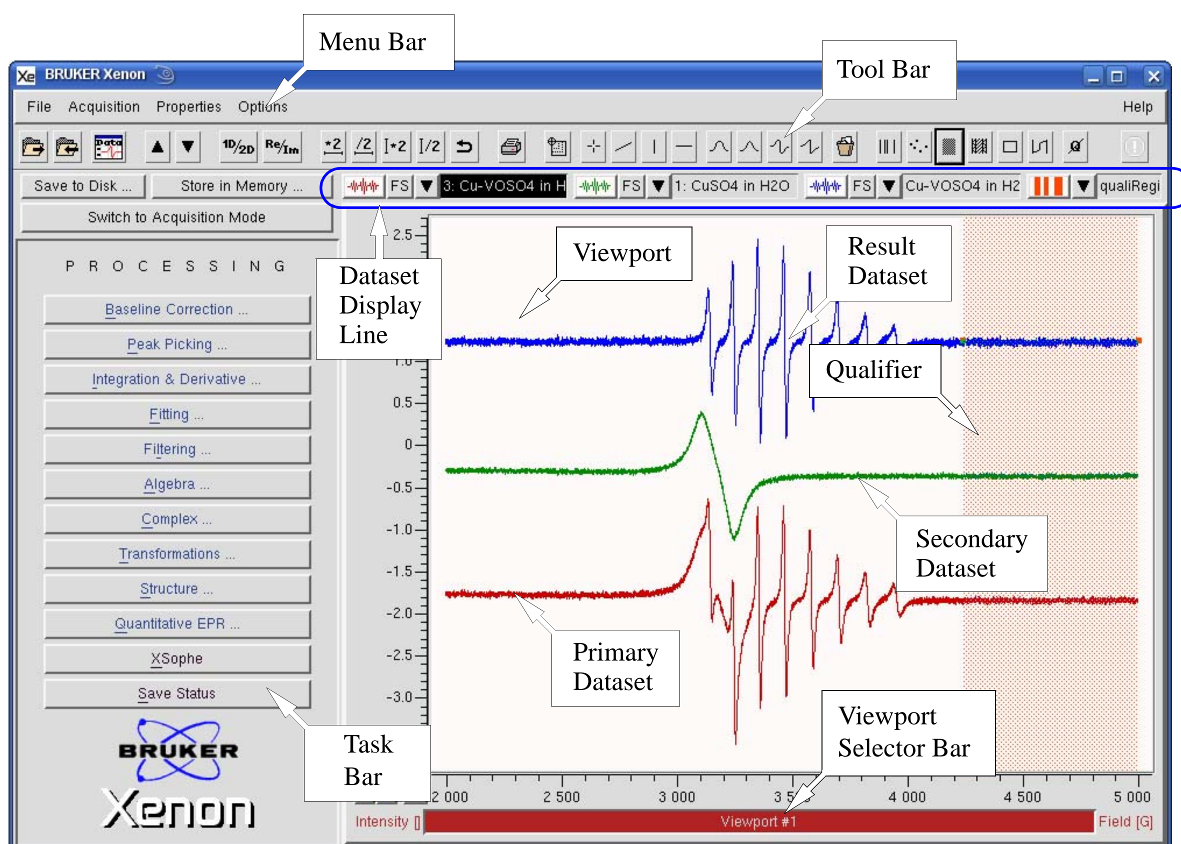


Figure 2-1 The basic components of a Xenon window in processing mode.

Menu Bar The menu bar groups several menus together. The File menu deals with input and output of files. The Acquisition menu lets you configure the spectrometer. The Properties menu sets up the Xenon window's features. In the Options menu you can modify the behavior and properties of tools and load external ProDEL programs. Under the Help menu you can find a getting started document for Xenon and information about the Xenon software.

Tool Bar Buttons for frequently used commands and operations are grouped here for your convenience.

Task Bar Tasks are macros which organize and streamline the individual processing steps required to perform common operations such as Baseline Correction,

Integration, and Peak Picking. These routines are grouped together in the Task Bar.

Viewports The window in the center is called the Viewport. It displays your data. We will discuss viewports in the next section.

Acquisition Mode There are two modes for Xenon. We have already seen the processing mode. The second mode is acquisition mode. In this mode, the left side of the application window displays the acquisition parameters. The switch button switches between the two modes. It shares many of the same features as the processing mode window but also has a spectrometer parameters panel for setting parameters and a spectrometer control panel for monitoring spectrometer conditions, selecting experiments, and starting, stopping, and pausing experiments.

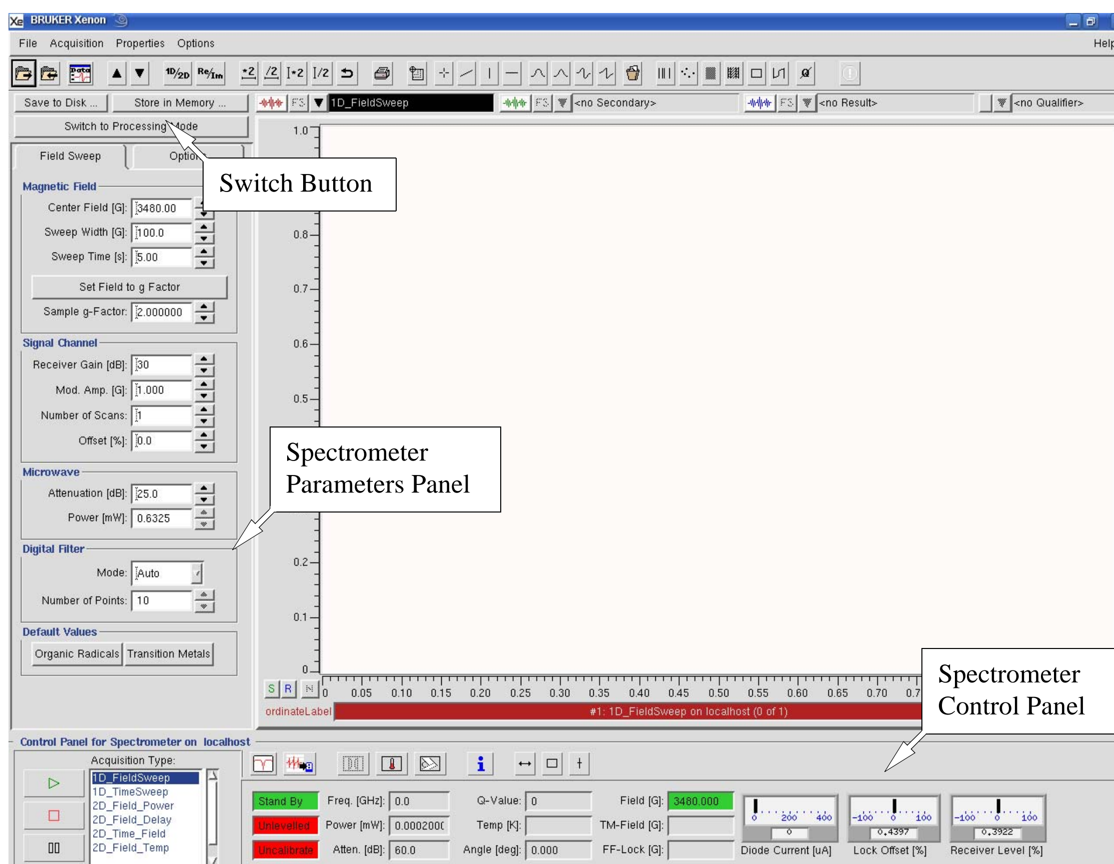


Figure 2-2 The basic components of a Xenon window in acquisition mode.

Viewports 2.2

The viewport is the central feature of the Xenon software. All datasets are presented and processed in a viewport. When you start the Xenon software, a single viewport appears by default. A viewport can show 1D or 2D datasets in the display area with a multitude of options. (See Section 2.2.1.) You can control which datasets are displayed and their options with the dataset display line. (See Section 2.2.2.) When you have more than one spectrum in the viewport, they can be individually scaled by using the individual scale buttons. (See Section 2.4.6.)

Display Area 2.2.1

The center part of a viewport is the display area. By default, the background is white.

Viewport Selector Bar

There may be some instances when there are more than one viewport. A red or highlighted bar indicates the active viewport. You can switch active viewports by clicking this bar. It is also used sometimes to display readout information.

There are four types of datasets that can be displayed:

Primary

If you only have one dataset, it is normally in the Primary dataset. This is the spectrum which you process or analyze. Its default display color is red.

Result

After you process the dataset in Primary, the results of your operation are temporarily stored in the Result dataset. The Result and Primary datasets appear simultaneously in the Viewport. Its default display color is blue.

Secondary

Some operations require two datasets, such as subtracting two spectra from one another. In this case, the second spectrum should be loaded into the Secondary dataset. Its default display color is green.

Qualifier

The qualifier allows you to define or qualify the region of a dataset which is affected when you perform an operation. By default, the whole dataset is qualified. Its default display color is orange.

Dataset Display Line 2.2.2

Below the tool bar is the Dataset Display Line. It is separated into four sections corresponding to the dataset which it controls. Most of the sections consist of four elements which are described below.

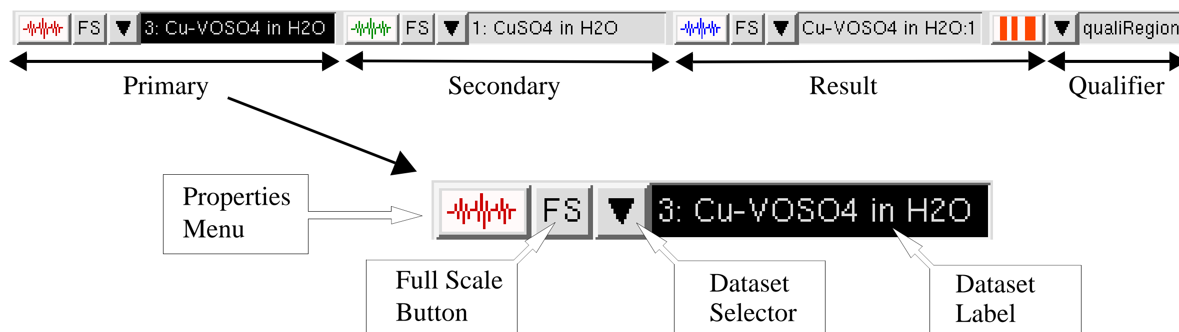


Figure 2-3 The Display Line and its elements.

Dataset Label

The Dataset Label indicates which spectrum is selected, for example Cu_VOSO4 in H2O in Figure 2-3 is selected as the Primary dataset. The inverse video highlighting indicates that the Primary dataset is active, *i.e.* that it is the input for any data processing. For example, if we wish to multiply the Secondary dataset by three, we would first click the Secondary Dataset Label to make it active and then perform a multiplication. By default, the Primary dataset is active. Each type of dataset (Primary, Secondary ...) has its own Dataset Label.

Dataset Selector

In order to select a dataset, click the small triangle next to the Dataset Label. A menu drops down listing all of the datasets that are currently loaded in Xenon. (See Figure 2-4.) To select the dataset to display in the viewport, click the desired dataset. You can also choose not to show any dataset by clicking <no Primary> (or <no Secondary>...). A particularly useful feature is <Result>. When you click it, it loads the latest Result dataset.

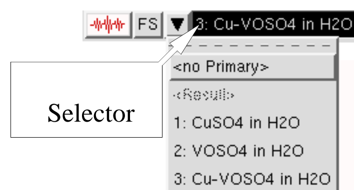


Figure 2-4 Selecting a dataset to display.

Full Scale Button

To the left of the Dataset Selector is a button labeled FS. (See Figure 2-4.) When clicked, it resizes the spectrum so that it completely fills the viewport.

Properties Menu

The Properties menu allows you to choose the Display Type. (See Figure 2-5.) 1D datasets can be presented as points, line, histogram, numeric, or distance. For 2D datasets you can choose from density, contour, dot plot, stackplot, transparent, and hidden lines.

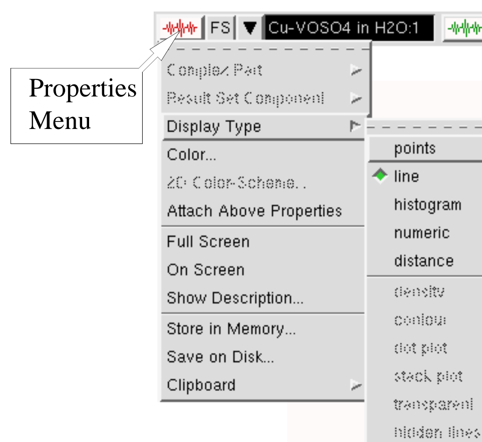


Figure 2-5 Choices for the display type of a dataset.

Under Color you can choose the color of the display to distinguish Primary, Secondary, and Result datasets. (See Figure 2-6.)

Figure 2-6 Choosing the dataset color.

Show Description allows you to view the parameters of the dataset you select.

You can store or save the currently displayed dataset. Store in Memory temporarily saves the data in memory. When you quit Xenon the data is lost. The Save on Disk function writes the data onto the hard disk and makes the data permanent.

The Result Section 2.2.3

The Result section is similar in structure to the Primary section. In the property menu most of the submenus are the same except that there is Show History to allow you to view the data processing history. (See Figure 2-7.) The FS button is the same. The select menu is a little different. The menu lists the results of each dataset processing operation. It has a <no Result> button to clear the Result from the viewport.

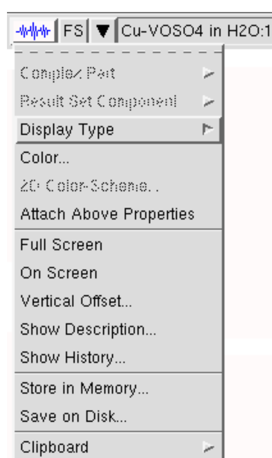


Figure 2-7 The properties menu of the Result section.

Sometimes when much data is processed the huge amount of data could overload the memory and slow down the computer. The Clear List button removes all the temporary result data. (See Figure 2-8.) Store or Save the useful results and use Clear List frequently when you process data intensively.

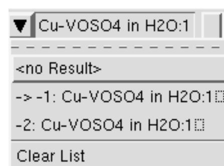


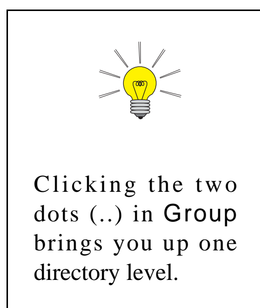
Figure 2-8 The selection menu of the Result section.

Tools 2.3

The tool bar lies underneath the menu bar. (See Figure 2-1.) It contains 31 commonly used tools arranged in eight groups. In order from left to right, we list the name and the function of each tool button.

Management 2.3.1

Load Dataset Clicking this button opens a dialog box for choosing the dataset (and its path) you want to load into Xenon. (See Figure 2-9.)



Clicking the two dots (..) in Group brings you up one directory level.

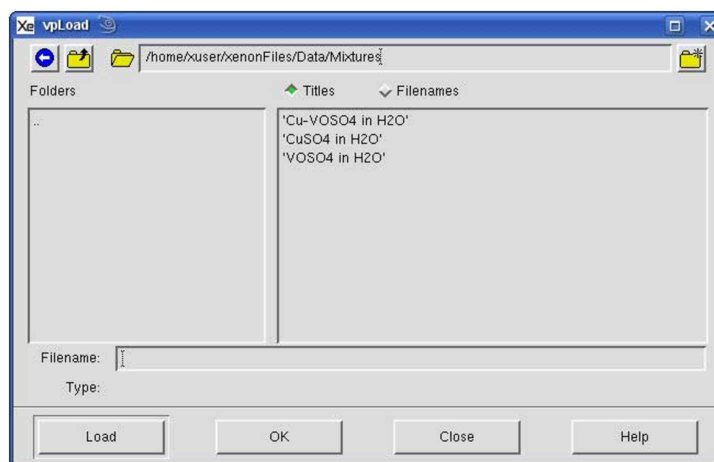


Figure 2-9 Loading datasets.

Save Dataset Clicking this button opens the save file dialog box so that you can save the dataset onto the hard drive. On top, you can select the source (*e.g.* Primary, Secondary, ...) as well as enter a title for the dataset. Below, you can choose the path and filename for the saved dataset. (See Figure 2-10.)

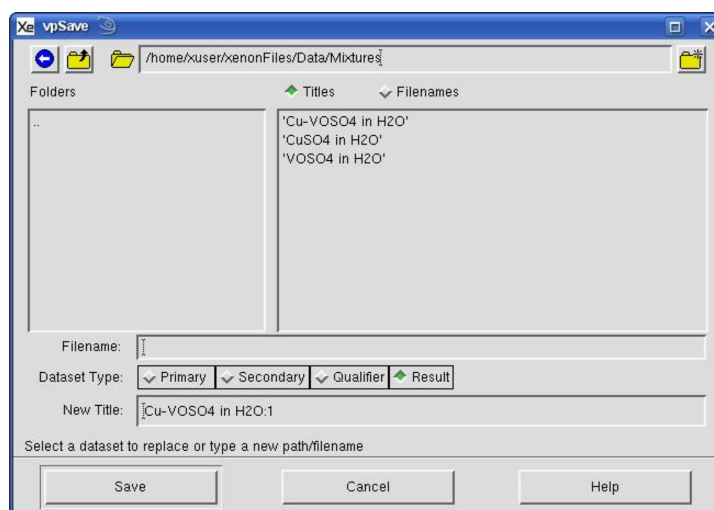
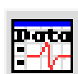
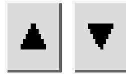


Figure 2-10 Saving a dataset.



Dataset Table This dataset table lists all the datasets loaded or currently stored in memory.

Selection 2.3.2



Previous/Next Dataset Clicking this button displays the dataset listed before/after the current dataset in the dataset table.

Display Toggling 2.3.3

Toggle Dimension Clicking this button toggles the current active viewport between 1D and 2D views.

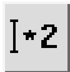
Toggle Complex Part Clicking this button toggles the current display between the real and imaginary part of the dataset if the dataset is a complex dataset.

Zooming 2.3.4

Note that these operations only change the display of the data and not the actual data.

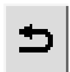
X-Range * 2 This button expands the X-axis by a factor of 2.

X-Range / 2 This button shrinks the X-axis by a factor of 2.



Y-Range * 2 This button expands the Y-axis by a factor of 2.

Y-Range / 2 This button shrinks the Y-axis by a factor of 2.



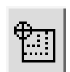
Previous Range Clicking this button brings you back to the previous range.

Printing 2.3.5

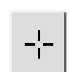
Print Viewport Click this button to print the spectra in the currently active viewport.

Graphics and Measurement Tools 2.3.6

The following tools allow you to measure distances, linewidths and amplitudes in your data. The expand tool also allows you to zoom in and out.



Expand With this button selected you can select regions to zoom or expand with the mouse. Note that these operations only change the display of the data and not the actual data. Zooming is further discussed in Section 2.4.4.



Dot Marker A point marker appears in the current viewport when you click this button. You can use the mouse to move it to where you want. The x and y coordinates are displayed next to the marker. Measuring positions is further discussed in Section 2.4.2.

Free Line This marker provides you with a straight line of arbitrary angle and length. Both ends can be moved by clicking the control points (appearing as small squares) at the ends and dragging with the mouse. The line can be moved by clicking towards the middle of the line and dragging the mouse. When any part of the tool is clicked, the height and width of the line are displayed in the viewport selector bar. The line can be deleted by dragging one end over to the other end (*i.e.* the two end squares overlap).

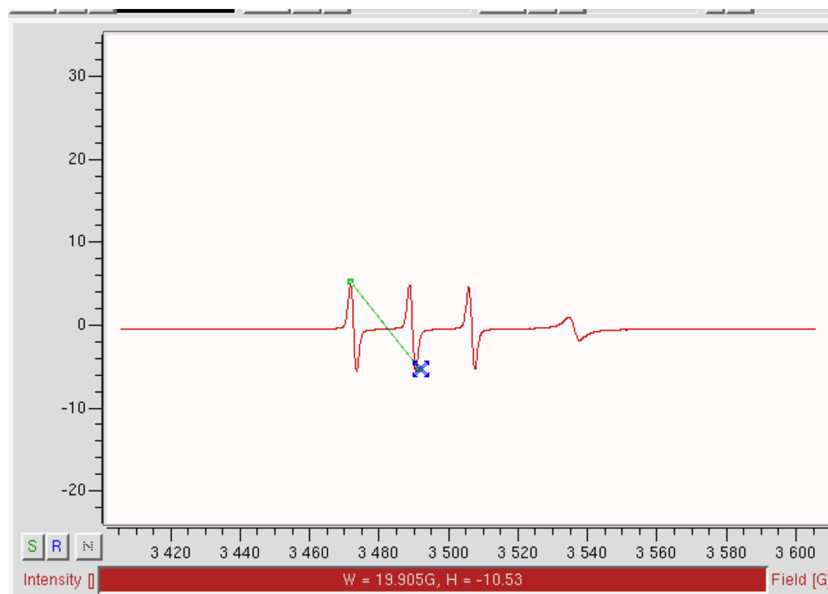
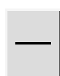
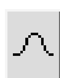


Figure 2-11 Using the Free Line tool.

Vertical Line This tool provides you with a vertical line. It works much the same way as the Free Line tool except it is constrained to be vertical.



Horizontal Line This tool is similar to the vertical line except that it is horizontal.



Gaussian This tool provides you with a gaussian lineshape. You can change its height and width by dragging its control points. If you click any part of the tool, the height and width are displayed in the viewport selector bar. The equation for this lineshape is:

$$f(x) = \mathrm{H} \cdot e^{(-2 \cdot ((x-\mathrm{b})/\mathrm{W})^2)} \qquad [2\text{-}1]$$

and the W displayed in the viewport selector bar corresponds to the width and the H corresponds to the height. Note that W does not correspond to the full width at half maximum, Γ, but can be calculated with the following equation:

$$\Gamma = \sqrt{2\ln 2} \cdot \mathrm{W} \approx 1.1775 \cdot \mathrm{W} \qquad [2\text{-}2]$$

If you click the tool in a region other than the control points and dragging, the tool can be moved in the viewport. There are five control points (appearing as small squares) for this tool. The first is at the center of the tool. Clicking and dragging it vertically changes the height.

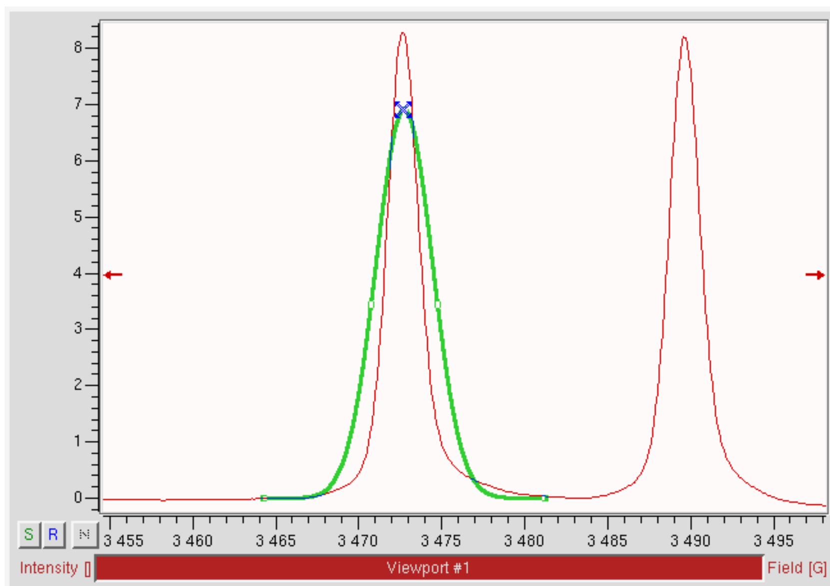


Figure 2-12 Adjusting the height of the gaussian tool by clicking and dragging the center control point.

Clicking and dragging the control points on either side of the center control point horizontally changes the width.

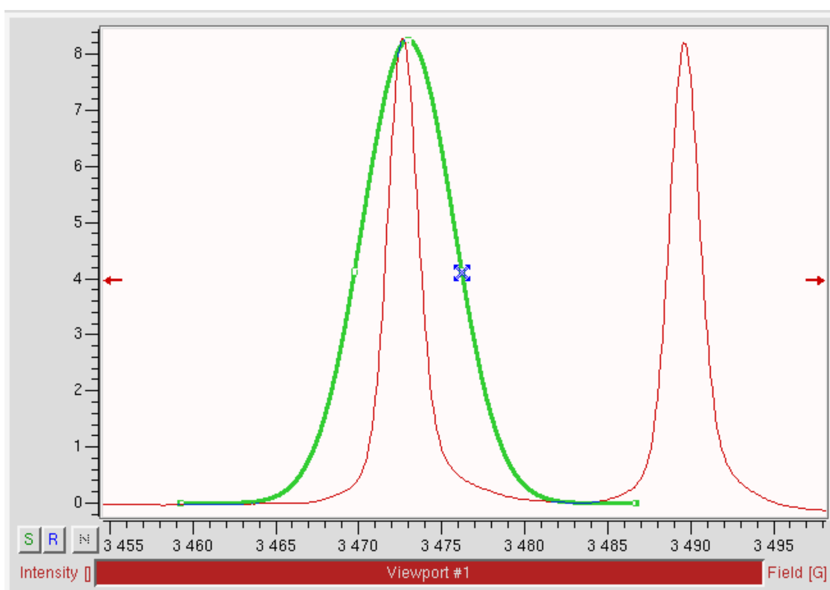


Figure 2-13 Adjusting the width of the gaussian tool by clicking and dragging the control points on either side of the center control point.

Clicking and dragging the end control points allows you to change both the width and height simultaneously.

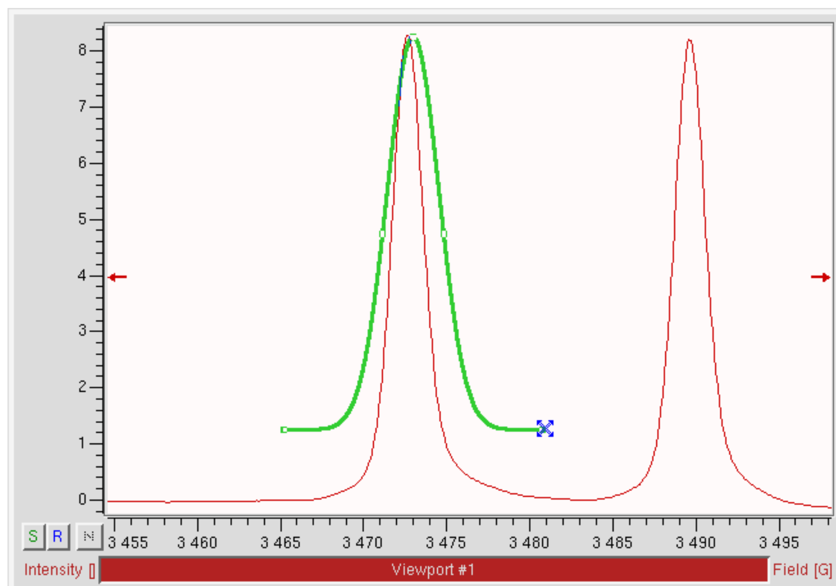


Figure 2-14 Adjusting the width and height of the gaussian tool simultaneously by clicking and dragging the end control points.

This tool can be deleted by decreasing the width to zero.

Lorentzian This tool is similar to the gaussian tool except that it is a lorentzian lineshape. The equation for this lineshape is:

$$f(x) = \frac{H}{1 + \left(\frac{x}{W/2}\right)^2} \quad [2\text{-}3]$$

where the W displayed in the viewport selector bar corresponds to the full width at half maximum and the H corresponds to the height.

Derivative Gaussian This tool provides you with a first derivative gaussian lineshape. You can change the height and width with the mouse. If you click any part of the tool, the height and width are displayed in the viewport selector bar. The equation for this lineshape is:

$$f(x) = -H \cdot e^{1/2} \cdot \frac{x}{W} \cdot e^{-2\left(\frac{x}{W}\right)^2} \quad [2\text{-}4]$$

where the W displayed in the viewport selector bar corresponds to the peak to peak width and the H corresponds to the peak to peak height.

If you click the tool in a region other than the control points and dragging, the tool can be moved in the viewport. There are four control points (appearing as small squares) for this tool. Clicking and dragging the innermost ones changes both the height and width of the tool simultaneously.

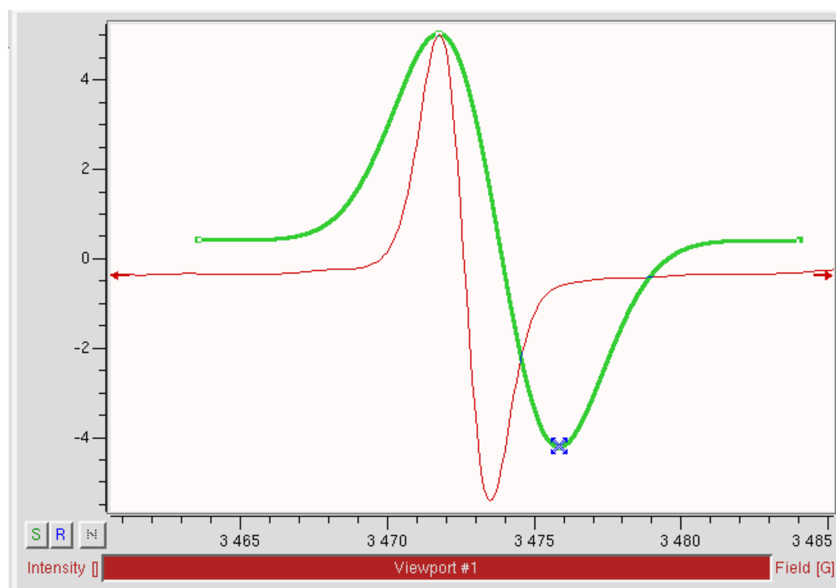


Figure 2-15 Adjusting the width and height of the gaussian derivative tool simultaneously by dragging the innermost control points.

Clicking and dragging the end control points changes the width and the y coordinates simultaneously.

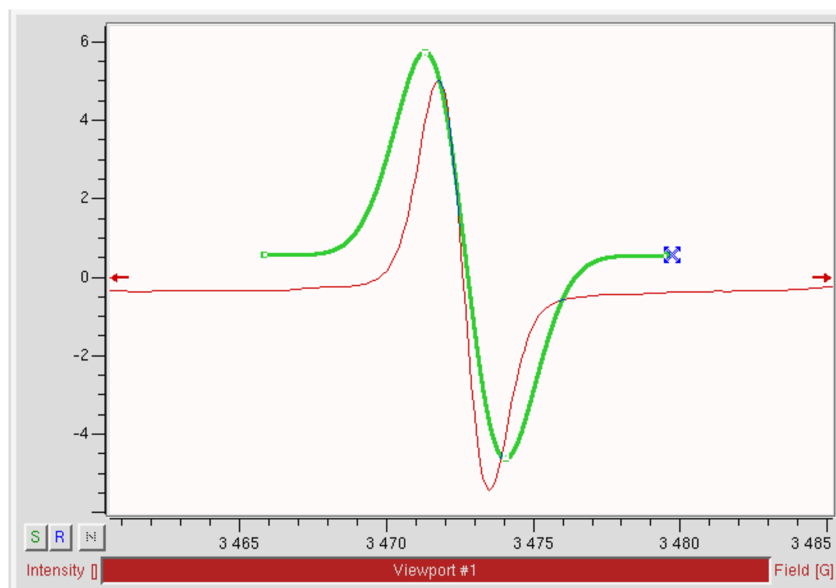


Figure 2-16 Adjusting the width and y coordinates of the gaussian derivative tool simultaneously by dragging the end control points.

This tool can be deleted by decreasing the width to zero.

Derivative Lorentzian This tool is similar to the derivative gaussian tool except that it is a first derivative lorentzian lineshape. The equation for this lineshape is:

$$f(x) = -\mathrm{H} \cdot \frac{16}{9} \cdot \frac{\frac{x}{\mathrm{W}}}{\left(1 + \frac{4}{3} \cdot \left(\frac{x}{\mathrm{W}}\right)^2\right)^2} \quad [2\text{-}5]$$

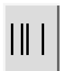
where the W displayed in the viewport selector bar corresponds to the peak to peak width and the H corresponds to the peak to peak height.

Remove Tool Click the tool you wish to remove with the mouse. Then click this remove tool button to delete the selected tool.

Qualifiers 2.3.7

The last group consists of six qualifier buttons. The various types of qualifiers allow you to select or limit certain parts of the dataset for processing. Qualifier definition is started by clicking the desired qualifier type button. (There are further examples of the use of qualifiers in the sections to follow.)

When the qualifier is active, one or several control points (appearing as small squares) appear for changing the size or position of the qualifier by clicking and dragging the handles. Clicking the edges also allows resizing of the qualifier. Clicking on a qualifier selects that qualifier.



Position Qualifier This qualifier provides you with a vertical line and allows you to select an x-axis position for processing. First click the position qualifier button and then click on the position in the dataset you wish to qualify. It can be removed by clicking and dragging the qualifier so that its control point no longer lies on the spectrum. Position qualifiers can be moved by clicking and dragging them but care must be taken to not delete them by keeping the control point on the spectrum.

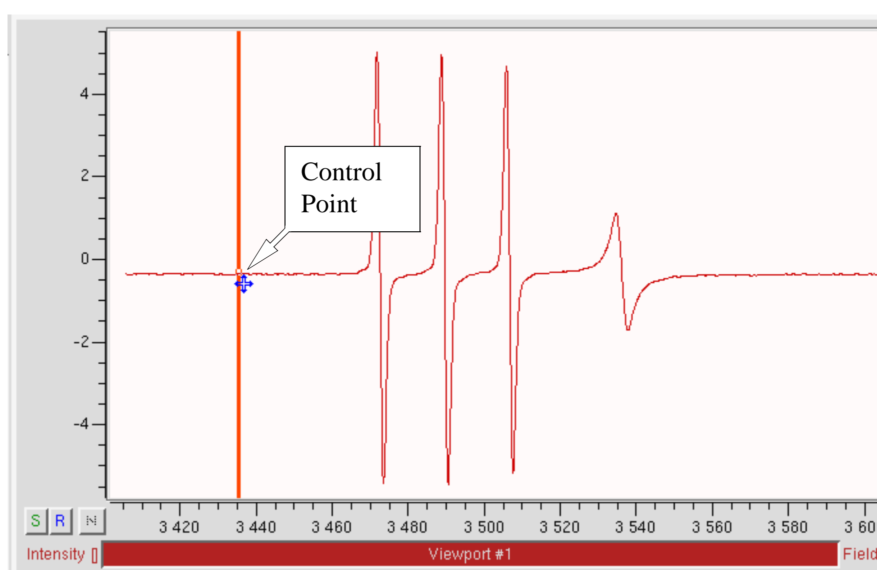
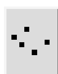


Figure 2-17 The position qualifier and its control point.



Point Qualifier You can select a specific point for processing with this tool. First click the point qualifier button. Place the mouse over the point in your data you wish to qualify and click. It can be removed by clicking and dragging the qualifier so that it no longer lies on the spectrum. Point qualifiers can be moved by clicking and dragging them but care must be taken to not delete them by keeping them on the spectrum.

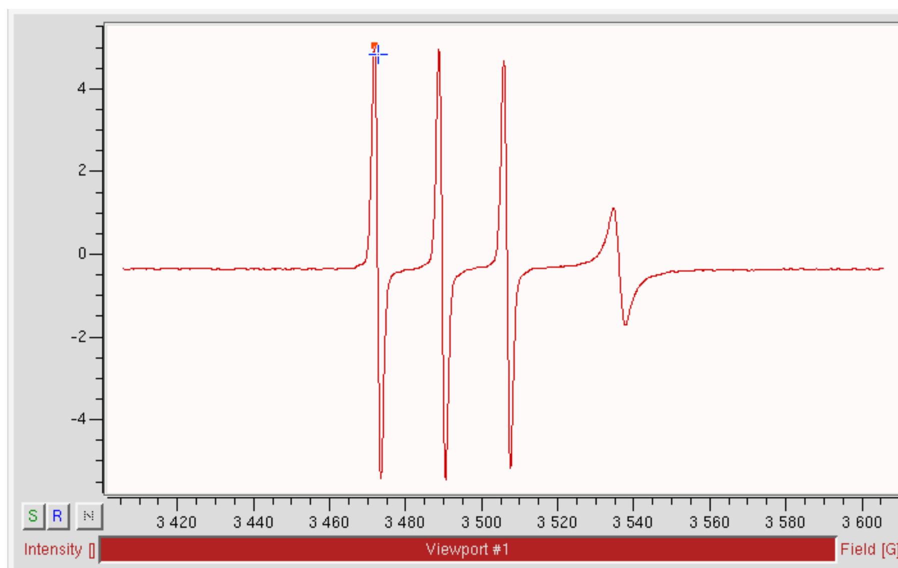
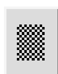


Figure 2-18 The point qualifier.



Region Qualifier Using this qualifier you can select a region of the x-axis for processing. First click the region qualifier button. Click and drag over the region you wish to qualify. By clicking and dragging the left or right edge of the qualifier, you can move the region qualifier's position and width. The qualifier can be deleted by clicking and dragging to set the width to zero.

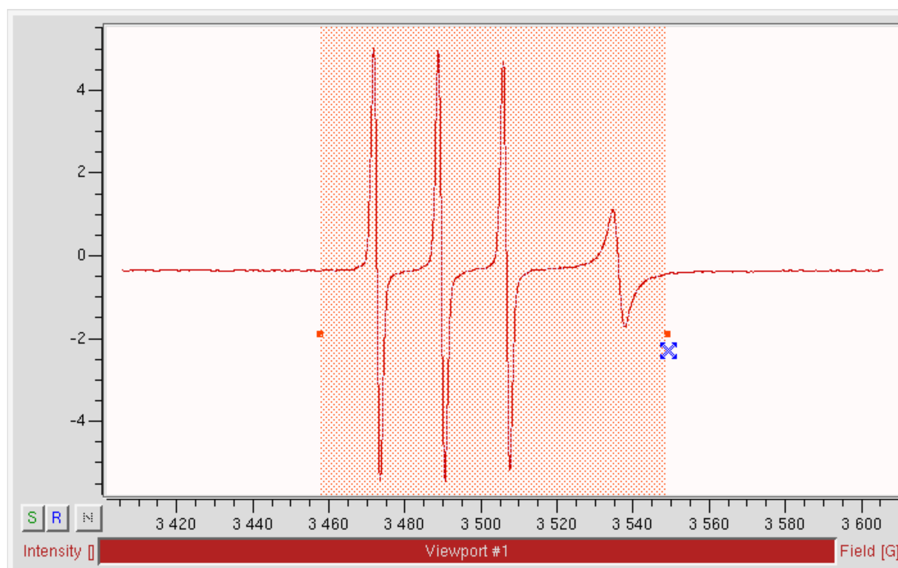
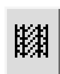


Figure 2-19 The region qualifier and its control points.



Baseline Qualifier Using this qualifier you can select a region of the x-axis for background or baseline fitting as well as for integration. This works in a similar fashion as the region qualifier. (See Section 6.2 for an example.) First click the baseline qualifier button. Click and drag over the region you wish to qualify. Clicking and dragging the mouse cursor creates a baseline qualifier consisting of four lines and three shaded areas. The center region is the region to be integrated. The outer two regions are selected as baseline. A straight line is fitted to the baseline regions and this fitted line is then subtracted from the EPR dataset when it is integrated. To change the widths, click and drag the lines to change their positions and thereby change the regions. You may notice that the widths of the baseline regions do not change when moving the second and third lines. The first line follows the second line and the fourth line follows the third line. The baseline regions can be changed by clicking the first and fourth lines. Note that the two are linked, so that changing the width of the left baseline region also changes the width of the right baseline region. The qualifier can be deleted by clicking and dragging to set the width to zero.

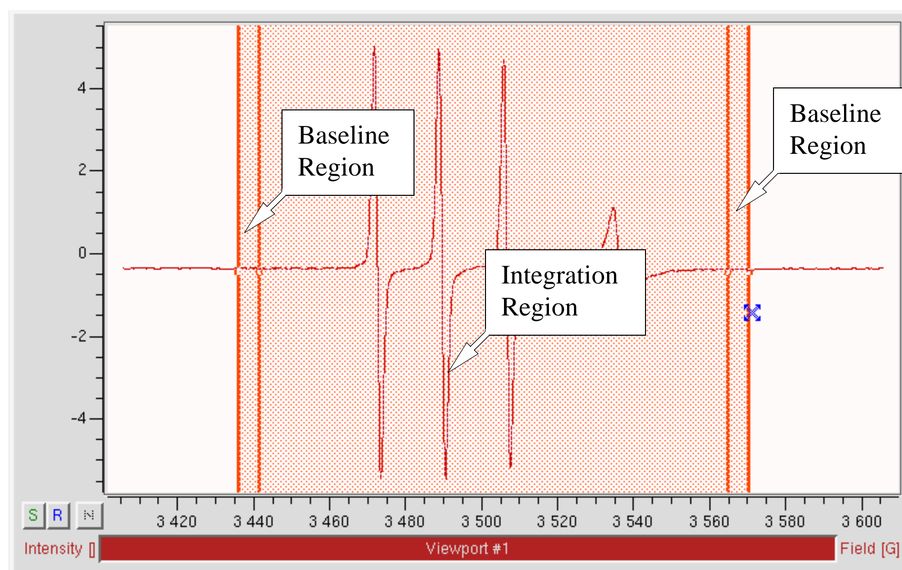
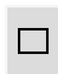


Figure 2-20 The background qualifier and its control points.



Area Qualifier This tool provides you with a rectangle to cover the area you wish to process. (See Section 5.2 for an example.) First click the area qualifier button. Click and drag over the area you wish to qualify. Clicking and dragging the corners of the qualifier changes its size. The position can be changed by clicking and dragging the edges of the rectangle. The qualifier can be deleted by clicking and dragging to set the width and height of the rectangle to zero.

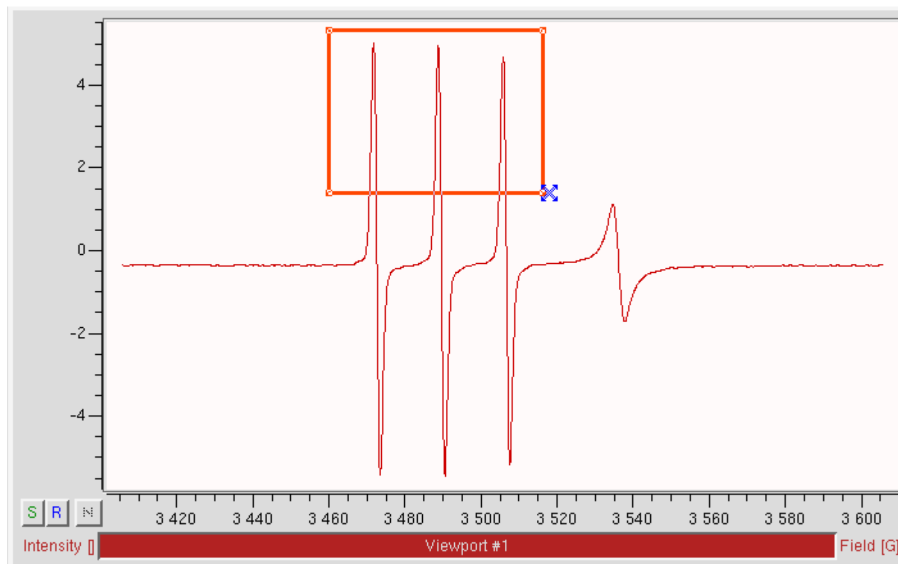


Figure 2-21 The area qualifier and its control points.

Integral Qualifier This tool consists of two vertical lines and a free line. The vertical lines indicate the starting and ending points for integration respectively. The free line indicates the offset and the slope and these are used to baseline correct the data. It is designed for integration, though in most cases the background qualifier is used. Moving the left control point up or down increases and decreases the offset. Moving the right control point up or down increases and decreases the slope. The qualifier can be deleted by clicking and dragging to set the width to zero.

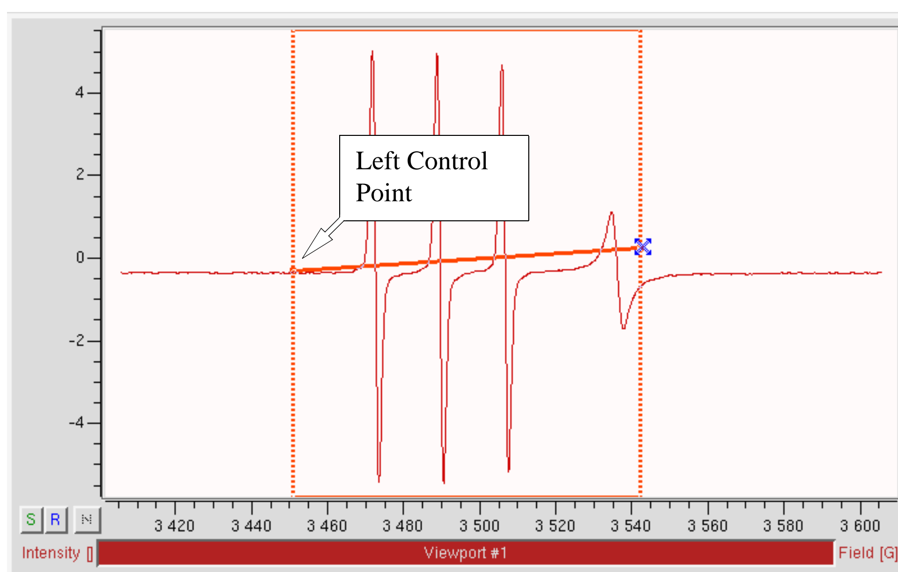


Figure 2-22 The integral qualifier and its control points.

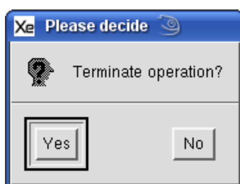
No Qualifier Click the qualifier you wish to remove with the mouse. Then click this no qualifier button to delete the selected qualifier.

Terminate Operation 2.3.8

Some operations or calculations may take a long time such as SpinFit simulations. The terminate operation button allows you to stop an operation.

Terminate operation This button stops a process or operation that is running.

Normally the button is grayed out. When a process is running, it turns red. Clicking the red button brings up a dialog box. Click Yes to stop the process. Once the process is terminated, a new window appears confirming the process has been terminated.



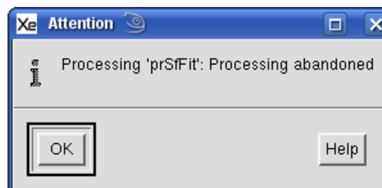


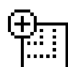
Figure 2-23 Stopping a running operation or process.

Mouse Functions 2.4

Depending on which buttons you pressed, the mouse performs many different functions such as resizing, moving, and measuring. By default, the mouse is in auto select mode and changes its function and mouse cursor according to the buttons pressed and location of the cursor. The following examples illustrate the various mouse functions.

The Cursors 2.4.1

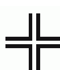
The mouse cursor indicates the current mouse function in Xenon.



Expand When this cursor is present you can click the left mouse button on the position you want to expand and drag the mouse to the place you want to end. A rectangle shows up indicating the area you want to expand. The area covered by the square expands to fill the screen of the viewport.

Zoom This symbol indicates that the zooming function is activated. Clicking with the right mouse button in the display area of the viewport displays this symbol. Dragging the cursor upwards or downwards vertically zooms in or out the area you point to. Dragging towards the right or left horizontally zooms in or out the area you point to. Dragging at an arbitrary angle zooms both horizontally and vertically at the same time. If you click the right mouse button in the x- or y-axis area, you zoom either only horizontally or vertically.

Moving Clicking on the middle mouse button changes the cursor to this symbol. The spectrum moves in the direction you drag the mouse. When you click the middle mouse button in the axis area you only move the spectrum either up and down or to the left and right.



Read Out With this mouse function you can read out the X-, Y-, and other values of the point where the mouse is in the spectrum. See the next section for details.

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Reading Out Coordinates 2.4.2

Move the mouse cursor close to the spectrum curve. The mouse cursor changes from the Expand to the Read Out cursor. Left click on a point of the spectrum: the coordinates of the cursor are displayed next to the cursor. The field value, intensity, and the g factor value are also displayed inside the Viewport Selector Bar if the spectrum is an EPR field sweep spectrum. (See Figure 2-24.)

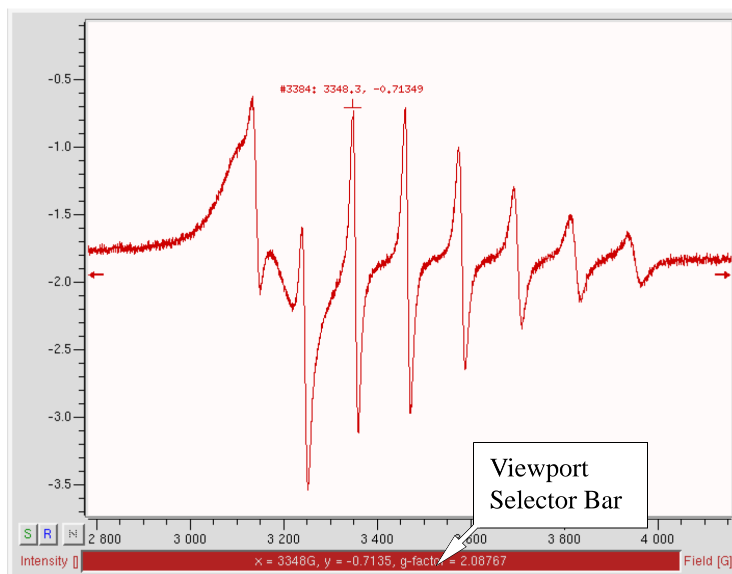


Figure 2-24 Using the Read Out mouse function.

Measuring Distances 2.4.3

Move the mouse cursor to the starting point on the spectrum. Press the left button and the right button simultaneously. Hold the mouse buttons and drag the cursor to the point where you want to end the measurement. The distance between the starting point and the ending points along the x and y axes are displayed in the Viewport Selector Bar. (See Figure 2-25.)

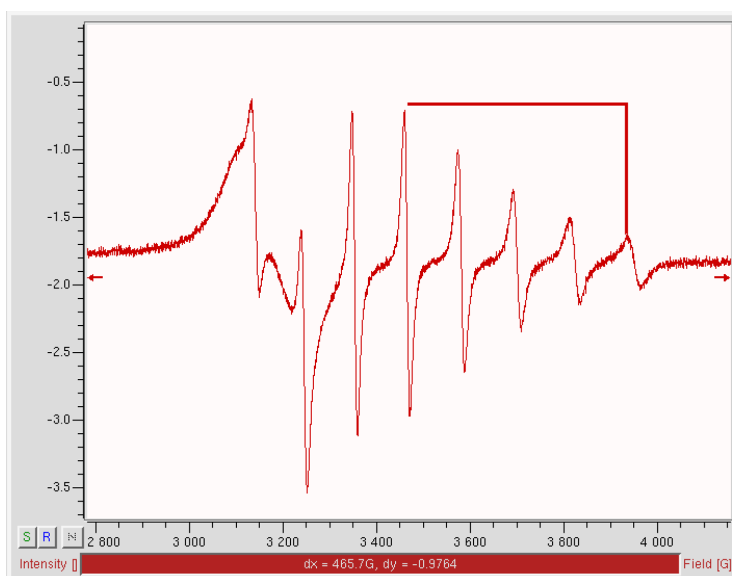


Figure 2-25 Measuring distances.

Zooming Spectra 2.4.4

You can zoom in on a specific area of a spectrum by using the rectangular scaling option. Move the mouse pointer to the display area of the viewport. The mouse pointer changes to the expand cursor. Click the left mouse button and drag the rectangle until it encompasses the region of interest. Release the mouse button and the region of interest then expands to fill the viewport. (See Figure 2-26.)

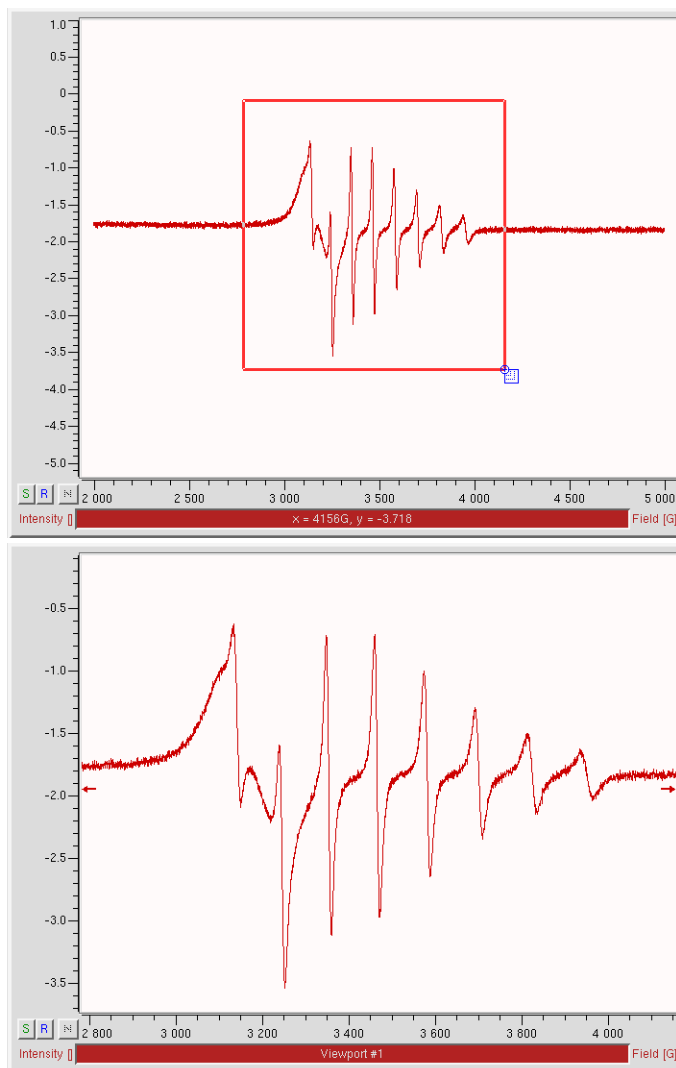


Figure 2-26 Zooming in with the rectangle scaling option.

A second means of zooming not only allows you to zoom in but also to zoom out. Place the mouse pointer in the spectrum area or in the axes area where you want to zoom. Click the right mouse button. The mouse pointer changes into a zoom cursor. Dragging up or right zooms in the spectrum or axis. Dragging down or left zooms out the spectrum or axis. (See Figure 2-27.)

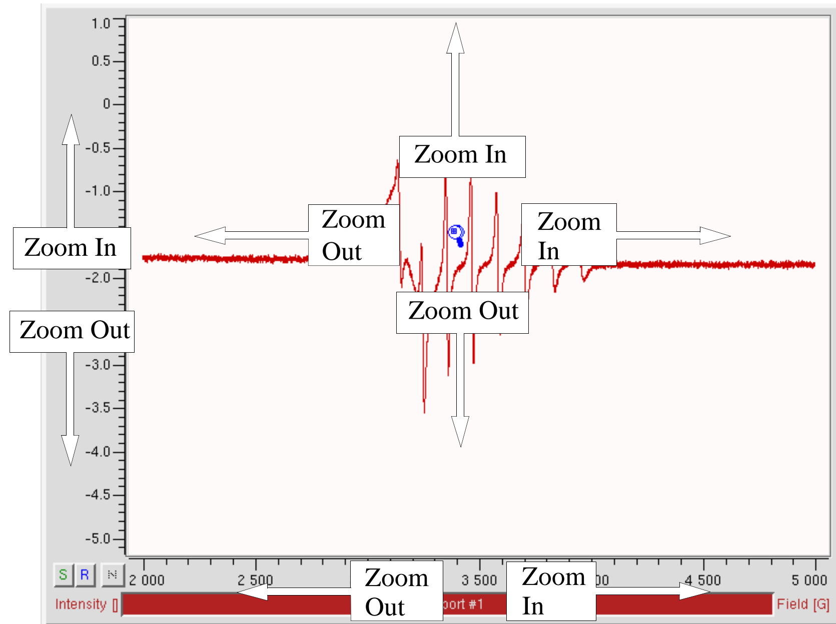
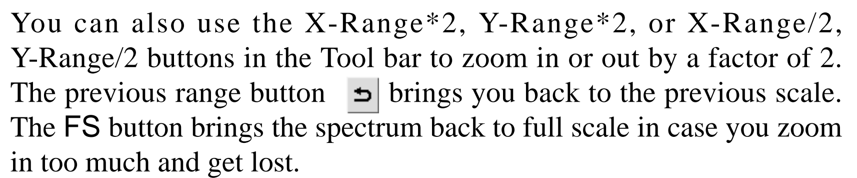


Figure 2-27 Zooming the spectrum.



You can also use the X-Range*2, Y-Range*2, or X-Range/2, Y-Range/2 buttons in the Tool bar to zoom in or out by a factor of 2. The previous range button brings you back to the previous scale. The FS button brings the spectrum back to full scale in case you zoom in too much and get lost.

To display a precisely defined area you can click Properties from the menu bar and then Display Range. (See Figure 2-28.)

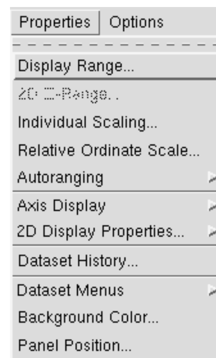


Figure 2-28 Activating the display range function.

A dialog box appears in which you can then select precisely the X- and the Y- range for display. Click the Set button in the dialog box to execute the selected range. (See Figure 2-29.)

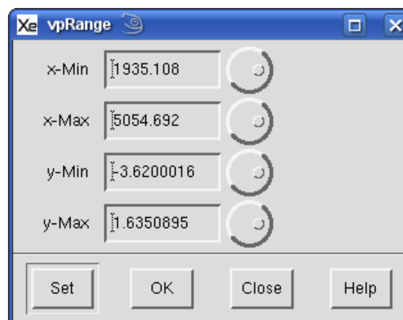


Figure 2-29 The set display range dialog box.

Moving a Spectrum Around 2.4.5

You can move the spectrum around by clicking the middle mouse button while the cursor is in the viewport display area and dragging the spectrum. The cursor changes to a move cursor. You can also place the cursor on either axis area to constrain the movement along one axis. (See Figure 2-30.)

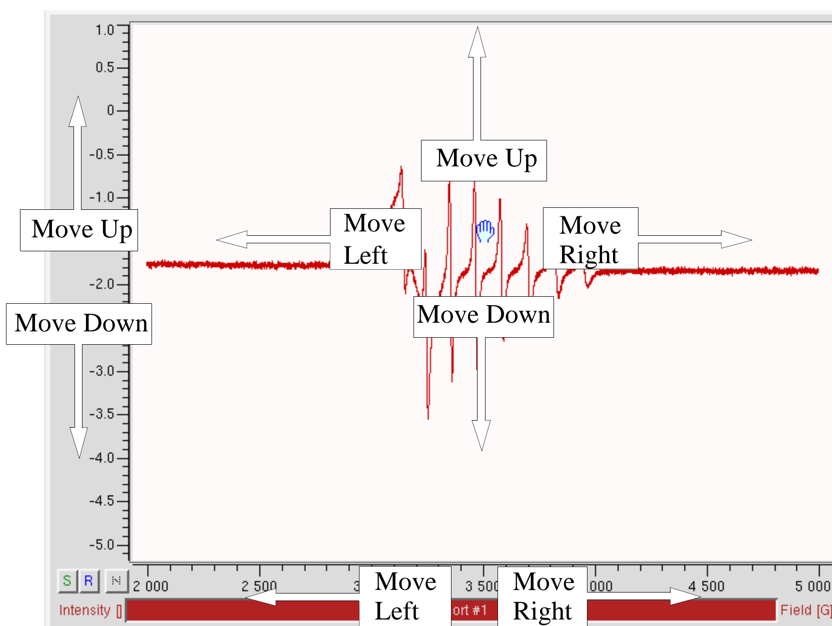


Figure 2-30 Moving the spectrum.

Individual Scale Buttons 2.4.6

You can set different scales for the Primary, Secondary, and Result datasets by clicking these buttons. (See Figure 2-31.) Once selected, the zooming and moving described in the previous sections is only applied to the selected dataset. Clicking S adjusts the scale for the Secondary dataset only. Clicking R allows you to independently adjust the Result dataset scale. A * appears next to S or R when the Secondary or Result dataset scales are different. Clicking N brings them back to the same scale as the Primary.

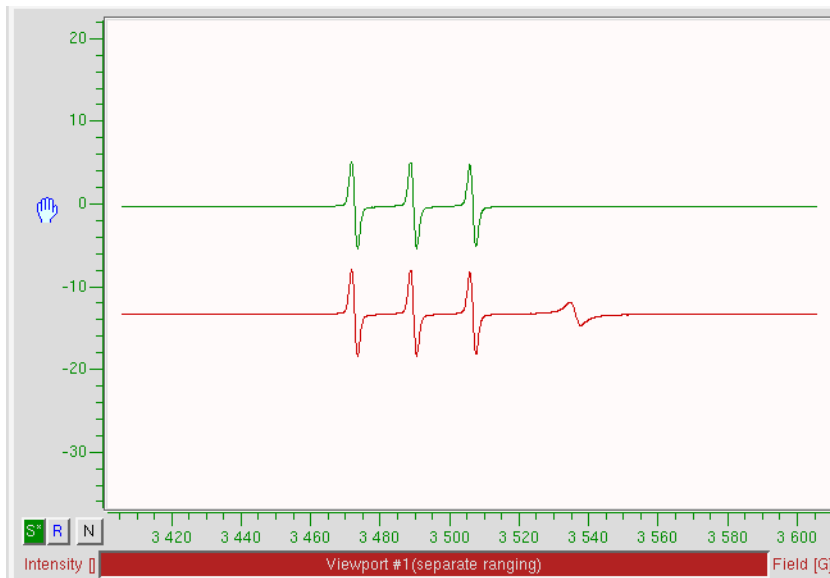


Figure 2-31 Moving only the Secondary dataset.

The Task Bar 3

This chapter describes the Processing task bar from which all processing tasks are started.

Accessing the Task Bar 3.1

Data processing tasks are accessed via the Processing task bar. If you are still in acquisition mode, this task bar is displayed when you click Switch to Processing Mode. The different classes of processing tasks are accessed by clicking its button. Some tasks may also have sub-task bars splitting the task into further sub-tasks.

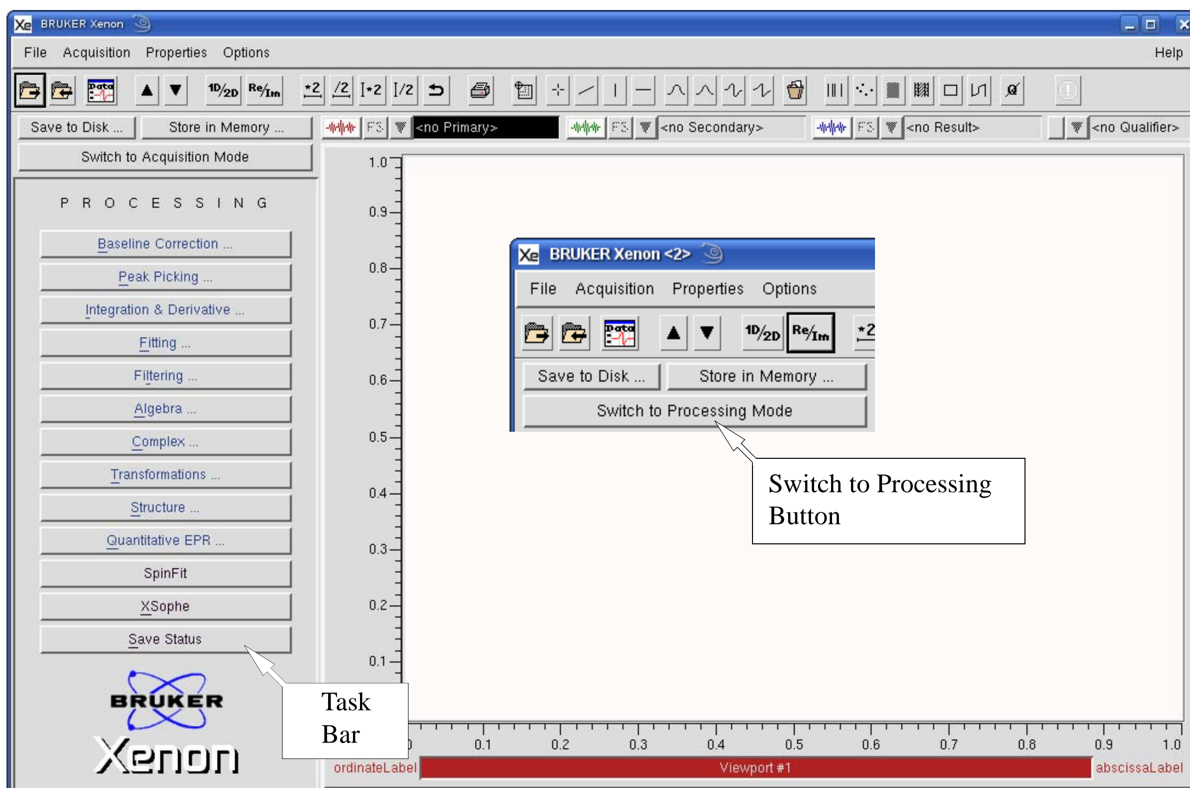


Figure 3-1 The Processing task bar.

Task bar Common Elements 3.2

Most of the tasks share some common elements.

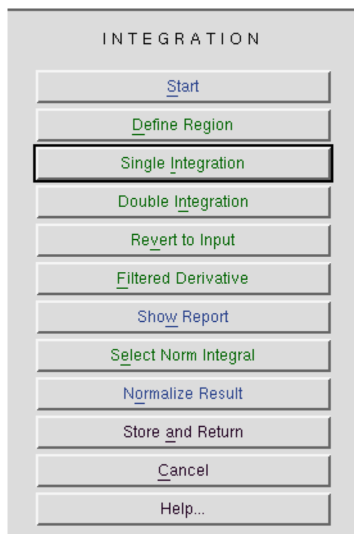


Figure 3-2 A representative task bar.

Start The first button is usually Start. When clicked, it clears the qualifiers.

Define and Change The second button is usually Define Region, area, or Points, depending on the qualifier type. When clicked you enter Define mode, the mouse cursor changes, and the caption of the button changes to Change Region, area, or Points. Qualifiers are added by clicking and dragging the mouse cursor in the viewport to select the desired area/region/point. You remain in Define mode and you can add multiple qualifiers. Once finished you click Change Region, area, or Points to adjust the qualifiers. Clicking this button toggles between the two modes. Area qualifiers can be moved by clicking and dragging the edges. They can be resized by clicking and dragging the corners. Region qualifiers are resized by clicking and dragging the vertical edges. Position qualifiers cannot be moved, but they can be removed and added.

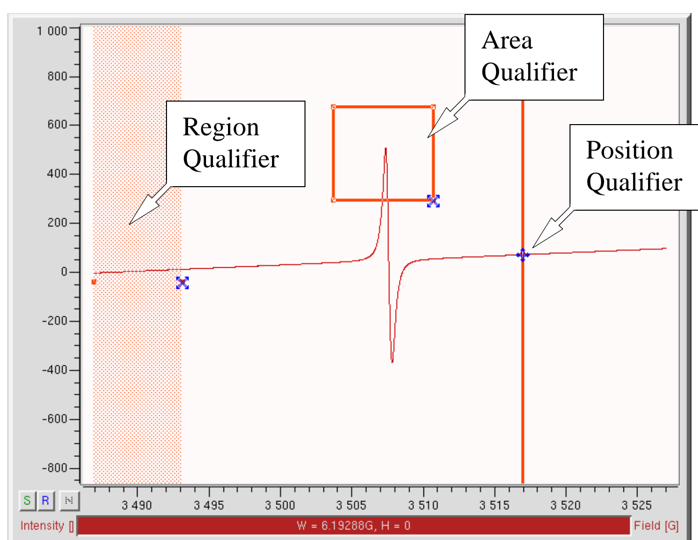


Figure 3-3 The most common qualifier types.

Store and Return

Note that this does not save the data to the hard disk.

After clicking Store and Return you are prompted to Store the Result data-set in memory. Enter a Title and then click Store. You are then returned to the Processing task bar.

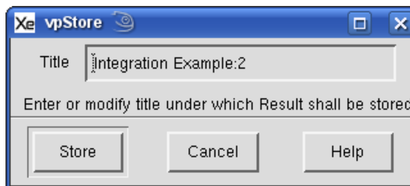


Figure 3-4 Storing the integral result in memory.

Return Clicking Return returns you to the Processing task bar.

Help Clicking Help causes a Help window to appear. Click More and Back navigates you between the different Help entries. Clicking Close closes the window.

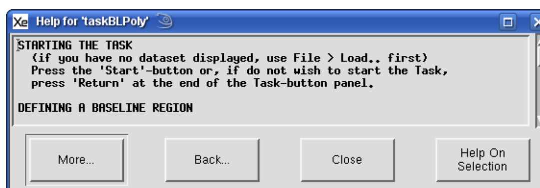


Figure 3-5 The Help window.

Revert to Input With some operations, datasets may be replaced by results. Clicking this button resets the datasets to what they were before the operation.

2D Datasets For 2D datasets, certain operations prompts you for further choices.

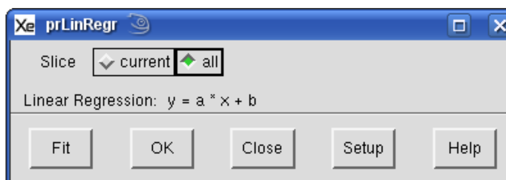
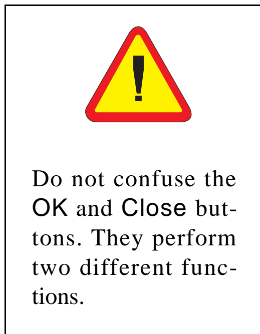


Figure 3-6 Selecting current or all for 2D datasets.

If the all radio button is clicked, the operation is carried out on all slices of the 2D dataset. If current is selected, the operation is only carried out on the current slice displayed in the viewport and the Result is a 1D dataset.

OK This button appears in many dialog boxes (See Figure 3-6.) and what it does can be a bit confusing at first. If you were to click Fit, a curve is fitted to the defined region and the result appears in the Result dataset. This is precisely what you want if you wish to proceed further to subtract this fitted curve from the Primary dataset. Clicking Close then closes the window.



Do not confuse the OK and Close buttons. They perform two different functions.

If you were to click OK, the curve is fitted and the result is transferred to the Primary dataset, displacing the original Primary dataset. This is what you want if you are only interested in the fitted curve. This is NOT what you want if you wish to subtract the fitted curve from the Primary dataset. The OK button does not close the window.

Baseline Correction 4

Sometimes EPR spectra may have offsets, sloping baselines, or background signals from other species. This can be particularly problematic for integrations. (See Section 6.1 and Section 6.2.) Often these problems can be remedied by performing a baseline correction. Portions of the EPR spectrum are selected as “baseline”, *i.e.* not part of the EPR signal we are interested in. A polynomial or spline is fitted to the selected portion and then subtracted from the EPR spectrum to yield the baseline corrected spectrum. The Baseline Correction task bar is started by clicking Baseline Correction in the task bar. A new task bar then appears.

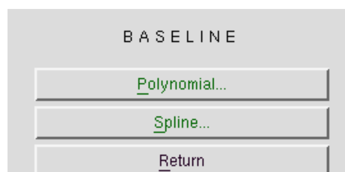


Figure 4-1 The two choices for baseline correction.

How to Fit a Polynomial 4.1

1. **Click the Polynomial button.** A new task bar then appears.

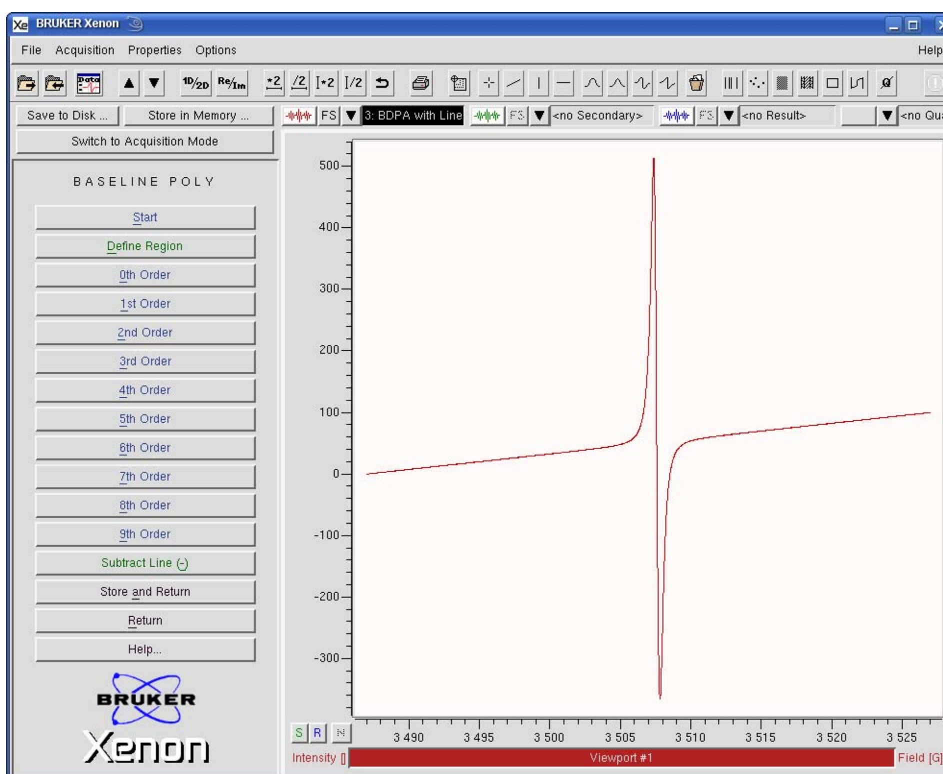


Figure 4-2 A spectrum requiring baseline correction.

2. **Define the baseline region.** Click Define Region. Click and drag the cursor to select the region or regions that you wish to define as

baseline. By default, the Qualifier is a Region Qualifier. Resize the qualifiers if necessary.

3. **Fit a polynomial.** Click one of the Nth Order buttons to fit a polynomial of order N to the baseline signal. (In this case, 1st Order works very well.) The fitted polynomial then appears in the Result dataset and is the blue trace in the viewport.

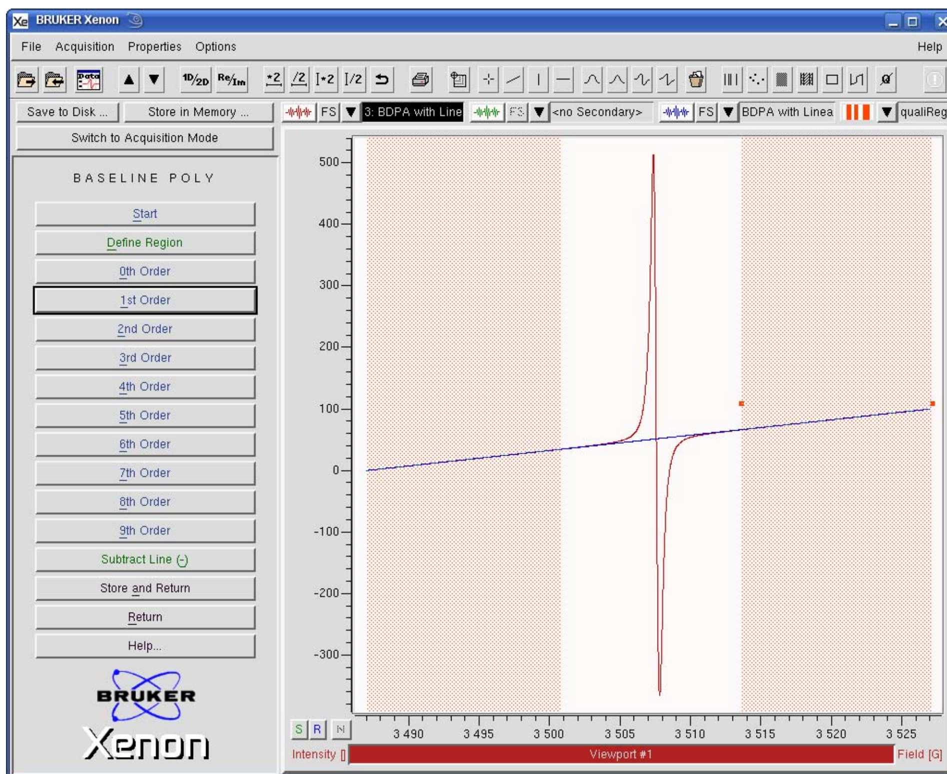


Figure 4-3 Two Region Qualifiers and a fitted line.

If your first choice is not satisfactory, you can try again by choosing another order polynomial. You can also modify the region qualifiers.

If you have a 2D dataset, a new window pops up. If you wish to fit and subtract the baseline from each slice, it is important that you select all. Otherwise when you perform the subtraction in the next step, the result is a 1D baseline corrected spectrum of the currently active slice.

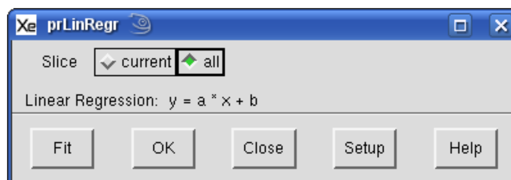


Figure 4-4 Selecting all for 2D datasets.

Clicking Close closes the window and the fitted baseline appears in the Result dataset. Do not click OK if you wish to subtract the baseline.

4. **Subtract the baseline.** Click Subtract Line. The baseline corrected signal then appears in the Result dataset.

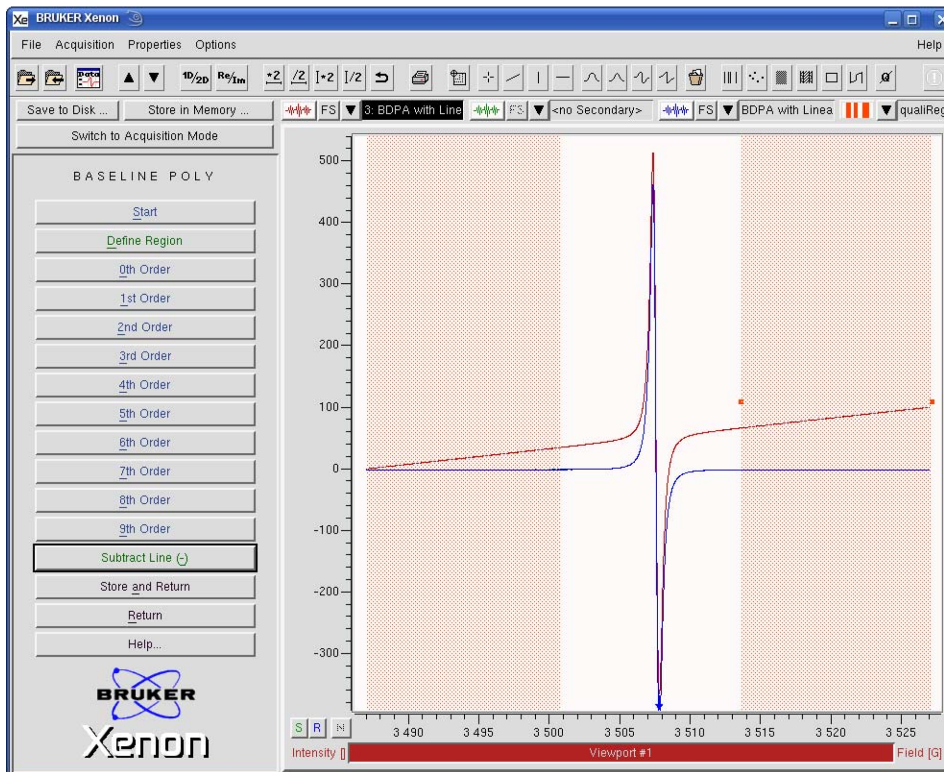


Figure 4-5 The baseline corrected spectrum.

5. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant baseline corrected dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the baseline corrected dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

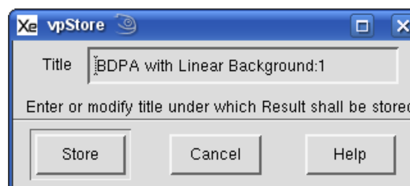


Figure 4-6 Storing the baseline corrected spectrum in memory.

How to Fit a Cubic Spline 4.2

Often, polynomials are not the best basis set for accurately fitting baselines. Even a sixth order polynomial does not work well for the example shown below.

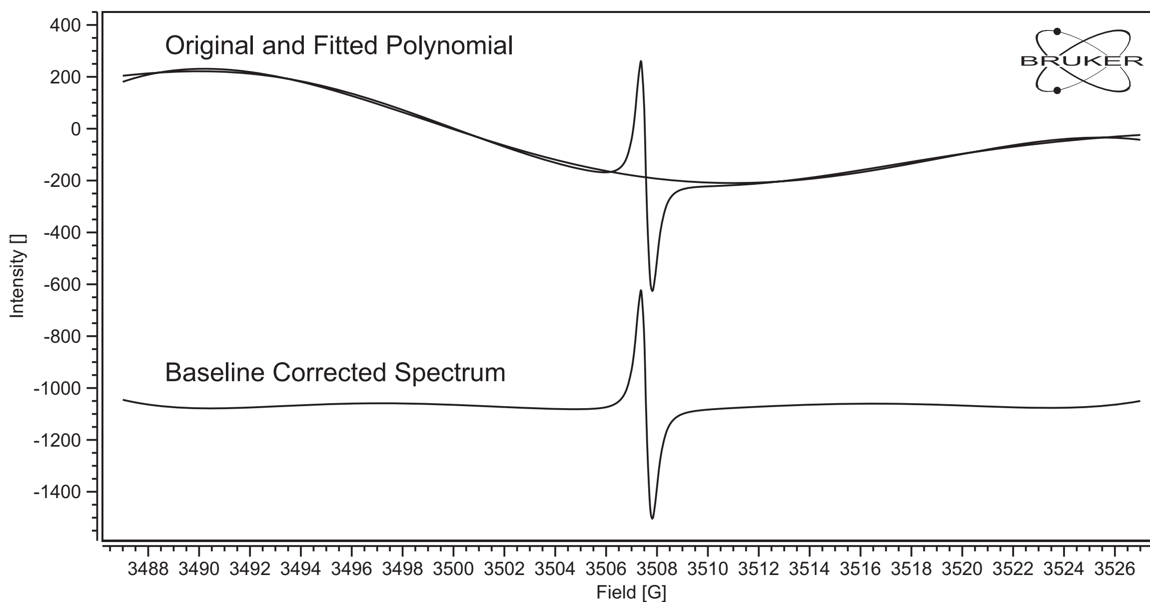


Figure 4-7 A spectrum with a baseline not easily fitted by a polynomial.

An alternative is to select a set of individual points as baseline and fit a cubic spline. A cubic spline smoothly interpolates the data points between the individual points. It is a bit more work than the polynomial baseline correction because individual points need to be defined, however it can usually fit baselines better than polynomials.

1. **Click Spline.** A new task bar then appears.

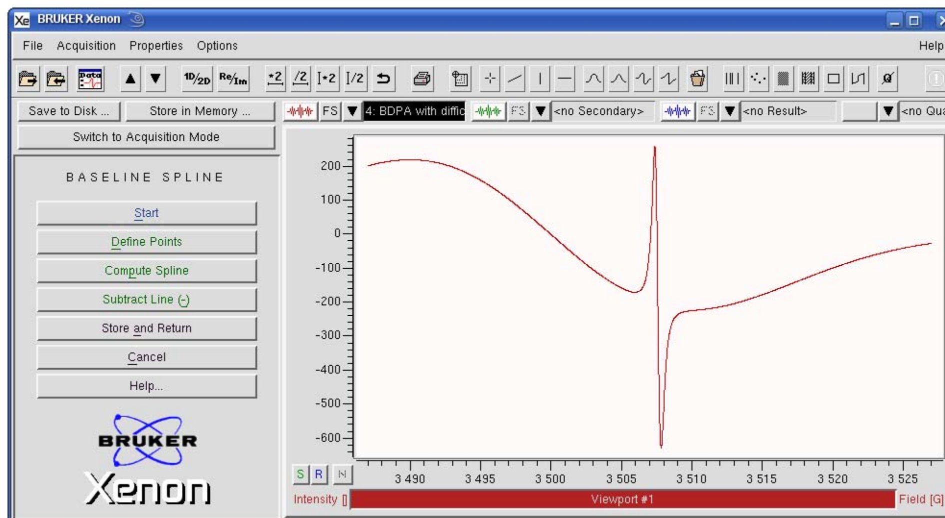


Figure 4-8 A spectrum requiring baseline correction more complicated than a polynomial.

2. **Define the baseline points.** Click Define Points. Left click individual points with the mouse to define the baseline points. Your cursor must be on the spectrum in order for the qualifier to be set. In contrast to the polynomial fitting, the default is a Position Qualifier and not a Region Qualifier. The corrections are performed only between the left most and right most qualifier. In order to ensure the corrections are performed across the complete dataset, the first and last point of the dataset must be qualified.

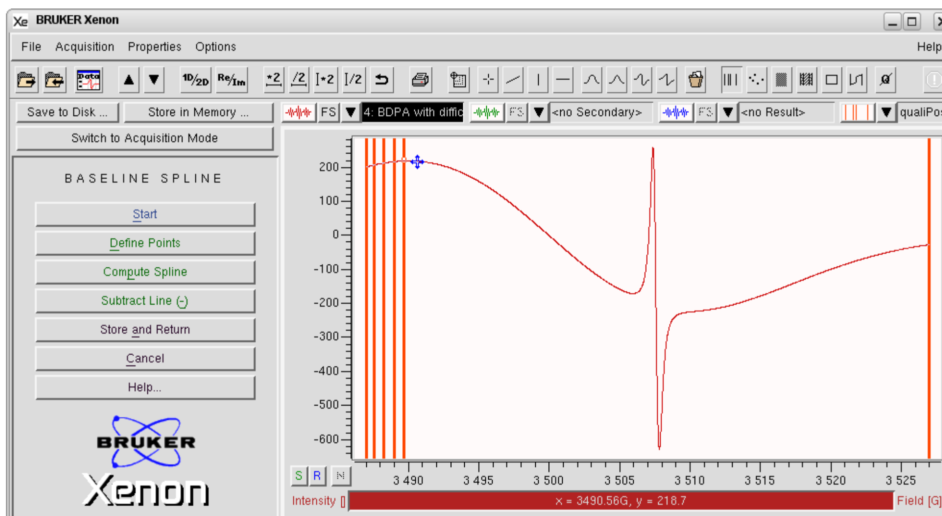


Figure 4-9 Setting baseline points.

3. **Fit a spline.** Click Compute Spline to fit a spline to the selected baseline points. A new window pops up. If you have a 2D dataset and if you wish to fit and subtract the baseline from each slice, it is important that you select all. Otherwise when you perform the subtraction in the next step, the result is a 1D baseline corrected spectrum of the current active slice.

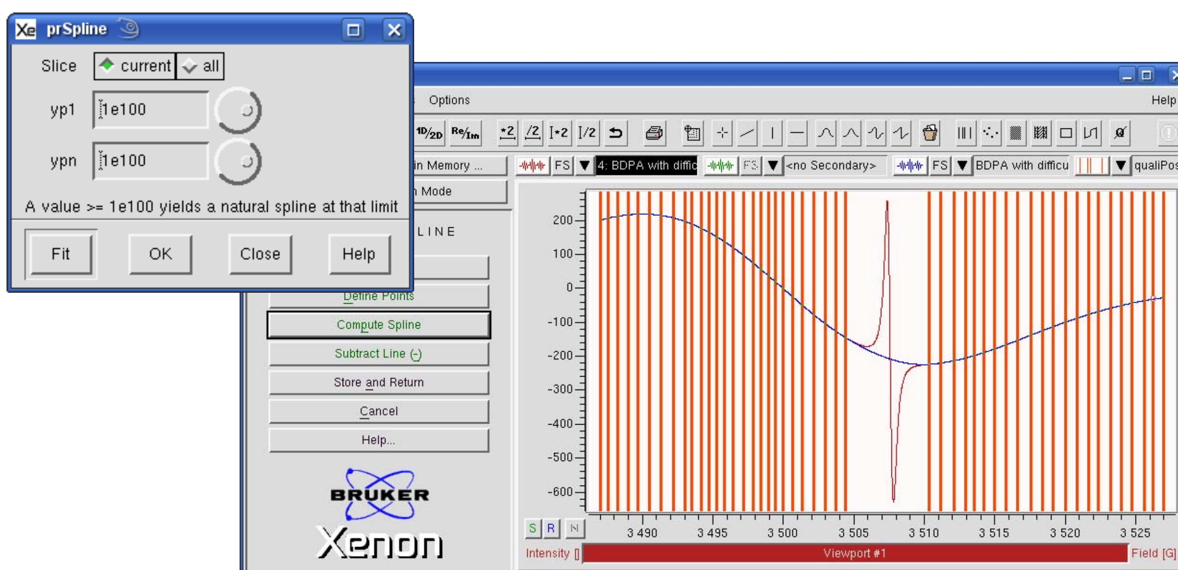


Figure 4-10 Fitting a spline to the baseline.

Click Fit. The fitted spline appears in the Result dataset and is the blue trace in the viewport. If your first choice is not satisfactory, you can try again by adding more qualifier points or removing some qualifier points. A position qualifier is removed by left-clicking it.

Clicking Close closes the window and the fitted baseline appears in the Result dataset.

4. **Subtract the baseline.** Click Subtract Line. The baseline corrected signal then appears in the Result dataset.

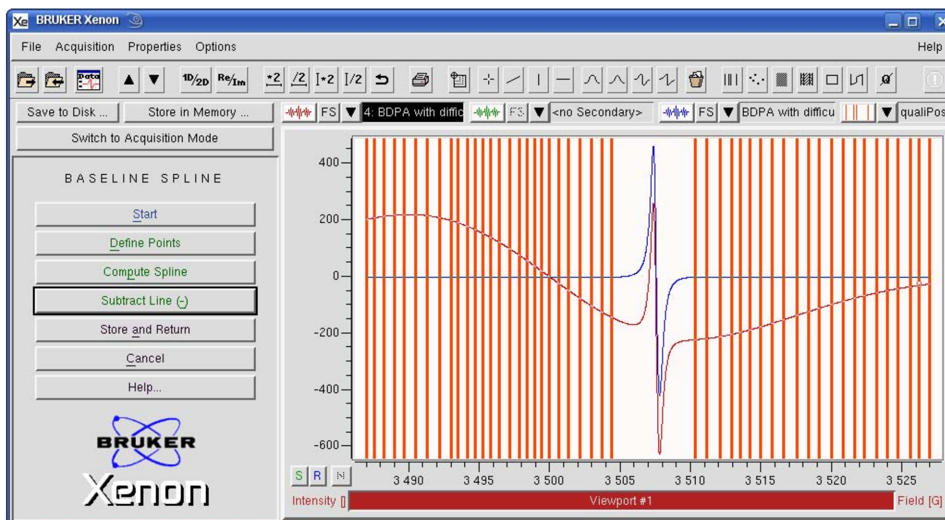


Figure 4-11 The baseline corrected spectrum.

5. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant baseline corrected dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the baseline corrected dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

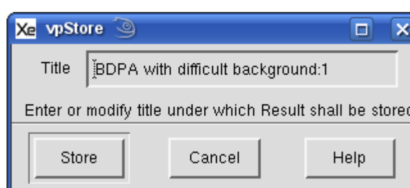


Figure 4-12 Storing the results in memory.

Peak Picking 5

The line positions in an EPR spectrum are important for interpretation of the spectrum. Peak picking is a means of automating the measurement of line positions. Peaks and troughs (maxima and minima) are searched for in the qualified area. In the case of first derivative spectra, it is the zero crossover that is the picked quantity. In this case, the peak pick values of the peak and associated trough is calculated as:

$$x = \frac{x_{Peak} + x_{Trough}}{2} \qquad [5\text{-}1]$$

$$y = y_{Peak} - y_{Trough}$$

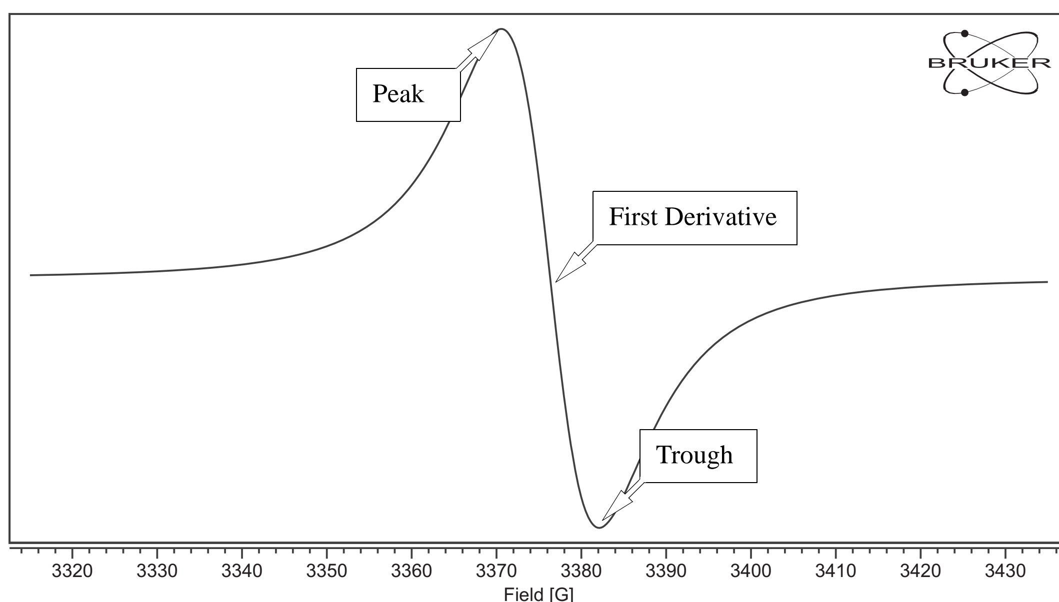


Figure 5-1 Options for peak picking.

Approaches to Peak Picking 5.1

Noise can interfere with the peak picking process. One means of dealing with this problem is to introduce a threshold. In order to be picked, the peak must be at least a certain amplitude. This problem can be remedied by using an area qualifier. With no qualifier defined, all points are valid for peak picking. Once a qualifier has been defined, the peak must lie within the qualifier in order to be picked.

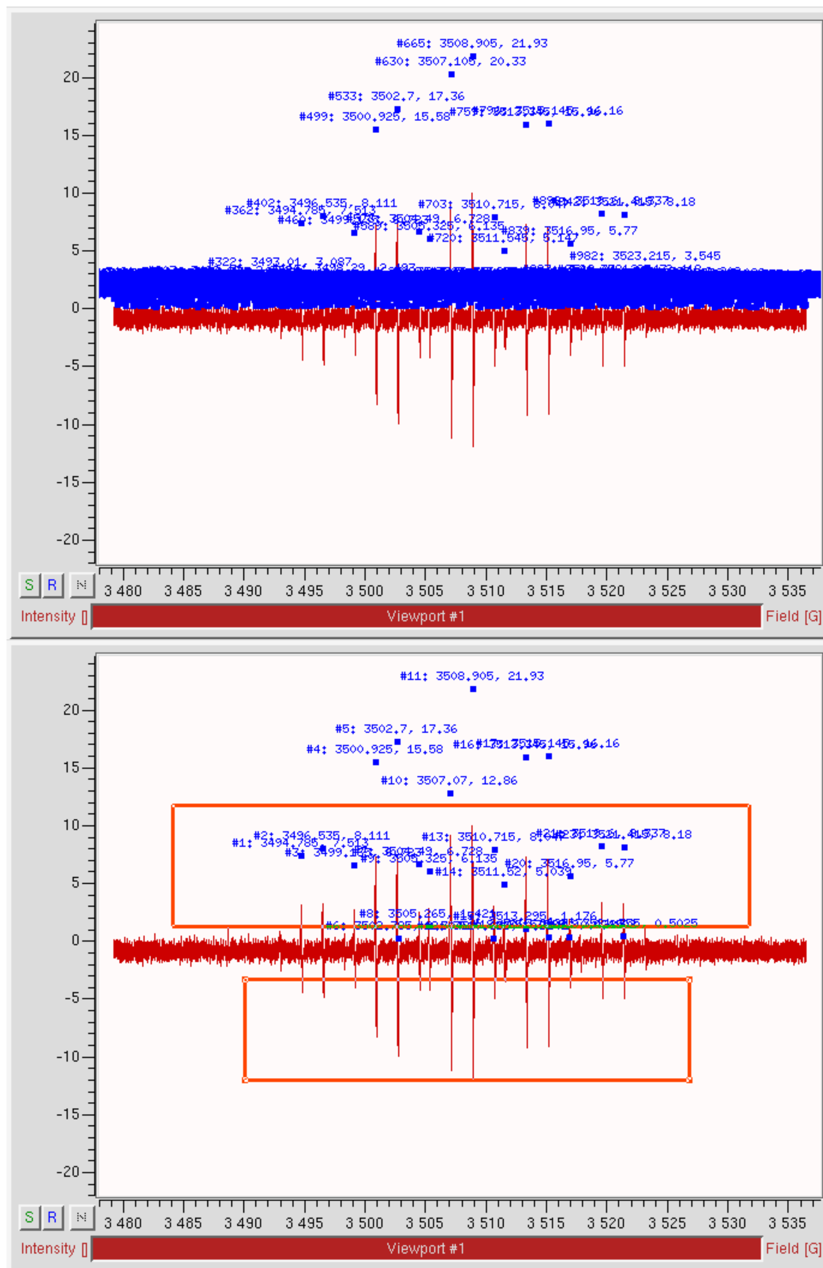


Figure 5-2 Suppressing excess noise peaks by using area qualifiers to define a threshold.

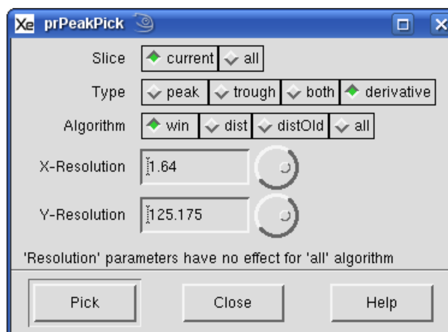


Figure 5-3 The Peak Picking menu.

There are four algorithms for finding peaks. The first is the all method. It simply looks for minima and maxima in the qualified area. This method may still pick too many peaks. To filter more of the peaks, there are three other algorithms.

The win (window) algorithm selects only peaks that are local maxima (or minima) within a window of width X-Resolution and height Y-Resolution and both the spectrum lines exit through the bottom (or top for troughs) of the window.

In Figure 5-4 both peaks are picked. In both cases, the spectrum lines exit through the bottom of the window.

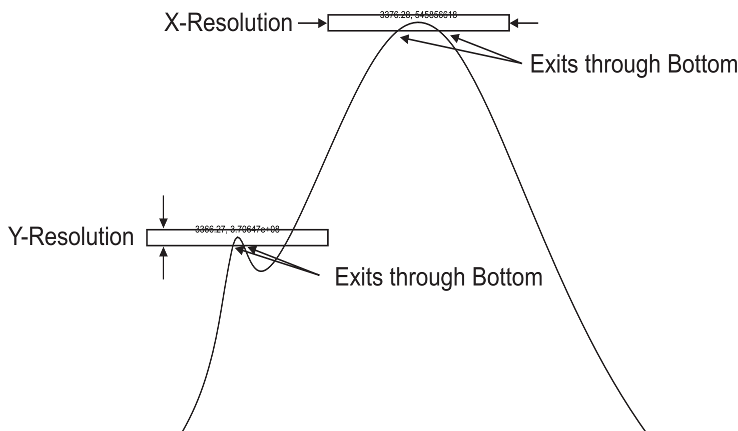


Figure 5-4 The win peak picking algorithm. The rectangle represent the window whose width is equal to X-Resolution and height is equal to Y-Resolution.

In Figure 5-5 only the smaller peak is picked because in the case of the larger peak, the spectrum lines exit through the sides of the window.

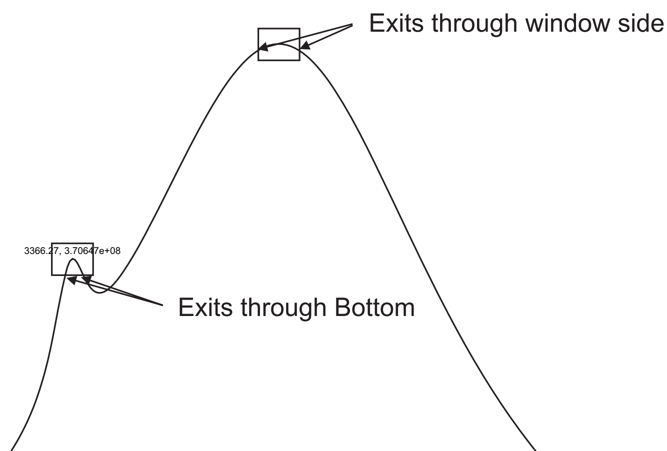


Figure 5-5 An example of peak filtering using the win peak picking algorithm.

In Figure 5-6 only the large peak is picked because the smaller peak's right spectrum line is exiting out the top of the window. Increasing the Y-Resolution also acts in a similar fashion as increasing the threshold for noise suppression.

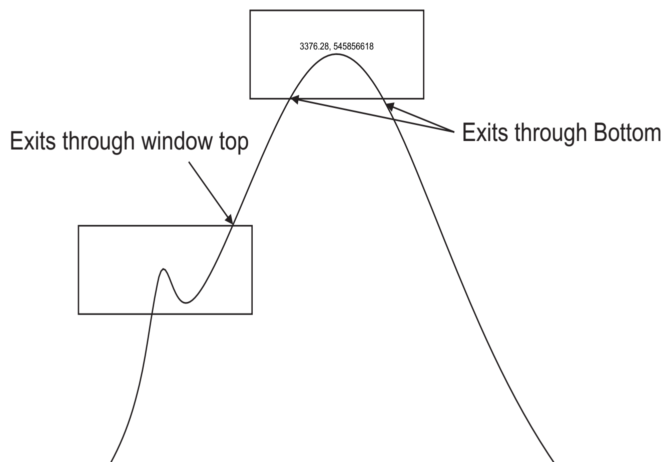


Figure 5-6 Another example of peak filtering using the win peak picking algorithm.

Another algorithm is the dist (distance) algorithm. Only local maxima that are separated by at least X-Resolution from the next local maximum and the trough depth between the two maxima is greater than Y-Resolution are picked. Only local minima that are separated by at least X-Resolution from the next local minimum and the peak height between the two minima is greater than Y-Resolution are picked.

Both peaks in the example shown in Figure 5-7 are picked because they are separated by more than X-Resolution and the difference between the peak and trough is greater than Y-Resolution.

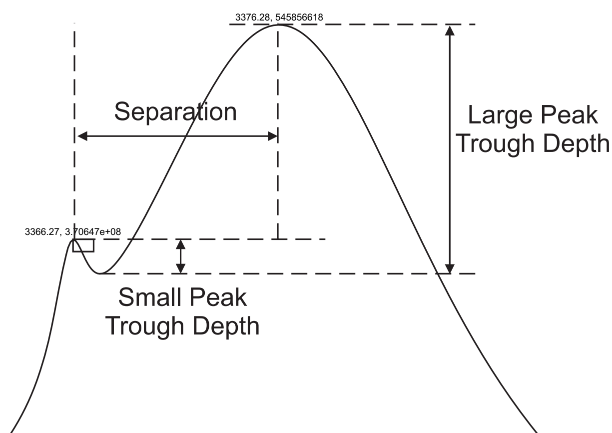


Figure 5-7 The dist peak picking algorithm. The rectangle's width represents the X-Resolution and its height, Y-Resolution. Both peaks are picked because their size and separation are larger than X-Resolution and Y-Resolution respectively.

Only the large peak is picked in the example shown in Figure 5-8 because the smaller peak has a peak-trough difference less than Y-Resolution.

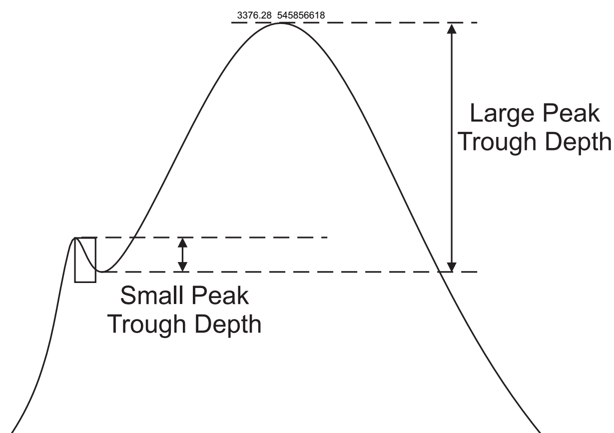


Figure 5-8 The dist peak picking algorithm. The rectangle's width represents the X-Resolution and its height, Y-Resolution. The smaller peak is not picked because the trough depth is less than Y-Resolution.

The examples below have the same X-Resolution and Y-Resolution but use two different algorithms, dist Old and dist. Only one peak is picked because the separation along the x axis is smaller than X-Resolution. For the dist Old algorithm, the smaller peak is picked. For the dist algorithm, the larger peak is picked.

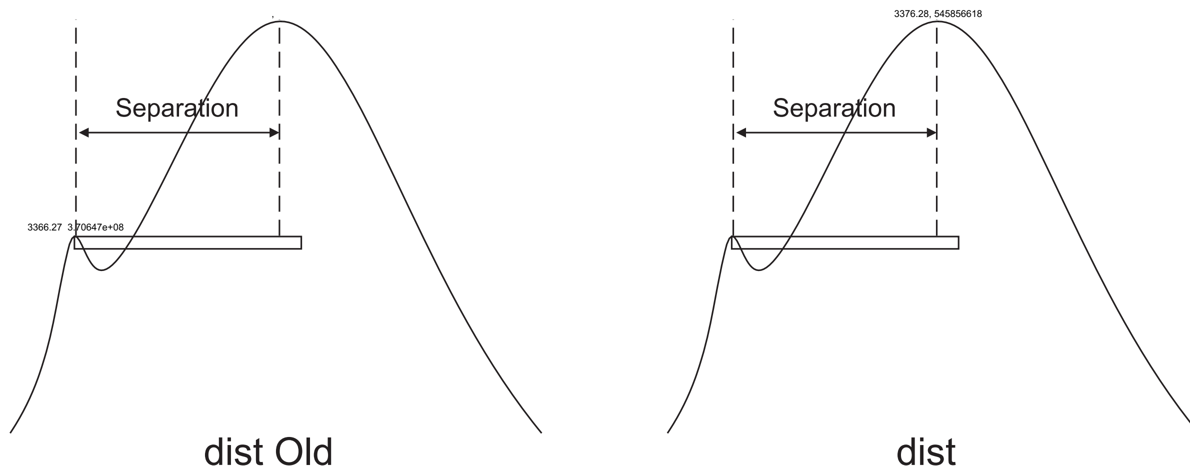


Figure 5-9 The dist peak picking algorithm. The rectangle's width represents the X-Resolution and its height, Y-Resolution. Only one peak is picked because the separation between the peaks is less than X-Resolution. The difference in results stems from the different algorithms used. The larger peak is picked with dist and the smaller with dist Old.

How to Peak Pick 5.2

The Peak Picking task is started by clicking the Peak Picking button in the task bar. A new task bar then appears.

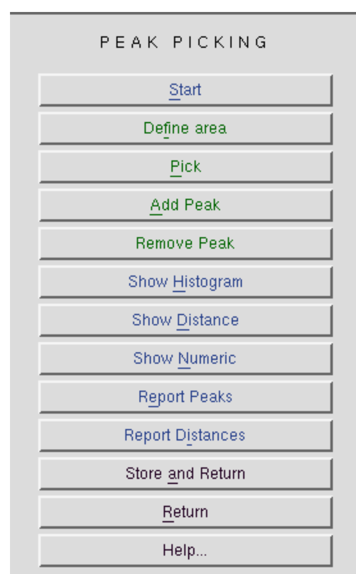


Figure 5-10 The available operations for peak picking.

It is assumed you are already in the Peak Picking task bar and the spectrum is in the Primary dataset. Peak Picking performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Section Figure 2-3.) Then the operation is performed on the Secondary dataset. Here is how to pick peaks:

1. **Load a dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Click Pick.** Click Pick in the Peak Picking task bar. (See Figure 5-11.) A peak picking dialog box appears. If you have a 2D spectrum you have an option to pick peaks on the current slice or apply the operation to all the slices. Select Derivative for the Type. Then click Pick.

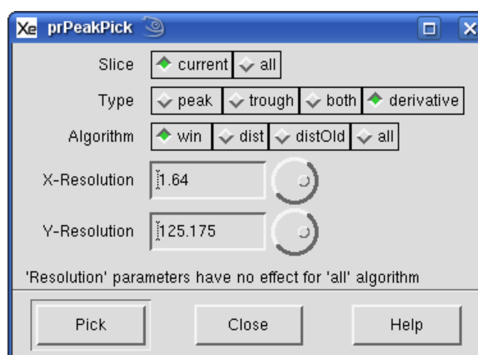


Figure 5-11 The Peak Picking menu.

The Peak Picking results are then displayed in the viewport.

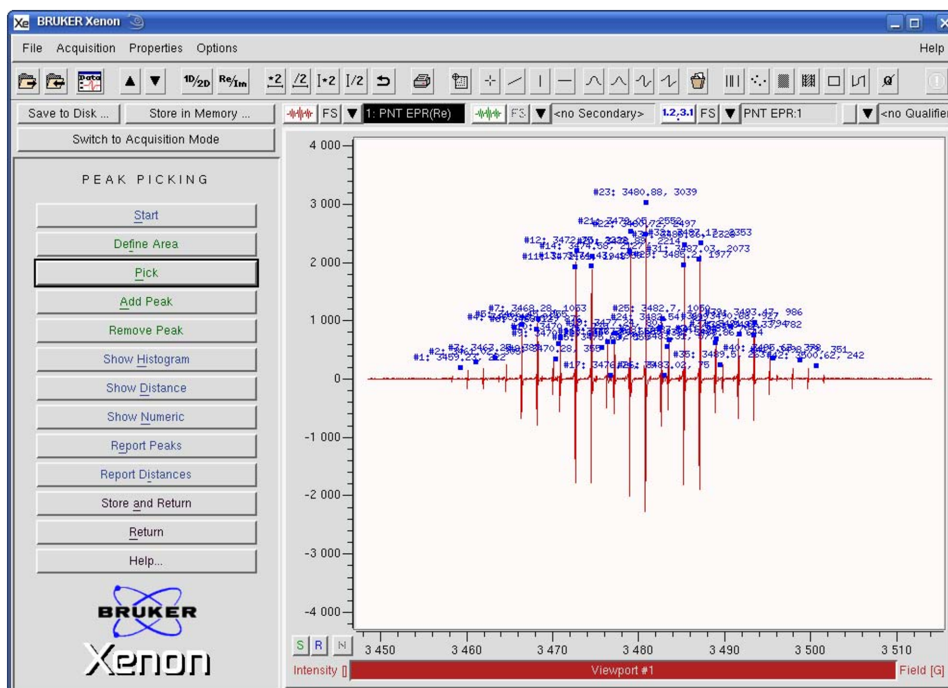


Figure 5-12 Peak Picking results.

4. **Change the resolution.** The X- and Y-resolutions set the threshold for picking the peaks. In Figure 5-12, the small peaks are still not picked. Reduce the Y-Resolution by turning the knob counter-clockwise in the dialog box. The small peaks are now picked by the Peak Picking function.

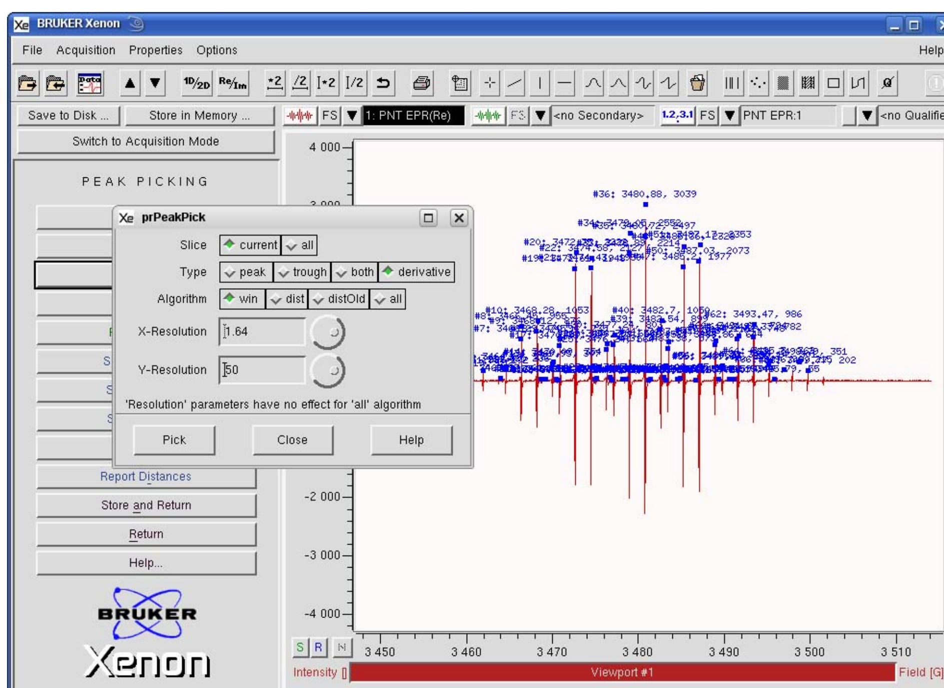


Figure 5-13 Changing the resolution.

5. **Define the Peak Picking area.** If you are only interested in the peaks of only a specific area of the spectrum, you can define the area for the peak picking function. Click the Define Area button. Click and drag with the mouse cursor to enclose the area for peak picking and then click the Pick button. The peaks within the area you defined are now picked. If you are picking the Derivative Type, you need to add an additional qualifier so that both the peaks and troughs are picked. Resize the qualifiers if needed.

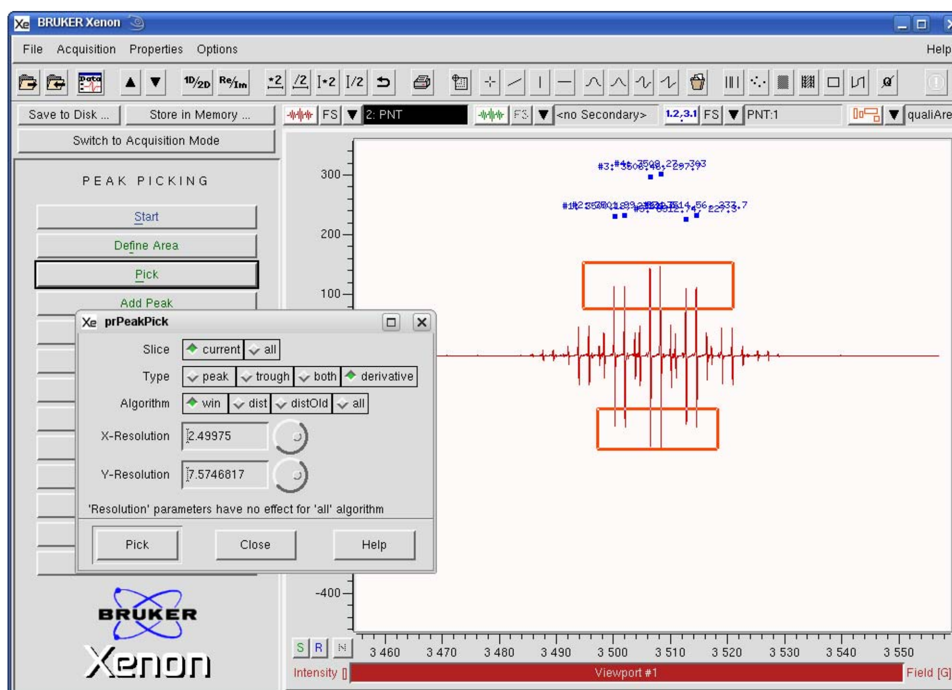


Figure 5-14 Defining the Peak Picking area.

6. **Add and Remove Peaks.** When dealing with multiple species or noisy spectra, it is difficult to pick only the peaks you want by adjusting the resolutions or qualifier area. Add and Remove Peak allow you to add the peaks that the program missed or remove the unwanted peaks. Click Add Peak (or Remove Peak) in the Peak Picking menu. Move the mouse pointer to where the peak is or would like one to be and click the left mouse button. The peak is added (or removed).

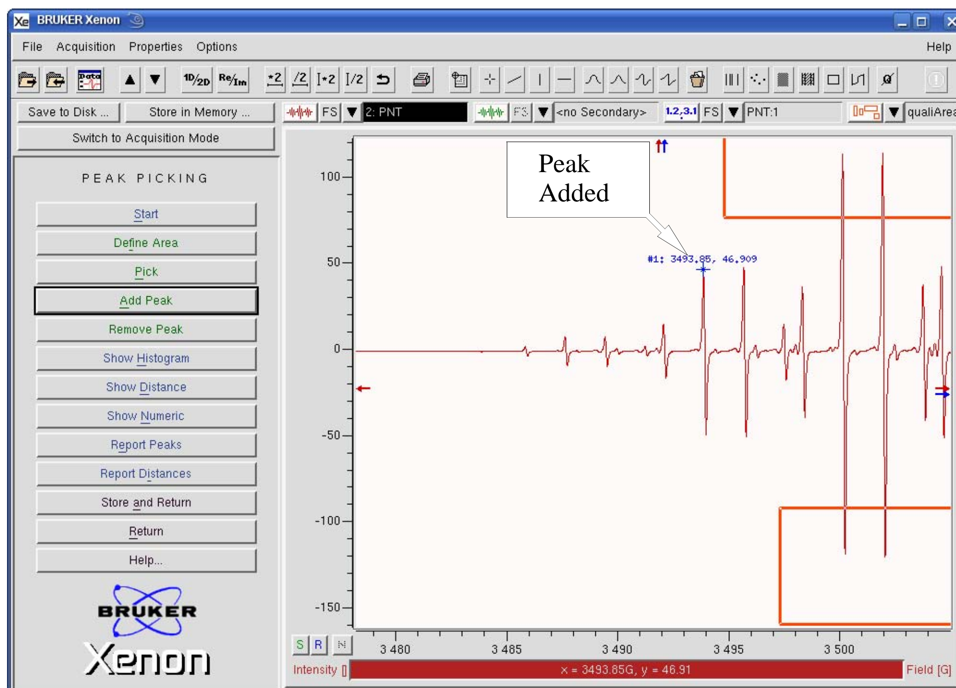


Figure 5-15 Adding a peak.

7. **Show a Histogram.** You can select to display the peaks picked in numerical (default) or histogram format. Click Show Histogram in the Peak Picking menu. The numbers change to vertical bars.

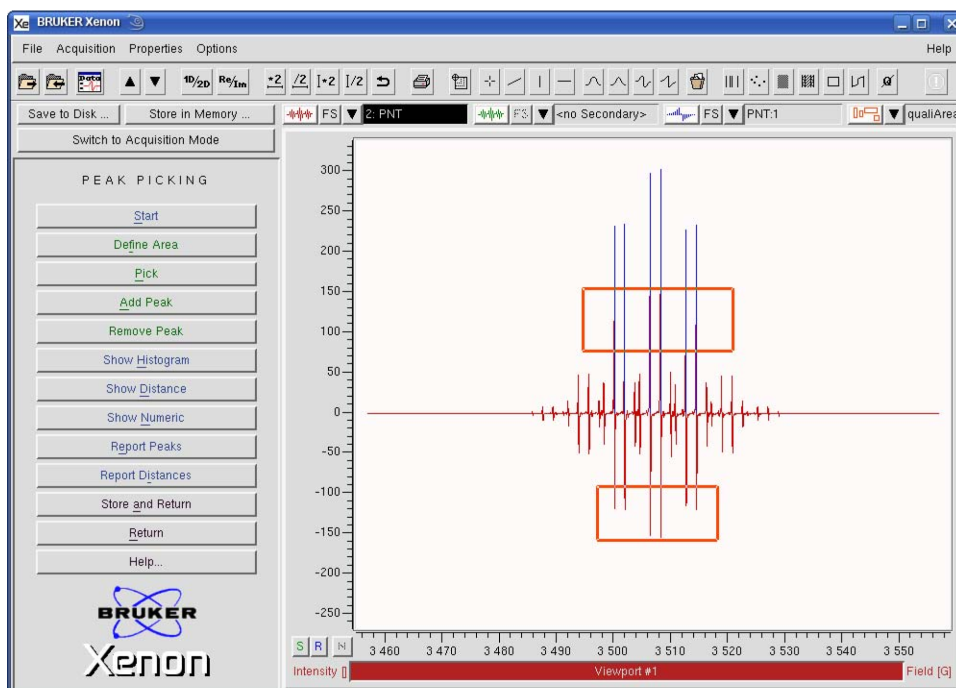


Figure 5-16 A Histogram display of the Peak Picking results.

8. **Show Distance.** You can also display the distances or splittings between the peaks. Click Show Distance and the splittings are then displayed.

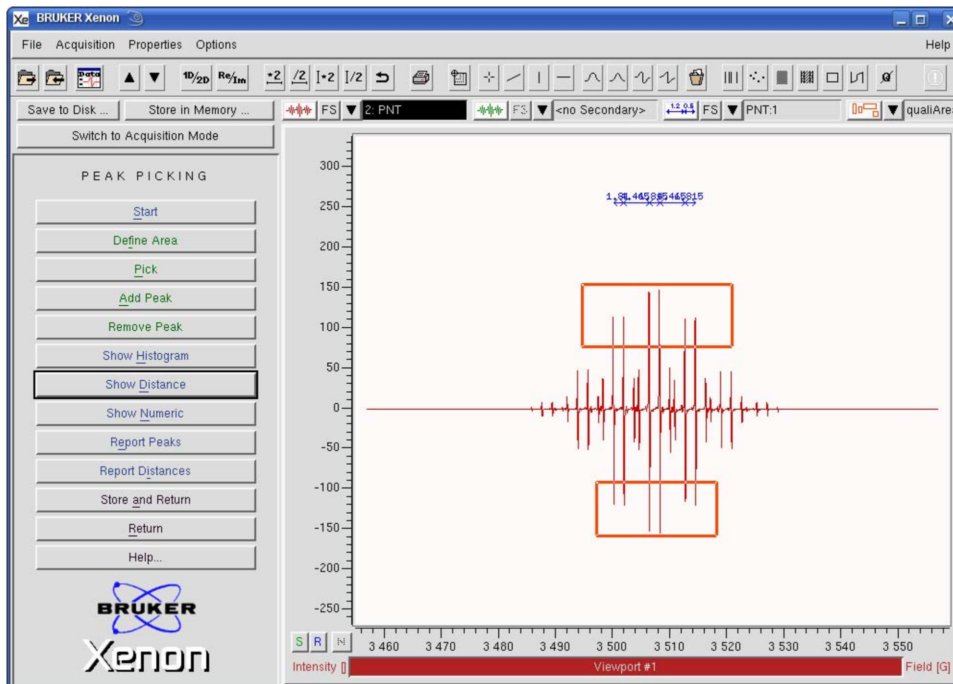


Figure 5-17 A Distance display of the Peak Picking results.

9. **View the peaks report.** Click Report Peaks and a window with a list of all the picked peaks appears. You can save the results in an ASCII file by simply clicking Save.

Contents of 'Peak List'

index	Field [G]	Intensity []
1	3500.185	232.22877967354
2	3501.995	234.537450159621
3	3506.46	297.696197059887
4	3508.275	302.987266362503
5	3512.74	227.250822823701
6	3514.555	233.694365086815

Close Save Help On Selection

Figure 5-18 Report of the peak picking result.

A new dialog box appears to prompt you for a filename and folder where results are to be saved. Click Save to continue.

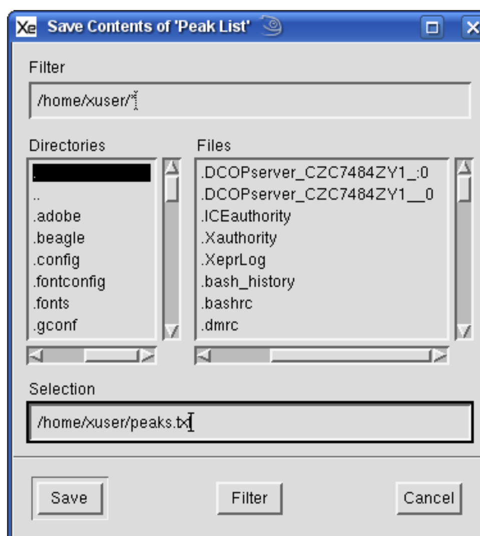


Figure 5-19 Saving the peak picking results.

10. **View the distances report.** Click Report Distances. The distance results are then displayed. This window works in a similar fashion as the Report Peaks window.

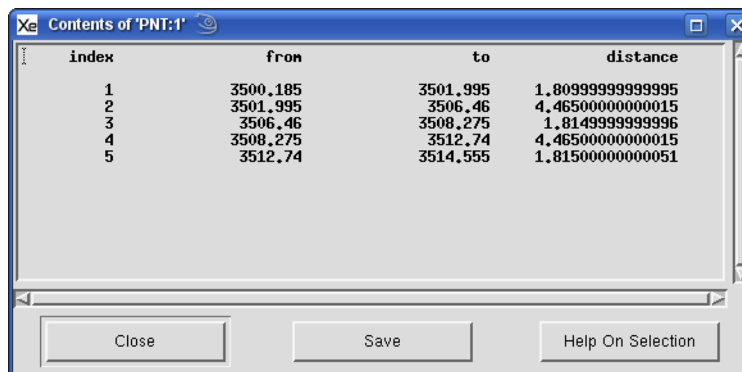


Figure 5-20 Viewing the distance results.

11. **Store the peak picking results in memory.** Click Store and Return and enter a Title. Then click Store. The picked peaks are stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the peak picked dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

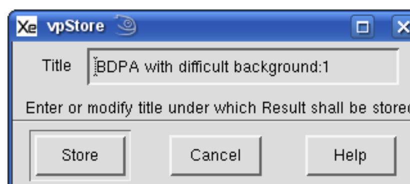


Figure 5-21 Storing the results in memory.

Integration and Differentiation 6

Double integration of the first derivative EPR spectrum is commonly used to quantitate EPR samples. Because most spectrometers record the EPR signal as a first derivative of the absorption signal, we have to integrate the spectrum once to recover the absorption spectrum and then integrate a second time to obtain the area under the absorption curve.

The data are normalized with respect to Receiver Gain, Conversion Time, and number of averages. They are not normalized with respect to Microwave Power and Modulation Amplitude. It is therefore recommended that you use the Quantitative EPR task described in Section 13.5 for counting spins or measuring concentrations.

The Integration and Derivative task is started by clicking the Integration and Derivative button in the task bar. A new task bar then appears.

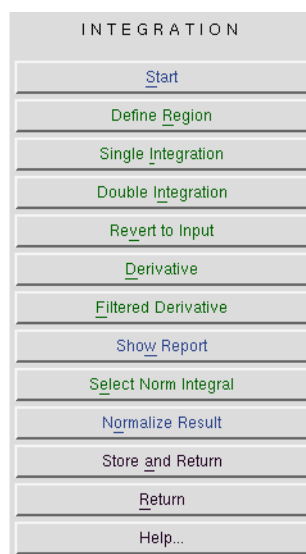


Figure 6-1 The available operations for integration and differentiation.

Approaches to Integration 6.1

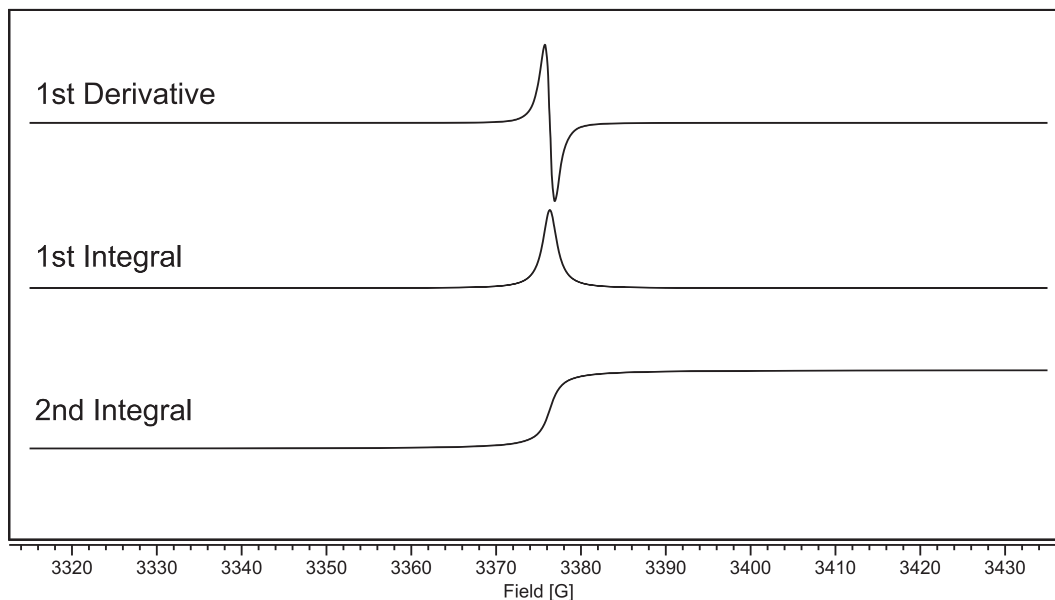
It is important to realize that even slight baseline drifts, background signals, or a very low signal to noise ratio can be detrimental to the accuracy of your double integrations. Fortunately, there are many ways to avoid these problems.

One means is to use signal averaging with a lower time constant as opposed to a signal sweep with a long time constant as shown in Figure 2-47 of the Xenon User’s Guide to suppress baseline variations.

The Integration task includes techniques to correct for offsets and slopes in the backgrounds, however you may have a more complicated background. For such cases, you need to use the Baseline Correction routines described in Section 4.

Why is the integral so sensitive to backgrounds? If we look at a slight offset in the level of a spectrum, when we integrate this constant offset along with the spectrum, it introduces a linear baseline. If we integrate twice, it results in a quadratic baseline which can become large quickly.

The upper part of Figure 6-2 shows what to expect with no offset or baseline. The first integral returns to zero at the end. The double integral becomes a straight line leveling off at the value of the integral. If there is a slight constant offset, then the first integral has a slight negative linear baseline. The double integral is very distorted and cannot be use for proper quantitation.



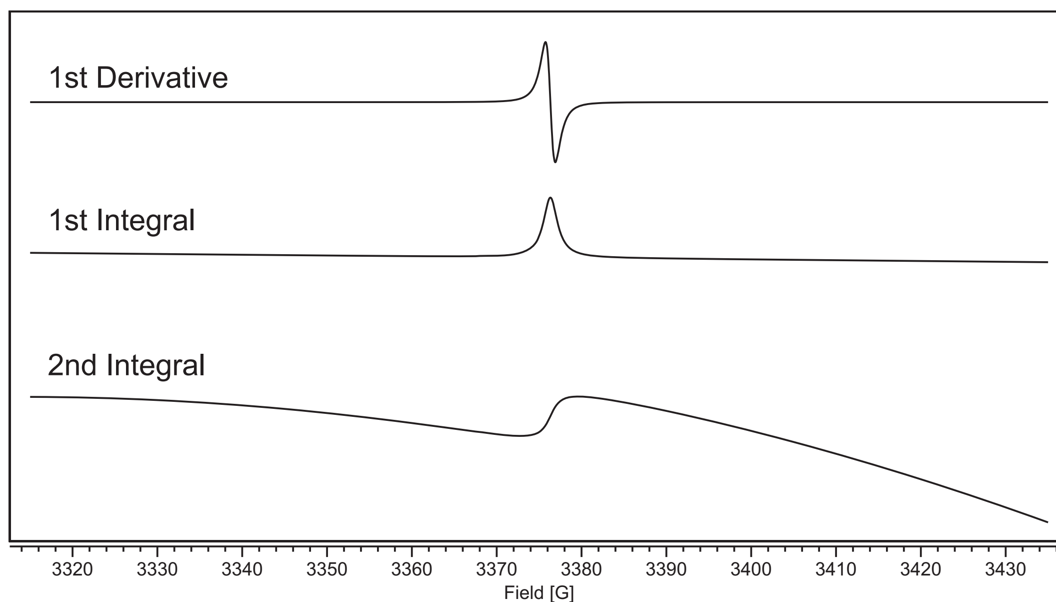


Figure 6-2 The effect of a small negative offset on the integrated EPR spectrum.

How to Integrate 6.2

It is assumed you are already in the Integration and Derivative task bar and the spectrum is in the Primary dataset. Integration performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Section Figure 2-3.) Then the operation is performed on the Secondary dataset. Here is how to pick peaks:

1. **Load a dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Define the baseline and integration region.** Clicking on the Define Region button activates the Baseline qualifier definition mode. The mouse cursor changes into an x shape. Clicking and dragging the mouse cursor creates a Baseline qualifier consisting of four lines and three shaded areas. The center region is the region to be integrated. The outer two regions are selected as baseline. A straight line is fitted to the baseline regions and this fitted line is then subtracted from the EPR dataset when it is integrated.

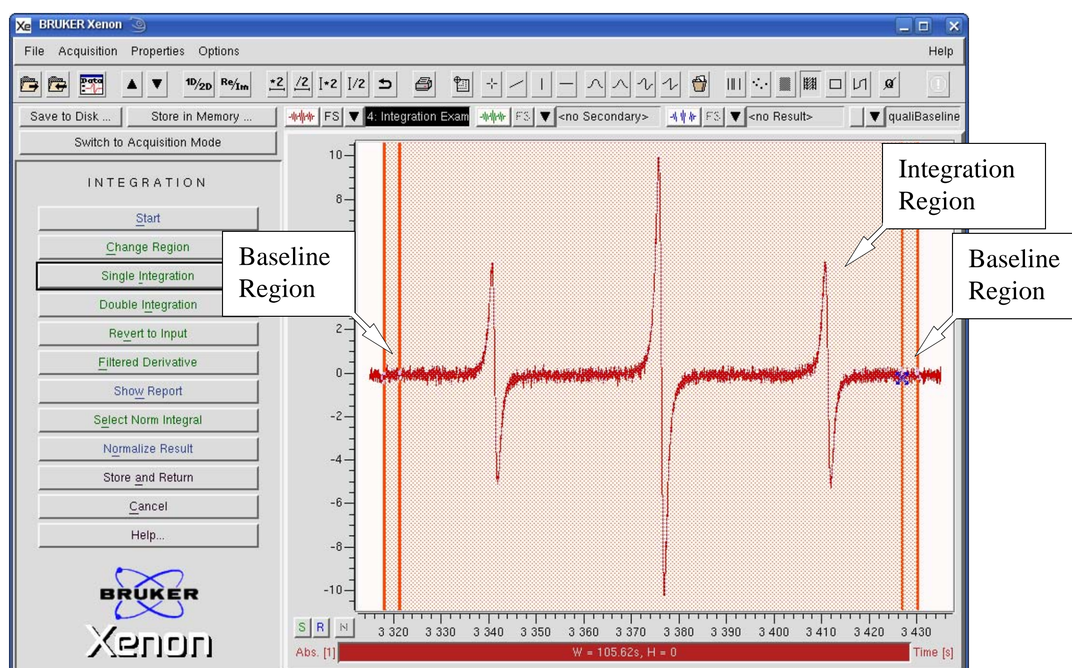


Figure 6-3 Defining the integration region.

Don't be worried if the region selection was not perfect. We now edit the regions. Click the Change Region button; now we can click and drag the lines to change their positions and thereby change the regions. The second and third lines control start and end points of the integration region. You may notice that the widths of the baseline regions do not change as these lines are dragged. The first line follows the second line and the fourth line follows the third line.

The baseline region widths are changed by clicking and dragging the first or fourth line. You may notice that both widths are linked. Changing the position of the first line changes the position of the fourth line and vice versa.

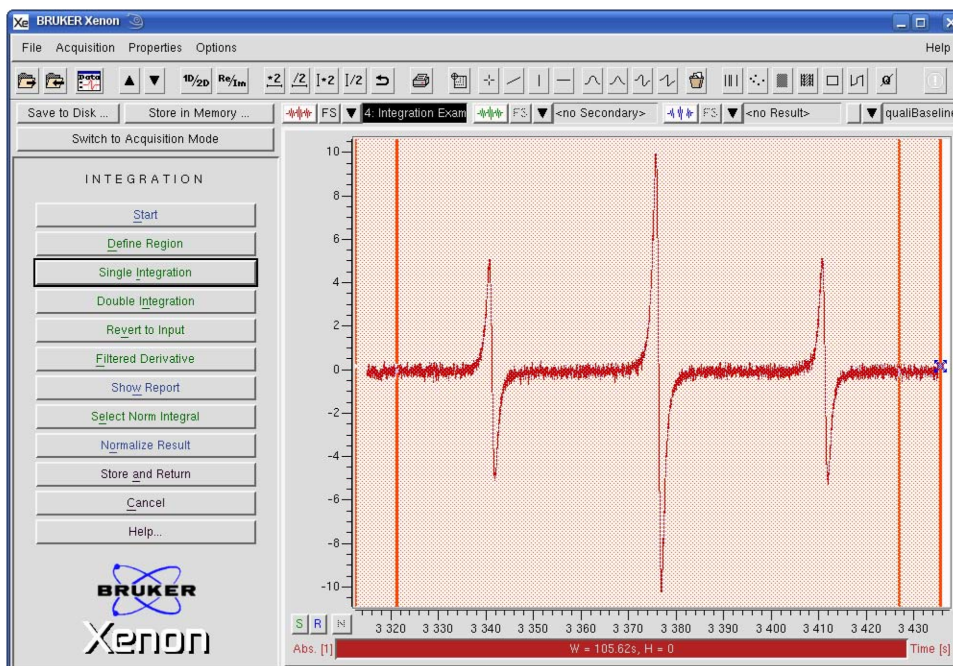


Figure 6-4 Defining the baseline regions.

It may take a few iterations to get what you want. Once you are satisfied, proceed to integration.

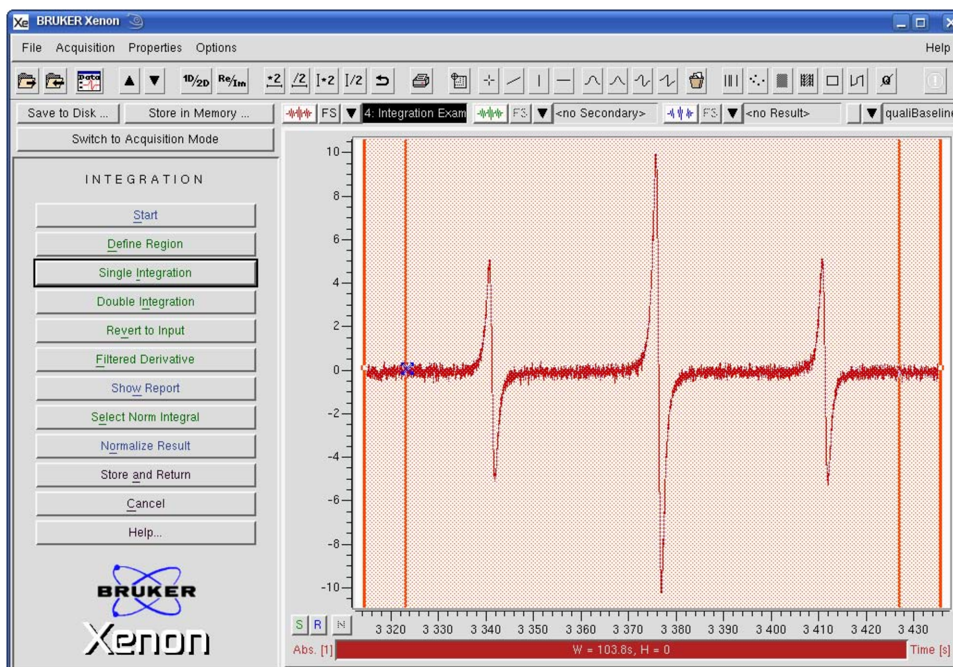


Figure 6-5 Well chosen integration and baseline regions.

4. **Integrate the spectrum.** Click Single Integration. The region of the spectrum you selected is integrated once and displayed in the Result dataset.

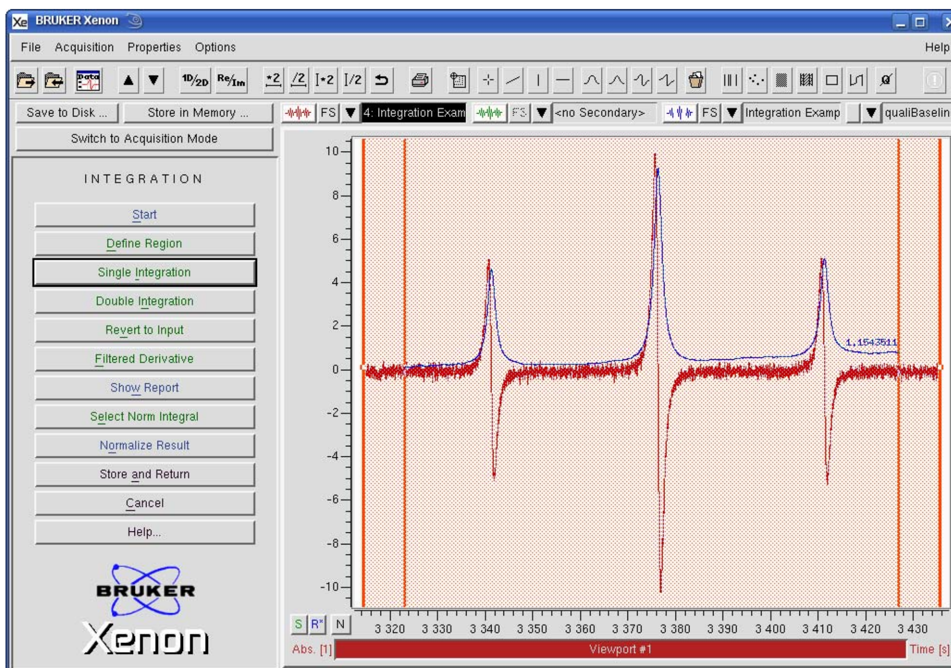


Figure 6-6 The first integral of the selected region.

5. **Double integrate the spectrum.** Click Double Integration. The region of the spectrum you selected is integrated twice and displayed in the Result dataset.

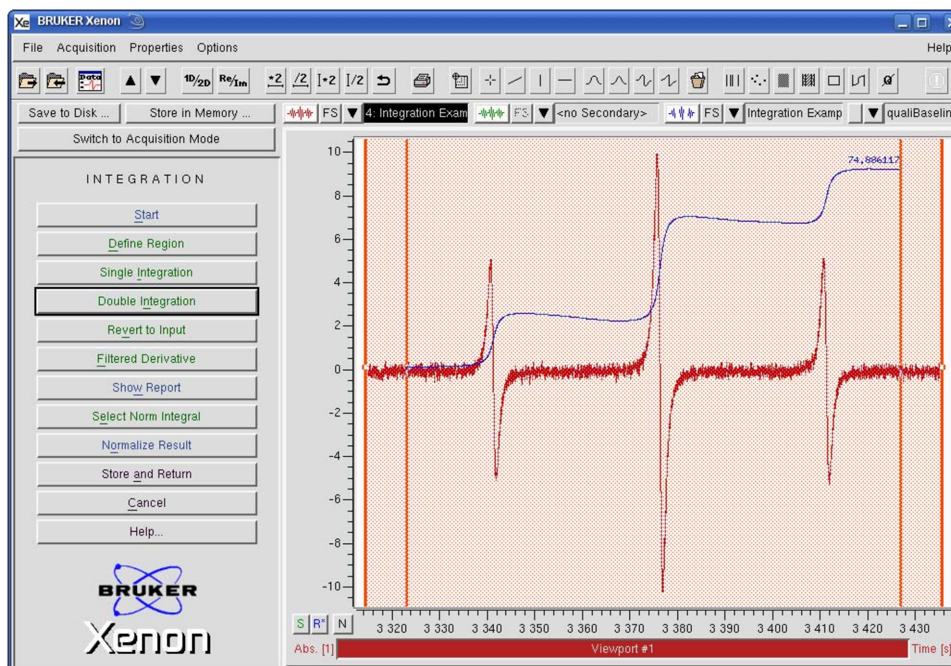


Figure 6-7 The double integral of the selected region.

6. **Select individual peaks for integration.** You can integrate each EPR peak separately with Xenon. Click Define Region and move the qualifier to cover the EPR peaks you want to integrate. Repeat this procedure until all the peaks you want to integrate have been covered by individual qualifiers. Note that regions can overlap. The overlapping regions appear as white areas. Click Change Region and fine adjust each qualifier.

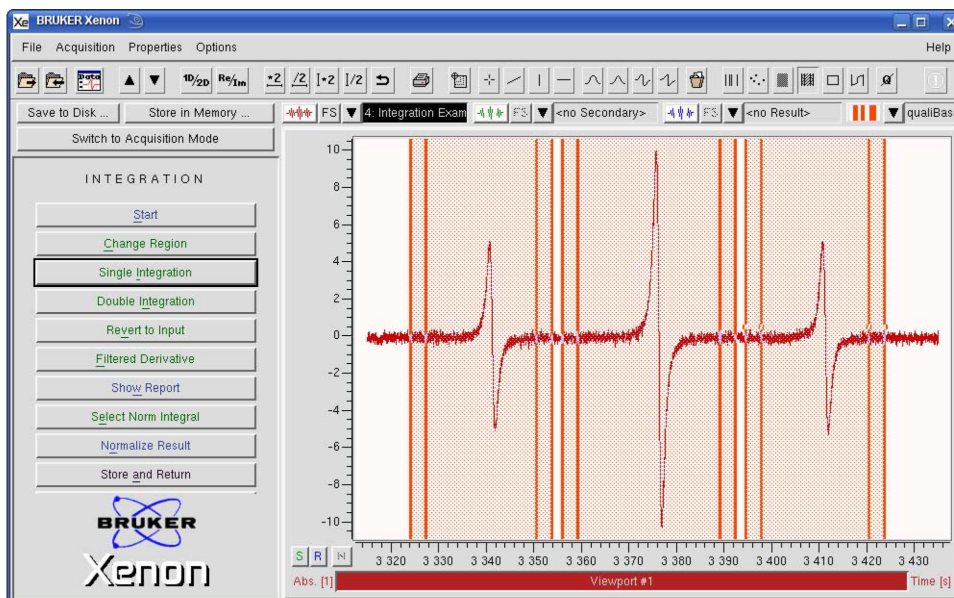


Figure 6-8 Selecting regions with individual qualifiers.

7. **Double integrate the individual peaks.** Click Double Integration. The double integrals of all the regions you selected are displayed in the viewport.

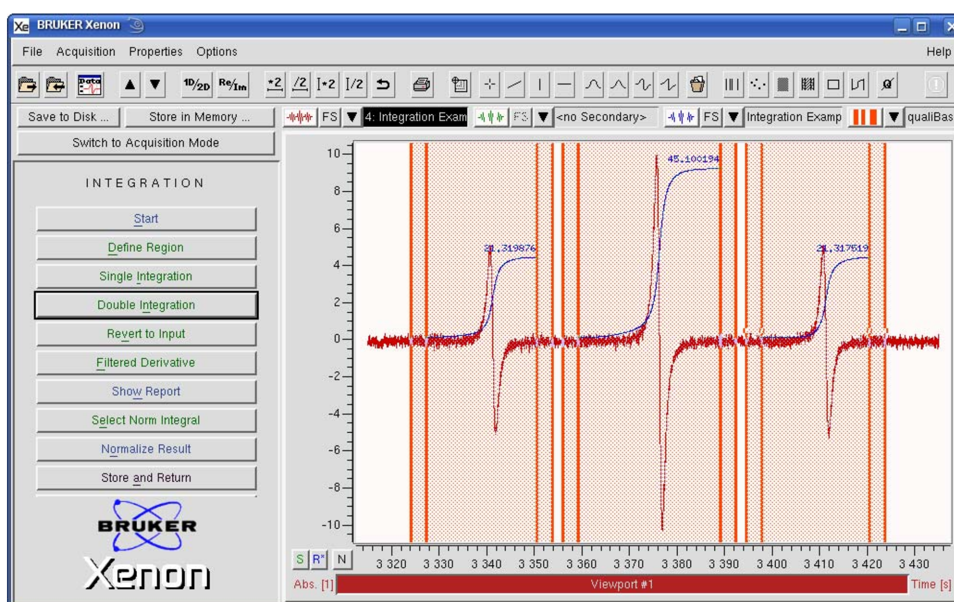


Figure 6-9 Double integration of individual peaks.

8. **Normalization to one of the integrals.** Quite often, the relative ratios of the integrals is important in the data analysis. (*E.g.* hyperfine patterns and quality of a spectrum.) You can normalize the integrals with respect to one of the integrals to obtain these relative ratios. There are three ways to do this. If you simply click Normalize Result, the highest value integral is selected as the normalization constant to normalize the rest of the integrals.

$$\text{Normalized Integral} = \frac{\text{Integral Value} \times 100\%}{\text{Normalization Constant}} \qquad [6\text{-}1]$$

Should you wish another integral for normalization, first click Select Norm Integral and then Normalize Result. A dialog box appears allowing you to select the integral for normalization. Click the up or down arrow to select the integral. Integrals are labeled numerically increasing from left to right. Click the Normalize button in the dialog box. All the integrals are normalized with respect to the integral you select.

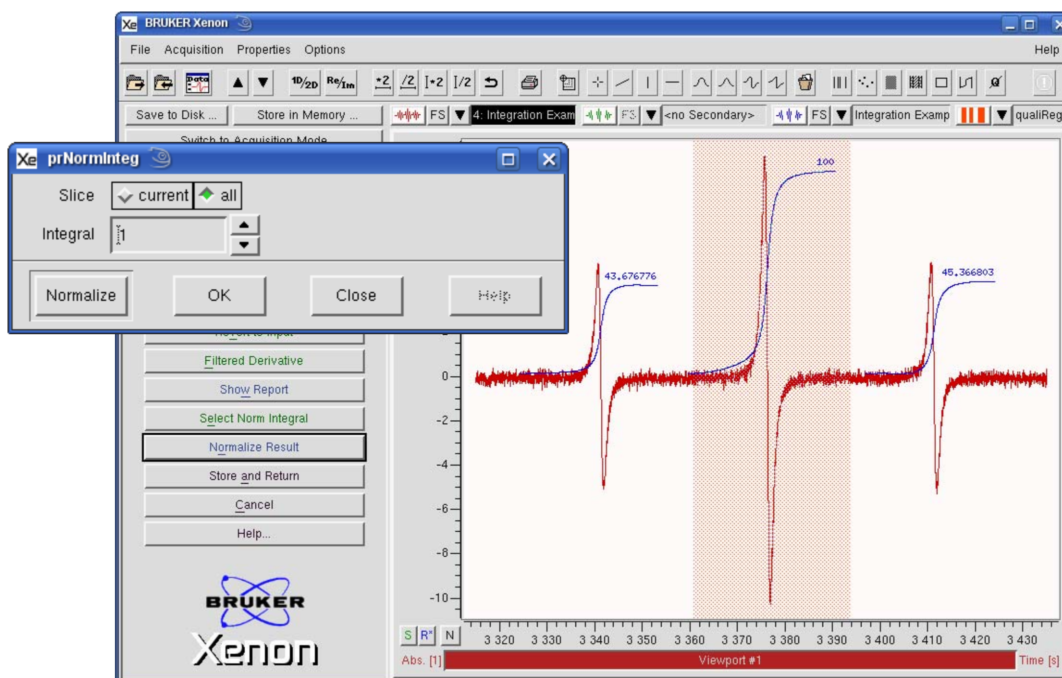


Figure 6-10 Normalizing with respect to the first integral.

Alternatively, you could click Select Norm Integral and use the Region qualifier to select the desired integral. First remove the qualifiers for integration. Then select the region of the integral to be used for normalization. Click Normalize and the integrals are normalized without the dialog box appearing.

9. **Inspect and save the integral values.** Click Show Report. You now have a list of the integral values displayed.

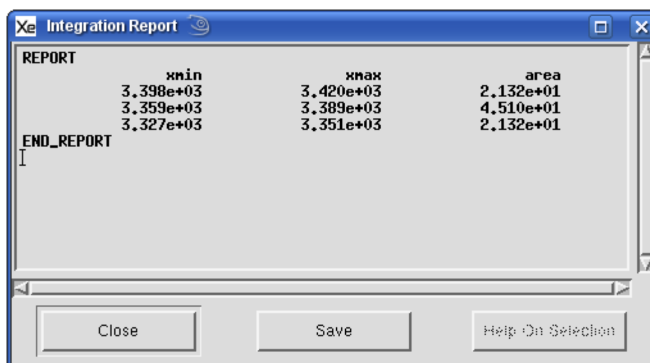


Figure 6-11 Viewing the integration list.

If you wish to save these values in a text file, click Save. A dialog box appears prompting you where to save the file.

Figure 6-12 Saving the integration list.

10. **Store the integration result.** Click Store and Return and enter a Title. Then click Store. You are then returned to the main Processing task bar. The integral is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the integral dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

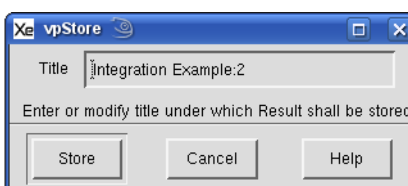


Figure 6-13 Storing the integral result in memory.

Differentiating EPR Spectra 6.3

There may be cases when you need to take the derivative of a spectrum. Differentiating a first derivative can offer you higher resolution.

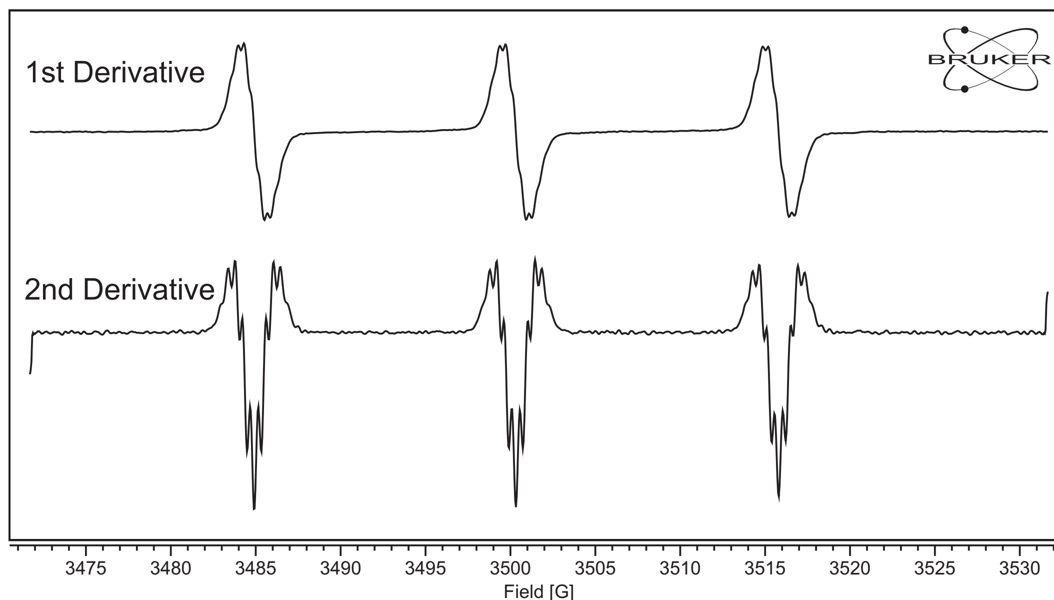


Figure 6-14 Increased resolution via differentiation of a TEMPOL EPR spectrum. The methyl group protons become apparent in the second derivative.

Unfortunately taking a derivative enhances the noise in your spectrum. One means of suppressing this noise is to use a Filtered Derivative.

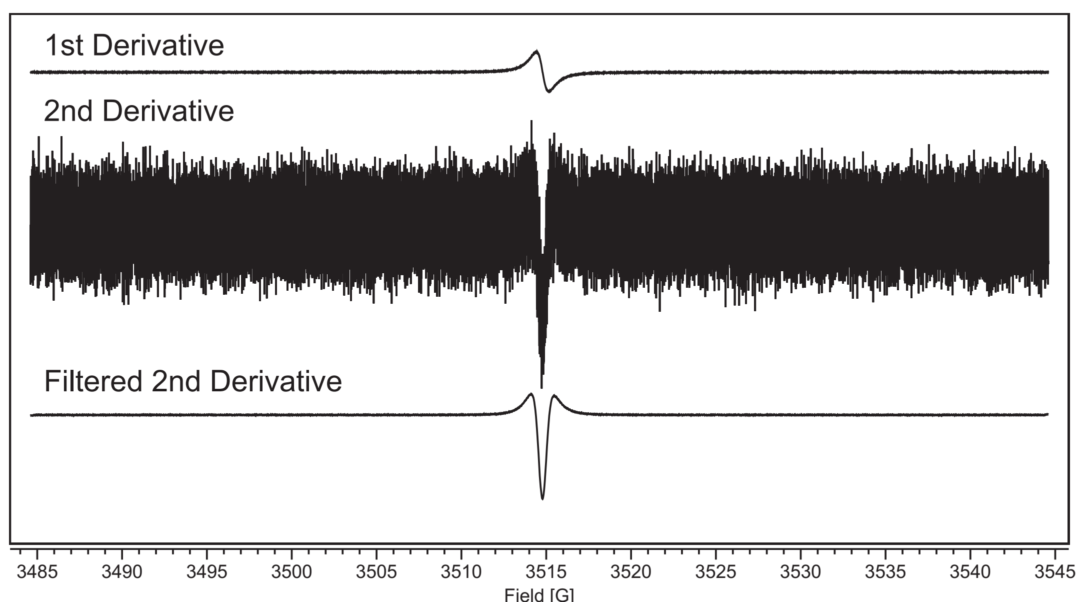


Figure 6-15 Noise suppression using a Filtered Derivative.

This operation is based on pseudo-modulation which is analogous to performing a second phase sensitive detection on the acquired EPR spectrum. As such the Amplitude parameter must be optimized to obtain the desired noise suppression while avoiding overmodulation distortion.

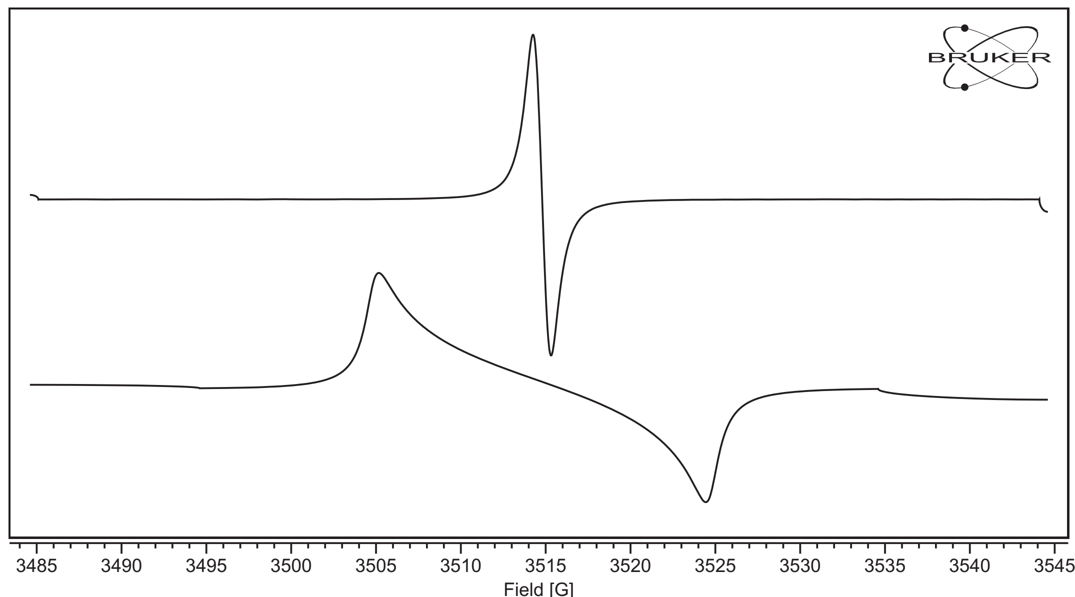


Figure 6-16 Just as in acquiring EPR spectra, overmodulation also creates distortion and broadening in a filtered derivative.

There is also a Harmonic parameter that is identical to the parameter for the signal channel. The first harmonic takes the first derivative, the second takes the second derivative, and so forth.

How to Differentiate 6.4

Here is how to take the filtered derivative of the EPR spectrum. It is assumed you are already in the Integration and Derivative task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset.

1. **Take the derivative (unfiltered).** Click Derivative. The derivative replaces the original spectrum in the Primary dataset.
2. **Take the derivative (filtered).** Click Filtered Derivative. A window appears with controls for varying the Amplitude and Harmonic. After proper adjustment of the parameters, click Transform and the derivative appears in the Result dataset. Click Close and the differen-

tiated spectrum remains in the Result dataset. If you click OK, the derivative replaces the original spectrum in the Primary dataset.

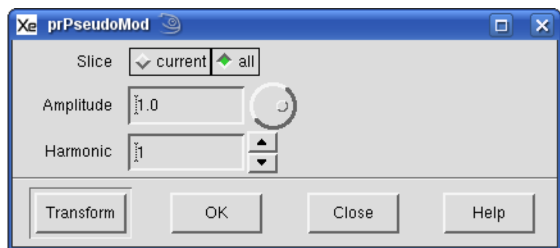


Figure 6-17 Performing a filtered derivative.

3. **Store the derivative result in memory.** Click Store and Return and enter a Title. Then click Store. The differentiated dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the derivative dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

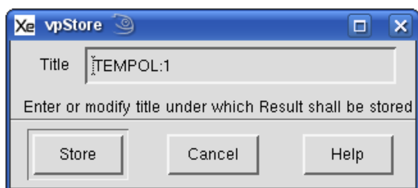


Figure 6-18 Storing the filtered derivative.

Fitting 7

Fitting refers to using least squares analysis to fit parameters of a model function to the experimental data. This is useful for determining, relaxation times, reaction kinetics, activation energies, and many other useful parameters that give us insight regarding our samples. The Fitting task is started by clicking the Fitting button in the task bar. A new task bar then appears with several sub-tasks.

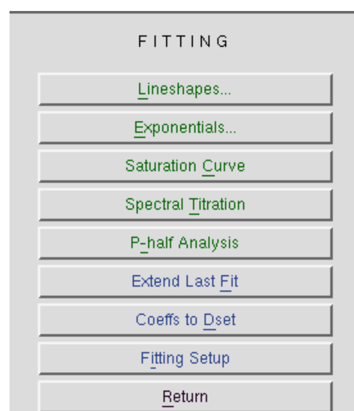


Figure 7-1 The Fitting task bar.

Least squares fitting is the minimization or optimization of Chi-squared, defined as:

$$\chi^2 \equiv \sum_{i=1}^{N} \{y_i - f(x_i;p_1 \ldots p_m)\}^2 \qquad [7\text{-}1]$$

where y_i are the experimental ordinate values, f is the function to be fitted, x_i are the experimental independent variable or abscissa values, p_i are the function parameter values and N is the number of data points.

Common Elements in the Fitting Task 7.1

Qualifiers Only the section of the dataset that is selected via a qualifier is fitted. This allows you to filter out any extraneous signals or time shifts in signals. The fit is extrapolated outside the region of the qualifier to fill the full x-axis of the dataset.

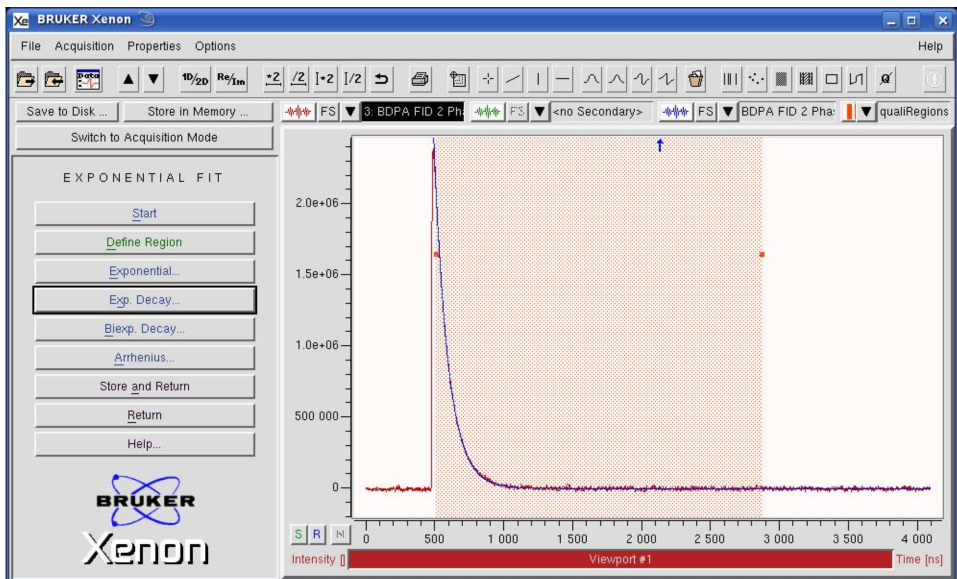


Figure 7-2 Selecting a portion of the dataset for fitting via a qualifier.

Slice current/all When current is selected only the current slice of a 2D dataset is fitted. When all is selected all the slices of a 2D dataset is fitted.

Setup The setup for fitting is accessed from the Fitting Setup button in the Fitting task bar. Also each fitting window has a Setup button. When clicked, a new window appears that allows you to configure how the functions are fitted.

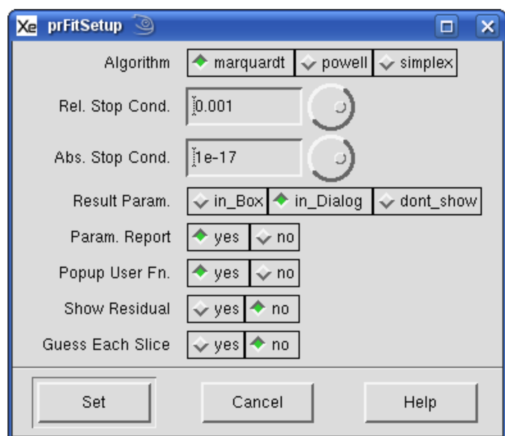


Figure 7-3 The Fitting Setup window.

Algorithm The algorithm refers to the technique used to minimize χ^2. Since what is often fitted are nonlinear functions, all of the algorithms are iterative techniques and the closer the initial guesses for the parameters are to the actual values, the faster the fit is finished. The Marquardt algorithm is usually the best choice as the iterations typically converge to the optimum values the most quickly. It does require the inversion of a matrix that can sometimes become singular, thereby failing to optimize the parameters. You are then be greeted by a warning messages as shown in Figure 7-4.

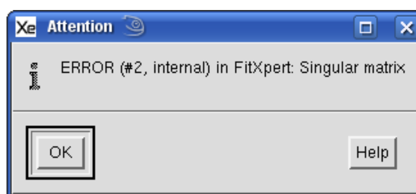


Figure 7-4 The singular matrix warning.

This can sometimes be remedied by entering better initial guesses for the parameters. You can also constrain parameter values by selecting to not fit a particular parameter but instead keep its value fixed. This is selected by clicking the no button in the fitting dialog box.

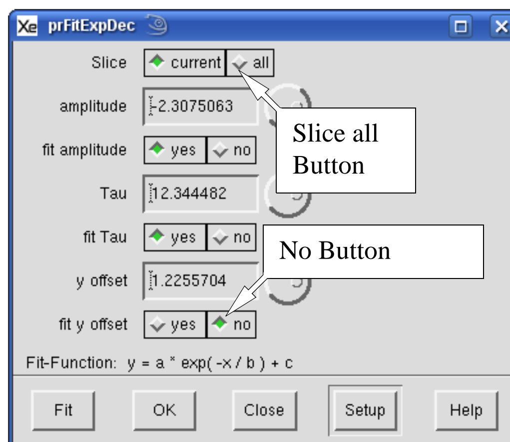


Figure 7-5 Selecting not to fit a parameter.

The Powell algorithm is a more stable but not as efficient algorithm as the Marquardt. Even more stable but even less efficient is the Simplex algorithm.

Rel. Stop Cond. Because an iterative technique is used, we must specify criteria for stopping the iterations. The first criterion is that the relative change in χ^2 between iteration satisfies:

$$\frac{\Delta\chi^2}{\chi^2} < \text{Rel. Stop Cond.} \qquad [7\text{-}2]$$

Abs. Stop Cond. The second criterion is that the absolute change in χ^2 between iteration satisfies:

$$\Delta\chi^2 < \text{Abs. Stop Cond.} \qquad [7\text{-}3]$$

Result Param. There are three options for displaying the resultant fitted parameters. The default is in_Dialog in which the fitted parameters values are displayed in the fitting dialog box. The second options is in_Box. The results are displayed in a window with the uncertainties of the parameters and the reduced chi-square:

$$\text{reduced chi-square} \equiv \sum_{i=1}^{N} \frac{\{y_i - f(x_i;p_1 \ldots p_m)\}^2}{N} \qquad [7\text{-}4]$$

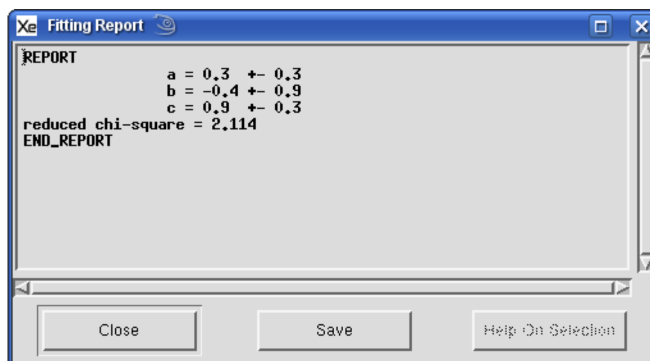


Figure 7-6 The in_Box parameter display.

If you wish to save these values in a text file, click Save. A dialog box appears prompting you where to save the file.

Figure 7-7 Saving the fitted parameters results.

For the dont_show option, the fitted parameters are not displayed. This is commonly used if you are only interested in subtracting the fitted curve from your dataset for background correction.

Param. Report Each dataset has the processing history of the dataset documenting what the user has done to the data. If this option is yes, the fitted parameters values are stored in this processing history for future reference.

Popup User Fn This is not applicable to Xenon.

Show Residual By default, the fitted function is displayed in the Result dataset. There are some cases in which the Residual (difference between the experimental and fitted data) provides useful information such as how well the function fits. To display the Residual, click yes.

Guess Each Slice The fitting routines make an estimate of the initial parameter values based on the experimental data. For 2D data, it is in general best for the fitting routine to make this estimate for each individual slice as parameters may change dramatically from slice to slice. Click yes to enable this option.

How to Fit a Function 7.2

Here is how to fit any of the functions described in the following sections. It is assumed you are already in the Fitting task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 7-2.) Then the operation is performed on the Secondary dataset.

1. **Setup Fitting.** Choose the Fitting Setup parameters you wish to use. Section 7.1 describes the various options.
2. **Choose the function you wish to fit.** Click one of the function buttons to select the desired fit function.
3. **Define the fitted region.** Click Define Region. Click and drag the cursor to select the region or regions that you wish to define as the region to fit. By default, the Qualifier is a Region Qualifier. Resize the qualifiers if necessary. (See Figure 7-2.)
4. **Fit the function.** Click Fit. The fitted function appears in the Result dataset and is the blue trace in the viewport.

How to Fit Lineshapes 7.3

There are four main lineshapes that one commonly encounters in EPR. Note that it is assumed you have already performed a baseline correction as described in Section 4. Offsets and background signals can interfere with dataset fitting.

Figure 7-8 The Lineshape Fit functions.

Gaussian Derivative

Gaussian lineshapes are encountered for inhomogeneously broadened EPR spectra. Quite often it is the result of unresolved hyperfine interactions. The function that is fitted to the dataset is described by:

$$y = -\text{amplitude} \cdot e^{1/2} \cdot \frac{x - x\ \text{offset}}{\text{width}} \cdot e^{-2\left(\frac{x - x\ \text{offset}}{\text{width}}\right)^2} \qquad [7\text{-}5]$$

where amplitude and width are the peak to peak amplitude and width respectively of the first derivative signal. The x offset is the zero-crossover or center of the EPR line.

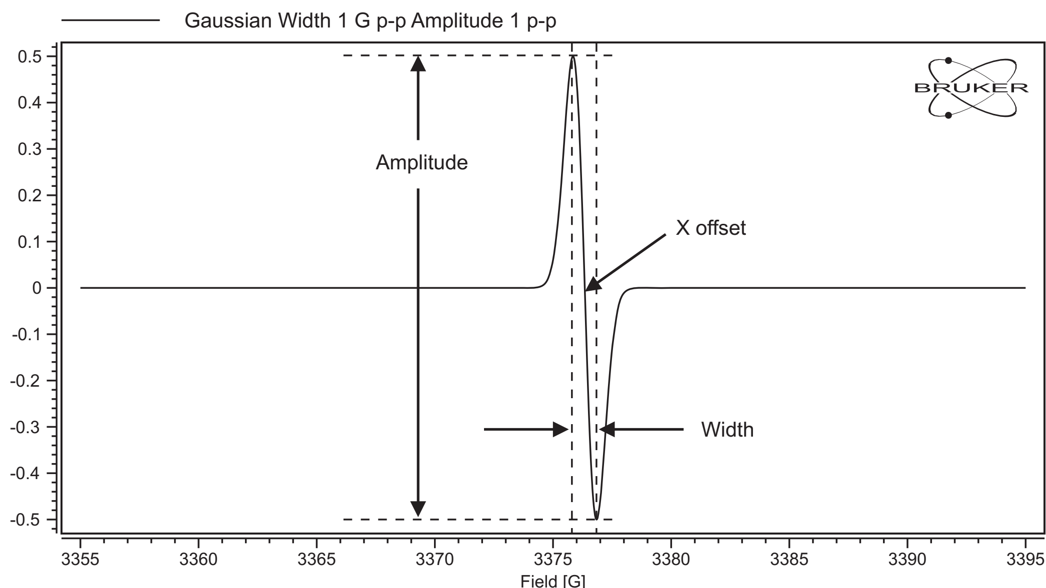


Figure 7-9 Definitions of the parameters for a Gaussian fit.

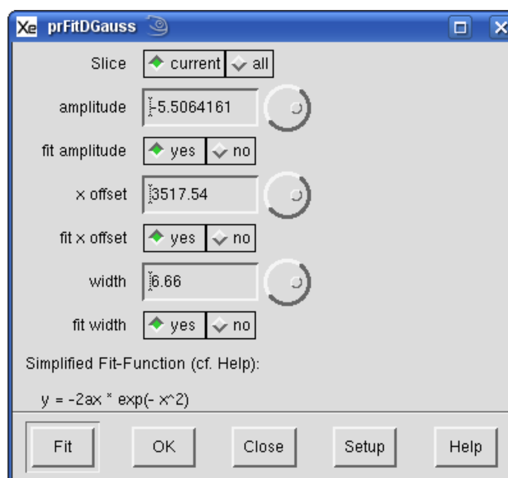


Figure 7-10 The Gaussian derivative fitting window.

Lorentzian Derivative

Lorentzian lineshapes are encountered for homogeneously broadened EPR spectra. The linewidth reflects the spin-spin relaxation time T_2 in the limit of no microwave power. The function that is fitted to the dataset is described by:

$$y = -\text{amplitude} \cdot \frac{16}{9} \cdot \frac{\frac{x - x\ \text{offset}}{\text{width}}}{\left(1 + \frac{4}{3} \cdot \left(\frac{x - x\ \text{offset}}{\text{width}}\right)^2\right)^2} \qquad [7\text{-}6]$$

where **amplitude** and **width** are the peak to peak amplitude and width respectively of the first derivative signal. The **x offset** is the zero-crossover or center of the EPR line.

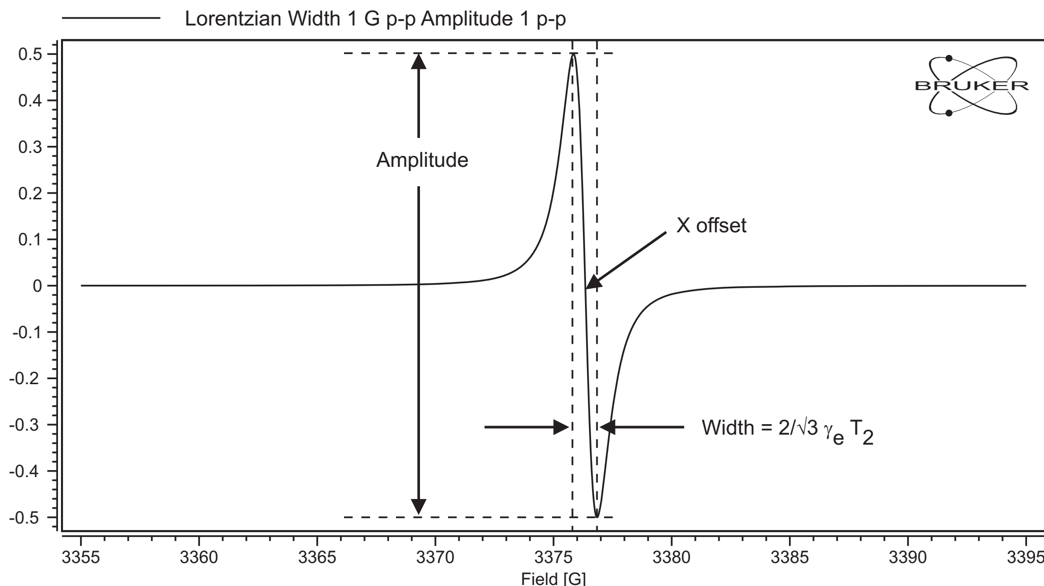


Figure 7-11 Definitions of the parameters for a Lorentzian fit. γ_e is the electronic gyromagnetic ratio and equals g x $8.7940981 \times 10^{10}\ s^{-1}\ T^{-1}$ where g is the g-value.

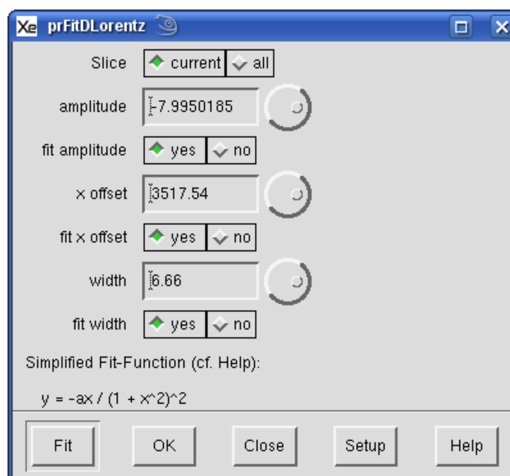


Figure 7-12 The Lorentzian derivative fitting window.

Mixture Deriv. In some cases, the dataset of the EPR signal may be intermediate between the Lorentzian and Gaussian lineshapes. In these cases the lineshape can expressed as a sum of both lineshapes. This is commonly called a Voigt lineshape. The function that is fitted to the to the dataset is described by:

$$y = \text{amplitude} \cdot \{\text{gauss-character} \cdot \text{Gaussian}(\text{x offset, width}) + (1 - \text{gauss-character}) \cdot \text{Lorentzian}(\text{x offset, width})\} \quad [7\text{-}7]$$

where amplitude and width are the peak to peak amplitude and width respectively of the first derivative signal. The x offset is the zero-crossover or center of the EPR line. It is assumed that these three parameters are identical for the Lorentzian and Gaussian contributions. The gauss-character indicates the amount of Gaussian contribution to the lineshape. 1 corresponds to purely Gaussian and 0 corresponds to purely Lorentzian.

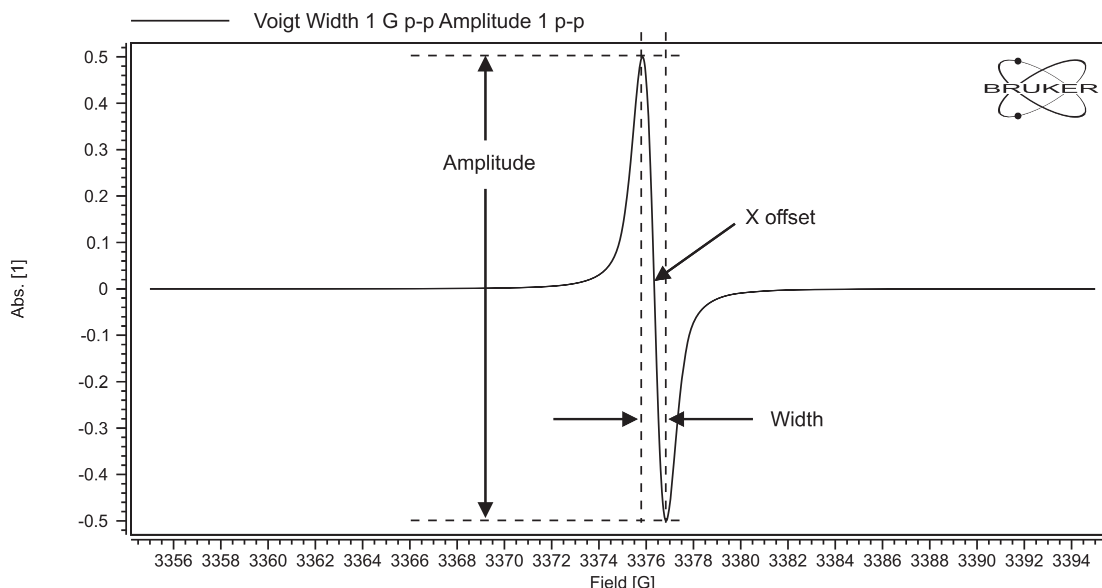


Figure 7-13 Definitions of the parameters for a Voigt lineshape fit.

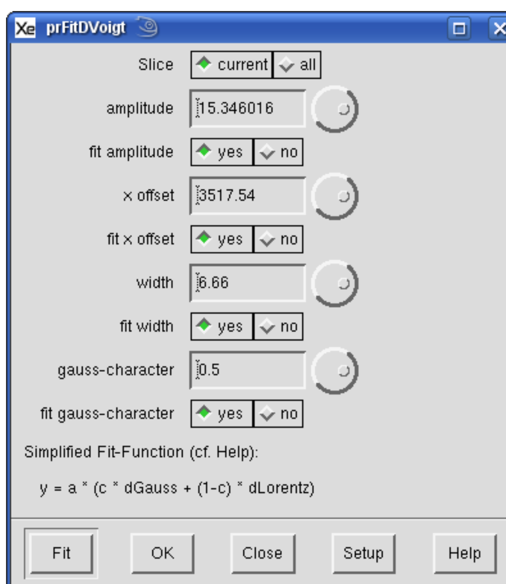


Figure 7-14 The Voigt derivative fitting window.

Dysonian Deriv. EPR spectra of conducting sample exhibit an asymmetric lineshape. The origins of the asymmetry is an admixture of absorption and dispersion owing to the skin depth and diffusion of the conducting electron. This is commonly called a Dysonian lineshape. The function that is fitted to the lineshape is described by:

$$y = \text{amplitude} \cdot \{f(p) + f(q)\} + \text{slope} \cdot x + \text{y-offset}$$
$$f(z) = \text{character} \cdot \frac{1 - z^2}{(1 + z^2)^2} + \frac{-2z}{(1 + z^2)^2} \qquad [7\text{-}8]$$
$$p = \frac{(x - \text{x-offset})}{\text{width}}$$
$$q = \frac{(x + \text{x-offset})}{\text{width}}$$

The last two terms for y are simply a linear baseline fit. f(z) is a weighted sum of the dispersion (first term) and absorption (second term). The character parameter is the weighting factor. For a pure absorption spectrum it is equal to zero. For a pure dispersion spectrum, it is a very large number. Typically the f(q) term can be ignored, but is included in the fitted function for completeness.

It is important to note that the width parameter does not refer to the peak to peak linewidth as it has with the other lineshape functions. The peak to peak width of the pure absorption Lorentzian EPR lineshape is given by:

$$\text{peak to peak width} = \text{width} \cdot 2/(\sqrt{3}) \qquad [7\text{-}9]$$

The amplitude parameter also does not correspond to the peak to peak amplitude as for the previous lineshape functions. The peak to peak height is given by:

$$\text{peak to peak height} = \text{amplitude} \cdot \frac{4}{3^{3/2}} \qquad [7\text{-}10]$$

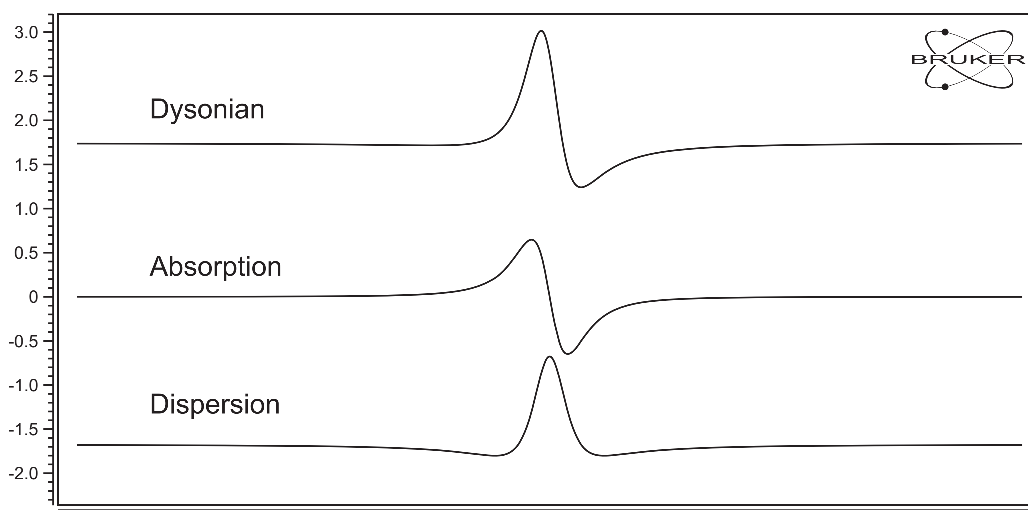


Figure 7-15 A Dysonian lineshape comprised of absorption and dispersion signals.

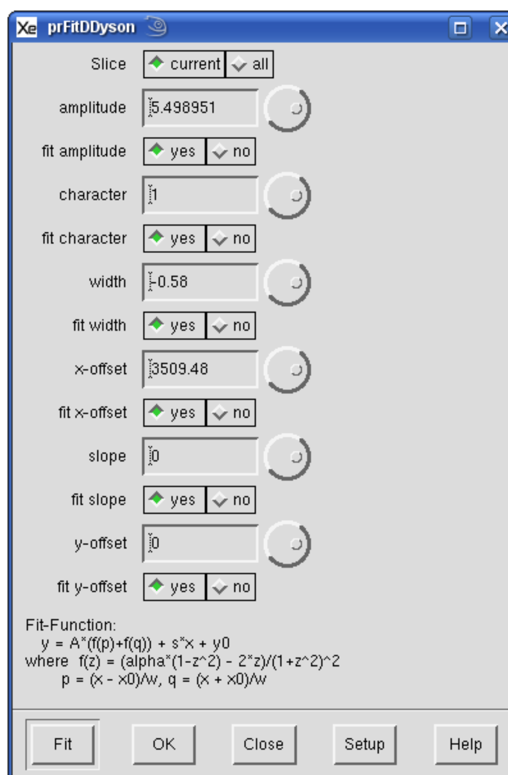


Figure 7-16 The Dysonian derivative fitting window.

How to Fit Exponentials 7.4

Often in kinetics and the study of activation energies you encounter exponential functions. The Exponentials sub-tasks facilitates the fitting of these functions to your data.

Figure 7-17 The Exponential Fit functions.

Exponential Sometimes signals grow exponentially. This sub-task fits a growing exponential. The function that is fitted to the dataset is described by:

$$y = \text{amplitude} \cdot e^{\text{Tau} \cdot x} + \text{y offset} \qquad [7\text{-}11]$$

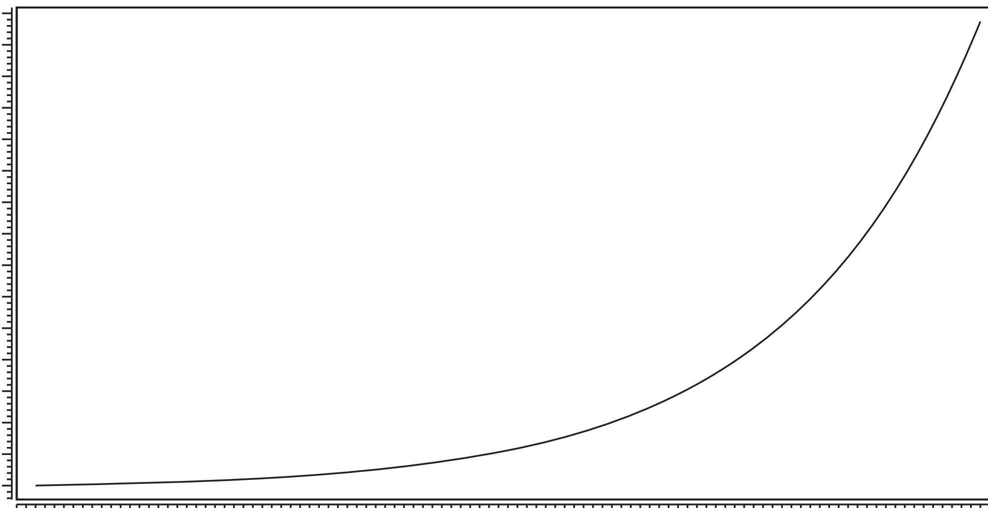


Figure 7-18 An exponentially growing curve.

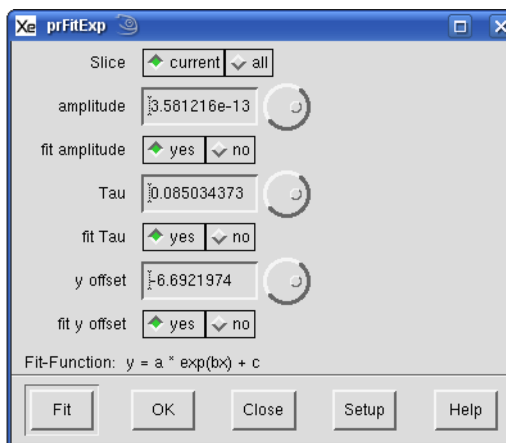


Figure 7-19 The Exponential fitting window.

Exp. Decay Sometimes signals decay exponentially. This sub-task fits a decaying exponential. The function that is fitted to the dataset is described by:

$$y = \text{amplitude} \cdot e^{-x/\text{Tau}} + y\ \text{offset} \qquad [7\text{-}12]$$

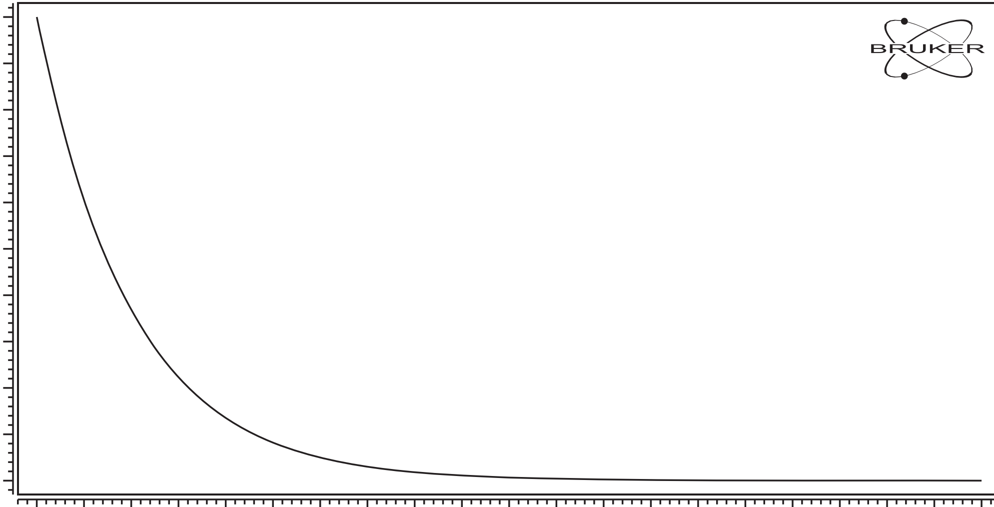


Figure 7-20 An exponentially decaying curve.

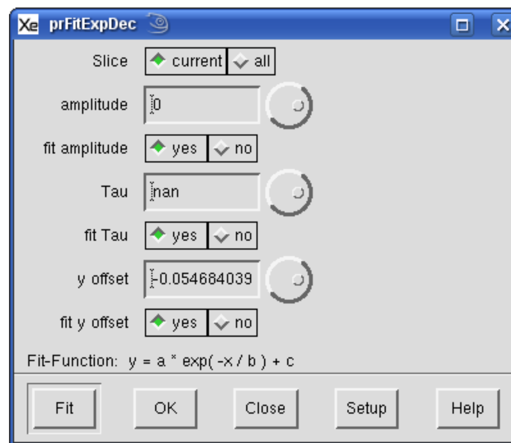


Figure 7-21 The Exp. Decay window.

Biexp. Decay

Often decays are not purely a single exponential but are comprised of the sum of two decaying exponentials. This sub-task fits two decaying exponentials. The function that is fitted to the dataset is described by:

$$y = \text{amplitude } 1 \cdot e^{-x/\text{Tau } 1} + \text{amplitude } 2 \cdot e^{-x/\text{Tau } 2} + \text{y offset} \quad [7\text{-}13]$$

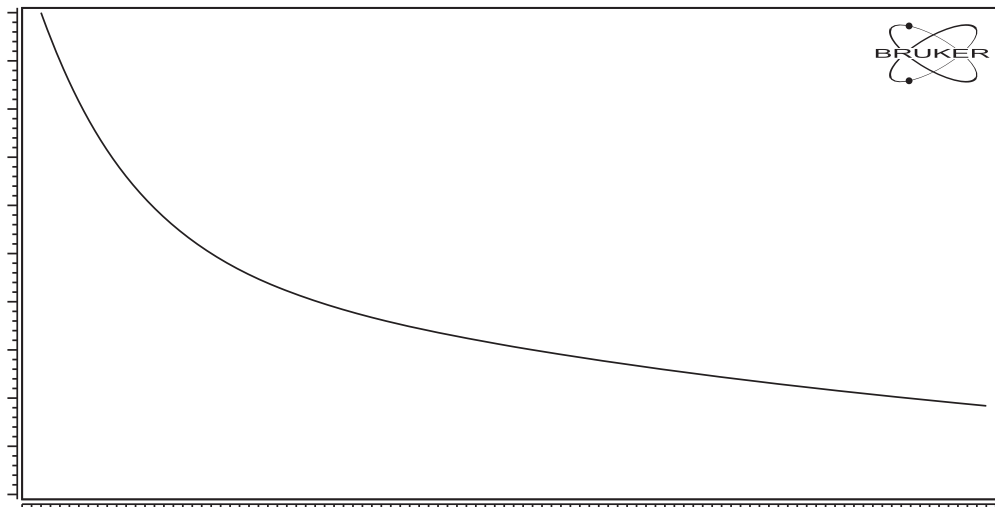


Figure 7-22 A biexponential decay curve.

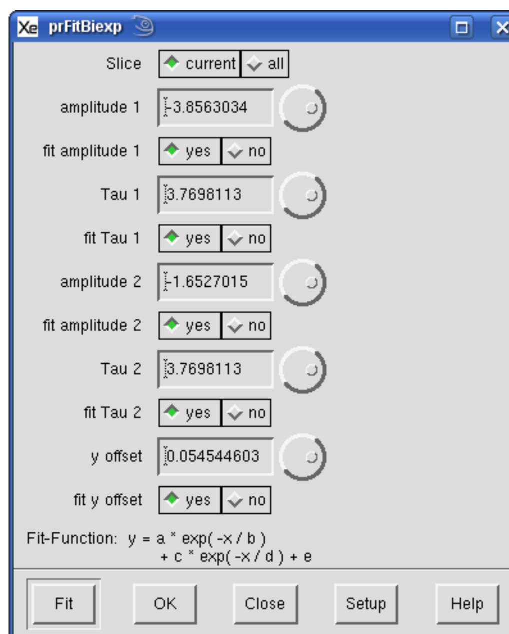


Figure 7-23 The Biexp. Decay window.

Arrhenius The Arrhenius equation describes the temperature dependence of rate constants:

$$k = Ae^{-E_a/RT} \quad [7\text{-}14]$$

where k is the rate constant, E_a is the activation energy, R is the gas constant and T is the temperature in Kelvin.

This sub-task fits this temperature dependence of rate constants. The function that is fitted to the dataset is described by:

$$y = a \cdot e^{-x/b} + c \quad [7\text{-}15]$$

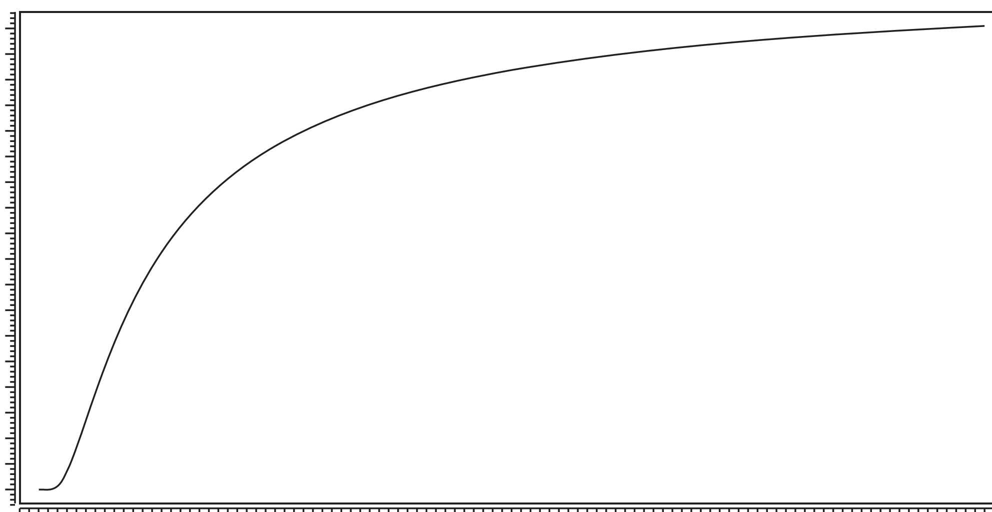


Figure 7-24 An Arrhenius plot.

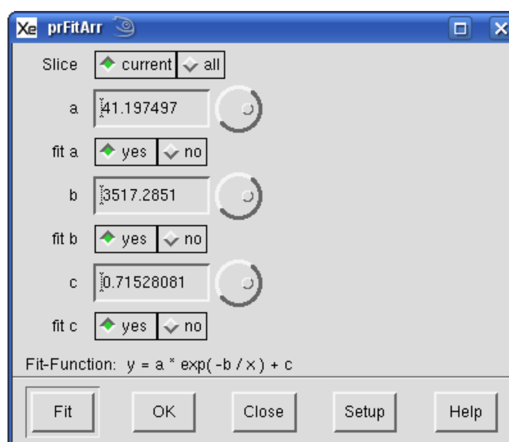


Figure 7-25 The Arrhenius fitting window.

How to Fit a Saturation Curve 7.5

Sometimes the signal intensity does not grow linearly with increasing experimental parameters such as concentration. Instead they level out showing saturation behavior. This sub-task fits a Saturation curve. The function that is fitted to the dataset is described by:

$$y = a \cdot \frac{x}{1 + 2bx} \qquad [7\text{-}16]$$

Note that this is not microwave saturation, which is described in the next section.

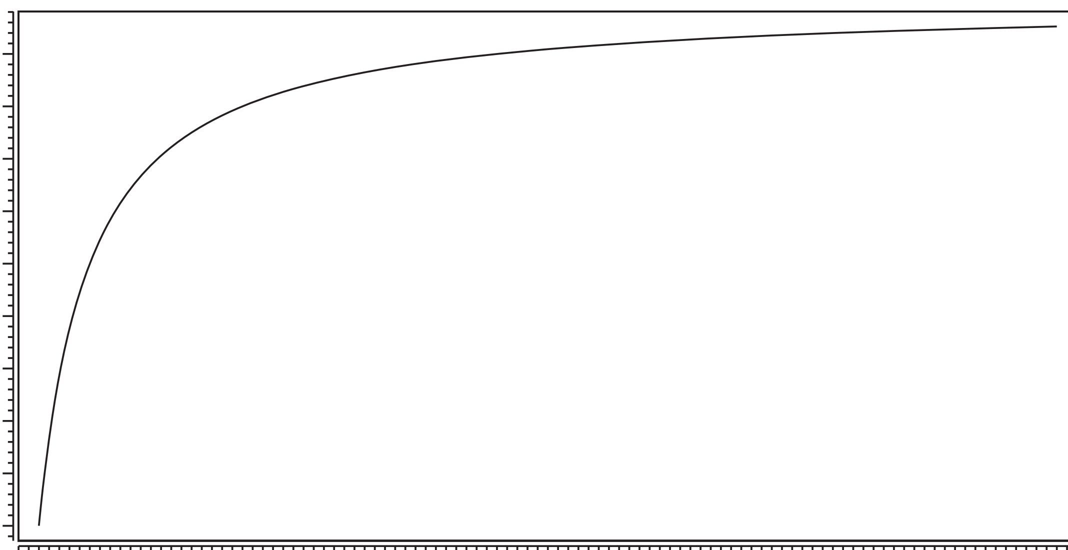


Figure 7-26 A Saturation curve.

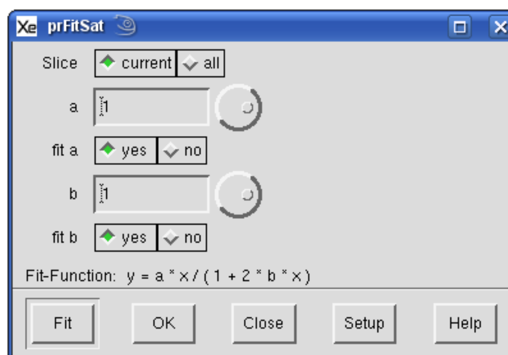


Figure 7-27 The Saturation Curve fitting window.

How to Perform a P-half Analysis 7.6

In the absence of saturation, the peak to peak EPR intensity increases with √P (the square root of the microwave power). At higher power, the intensity starts to drop off with increasing microwave power. This behavior can be fitted to the following function:

$$y = I0 \cdot \frac{\sqrt{x}}{(1 + x / \mathrm{Phalf})^{b/2}} \quad [7\text{-}17]$$

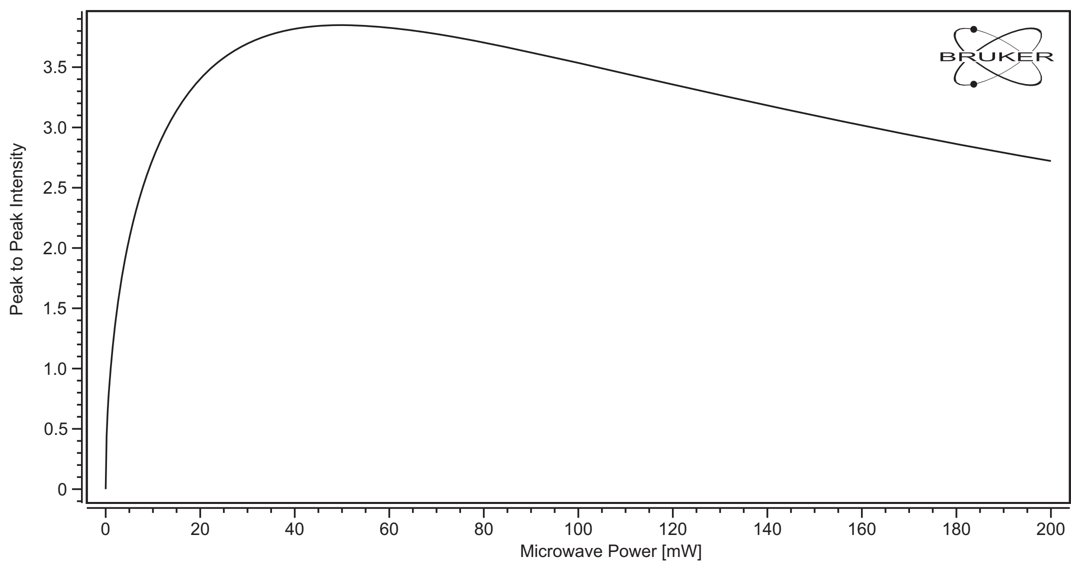


Figure 7-28 A microwave saturation plot.

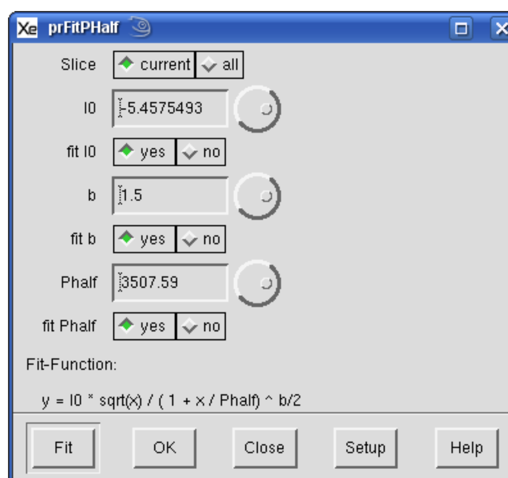


Figure 7-29 The Phalf analysis window.

For a homogeneously broadened line, b = 3 and the peak to peak amplitude of the EPR signal, H_{pp}, is given by:

$$H_{pp} = \frac{3^{3/2}M_0}{4}\frac{\gamma_e B_1 T_2^2}{(1+\gamma_e^{\,2} B_1^2 T_1 T_2)^{3/2}} \qquad [7\text{-}18]$$

where M_0 is the equilibrium magnetization, γ_e is the electronic gyromagnetic ratio, B_1 is the microwave magnetic field, T_2 is the spin-spin relaxation time, and T_1 is the spin lattice relaxation time. The microwave magnetic field is given by:

$$B_1 = c\sqrt{P} \qquad [7\text{-}19]$$

where c is the conversion factor in units of Gauss/√W. By substituting this expression into Equation [7-18], we start to see the similarities between Equation [7-17] and Equation [7-18]. First, the exponential factor b should be equal to three for a homogeneously broadened line. The term Phalf can also be solved for in terms of the relaxation times.

$$\gamma_e^{\,2} B_1^2 T_1 T_2 = \gamma_e^{\,2} c^2 P T_1 T_2 = \frac{P}{\text{Phalf}}$$
$$\text{Phalf} = \frac{1}{\gamma_e^{\,2} c^2 T_1 T_2} \qquad [7\text{-}20]$$

Depending on the information you have regarding the sample, Phalf can be used to measure the T_1 of the sample or the conversion factor of the resonator.

For inhomogeneously broadened lines, the exponential factor b can differ from three, approaching a value of one in the limit of a purely inhomogeneously broadened line.

How to Perform a Spectral Titration 7.7

This task moves and stretches the Secondary dataset according to

$$x = x\ \text{shift} + x \cdot x\ \text{factor} / 100$$
$$y = y\ \text{shift} + y \cdot y\ \text{factor} / 100 \qquad [7\text{-}21]$$

in order to fit or match the qualified region of the Primary dataset. This can be used to quantify the contribution of the Secondary dataset to the Primary dataset or to resolve overlapping EPR spectra. For example if we had a spectrum of a mixture of $CuSO_4$ and $VOSO_4$ in H_20 and a spectrum of pure $VOSO_4$ in H_2O, we could quantify the amount of vanadyl signal in the mixture. The resultant fitted vanadyl spectrum could then be used to subtract the vanadyl contribution from the mixture to yield the pure $CUSO_4$ in H_2O spectrum.

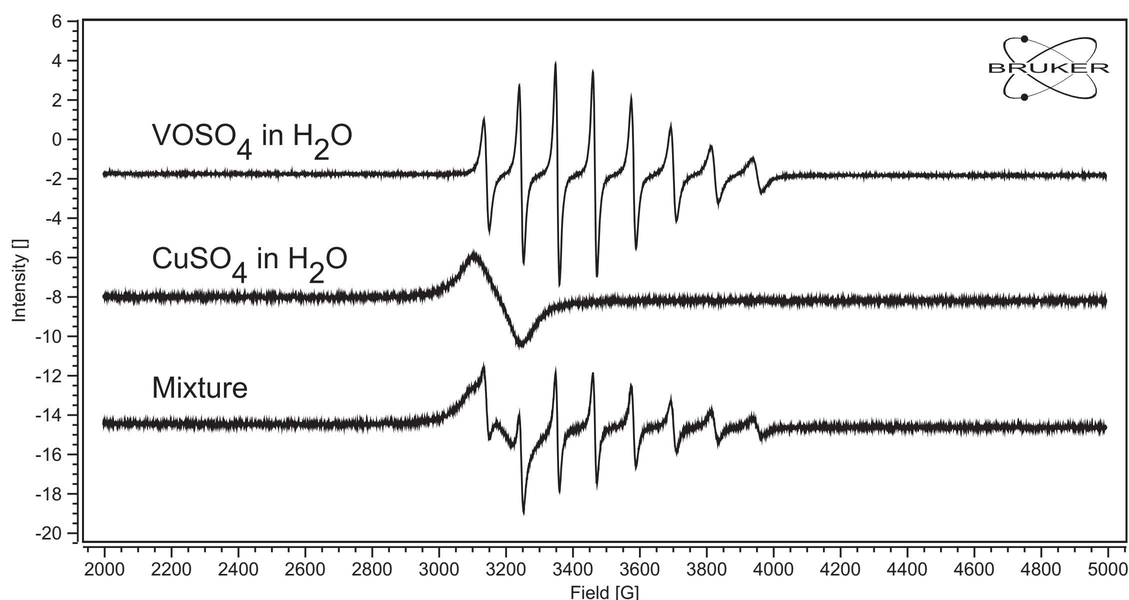


Figure 7-30 The EPR spectrum of a mixture of two species.

1. **Load the mixture spectrum in the Primary dataset.**
2. **Load the pure spectrum in the Secondary dataset.**
3. **Qualify the non-overlapping region of the pure spectrum.** Part of the vanadyl spectrum does not overlap with the copper spectrum. Select this region with a Region Qualifier.

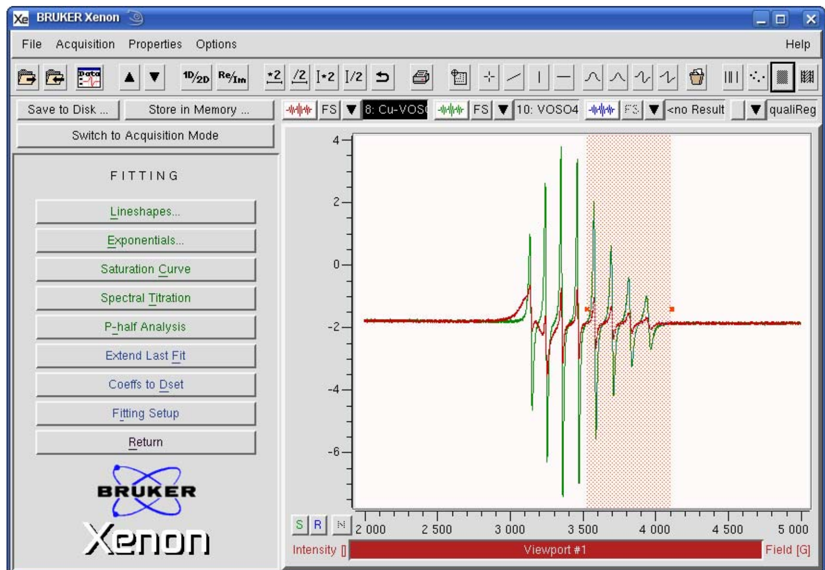


Figure 7-31 Qualifying the non-overlapping region of the two species.

4. **Click Fitting >Spectral Titration.** The Spectral Titration window appears. Click Fit. The Secondary is stretched and shifted to match its component in the mixture spectrum and the resultant fitted spectrum is displayed in the Result dataset. Note that the x shift is close to zero and the x factor is close to one because the microwave frequency does not differ much between the two EPR spectra. The y factor value of 22.424223 indicates that the component of the Secondary dataset in the Primary (mixture) dataset is 22.424223% of the Secondary dataset.

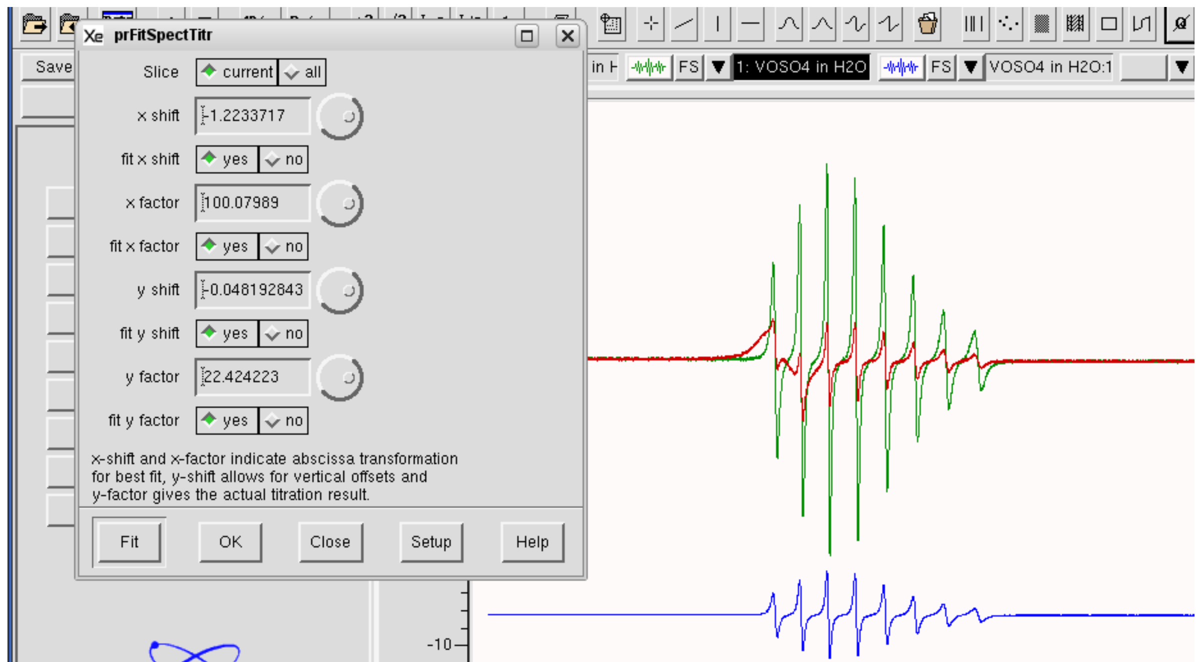


Figure 7-32 The EPR spectrum of a mixture of two species. The vanadyl spectrum contribution has been fitted and appears in the Result dataset. The Result has been offset for better visibility.

5. **Subtract the Result pure spectrum from the primary.** Transfer the Result dataset to the Secondary dataset. Refer to Section 9.3 for instructions on subtracting two datasets. The result is the pure copper spectrum.

Extend Last Fit 7.8

The fitted function is by default extrapolated to fill the full x-axis of the fitted dataset. In some cases you may want to extend or extrapolate the fit outside this range or you may want contract the fit to a narrower range or fewer data points. The Extend Last Fit performs this function.

1. **Fit a function to your dataset.** Once you are finished with fitting, click Close.
2. **Click Return or Store and Return.** You return to the main task bar. Store and Return stores your fitted function in memory.
3. **Click Fitting in the main task bar.** This brings you back to the Fitting task again.
4. **Click Extend Last Fit.** By entering values for xMin and xMax you can define the x-axis range for your fitted function. nrOfPoints defines the number of points in the fitted function. When finished, click Close.

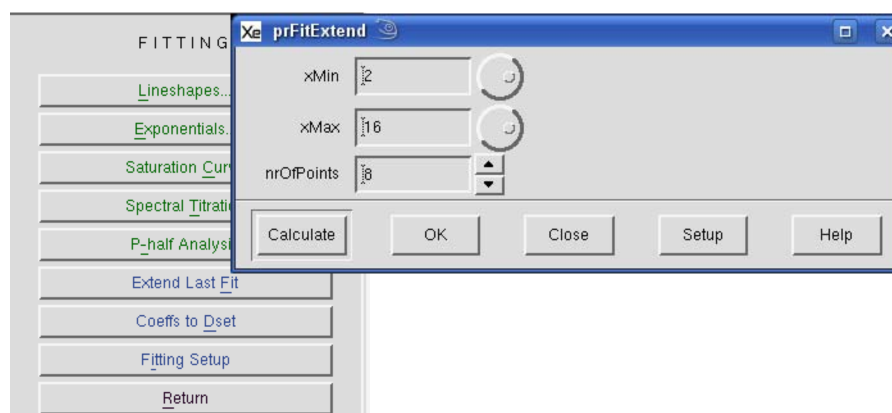


Figure 7-33 The Extend Last Fit window.

Coeffs to Dset 7.9

Often EPR spectra are acquired as a function of some second experimental parameter such as sample temperature or microwave power to form a 2D dataset. When fitting a function to this 2D dataset it would be desirable to create a dataset of fitted parameters vs. the second experimental parameter.

1. **Fit a function to your dataset.** Remember to click all to ensure that all the function is fitted to all the slices of the 2D dataset. Once you are finished with fitting, click Close.
2. **Click Return or Store and Return.** You return to main task bar. Store and Return stores your fitted function in memory.

3. **Click Fitting in the main task bar.** This brings you back to the Fitting task again.

4. **Click Coeffs to Dset.** The fitted parameters appear in the Result dataset. This 2D dataset has an x-axis corresponding to the second experimental parameter values. The second abscissa, the y-axis, corresponds to the fitted parameters. For example the first slice would correspond to the fitted amplitude, the second slice to the fitted Tau value, and so on.

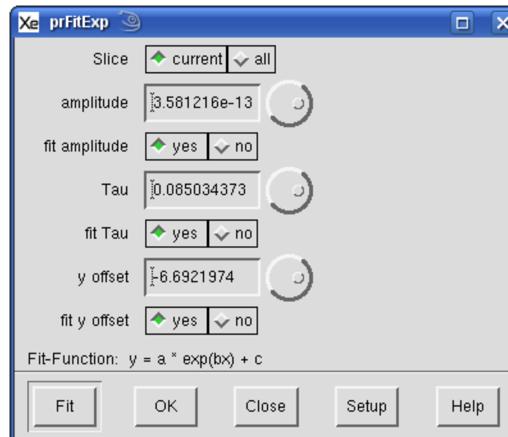


Figure 7-34 The Exponential fitting window.

Filtering 8

Sometimes, despite your best efforts, features in your EPR spectrum may still be obscured by noise. The Filtering task offers you some tools to filter some of the noise from the EPR spectrum. The Filtering task is started by clicking the Fitting button in the task bar. A new task bar then appears with several sub-tasks.

Figure 8-1 The Filtering tasks.

How to Filter a Dataset 8.1

Here is how to filter a dataset using the techniques described in the following sections. It is assumed you are already in the Filtering task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Section Figure 2-3.) Then the operation is performed on the Secondary dataset.

1. **Choose the technique you wish to use.** Click one of the buttons to select the desired technique.

2. **Define the filtered region.** By default the entire region is filtered. To filter only a portion of the region, click the Region Qualifier button. Click and drag the cursor to select the region or regions that you wish to define as the region to filter. Resize the qualifiers if necessary.

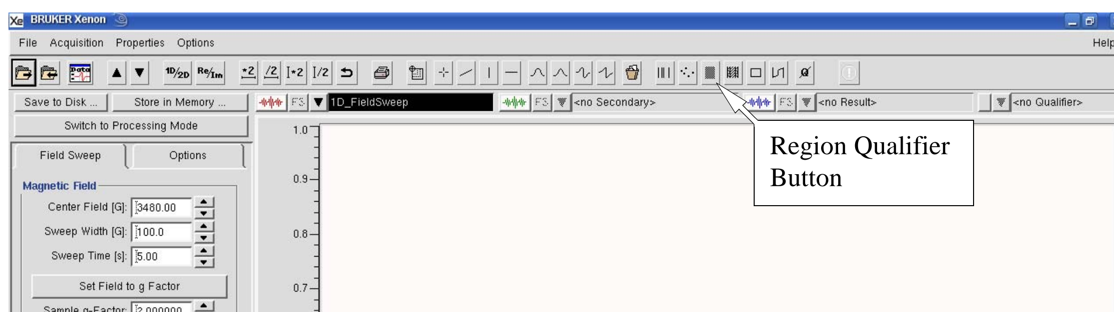


Figure 8-2 The Region Qualifier button.

3. **Filter the dataset.** Click Filter. The filtered data appears in the Result dataset and is the blue trace in the viewport.

4. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant filtered dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the filtered dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

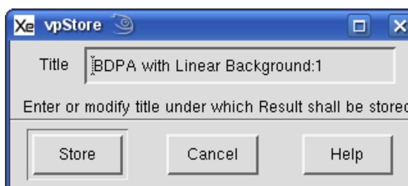


Figure 8-3 Storing the filtered spectrum in memory.

Filtering only affects how the spectrum looks and does not add information. In fact it removes information. It is important to compare the filtered and unfiltered data to determine if you have filtered out too much of the signal.

How to Filter with Smoothing and Savitzky-Golay Filtering 8.2

Often the signal and noise differ in their frequency components. Typically the EPR signal will have mainly low frequency components. The noise will have mainly high frequency components. The time constant of the signal channel takes advantage of this difference by filtering the higher frequency components, thereby filtering out noise, and passing through the EPR signal. There are ways of performing this filtering after the data has been acquired. We are removing the jagged noise thereby smoothing the data. The simplest is a moving average.

With this technique, a new filtered dataset is created from the original noisy dataset. For each point of the filtered data, information from the adjacent data points in the original dataset are used to calculate the filtered data. The Nr. of Points (See Figure 8-8.), otherwise known as the filter width is the number of points before and after the data point that is used in the calculation.

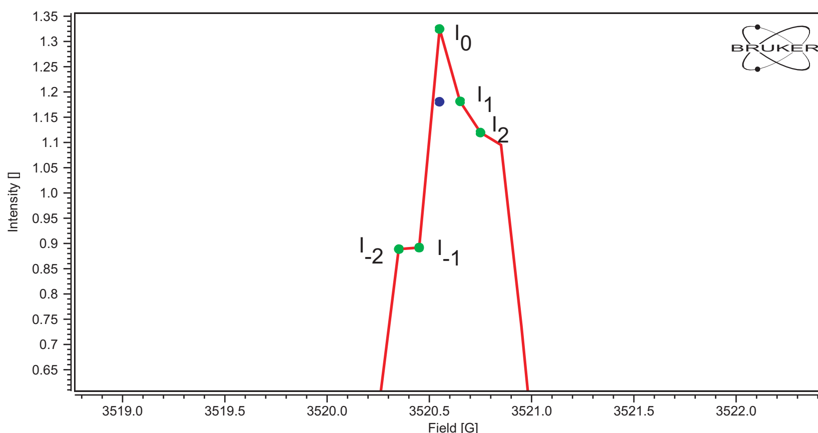


Figure 8-4 Labeling of points used for a moving average.

The point to be calculated is indexed as zero. The Nr. of Points before and after this point are used in the calculation. Therefore the number of points used in the calculation is equal to

$$\text{Number of Points} = 2 \cdot \text{Nr. of Points} + 1 \qquad [8\text{-}1]$$

As seen in Figure 8-4, Nr. of Points equal to two yields five points used in the calculation.

One approach to noise reduction is to calculate a moving average. The filtered intensity of the point I_0 is then substituted by:

$$I_0^{filtered} = \frac{I_0 + \sum_{i=1}^{\text{Nr. of Points}} I_i + I_{-i}}{2 \cdot \text{Nr. of Points} + 1} \qquad [8\text{-}2]$$

The noise will be reduced in a similar fashion to signal averaging. If the noise is random, the noise should decrease with the square root of the filter width. As can be seen in the figure below, the moving average is very effective at filtering noise. It is, however, a very blunt instrument; as the filter width gets large the signal is distorted. The lines become broader and the amplitude decreases.

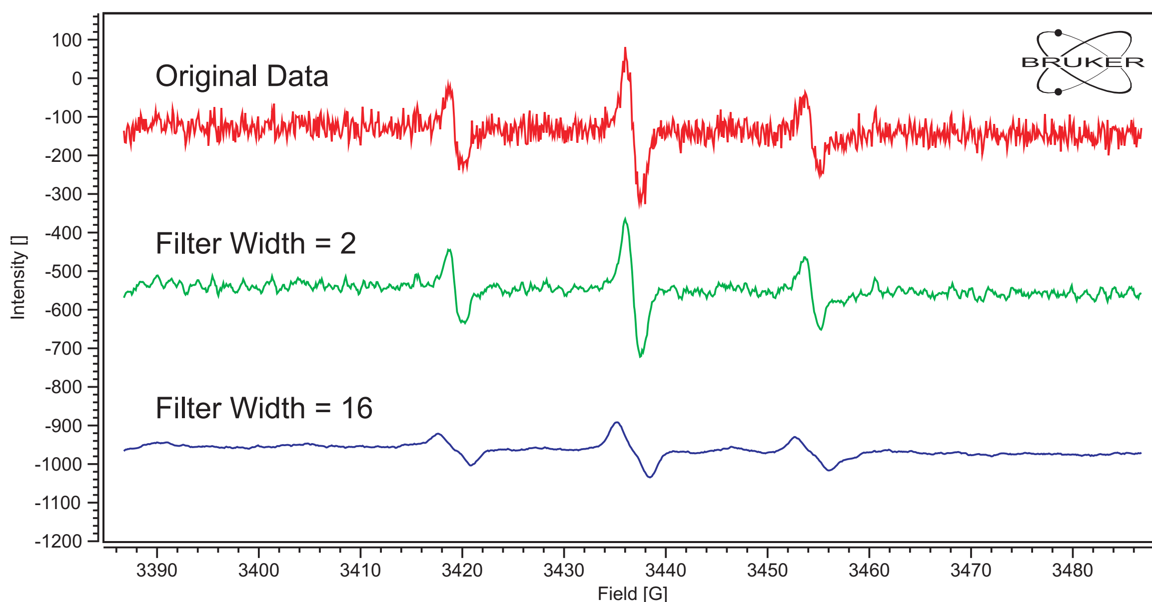


Figure 8-5 Noise filtering and distortion with different filter widths used in moving average filtering.

An alternative approach that does not distort the signal as much as the moving average is a weighted moving average. The most common weighting is a binomial weighting. The weighting coefficients are given by the binomial coefficients that are the polynomial coefficients when $(1+x)^{n}$ is expanded where n is the filter width. For $n = 2$, this is 1:4:6:4:1. After filtering, the filtered intensity at the center (the blue point in Figure 8-6) is given by:

$$I_0^{\text{filtered}} = (1 \cdot I_{-2} + 4 \cdot I_{-1} + 6 \cdot I_0 + 4 \cdot I_1 + 1 \cdot I_2)/16 \;, \qquad [8\text{-}3]$$

where the factor of 16 is required for normalization. This filtering procedure is then repeated for each individual data point of the spectrum.

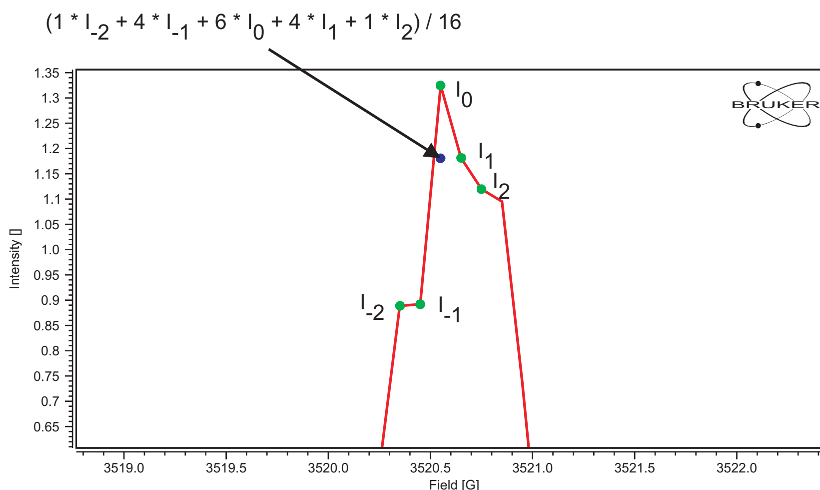


Figure 8-6 Binomial smoothing of the EPR data.

Below we compare a simple and binomial weighted moving average for a filter width of 16. The binomial weighting preserves the lineshapes much better than the simple average.

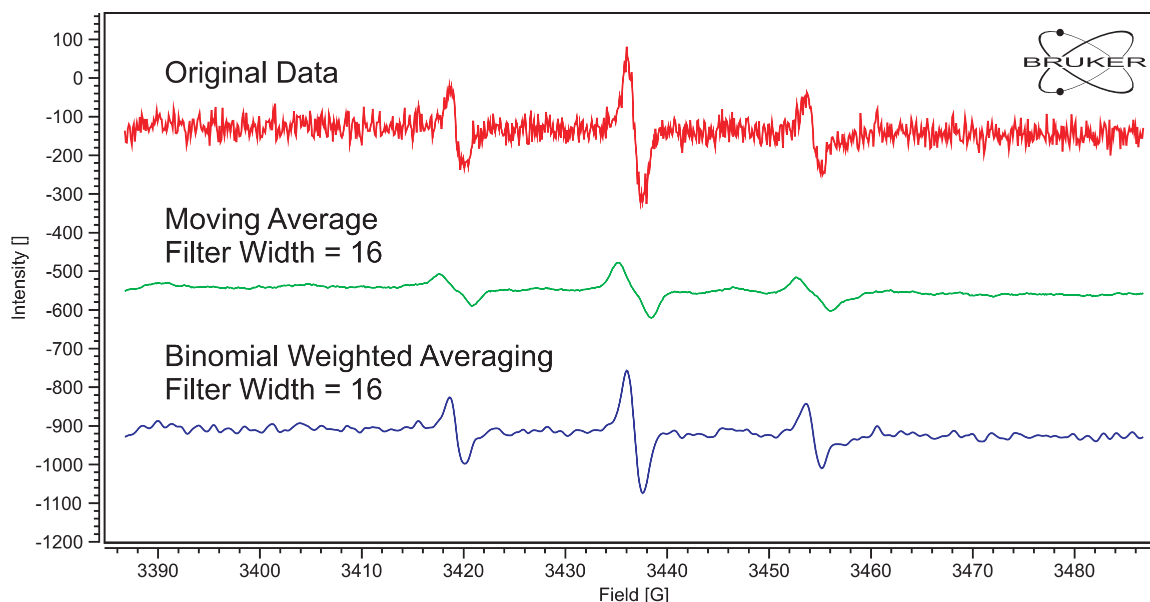


Figure 8-7 Noise filtering and distortion with a given filter width. The binomial weighting substantially improves the lineshapes in the filtered dataset.

These filtering techniques are supplied in the Smoothing sub task. Nr. of Points corresponds to the filter width. equal corresponds to a simple moving average whereas binomial corresponds to a binomial weighted moving average.

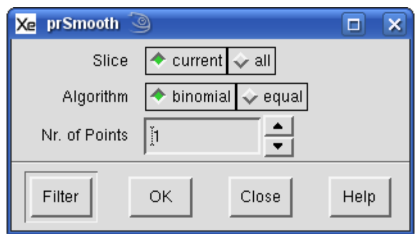


Figure 8-8 The Smoothing window.

Related to moving averages are the Savitzky-Golay filters. These filters fit a polynomial to the points adjacent to the point to be filtered and use the polynomial coefficients to calculate the filtered intensity. If the polynomial order is zero, this corresponds to a simple moving average.

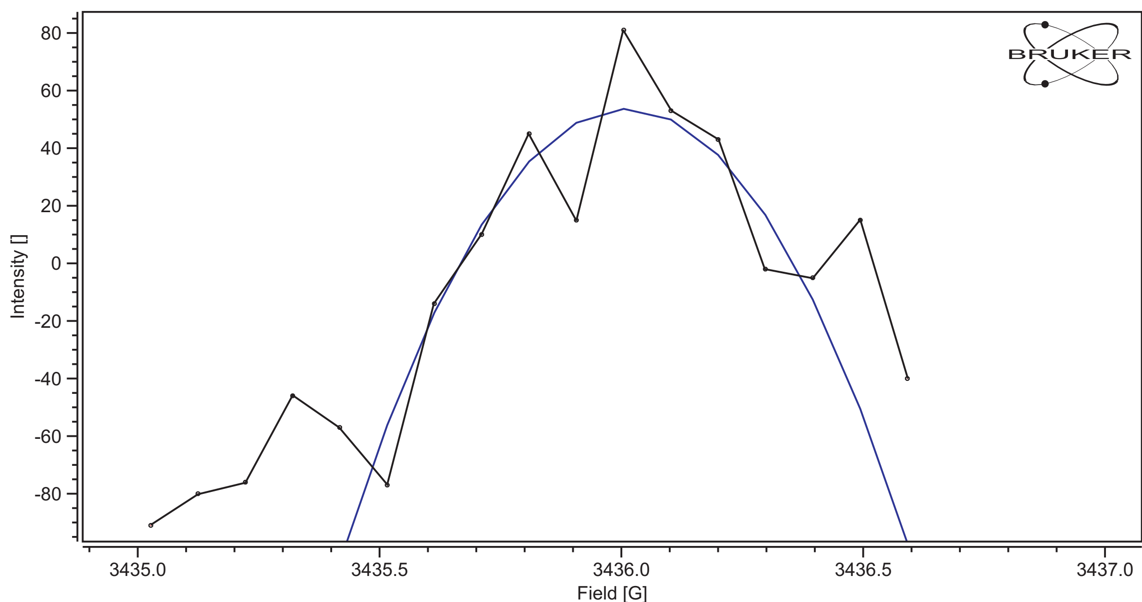


Figure 8-9 Fitting of a second order polynomial to noisy data.

Just as in moving averages, these filters also have a filter width corresponding to the number of points used to fit the polynomial. They also are characterized by the order of the polynomial. Because we are performing a fit the following condition must be met:

$$\text{Polynomial Order} < 2 \cdot \text{Nr. of Points} + 1 \qquad [8\text{-}4]$$

As the polynomial order increases the ability to fit sharper features in the data without distortion at the expense of not filtering the noise increases as well. Figure 8-10 shows the results of applying a 4th order polynomial filter with a filter width of 16.

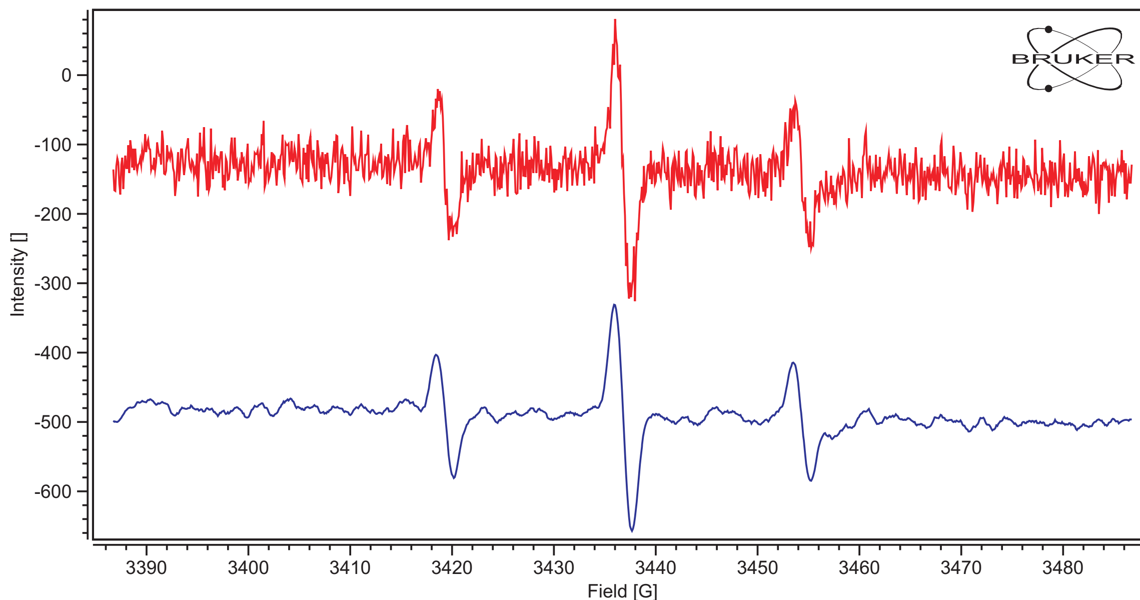


Figure 8-10 Smoothing of noisy data with a 4th order Savitzky-Golay filter with a filter width of 16 points.

The Savitzky-Golay filters are found in the Savitzky-Golay sub task. Order corresponds to the polynomial order. Nr. of Points corresponds to the filter width.

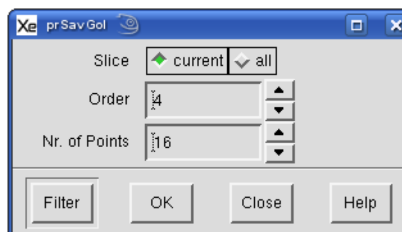


Figure 8-11 The Savitzky-Golay window.

How to Filter with the RC-Filter 8.3

Signal channels use a time constant to filter out noise from the EPR spectrum. This is also commonly called an RC filter. This filter can also be digitally implemented for post-processing to suppress noise in the EPR spectrum. The RC filter is found in the RC-Filter sub task.

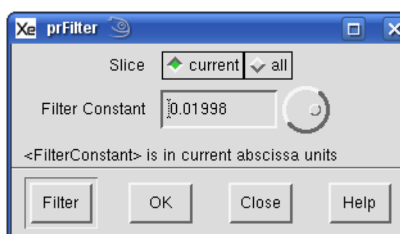


Figure 8-12 The RC-Filter window.

Just as with an analog time constant in a signal channel, the digital RC-Filter introduces the same type of distortion if the time constant is too long.

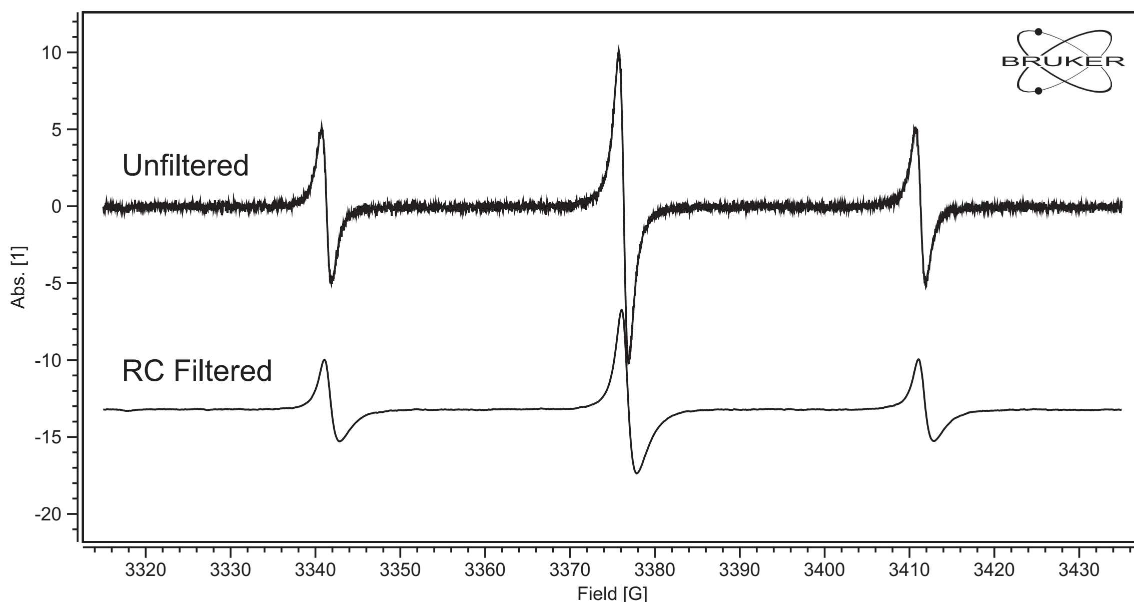


Figure 8-13 Distortion due to a time constant that is too long.

How to Filter Using Pseudo Modulation 8.4

We have already seen in Section 6.4 how pseudo-modulation can be used to suppress noise when differentiating an EPR spectrum If we select the Harmonic as 0, it can also function as a well behaved signal filter as well. The spectrum is not differentiated but the noise can be suppressed without introducing a great deal of distortion. The Pseudo Modulation filter is found in the Pseudo Modulation sub task.

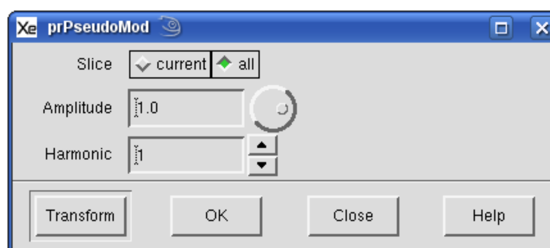


Figure 8-14 The pseudo-modulation window.

Note that the total region is always filtered. You can not use a Region Qualifier to just filter an isolated area of a dataset as with the other techniques.

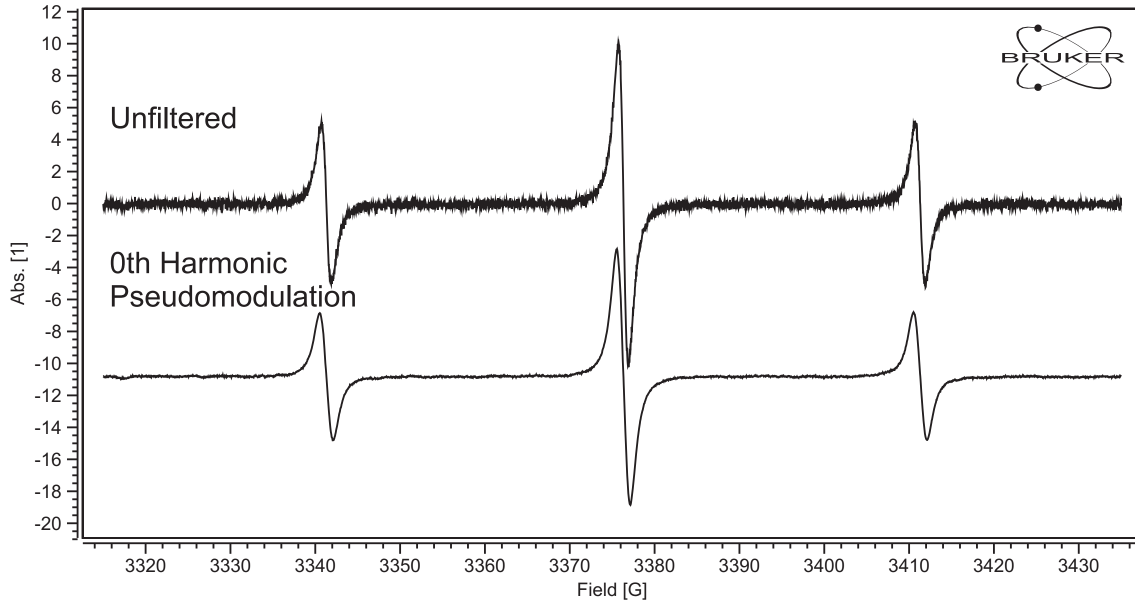


Figure 8-15 Using the 0th order Harmonic pseudo-modulation to filter noise from an EPR spectrum.

Algebra 9

The Algebra task facilitates a number of operations on both 1D and 2D datasets. Constant operation and f(ordinate) operate upon a single dataset, be it 1D or 2D. The rest of the operations require two datasets; both a Primary and Secondary. The Algebra sub task is invoked by clicking Algebra in the task bar.

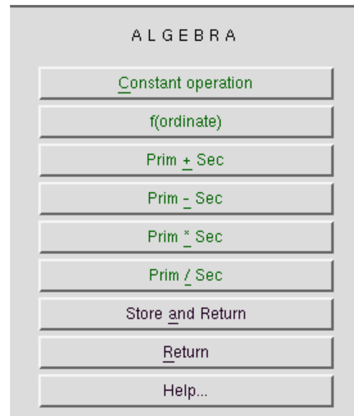


Figure 9-1 The Algebra task.

How to Perform a Constant Operation 9.1

It is assumed you are already in the Algebra task bar and the spectrum is in the Primary dataset. Constant operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Section Figure 2-3.) Then the Constant operation is performed on the Secondary dataset.

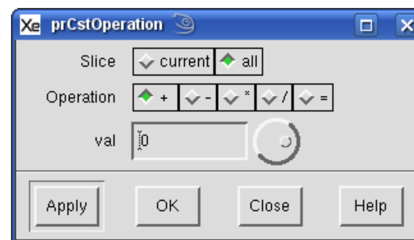


Figure 9-2 Performing a constant operation.

There are five different operations.

+ This operation adds the parameter val to the active dataset.

- This subtracts the parameter val from the active dataset.

***** This multiplies the active dataset by the parameter val.

/ This divides the active dataset by the parameter val.

= This substitutes the parameter val for all the ordinate values.

To perform a constant operation:

1. **Load a dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Define the operation region.** By default the entire region is operated on. To operate on only a portion of the region, click the Region Qualifier button. Click and drag the cursor to select the region or regions that you wish to define as the region to operate on. Resize the qualifiers if necessary.

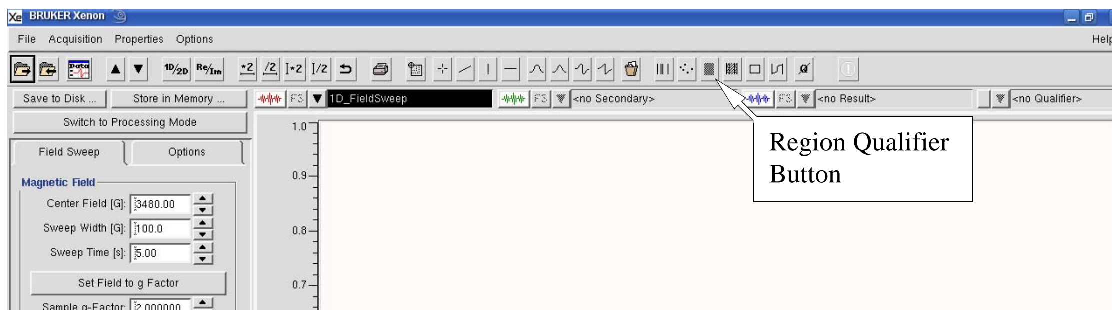


Figure 9-3 The Region Qualifier button.

4. **Click Constant operation.**
5. **Enter a value for val.**
6. **Select an Operation.**
7. **Select current or all.** If you have a 2D dataset, you probably want to select all so that the operation is performed on all the slices. Otherwise, the result is a 1D dataset based on the current slice.
8. **Click Apply.** The result of operation appears in the Result dataset. Click Close. If you click OK, the Result replaces the active dataset.
9. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

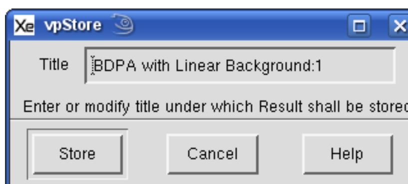


Figure 9-4 Storing the resultant spectrum in memory.

How to Perform an f(ordinate) Operation 9.2

In some cases you may want to transform to a nonlinear ordinate scale. For example if you have a decaying exponential. It is assumed you are already in the Algebra task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 4-3.) Then the transformation is performed on the Secondary dataset.

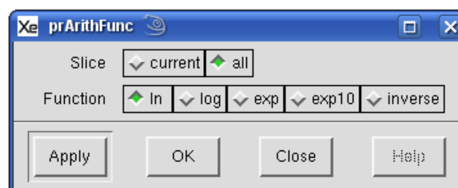


Figure 9-5 Performing an f(ordinate) operation.

There are five choices for nonlinear ordinates.

ln This operation takes the natural logarithm, ln(y), of the ordinate.

log This operation takes the base 10 logarithm, $\log_{10}(y)$, of the ordinate.

exp This exponentiates, e^y, the ordinate.

exp10 This exponentiates, 10^y, the ordinate.

inverse This takes the reciprocal, 1/y, of the ordinate.

To perform an f(ordinate) operation:

1. **Load a dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Define the operation region.** By default the entire region is operated on. To operate on only a portion of the region, click the Region Qualifier button. Click and drag the cursor to select the region or regions that you wish to define as the region to operate on. Resize the qualifiers if necessary.

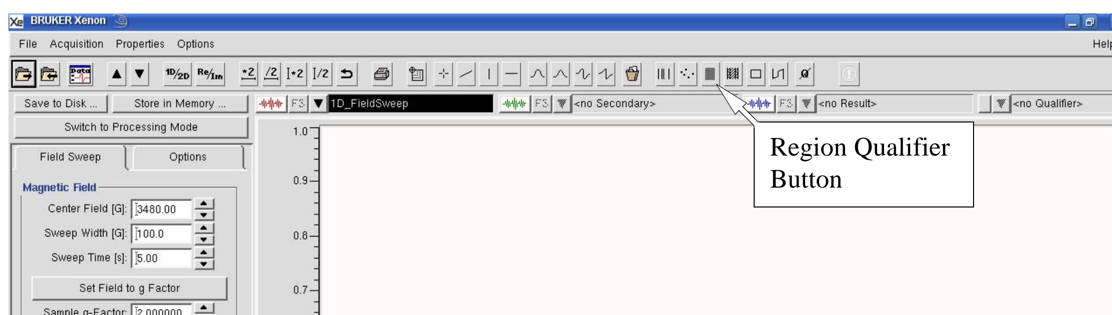


Figure 9-6 The Region Qualifier button.

4. **Click f(ordinate).**

5. **Select a Function.**
6. **Select current or all.** If you have a 2D dataset, you probably want to select all so that the operation is performed on all the slices. Otherwise, the result is a 1D dataset based on the current slice.
7. **Click Apply.** The result of operation appears in the Result dataset. Click Close. If you click OK, the Result replaces the active dataset.
8. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

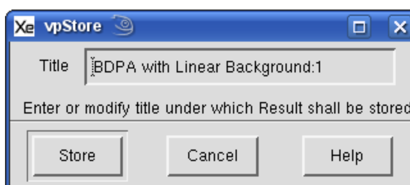


Figure 9-7 Storing the resultant spectrum in memory.

How to Perform Binary Operations 9.3

Many times you may need to operate on two datasets such as when subtracting a background or impurity signal. Because this operation involves two datasets, this is commonly referred to as a binary operation. These operations can performed on both 1D and 2D datasets. When one dataset is 1D and the other 2D, the 1D dataset must be in the Secondary dataset. Note that this operation operates on entire datasets. Qualifiers have no effect on the operation. It is assumed you are already in the Algebra task bar and the spectrum is in the Primary dataset.

Prim +/- Sec

The most common operation is adding or subtracting two datasets. To account for frequency shifts, the x axis of the Secondary dataset can be shifted and stretched. The amount of Secondary dataset added or subtracted from the Primary is defined by the Gain. The result dataset is defined by:

$$\text{Result} = \text{Primary} +/- \text{Gain} * \text{Secondary}(\text{x-Shift}, \text{x-Stretch}) \qquad [9\text{-}1]$$

Figure 9-8 The Prim - Sec dialog box.

1. **Load datasets into the Primary and Secondary datasets.**
2. **Click Prim+Sec or Prim-Sec.** A dialog box appears.
3. **Click Add or Subtract.**
4. **Select current or all.** If you have a 2D dataset, you probably want to select all so that the operation is performed on all the slices. Otherwise, the result is a 1D dataset based on the current slice.
5. **Adjust Gain, x-Shift and x-Stretch.** Adjust these parameters until the desired result is achieved. Below is the example we look at with regards to Spectral Titration in Section 7.7. The vanadyl EPR component has been successfully subtracted from the mixture spectrum, leaving only the copper spectrum.
6. **Click Close.** The result of operation appears in the Result dataset. If you click OK, the Result replaces the active dataset.

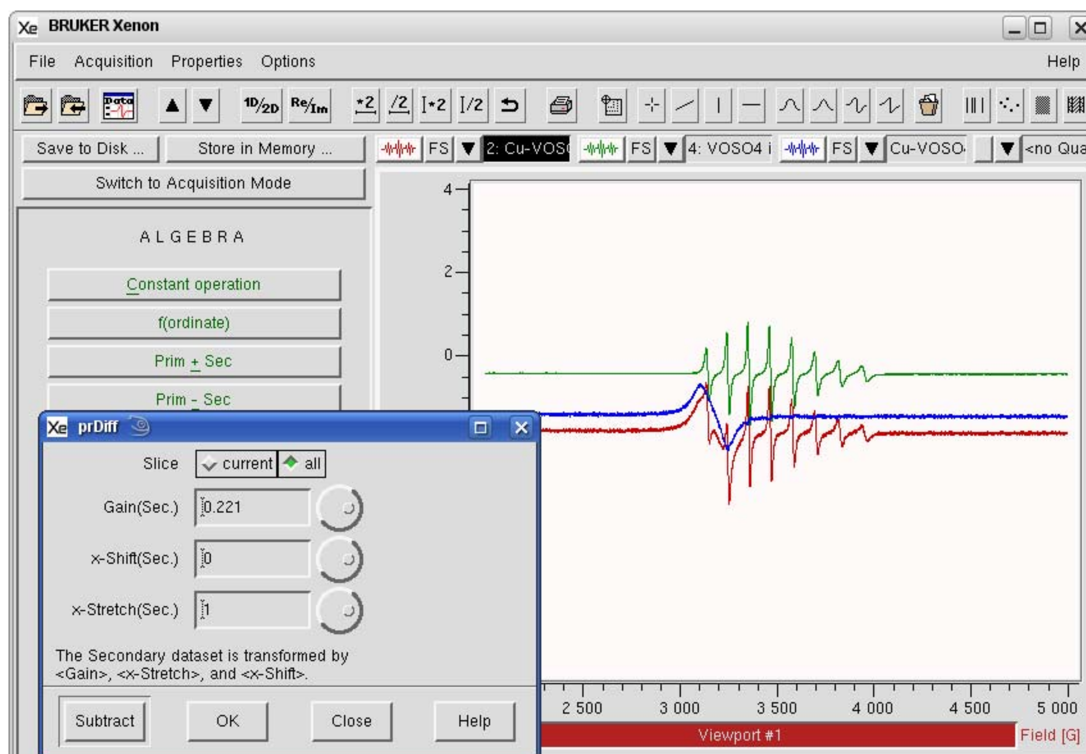


Figure 9-9 Subtracting a reference spectrum of a signal species from a spectrum of a mixture of two species.

7. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click

Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

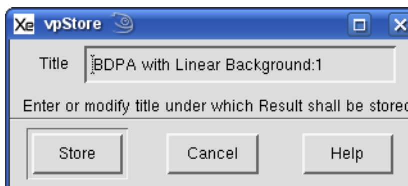


Figure 9-10 Storing the resultant spectrum in memory.

Prim */ Sec There may be some cases in which you need to multiply or divide two datasets. This operation is selected by clicking the Prim * Sec or Prim / Sec button. Its operation is similar to the +/- operations discussed in the previous section except there is no x-Shift or x-Stretch.

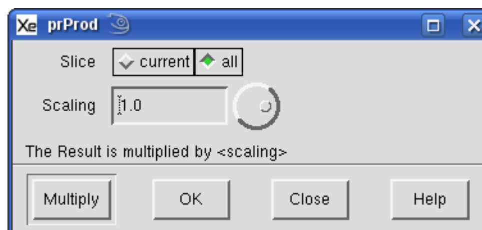


Figure 9-11 The Prim * Sec dialog box.

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Complex 10

Though complex data is usually encountered in pulse EPR experiments, there are a few cases in which it is important in CW experiments. In some experiments, the data may have two channels, such as in quadrature detection (first/second harmonic or 0°/90° field modulation measurements). The two channels can be represented as the real and imaginary part of a complex number.

$$y_i = a_i + ib_i \qquad [10\text{-}1]$$

There are a number operations required for dealing with complex data. The Complex sub task is invoked by clicking Complex in the task bar.

Figure 10-1 The Complex task.

Xenon displays the real or imaginary part in the viewport. In order to toggle between the real or imaginary display, click the Re/Im button in the toolbar. The (Re) and (Im) at the end of the title indicates the displayed component.

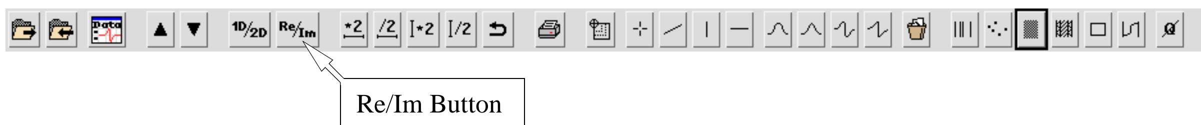

Figure 10-2 Toggling between the real and imaginary display.

It is assumed you are already in the Algebra task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. The following four operations replace the original data with the result. The original data is still in memory if they are Stored in Memory.

Absolute 10.1

This operation calculates the magnitude of the complex number.

$$\text{Absolute}(a_i + i \cdot b_i) = \sqrt{a_i^2 + b_i^2} \qquad [10\text{-}2]$$

Power 10.2

This operation calculates the magnitude of the complex number.

$$\text{Power}(a_i + i \cdot b_i) = a_i^2 + b_i^2 \qquad [10\text{-}3]$$

Real Part 10.3

This operation calculates the real part of the complex number.

$$\text{Real Part}(a_i + i \cdot b_i) = a_i \qquad [10\text{-}4]$$

Imag Part 10.4

This operation calculates the imaginary part of the complex number.

$$\text{Imag Part}(a_i + i \cdot b_i) = b_i \qquad [10\text{-}5]$$

How to Build a Complex Dataset 10.5

You can acquire the data as separate datasets and combine them into a complex dataset with this operation. Note that the abscissae of the two datasets must have the same number of points and span the same range.

1. **Load the real data into Primary dataset.**
2. **Load the imaginary data into the Secondary dataset.**
3. **Click Build Complex.** The resultant complex dataset appears in the Result dataset.

$$\text{Result}_i = \text{Primary}_i + i \cdot \text{Secondary}_i \qquad [10\text{-}6]$$

Transformations 11

A number of transformations may be required in order to analyze your data. The Transformations sub task is invoked by clicking Transformations in the task bar. Unlike many other task bars, this task bar immediately replaces the dataset to be transformed with the transformed dataset as opposed to the transformed dataset appearing in the Result dataset. The original data still remains in memory if it has been Stored in Memory.

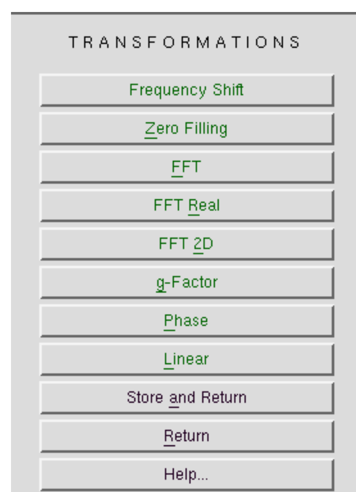

Figure 11-1 The Transformations task.

How to Frequency Shift Data 11.1

In some cases, you may need to compare data that have been acquired at two different microwave frequencies. There are two algorithms for performing this analysis. The first (g-factor) is a simple scaling of the field values:

$$\text{New Field}_i = \text{Old Field}_i \cdot \frac{\text{New Microwave Freq.}}{\text{Old Microwave Freq.}} \qquad [11\text{-}1]$$

Where i refers to the index of the data points. Figure 11-2 shows the spectrum of BDPA and the Bruker marker at 9.8 GHz. The two lines have different g-values. After the g-factor Frequency Shift for 1.0 GHz, the EPR spectrum is shifted to lower field. Also notice that the spectrum has been contracted if we overlay the two spectra. If we place our cursor on the zero cross-overs of the two EPR lines in both spectra, we read out the same g-values in both spectra.

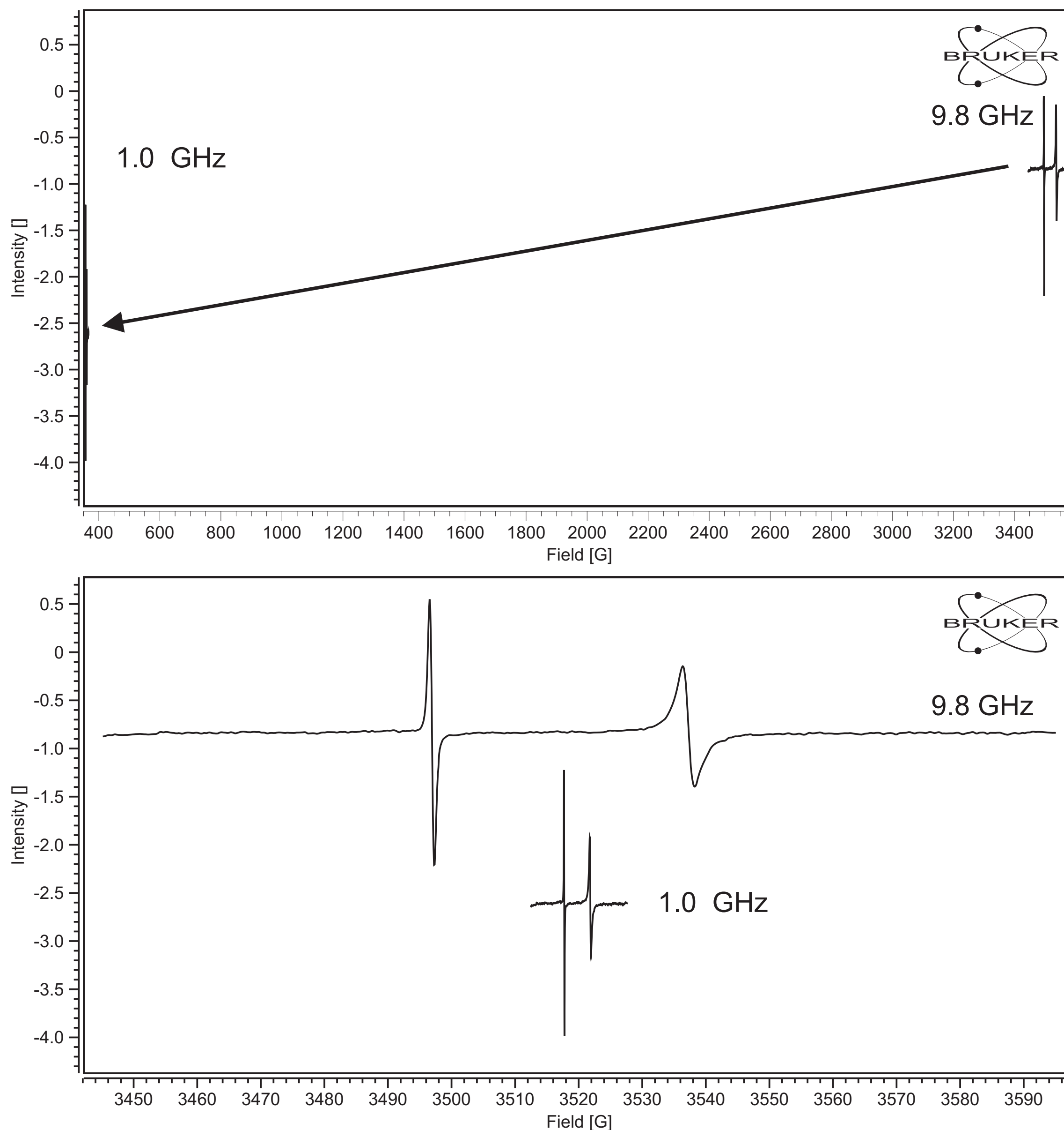

Figure 11-2 Transformation of an EPR spectrum via the g-factor Frequency Shift operation. Note for the bottom display the 1.0 GHz data has been shifted to the right clarity.

This may not be the best approach when you wish to compare hyperfine structure which is a field independent interaction. Figure 11-3 shows the contraction that happens when the g-factor Frequency Shift is performed; we can no longer compare the hyperfine splitting. If we select the hyperfine Frequency Shift, the center field is shifted to:

$$\text{New Center Field} = \frac{h\nu}{g|\mu_B|} \qquad [11\text{-}2]$$

where h is Planck's constant, ν is the microwave frequency, g is the Spectrum Center g-factor, and $|\mu_B|$ is the absolute value of the Bohr magneton. The sweep width remains constant in the hyperfine Frequency Shift. Now the hyperfine splitting can be compared properly.

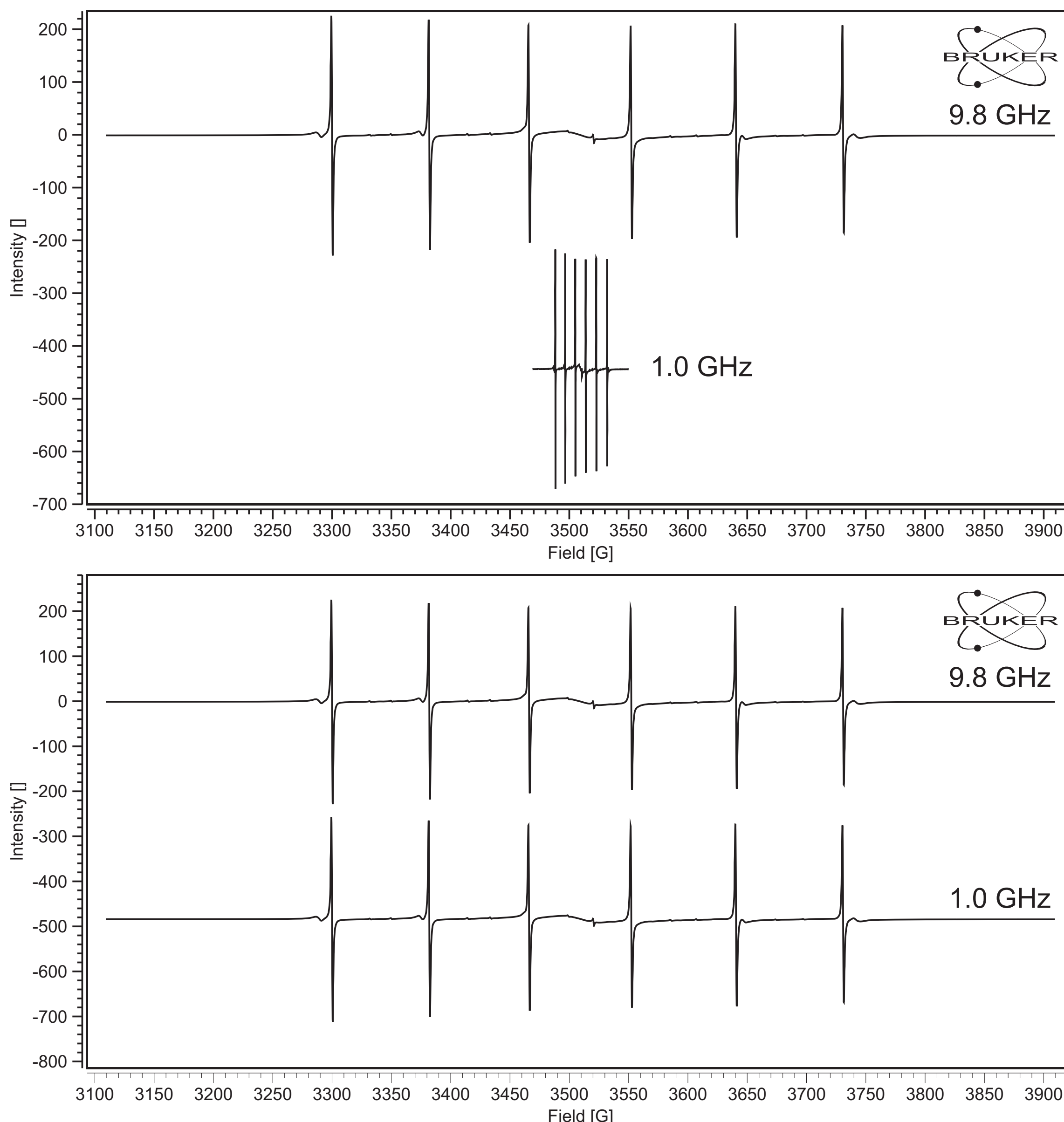

Figure 11-3 The g-factor Frequency Shift introduces an unwanted contraction in hyperfine splittings. The hyperfine Frequency Shift allows a better comparison of the hyperfine splittings. Note for the 1.0 GHz data has been shifted to the right clarity

It is assumed you are already in the Transformations task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. To perform a frequency shift:

1. **Load the Spectrum into the Primary or Secondary dataset.**

2. **Click Frequency Shift.** A new window appears.

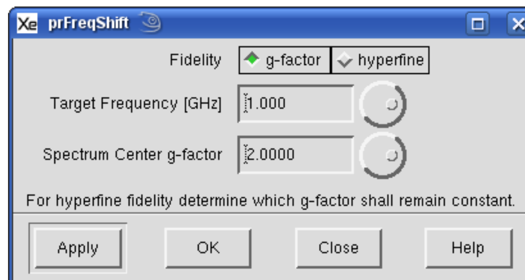

Figure 11-4 The Frequency Shift operation.

3. **Select the method.** Select g-Factor or hyperfine for Fidelity.
4. **Enter the Target Frequency.**
5. **Enter the Spectrum Center g-factor.** This only is used for the hyperfine method. This is the g-factor corresponding to the Center Field of the original EPR spectrum.
6. **Click Apply or OK to perform the transformation.** The transformed data replaces the original dataset.
7. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

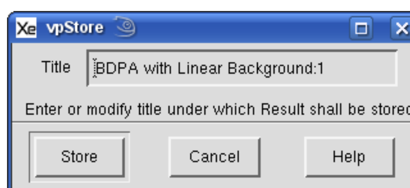

Figure 11-5 Storing the resultant spectrum in memory.

Fourier Transform Operations 11.2

The next four tasks in Transformations are related to Fourier transformation of the data. These operations are not typically used in CW EPR but may be of some use in looking for frequency components, reducing noise, or interpolating data.

Fourier transforms are usually applied to time or frequency data. We can represent a function either in the time domain or the frequency domain. It is the Fourier transform which converts between the two representations. The Fourier transform is defined by the expression:

$$F(\omega) = \int_{-\infty}^{+\infty} f(t)e^{-i\omega t}\, dt \qquad [11\text{-}3]$$

There is also an inverse Fourier transform:

$$f(t) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} F(\omega)e^{i\omega t}\, d\omega \qquad [11\text{-}4]$$

Field swept data can also be treated with Fourier transforms. The data can represented either in the field or 1/field domain.

The Fourier transformation is performed by a FFT (Fast Fourier Transformation) algorithm. This algorithm requires an integer power of 2 data points (2^N where N is an integer). If this is not the case, zerofilling will be applied (See Section 11.6.) to ensure this condition is satisfied.

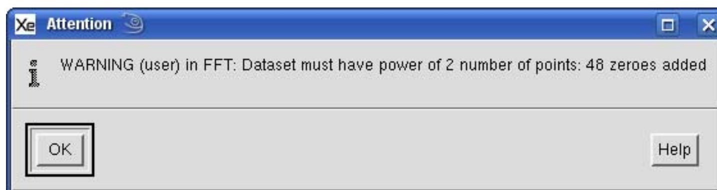

Figure 11-6 Zerofilling or adding points to ensure an integral power of two points for an FFT.

How to FFT Real and Complex Data 11.3

The complex FFT can transform both complex and real datasets. For a real dataset of length *n* points, the result of a forward transformation is a complex (both real and imaginary) dataset of length *n*. The result of the inverse transformation of this dataset is a complex dataset of length *n* with the imaginary values all equal to zero. For a complex dataset of length *n* points the transformation results in a complex dataset of *n* points. Inverse transformation yields a complex dataset of length *n*.

In order to make the inverse transformation work correctly after a forward transformation, Use History must be enabled. Choose Properties > Dataset History in the menu. Click yes to enable Use History. Now information for

proper reconstruction of the abscissa after the forward transformation is stored with the data.

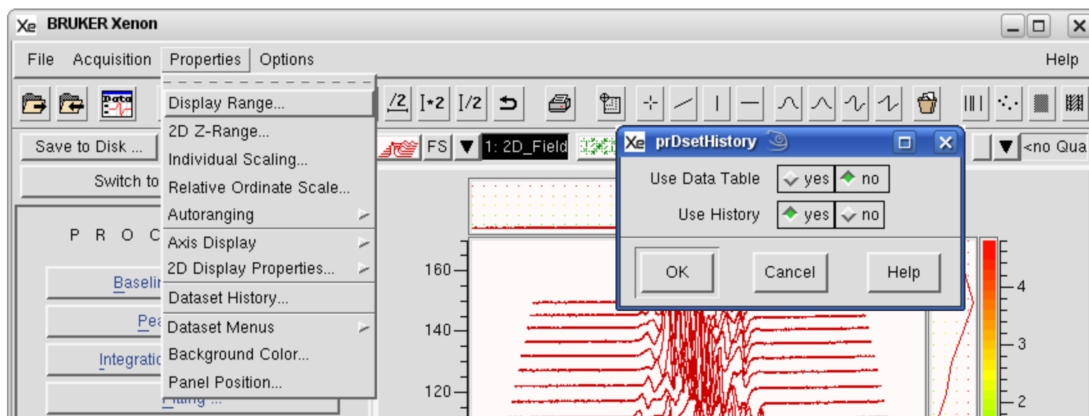

Figure 11-7 Enabling Use History.

It is assumed you are already in the Transformations task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. To perform an FFT:

1. **Load the Spectrum into the Primary or Secondary dataset.**
2. **Click FFT.** A new window appears.

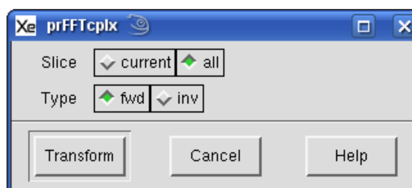

Figure 11-8 The FFT operation.

3. **Select the Type.** Select fwd for a forward FFT or inv for an inverse FFT.
4. **Select a Slice value.** By default current is selected for a 1D dataset and all for a 2D dataset in which case the FFT is performed for all the slices. If current is selected for a 2D dataset the FFT is performed only on the current slice in the display and results in a 1D result.
5. **Click Transform to perform the transformation.** The transformed data replaces the original dataset.
6. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click

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Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

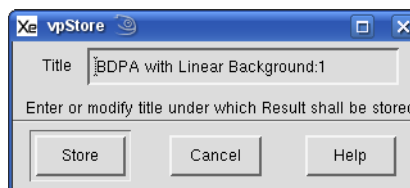

Figure 11-9 Storing the resultant spectrum in memory.

How to Perform an FFT Real Transformation 11.4

The real FFT is usually used to transform a purely real dataset. Transformation of a real dataset of length n results in a complex dataset of length $n/2$. Upon the inverse transformation of the complex data, a real dataset of length n results.

In order to make the inverse transformation work correctly after a forward transformation, Use History must be enabled. Choose Properties > Dataset History in the menu. Click yes to enable Use History. Now information for proper reconstruction of the abscissa after the forward transformation is stored with the data.

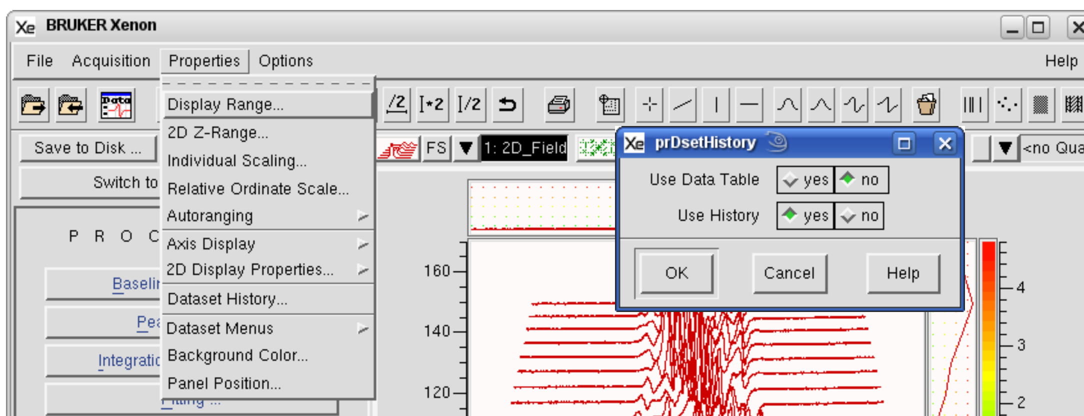

Figure 11-10 Enabling Use History.

Unlike the complex FFT described in Section 11.3, there is no distinction between forward and inverse transformations. There is also no choice for current or all. The appropriate choice is automatically made for 1D and 2D datasets.

It is assumed you are already in the Transformations task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. To perform a real FFT:

1. **Load the Spectrum into the Primary or Secondary dataset.**
2. **Click FFT Real.** The transformed data replaces the original dataset.

3. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

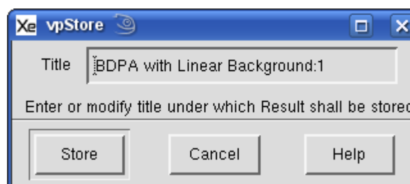

Figure 11-11 Storing the resultant spectrum in memory.

How to perform a 2D FFT 11.5

This task functions identically as the FFT described in Section 11.3 except it performs the transformation along both abscissae of a 2D dataset. By default if performs a forward transformation.

It is assumed you are already in the Transformations task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. To perform a 2D FFT:

1. **Load the Spectrum into the Primary or Secondary dataset.**
2. **Click FFT 2D.** A new window appears.

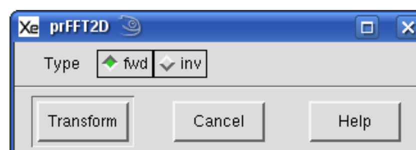

Figure 11-12 The FFT 2D operation.

3. **Select the Type.** Select fwd for a forward FFT or inv for an inverse FFT.
4. **Click Transform to perform the transformation.** The transformed data replaces the original dataset.
5. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click

Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

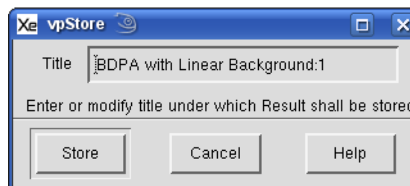

Figure 11-13 Storing the resultant spectrum in memory.

How to Zero Fill 11.6

This command adds a number of points of value zero to a dataset so that the total number of points is an integral power of two (2^N). This type of operation is commonly used for FFT (Fast Fourier Transformation) operations as 2^N points are required for such operations. There are two means of doing this operation; append the zeroes to the end of the dataset (append) or to the beginning and end of the dataset (symmetric). Select the New Length. Click Fill to perform the Zero Filling.

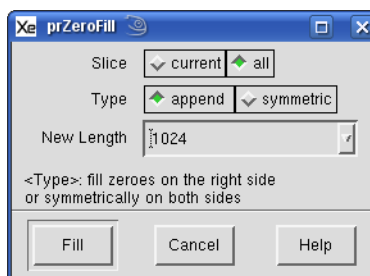

Figure 11-14

Interpolation is one application of this process. A dataset can be Fourier transformed, then zero filled, and then inverse transformed to increase the number of points in the dataset. Note that no further information is added to the dataset; it only affects the appearance of the dataset.

How to Display Data with a g-Factor Axis 11.7

The g-factor,

$$g = \frac{h\nu}{\mu_B B_0} \qquad [11\text{-}5]$$

is often a better representation for comparing data acquired at different frequencies.

It is assumed you are already in the Transformations task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then

the operation is performed on the Secondary dataset.To perform a g-factor transformation:

1. **Load the Spectrum into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Click g-Factor.** The EPR spectrum with the x-axis in g-Factor values replaces the original spectrum.

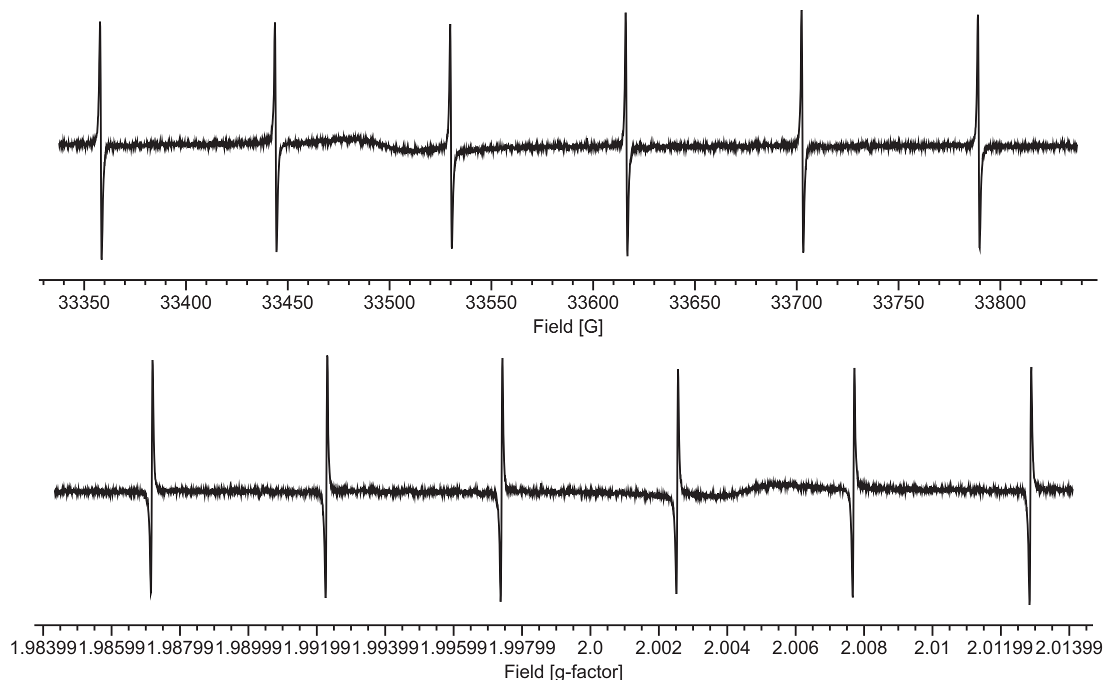

Figure 11-15 EPR data in both field and g-value displays. Note that the spectrum is mirrored in g-value mode because lower g-values correspond to higher magnetic fields owing to the reciprocal nature of the two parameters.

4. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original data

It should be noted that this transformation is one way. Pressing g-Factor again does not revert the data back to a field axis.

How to Phase Data 11.8

Though complex data is usually encountered in pulse EPR experiments, there are a few cases in which it is important in CW experiments. In some experiments, the data may have two channels, such as in quadrature detection (first/second harmonic or 0°/90° field modulation measurements). The two channels can be represented as the real and imaginary part of a complex number.

$$y_i = a_i + ib_i \quad [11\text{-}6]$$

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There may be cases in which we need to change the contributions from the real and imaginary channels. For example with 0°/90° field modulation measurements we wish to have all the signal in the real channel and minimized in the imaginary channel.

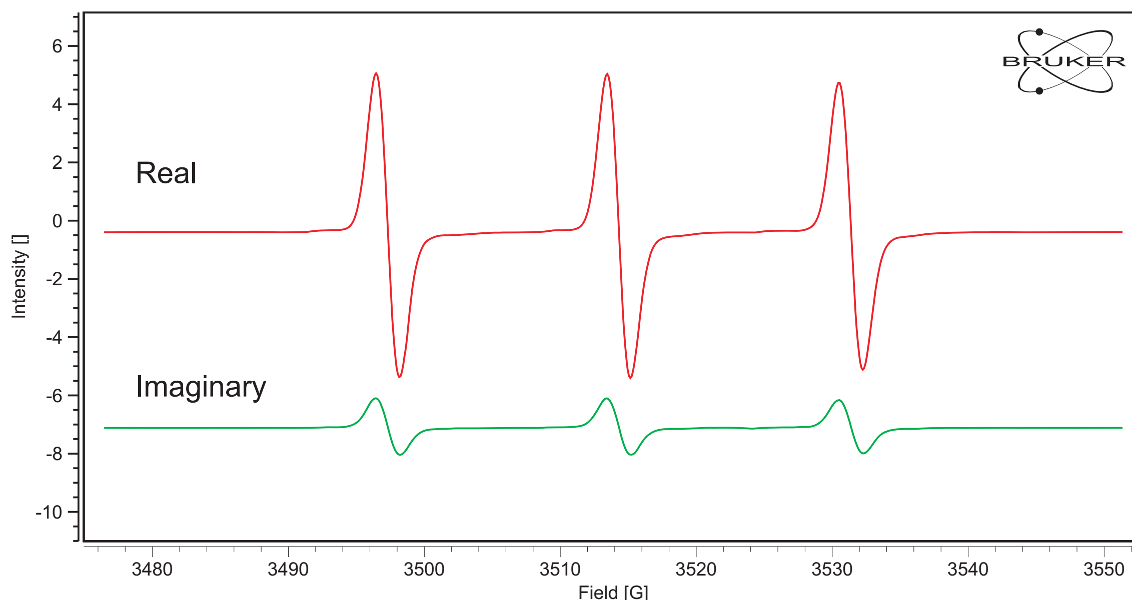

Figure 11-16 Complex dat from a 0°/90° field modulation measurement. The phase is not quite correct resulting in some signal in the imaginary channel.

This is accomplished via a phase transformation defined as:

$$a_i' = \cos\phi\varphi \cdot a_i + \sin\varphi \cdot b_i$$
$$b_i' = -\sin\varphi \cdot a_i + \cos\varphi \cdot b_i \qquad [11\text{-}7]$$

where a_i' and b_i' are the transformed values and φ is the phase angle.

It is assumed you are already in the Transformations task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. To perform a phase transformation:

1. **Load the Spectrum into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **View the imaginary part.** Click the Re/Im button in the toolbar to toggle between the real and imaginary components.

4. **Click Phase.** A new window appears.

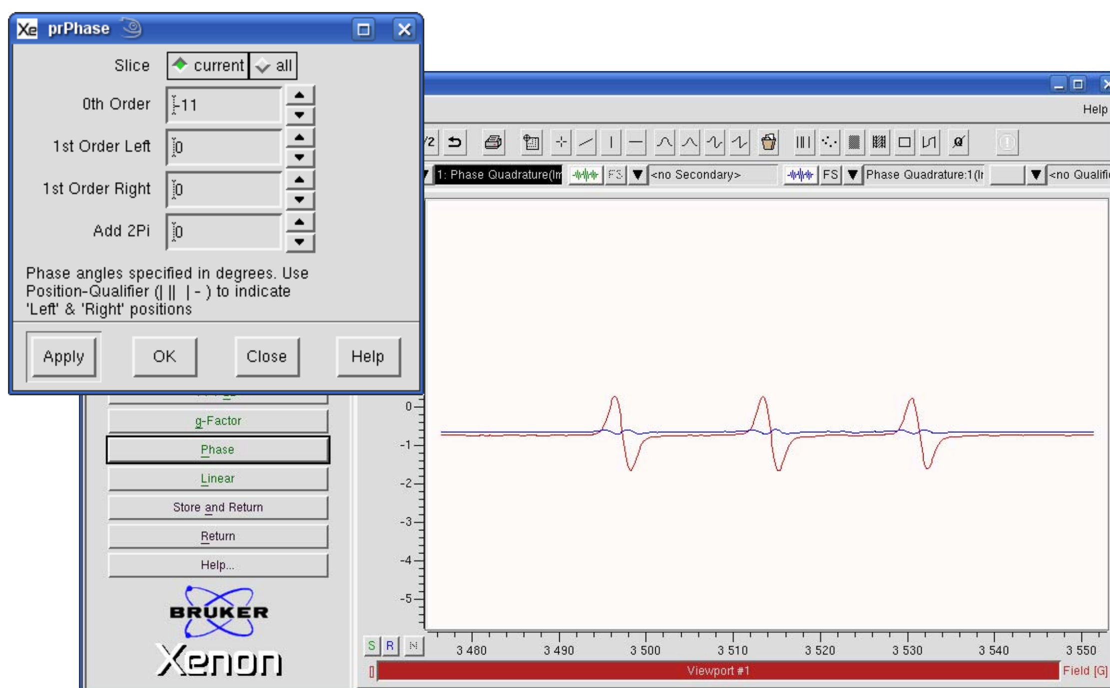

Figure 11-17 Phasing quadrature data.

5. **Vary the 0th Order phase.** Vary 0th Order until the Result (the blue trace) is minimized. The Result automatically updates.

6. **Click Apply or OK to perform the transformation.** The transformed data appears in the Primary or Secondary dataset.

7. **View the real part.** Click the Re/Im button in the toolbar to toggle between the real and imaginary components.

8. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original data

How to Perform a Linear Transformation 11.9

Sometimes you may have data that needs to be transformed by shifts or stretching along the x or y-axis. Linear accomplishes this task by calculating a new dataset with:

$$x' = x0 + (x - x0) \cdot \text{x-Stretch} + \text{x-Shift}$$
$$y' = y0 + (y - y0) \cdot \text{y-Stretch} + \text{y-Shift} \qquad [11\text{-}8]$$

where x' and y' are the transformed values.

The shift parameters simply move the axis value left and right for the x-axis or up and down for the y-axis. The stretching is a bit more complicated. It also involves the parameters x0 and y0. There are two options available: Relative to first point and Relative to zero. (See Figure 11-18.) Relative to

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zero chooses x0=y0=0 resulting in the simple formula:

$$x' = x \cdot \text{x-Stretch} + \text{x-Shift}$$
$$y' = y \cdot \text{y-Stretch} + \text{y-Shift} \quad [11\text{-}9]$$

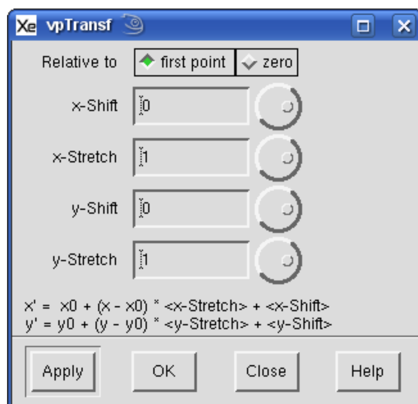

Figure 11-18 The Linear Transformation window.

An x-Stretch of two and Relative to zero multiplies the values of the x-axis by two.

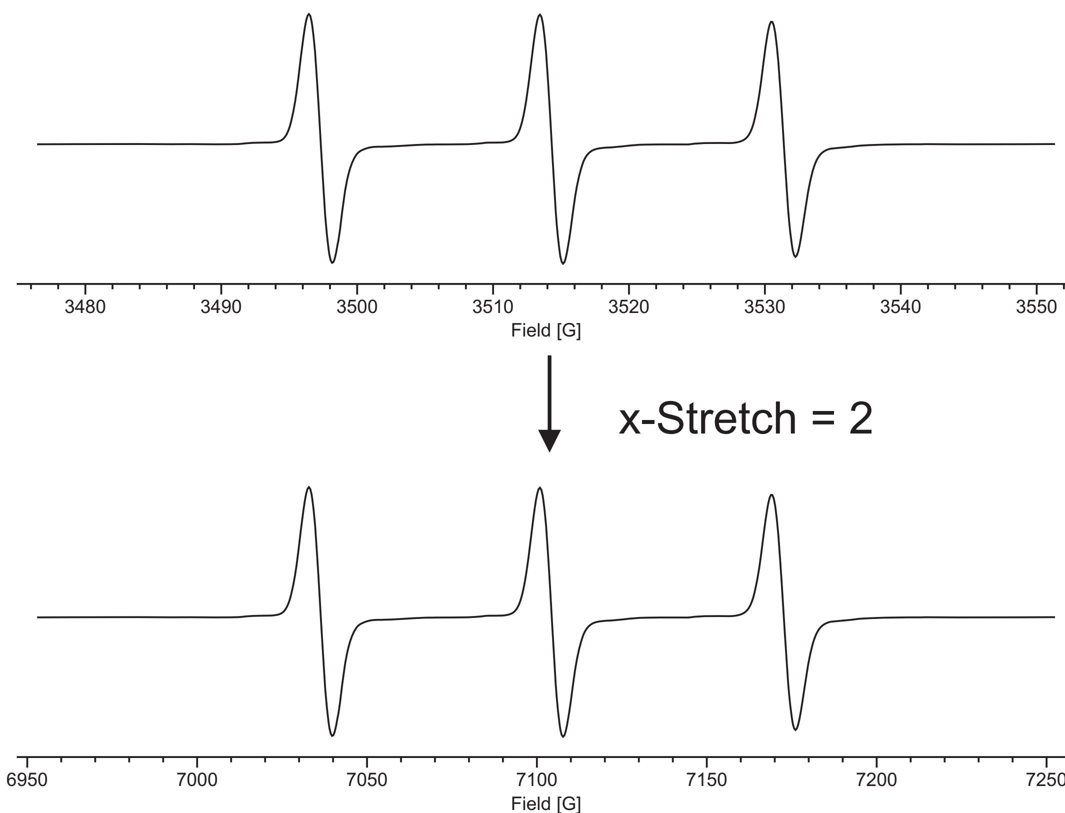

Figure 11-19 The result of an x-Stretch of two and Relative to zero.

If Relative to first point is selected x0 is set to the first x-value and y0 is set to the first y-value. All stretches are now performed with respect to the first

point. The first points of both the original and transformed data start at the same value and the data is stretched from that point.

The y-Stretch parameter behaves in a similar manner to x-Stretch.

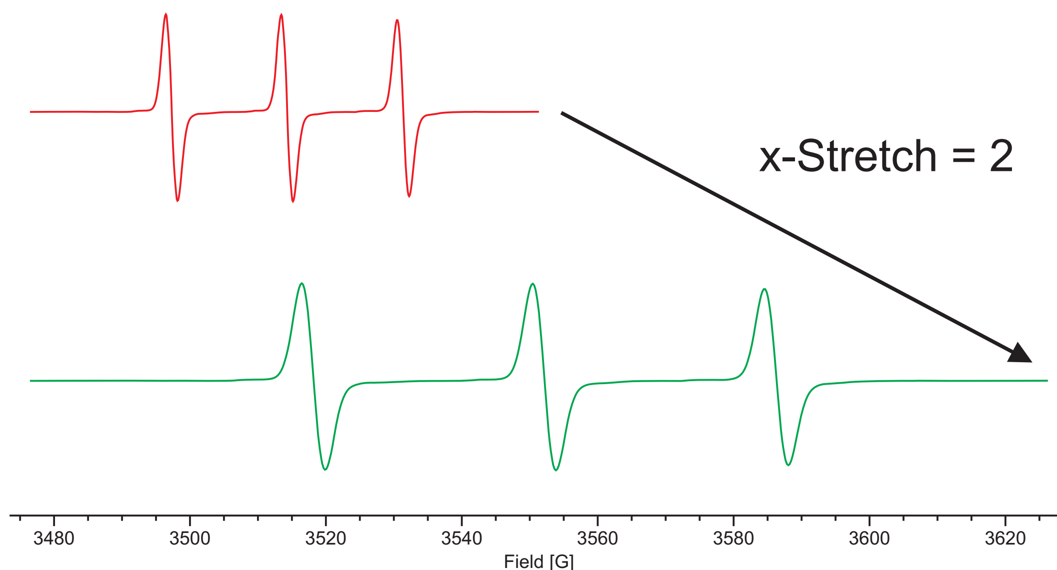

Figure 11-20 The result of an x-Stretch of two and Relative to first point.

It is assumed you are already in the Transformations task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. To perform a linear transformation:

1. **Load the Spectrum into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Click Linear.** Choose the Relative to value. Enter the desired values for the shift and stretch parameters. The transformed dataset replaces the original dataset.
4. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original data

Structure 12

Structure operations are used to change the size of datasets. It can be separated into two categories; those used for working with 2D datasets and those that work with both 1D and 2D datasets. The Structure sub task is invoked by clicking Structure in the task bar.

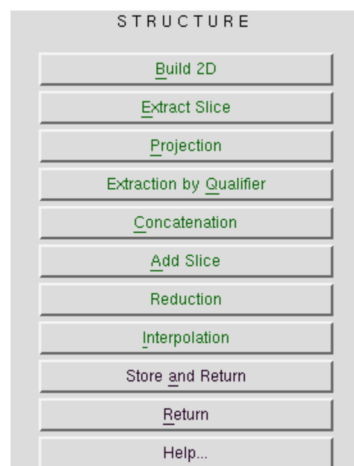

Figure 12-1 The Structure task.

How to Build a 2D Dataset 12.1

Often 2D datasets arise when an external variable is varied and these variable values are used to construct the second axis. In the 2D experiments this is done automatically. If you have acquired a series of 1D data and changed the external variable by hand, you can still combine the data into a 2D dataset by using Build 2D. It is assumed you are already in the Structure task bar. To build a 2D dataset:

1. **Load the 1D spectra into Xenon.** The spectra must be in memory in order to build the dataset.
2. **Click Build2D.** A new window appears displaying the 1D datasets in memory.

3. **Enter an Abscissa Name.** Type the name in the Abscissa Name field. In this case we have data collected at different times.

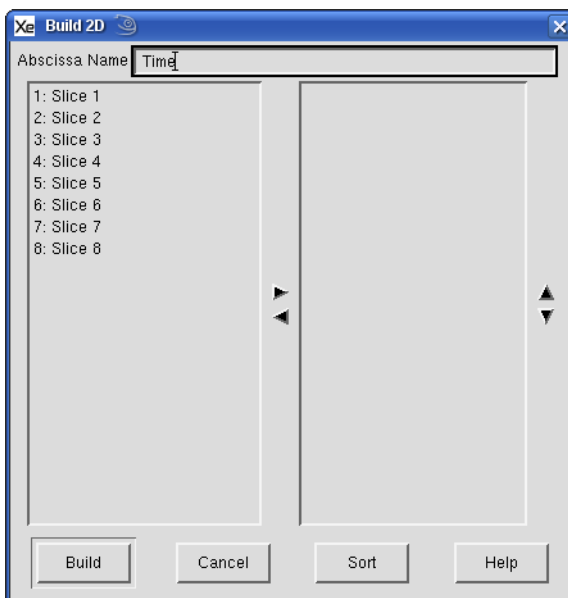

Figure 12-2 The Build 2D window.

4. **Select the data to include in the 2D dataset.** Click the dataset to select it and it will be highlighted. You can select multiple non-consecutive datasets by pressing the <ctrl> key while clicking. Consecutive dataset sets can be selected by clicking on the first desired dataset pressing the <shift> key and clicking the last desired dataset.

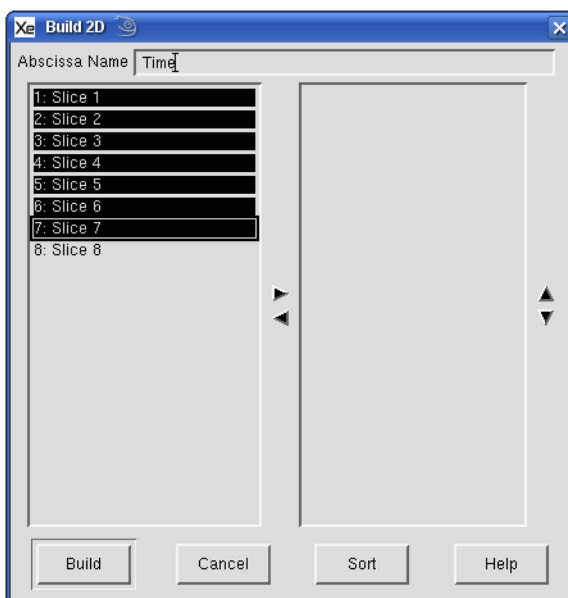

Figure 12-3 Selecting datasets for a 2D dataset.

5. **Add the datasets.** Click the arrow button pointed to the right. The data is then moved to the other display. A dataset can be removed by selecting it and clicking the arrow button pointed to the left. The data order can be changed by clicking a listed dataset and clicking the arrow buttons pointed up or down to move the dataset up or down in the list. Clicking the Sort button will sort the datasets in alphabetical order.

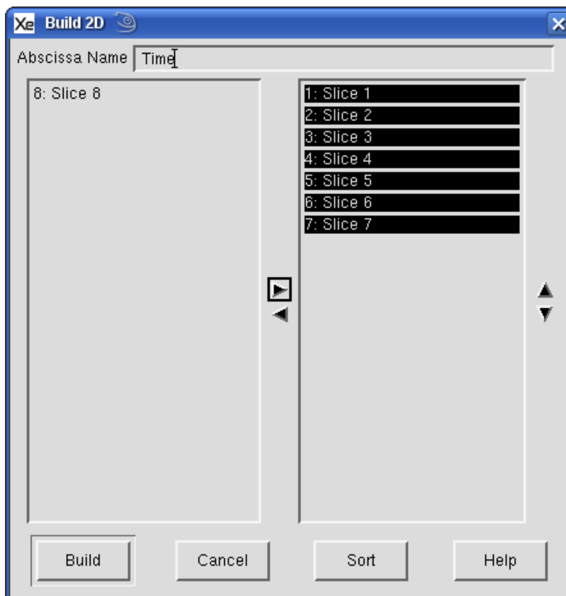

Figure 12-4 Adding datasets to a 2D dataset.

6. **Build the dataset.** Click Build. The 2D dataset appears in the Result dataset.

7. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

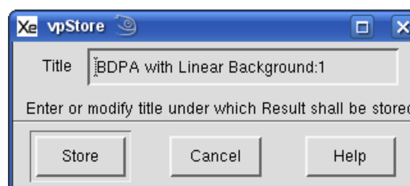

Figure 12-5 Storing the resultant spectrum in memory.

How to Add a Slice 12.2

It is assumed you are already in the Structure task bar. Adding a slice to a 2D dataset is easy:

1. **Load the 2D dataset into the Primary dataset.**
2. **Load the 1D dataset into the Secondary dataset.**
3. **Click Add slice. A new window appears.**
4. **Enter an Absc. 2 Name.** In this case it is Time.

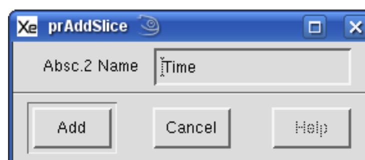

Figure 12-6 Adding a slice to a 2D dataset.

5. **Add the dataset.** Click Add. The new 2D dataset appears in the Result dataset.
6. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

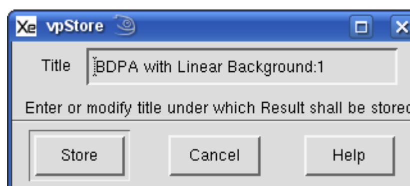

Figure 12-7 Storing the resultant spectrum in memory.

Note that you can use this operation to create and build a 2D dataset. Simply load 1D datasets in the Primary and Secondary dataset. Move the Result to the Primary and load the next slice in the Secondary. Repeat until all the slices are added.

How to Extract a Slice 12.3

There may be some cases in which you have a 2D dataset and you need one slice as a 1D dataset. It is assumed you are already in the Structure task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. This is easily accomplished:

1. **Load the 2D dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Scroll to the desired slice.** Click and drag the slider bar to the right of the dataset to select the slice to be extracted.
4. **Click Extract Slice.** The result appears in the Primary or Secondary dataset.
5. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

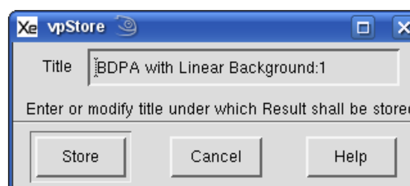

Figure 12-8 Storing the resultant spectrum in memory.

How to Create a Projection 12.4

It is often useful to view a projection of a 2D dataset. A projection is an operation in which the data along one of the axes is compressed into a 1D dataset. The most common is the roof projection. The value of the highest intensity point of a slice is stored in the projection analogous to the skyline silhouette of a city. In the figure below we see two roof projections along two sides of the display. The top projection is along the time axis. For each point along the magnetic field axis the highest intensity point in time at that field value is used to construct the projection. The right projection is along the magnetic field axis. For each time point the highest intensity point along the magnetic field axis is used to construct the projection.

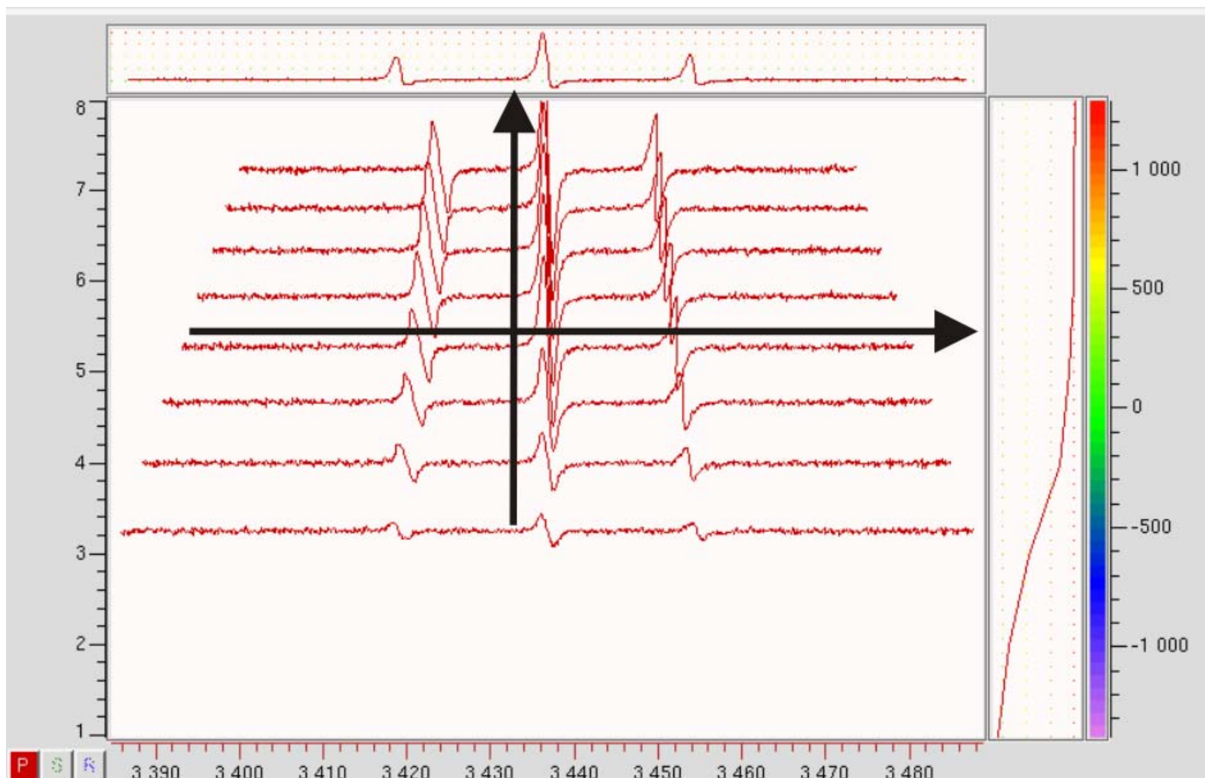

Figure 12-9 A roof projection of a 2D dataset.

Another common projection is the sum projection. In this case the values of a slice are summed and used to construct the projection.

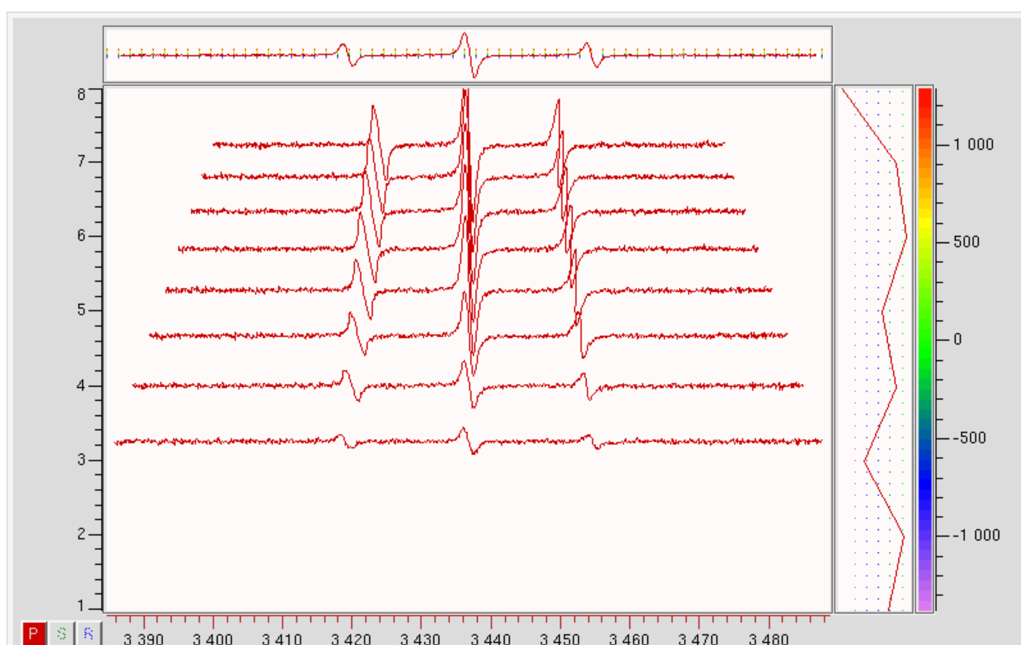

Figure 12-10 A sum projection of a 2D dataset.

A third type of projection is the floor projection. It is similar to the roof projection but the minimum instead of the maximum value is used for the projection.

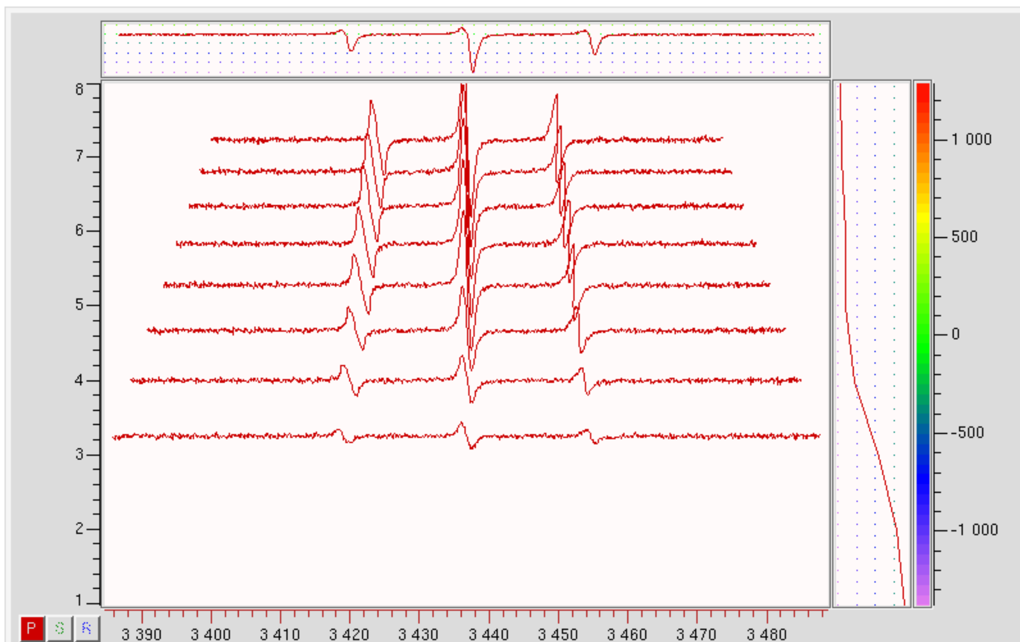

Figure 12-11 A floor projection of a 2D dataset.

It is assumed you are already in the Structure task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. A projection is calculated as follows:

1. **Load the 2D dataset into the Primary dataset.**

2. **Select 1D mode.** Click the 1D/2D toggle button to select 1D view.

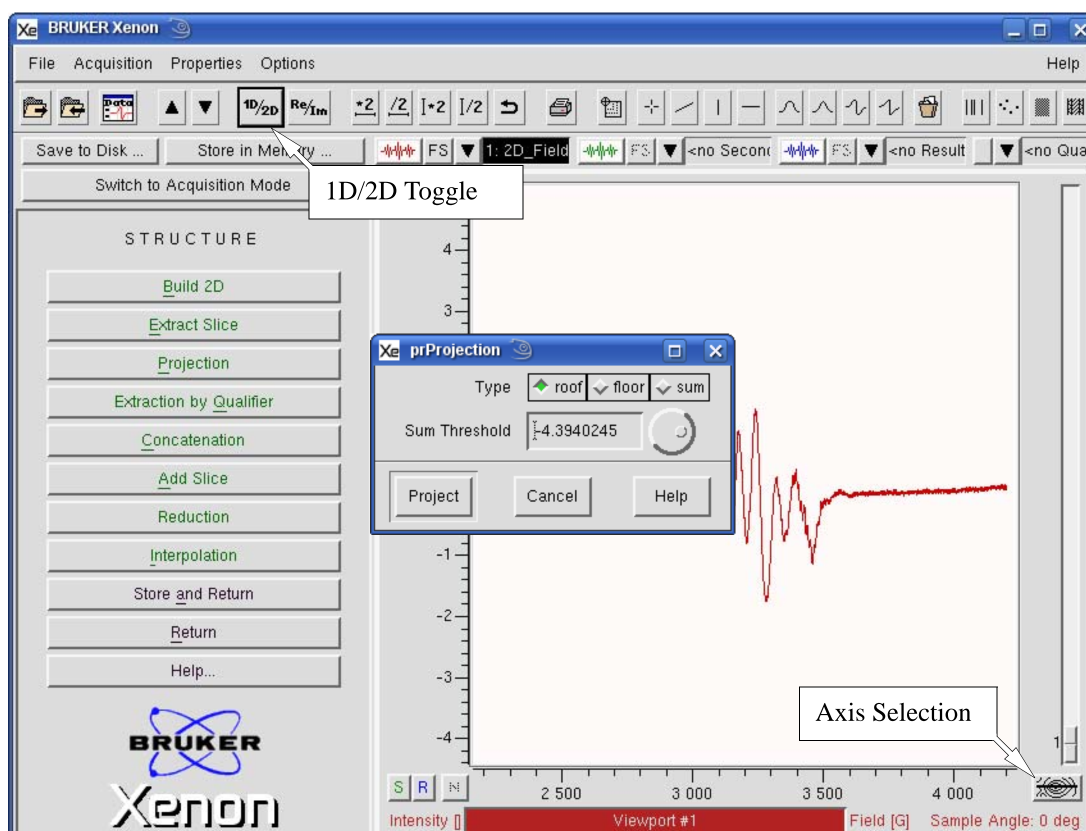

Figure 12-12 A floor projection of a 2D dataset.

3. **Select the axis for projection.** Click the axis selection button to select the desired axis along which you wish to project.
4. **Click Projection.** A new window appears.
5. **Choose the projection type.** Choose roof, floor, or sum.
6. **Enter a Sum Threshold.** If the value of a data point does not exceed the threshold, the data point is not used in the sum calculation. Note that this works only for sum projections.
7. **Click Project.** The result appears in the Primary dataset.
8. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

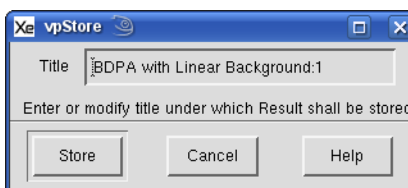

Figure 12-13 Storing the resultant spectrum in memory.

How to Extract by a Qualifier 12.5

Often only a small part of a dataset is needed. By using a qualifier to select the desired small part, this data can be extracted from the dataset. It is assumed you are already in the Structure task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset. This extraction can be performed as follows:

1. **Load the dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Select a section via a region qualifier.** For 1D data or with 2D data in 1D viewing mode, click on the region qualifier button and then click and drag to select the region of interest. You may select multiple regions.
4. **Click Extraction by Qualifier.** The result appears in the Result dataset (blue trace). For 2D data, this is performed for all slices.

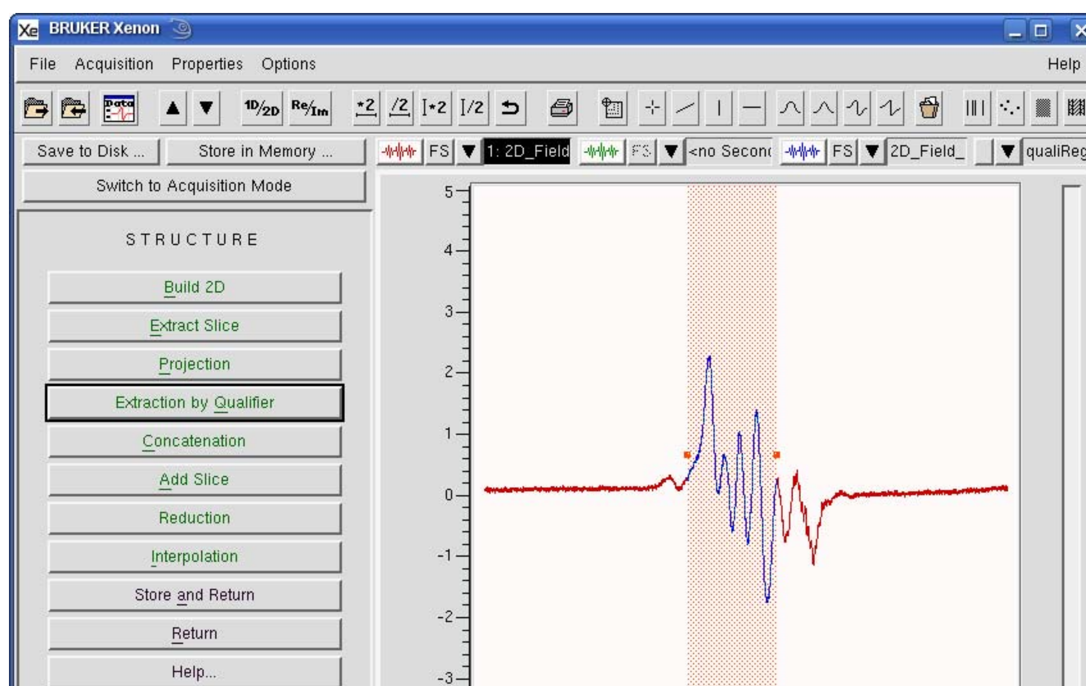

Figure 12-14 Qualifying a region with a region qualifier and the extraction result (blue trace).

5. **Select a section via an area qualifier.** For 2D data in 2D viewing mode, click on the area qualifier button and then click and drag to select the area of interest. You may select multiple regions.

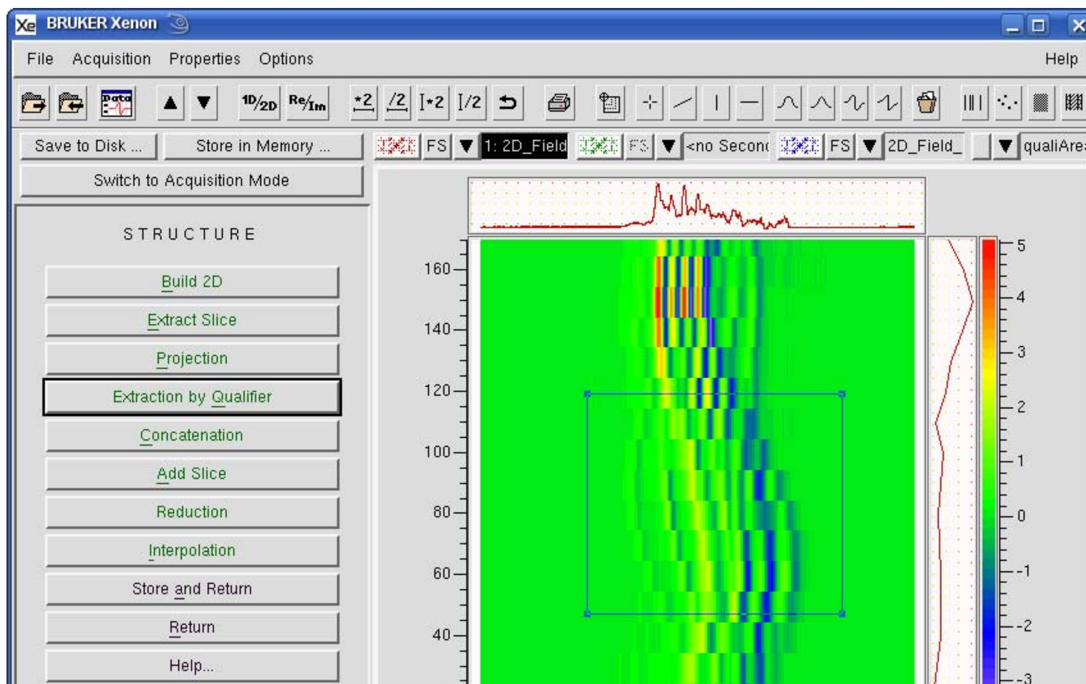

Figure 12-15 Qualifying an area with an area qualifier.

6. **Click Extraction by Qualifier.** The result appears in the Result dataset.

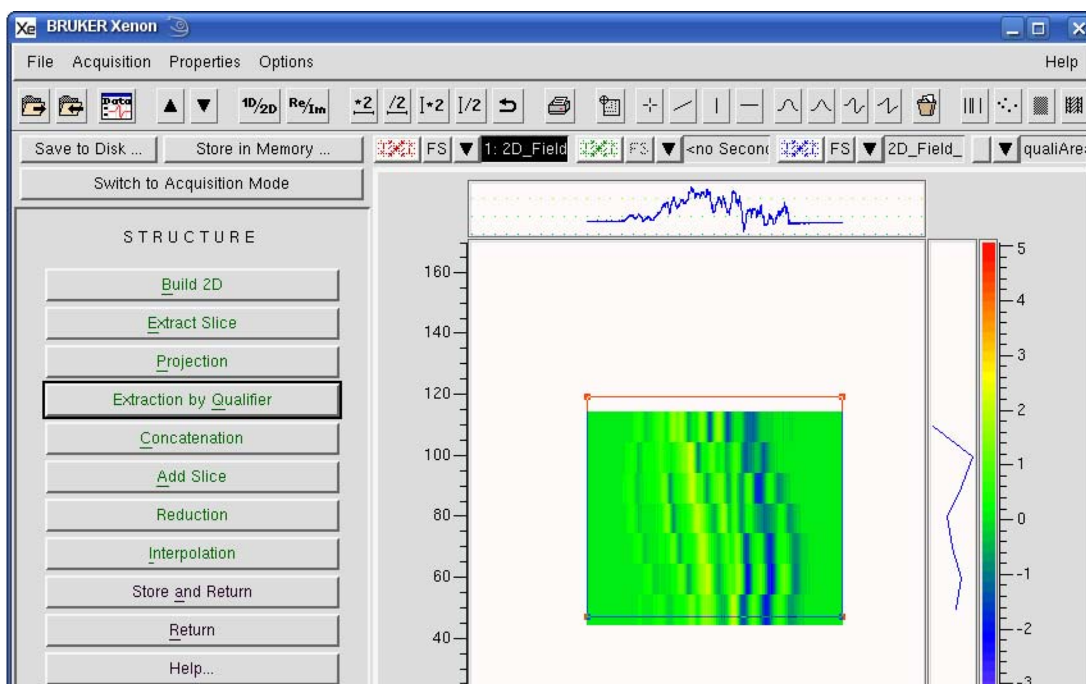

Figure 12-16 The result of extraction with an area qualifier.

7. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click

Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

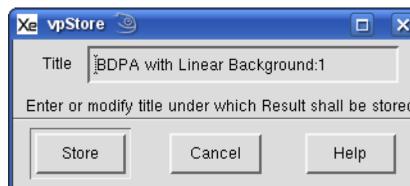

Figure 12-17 Storing the resultant spectrum in memory.

How to Concatenate Datasets 12.6

Two datasets can be combined into one larger dataset by concatenation. This involves joining the two dataset end to end. It is assumed you are already in the Structure task bar. This is accomplished as follows:

1. **Load the datasets into the Primary and Secondary dataset.**
2. **Click Concatenation.** The result will appear in the Result dataset. In this case the EPR spectrum was acquired from 0-5,000 G and from 5,000-10,000 G. After concatenation, the result is a continuous spectrum from 0-10,000 G.

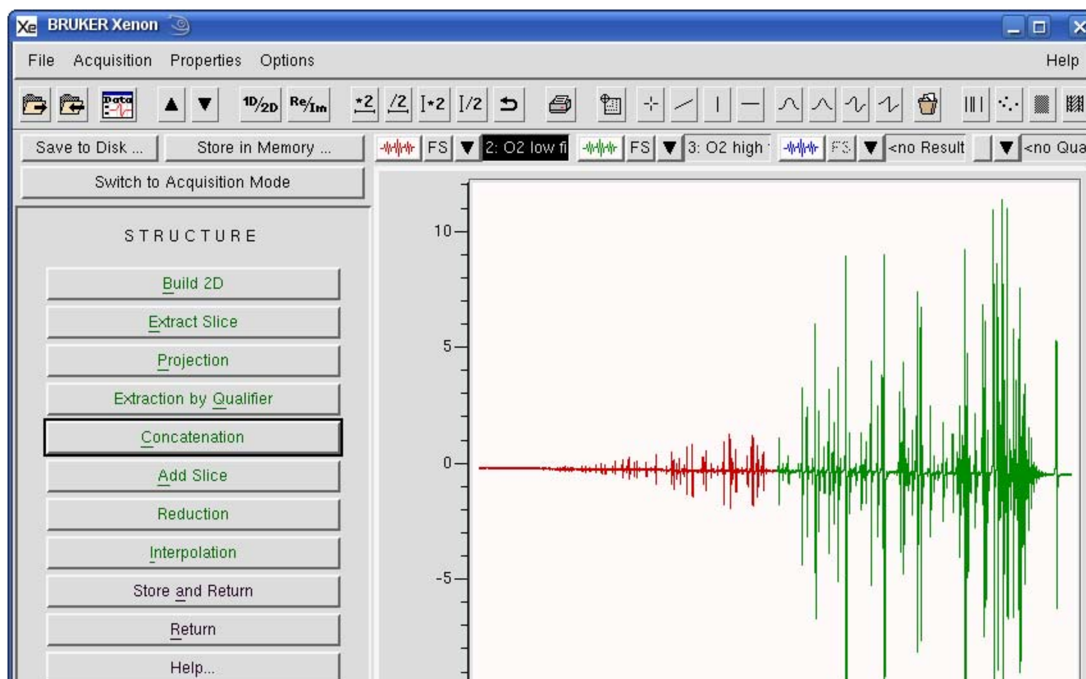

Figure 12-18 The result of concatenating two spectra.

3. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click

Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

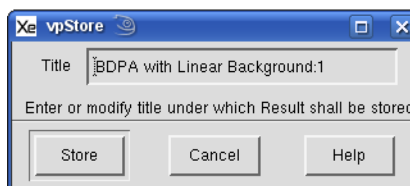

Figure 12-19 Storing the resultant spectrum in memory.

How to Reduce Datasets 12.7

In some cases the number of points in a dataset are too many. Reduction can be used to reduce the number of points.The important parameter is Nr. of Points. The original dataset P_i is grouped into subgroups of Nr. of Points and the average value of each subgroup is calculated. A new dataset P'_i is then constructed from these average values.

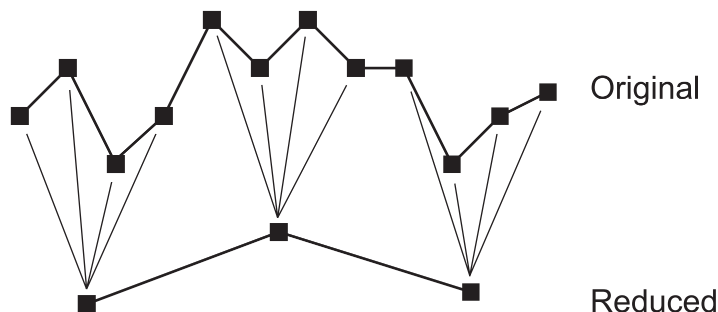

Figure 12-20 Reduction of a dataset using Nr. of Points = 4.

$$P'_i = \sum_{j=0}^{\text{Nr. of Points}} \frac{P_{i \cdot \text{Nr. of Points} + j}}{\text{Nr. of Points}} \qquad [12\text{-}1]$$

It is assumed you are already in the Structure task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset.

1. **Load the dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Click Reduction.** A new window appears.

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4. **Enter Nr. of Points and click Reduce.** The result appears in the Result dataset (blue trace).

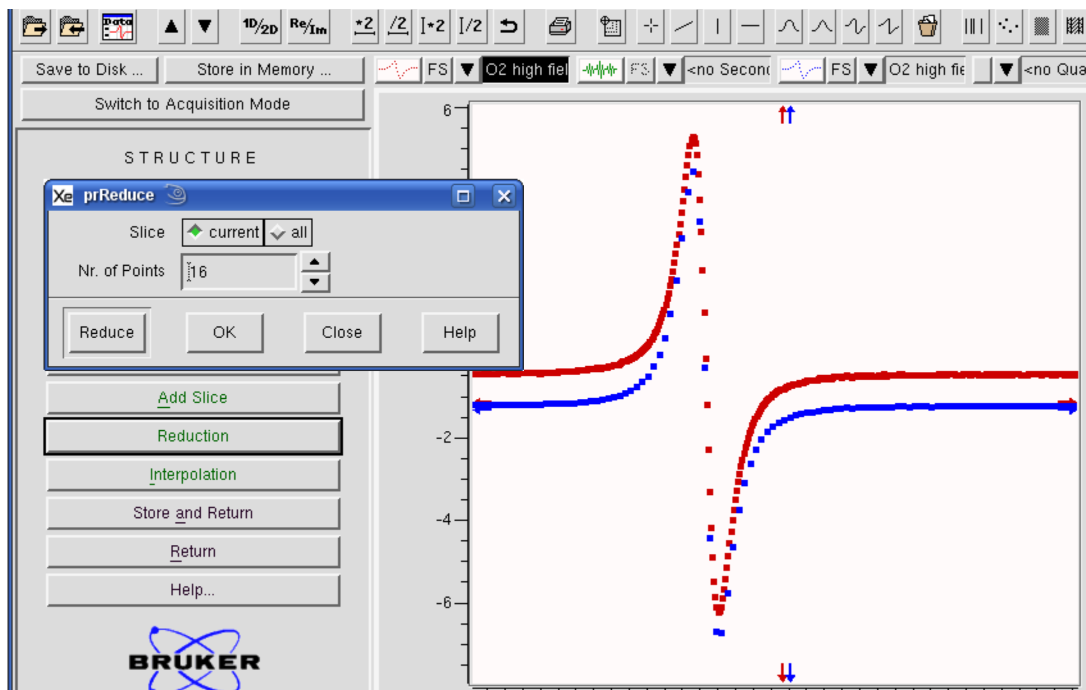

Figure 12-21 The result of reducing a dataset.

5. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

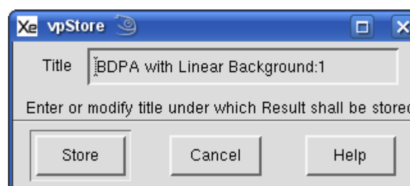

Figure 12-22 Storing the resultant spectrum in memory.

How to Interpolate Datasets 12.8

Xenon will change the number of points in a field sweep dependent on a number of parameters such as the modulation amplitude. If you need to analyze your data in another software application that requires a specific number of points, the interpolation process can give you the required number of points.

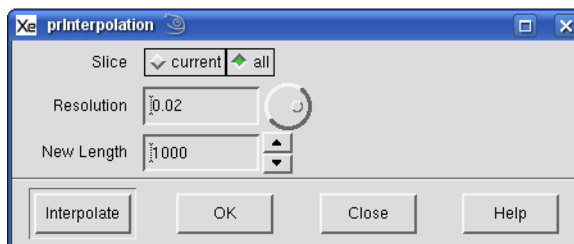

Figure 12-23 Interpolating data.

There are two interrelated parameters, the Resolution and the New Length. They are related by:

$$\text{Resolution} = \frac{\text{Sweep Width}}{\text{New Length-1}} \qquad [12\text{-}2]$$

Either parameter can be changed and the changes are then reflected in the other parameter.

It is assumed you are already in the Structure task bar and the spectrum is in the Primary dataset. The operation performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Figure 2-3.) Then the operation is performed on the Secondary dataset.

1. **Load the dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Click Interpolation.** A new window appears.
4. **Enter the parameters.** By default, New Length is equal to the original dataset length and Resolution is equal to the original resolution. Either parameter can be changed.

5. **Click Interpolate.** The result will appear in the Result dataset (blue trace).

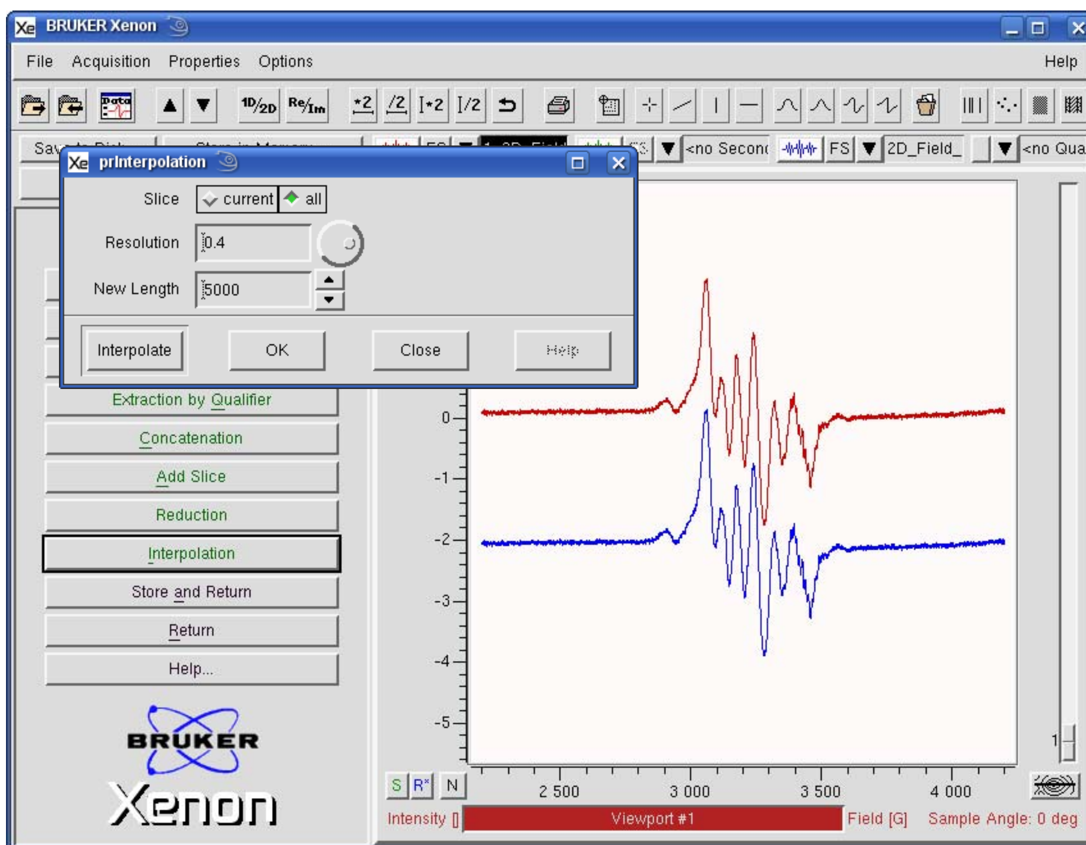

Figure 12-24 The result of interpolating a dataset.

6. **Store the dataset in memory.** Click Store and Return and enter a Title. Then click Store. The resultant dataset is stored in memory and this dataset is then transferred to the Primary dataset. If you click Return, the dataset remains in the Result dataset and the original dataset remains in the Primary dataset.

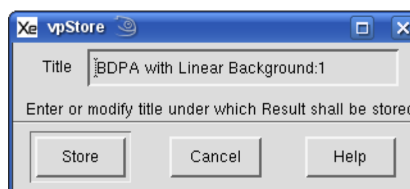

Figure 12-25 Storing the resultant spectrum in memory.

Quantitative EPR 13

Often the goal of EPR experiments is to quantitate the number of spins or concentration of radicals. The sub-tasks in the Quantitative EPR task assist you in performing these operations. The DR (Dual Resonator) sub-tasks are to be used for data in which an unknown sample is to be compared with a reference sample (marker) of known concentration or intensity. The two spectra are acquired separately, either in a dual resonator such as the ER 4105DR or by sequentially measuring the unknown and marker in a standard resonator. The Marker sub-tasks are used when both unknown and marker are measured simultaneously. The Absolute Number of Spins sub-task is used for quantitation without the use of a reference standard. The Quantitative EPR sub-task is invoked by clicking Quantitative EPR the taskbar.

Figure 13-1 The Quantitative EPR sub-tasks.

DR Integ 13.1

The DR Integ sub-task compares the integrated intensity of an unknown and known sample. Note that this operation is very similar to double integration described in Section 6.2 and most of what is described there is also applicable here. Note that it is necessary to baseline correct the data as described in Chapter 4 before performing the integrations. It is assumed you are already in the Quantitative EPR task bar. The comparison is accomplished as follows:

1. **Load the unknown sample's EPR spectrum into the Primary dataset.** This is referred to as the Signal.
2. **Load the reference sample's EPR spectrum into the Secondary dataset.** This is referred to as the Marker.

3. **Click DR Integ.** A new taskbar appears.

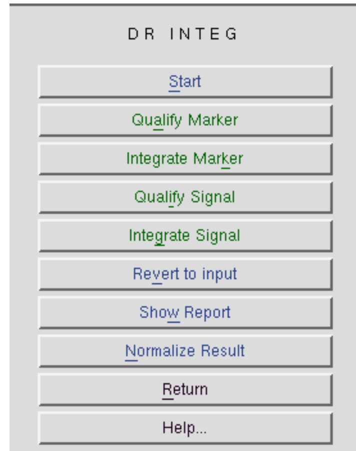

Figure 13-2 The DR Integ sub-task.

4. **Qualify the Marker.** Click Qualify Marker and then click and drag to select the desired integration range.

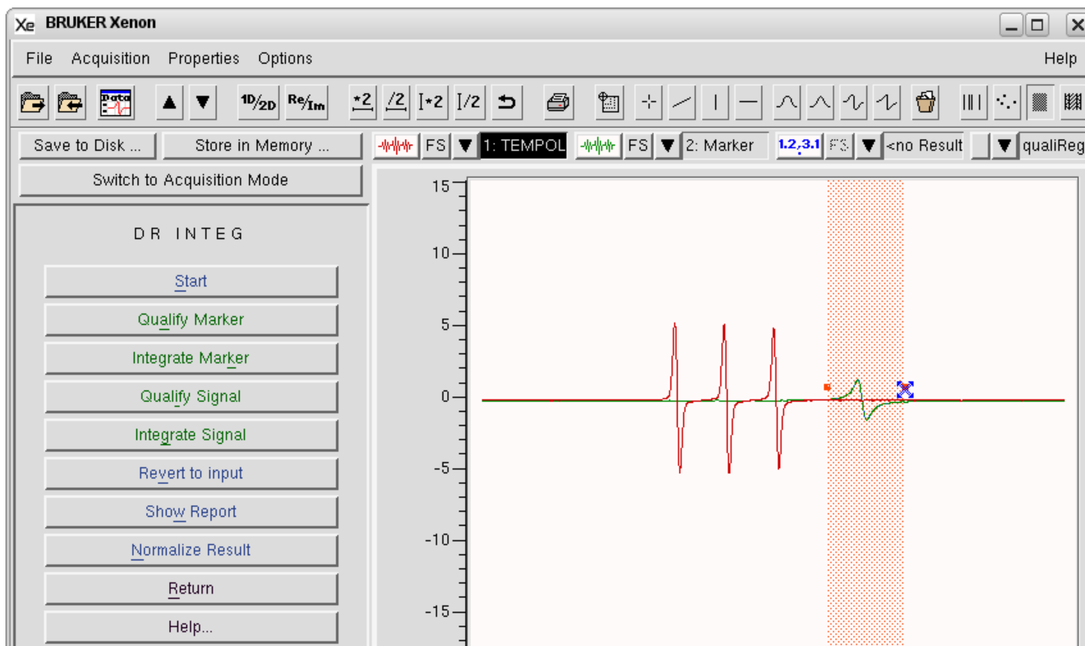

Figure 13-3 Qualifying the marker signal.

5. **Integrate the marker.** Click Integrate Marker. The double integral appears in the secondary dataset.

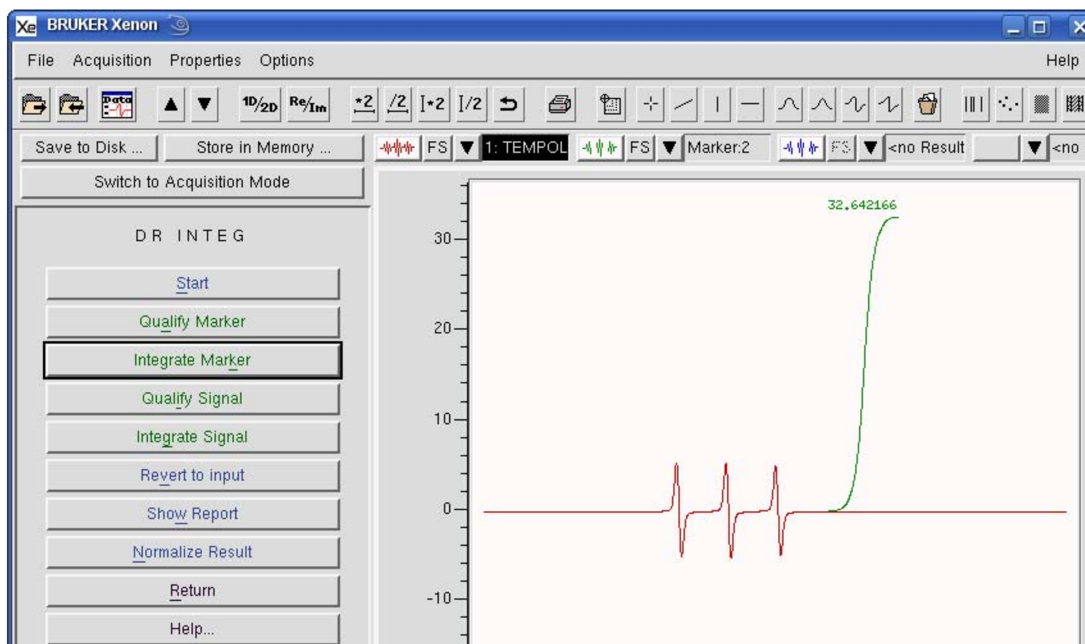

Figure 13-4 Integrating the marker signal.

6. **Qualify the signal.** Click Qualify Signal and then click and drag to select the desired integration range. You can select multiple ranges.

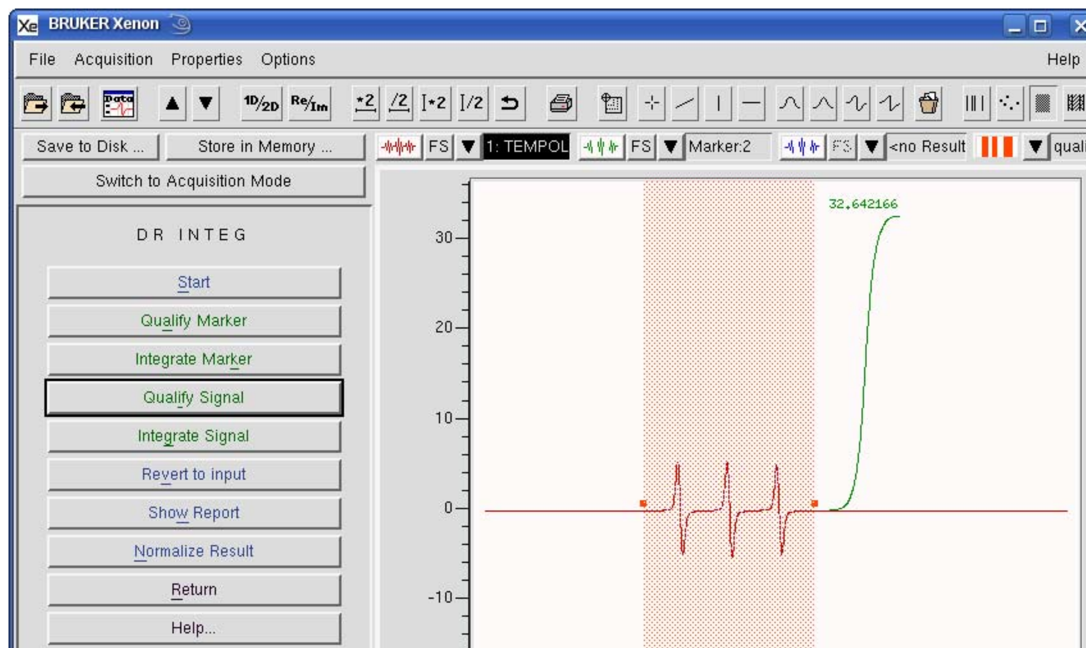

Figure 13-5 Qualifying the signal.

7. **Integrate the signal.** Click Integrate signal. The double integral appears in the primary dataset.

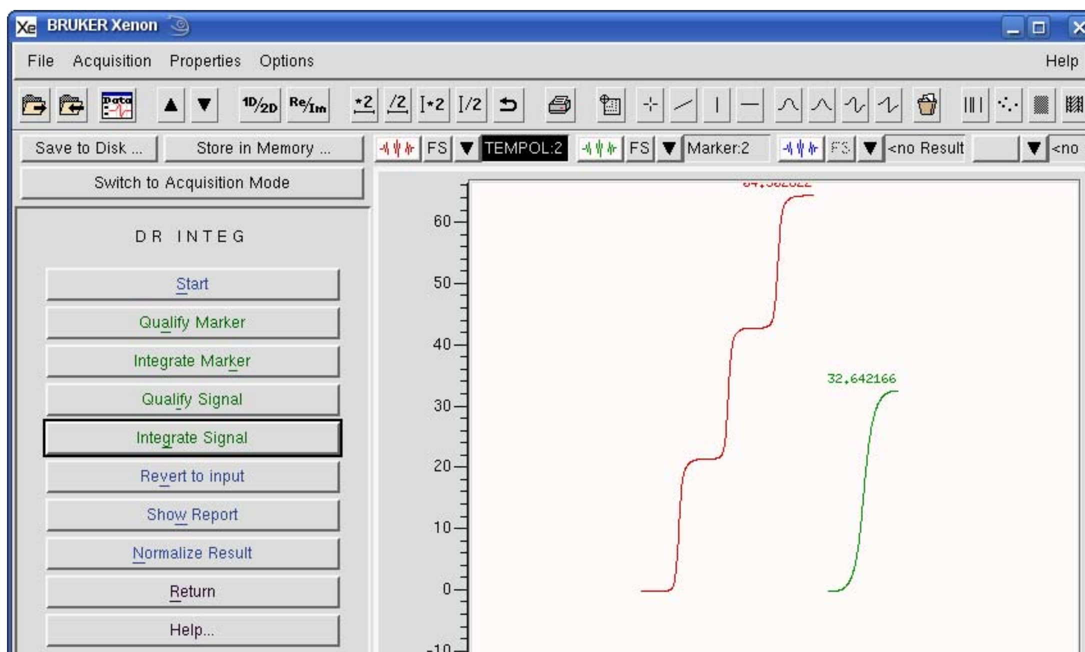

Figure 13-6 Integrating the signal.

8. **View the integration results.** Click Show Report. A new window appears

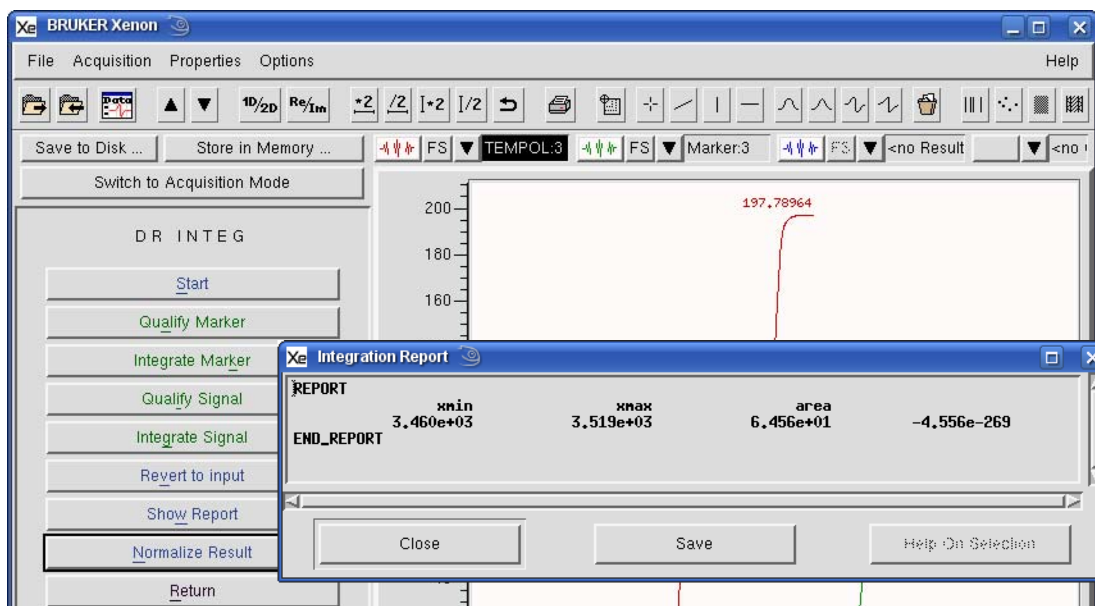

Figure 13-7 Viewing the integration results.

If you wish to save these values in a text file, click Save. A dialog box appears prompting you where to save the file.

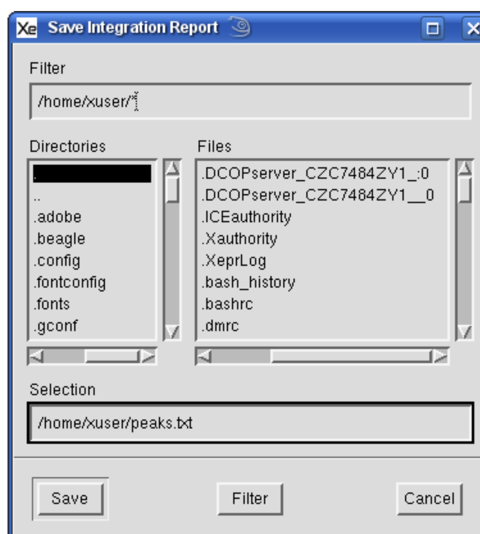

Figure 13-8 Saving the integration list.

9. **Normalize the integral.** Click Normalize Result. The signal integral is then normalized by the marker integral. The marker integral is set to 100 and the signal integral is changed accordingly. If you were to click Show Report again, the normalized integration intensities are displayed.

DR Peaks 13.2

The DR Peaks sub-task compares the intensity determined via peak-picking of an unknown and known sample. Note that this operation is very similar to peak picking described in Section 5.2 and most of what is described there is also applicable here. It is assumed you are already in the Quantitative EPR task bar. The comparison is accomplished as follows:

1. **Load the unknown sample's EPR spectrum into the Primary dataset.** This is referred to as the Signal.
2. **Load the reference sample's EPR spectrum into the Secondary dataset.** This is referred to as the Marker.

3. **Click DR Peaks.** A new taskbar appears.

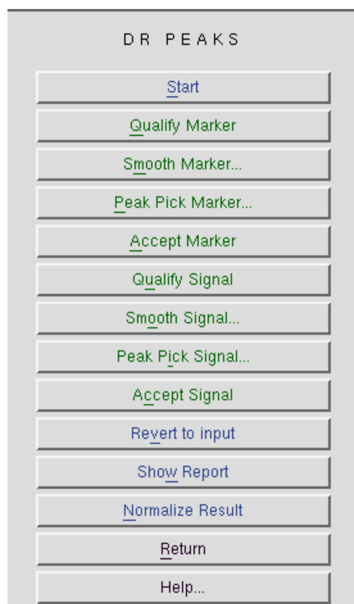

Figure 13-9 The DR Peaks sub-task.

4. **Qualify the Marker.** Click Qualify Marker and then click and drag to select the desired peak picking range.

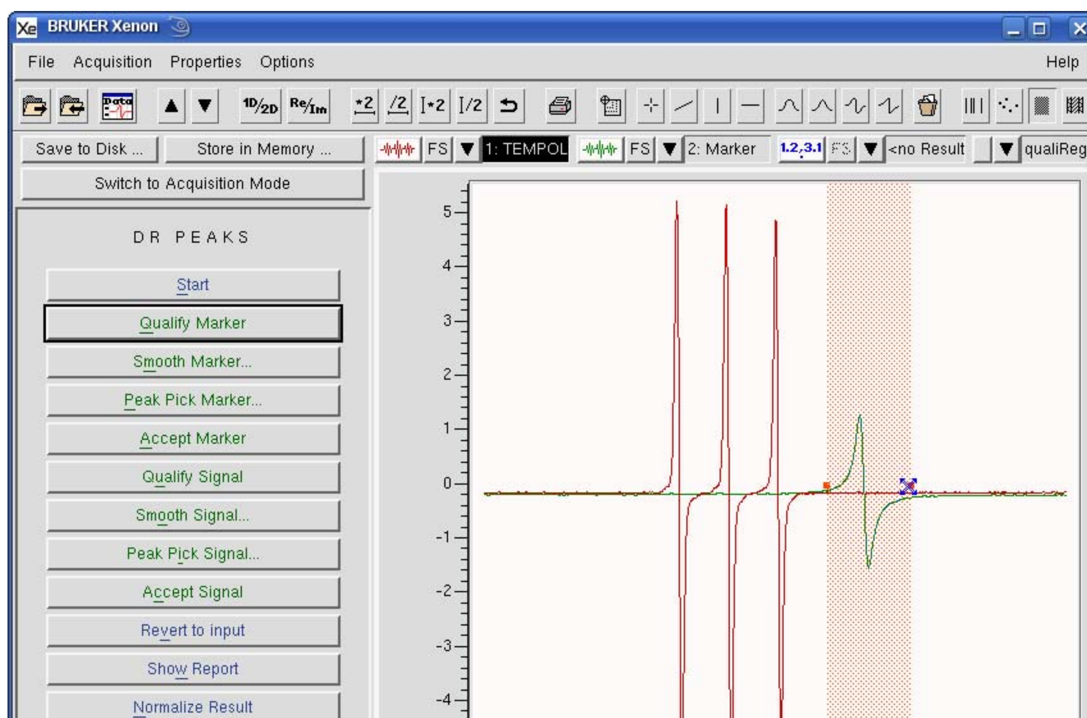

Figure 13-10 Qualifying the marker signal.

5. **Smooth the marker signal.** Click Smooth Marker. A new window appears. This is described in Section 8.2. The purpose of smoothing is to minimize the scatter in peak picking results due to noise. After the marker has been satisfactorily smoothed, click OK and the result appears in the secondary dataset. Note this an optional step.

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6. **Peak Pick the marker.** Click Peak Pick Marker. The peaks picked appears in the result dataset.

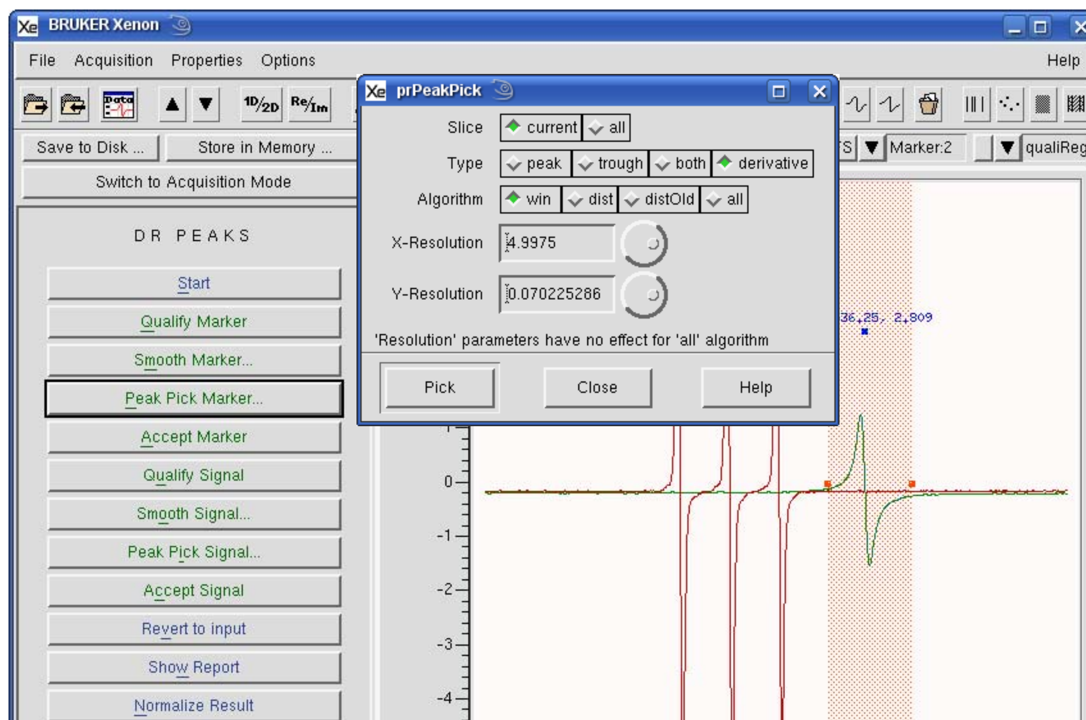

Figure 13-11 Peak picking the marker signal.

7. **Accept the marker.** Once peak picking has been satisfactorily performed, click Accept Marker. The peak picking result appears in the secondary dataset.

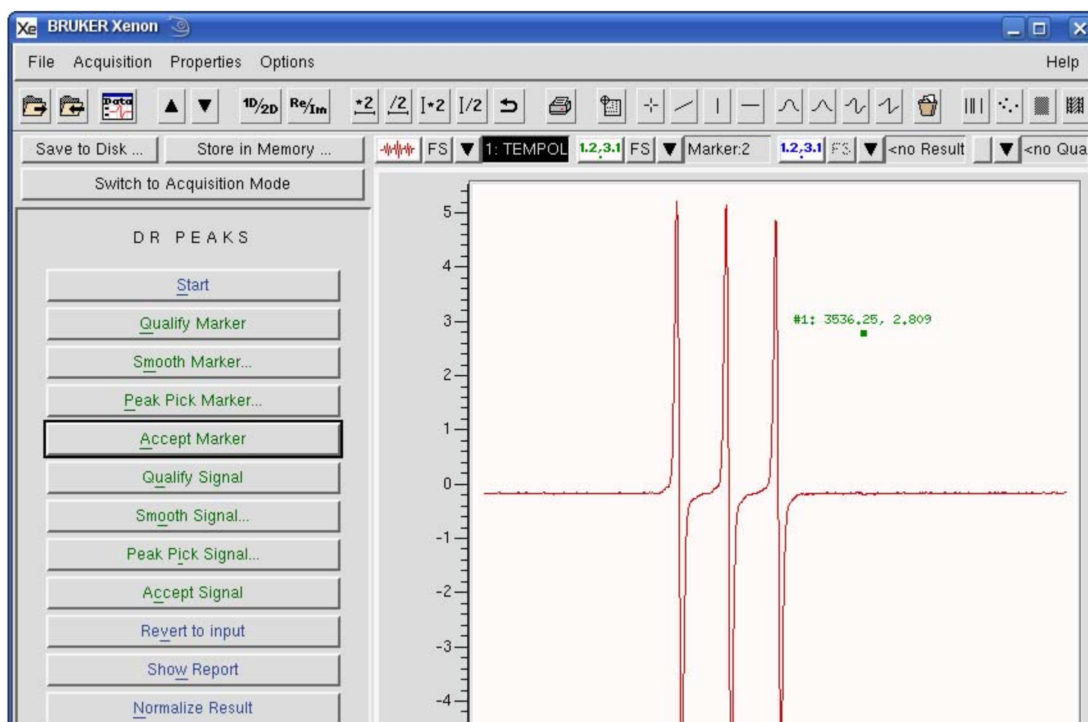

Figure 13-12 Accepting the marker signal.

8. **Qualify the signal.** Click Qualify Signal and then click and drag to select the desired peak picking range.You can select multiple regions.

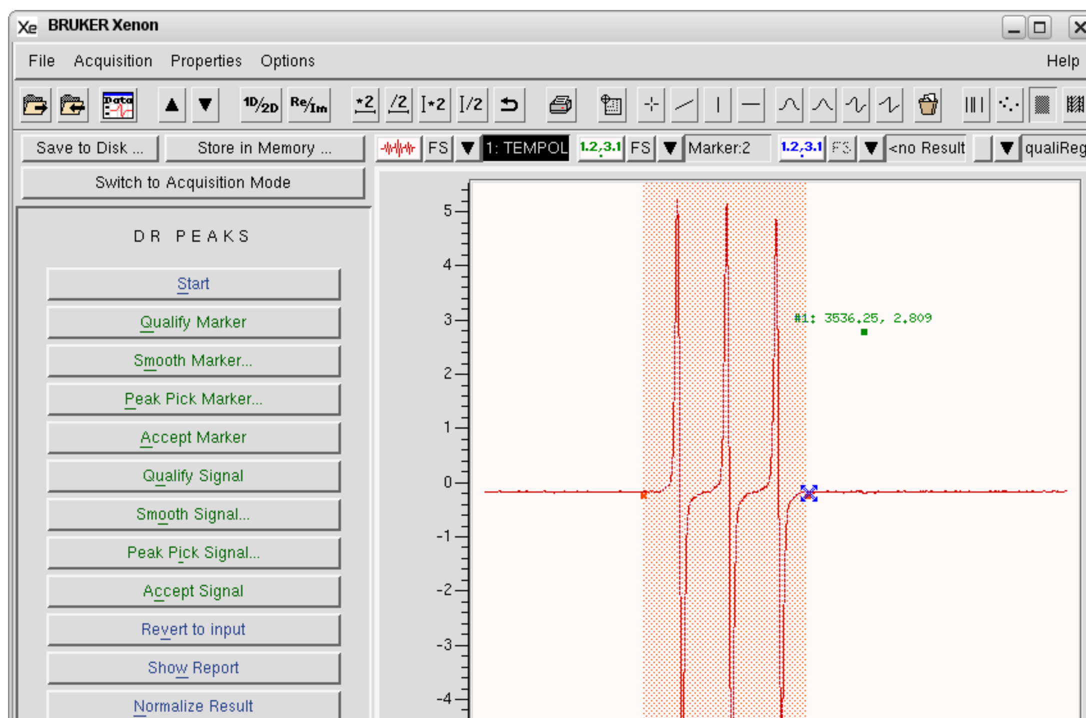

Figure 13-13 Qualifying the signal.

9. **Smooth the signal.** Click Smooth Signal. A new window appears. This is described in Chapter 8.2. The purpose of smoothing is to minimize the scatter in peak picking results due to noise. After the signal has been satisfactorily smoothed, click OK and the result appears in the primary dataset. Note this an optional step.

10. **Peak pick the signal.** Click Peak Pick Signal. The peak picking result appears in the primary dataset.

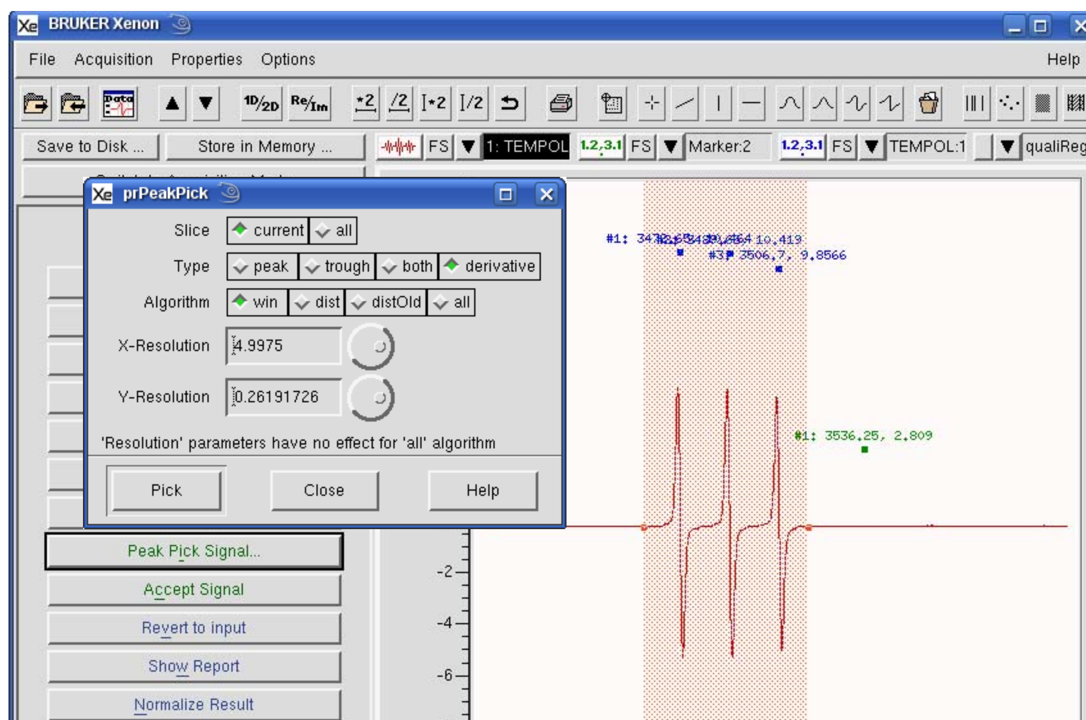

Figure 13-14 Integrating the signal.

11. **Accept the signal.** Once peak picking has been satisfactorily performed, click Accept Signal. The peak picking result appears in the primary dataset.

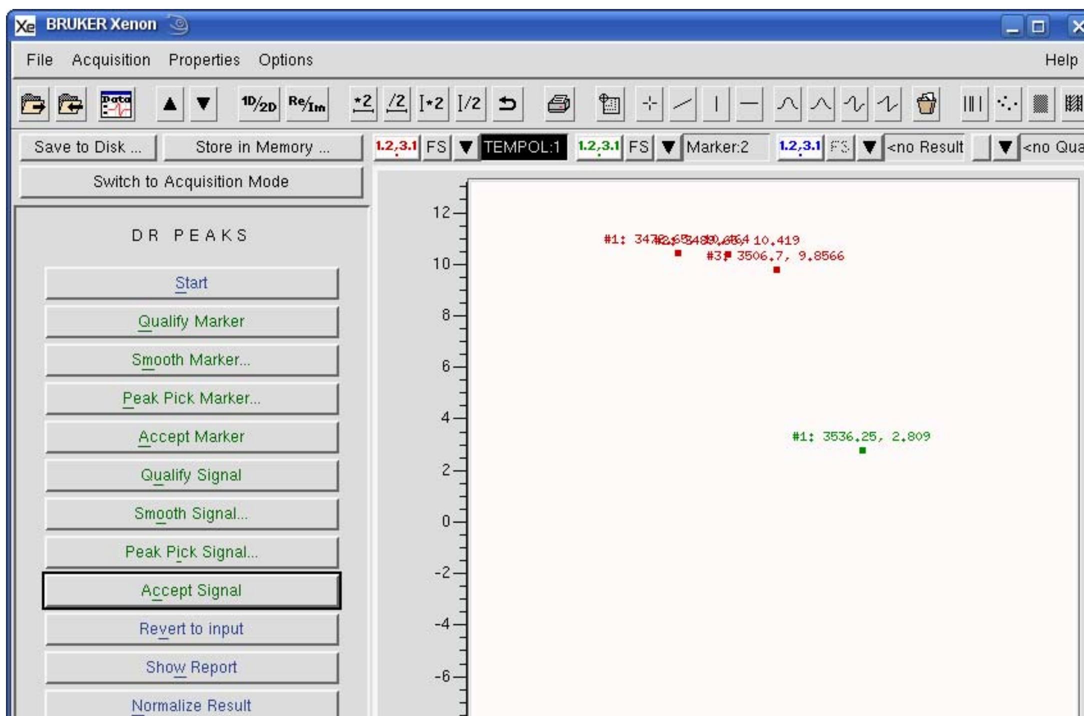

Figure 13-15 Accepting the marker signal.

12. **View the peak picking results.** Click Show Report. A new window appears You can save the results in an ASCII file by simply clicking Save.

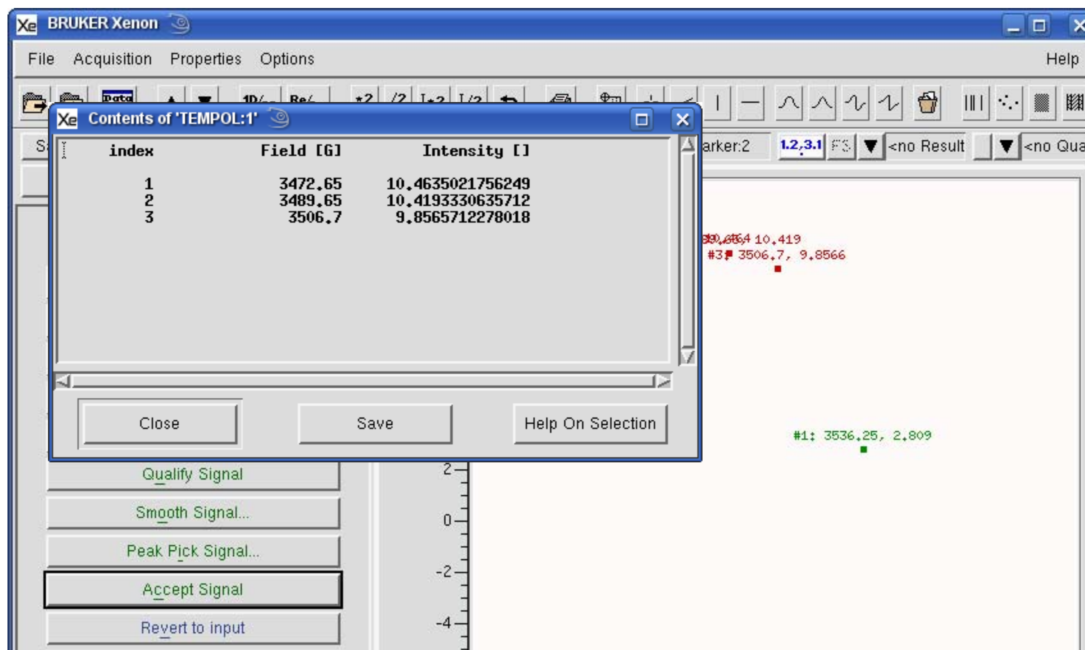

Figure 13-16 Viewing the peak picking results.

A new dialog box appears to prompt you for a filename and folder where results are to be saved. Click Save to continue.

Figure 13-17 Saving the peak picking results.

13. **Normalize the peak picking results.** Click Normalize Result. The signal peak picking results are then normalized by the marker peak picking results. The marker peak is set to 100 and the signal peaks are changed accordingly. If you were to click Show Report again, the normalized peak picking intensities are displayed.

Marker Integ 13.3

The Marker Integ sub-task compares the integrated intensity of an unknown and known sample. Note that this operation is very similar to double integration described in Section 6.2 and most of what is described there is also applicable here. Note that it is necessary to baseline correct the data as described in Chapter 4 before performing the integrations. It is assumed you are already in the Quantitative EPR task bar. The comparison is accomplished as follows:

1. **Load the EPR spectrum into the Primary dataset.**
2. **Click Marker Integ.** A new taskbar appears.

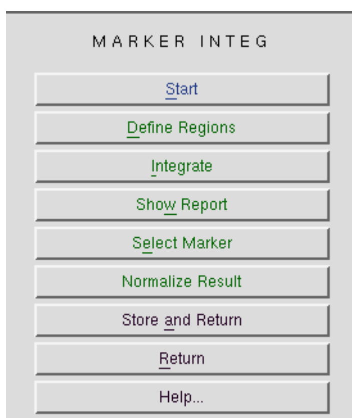

Figure 13-18 The Marker Integ sub-task.

3. **Qualify the integration ranges.** Click Define Regions and then click and drag to select the desired integration ranges. Perform this for both the signal and marker.

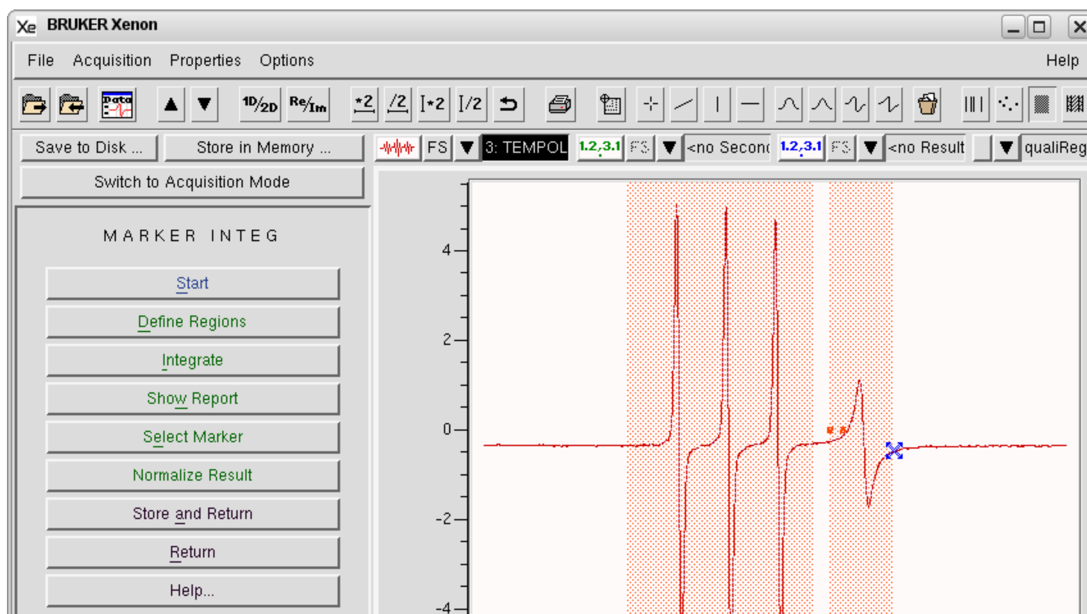

Figure 13-19 Qualifying the integration ranges.

4. **Integrate the spectrum.** Click Integrate. The double integral appears in the result dataset.

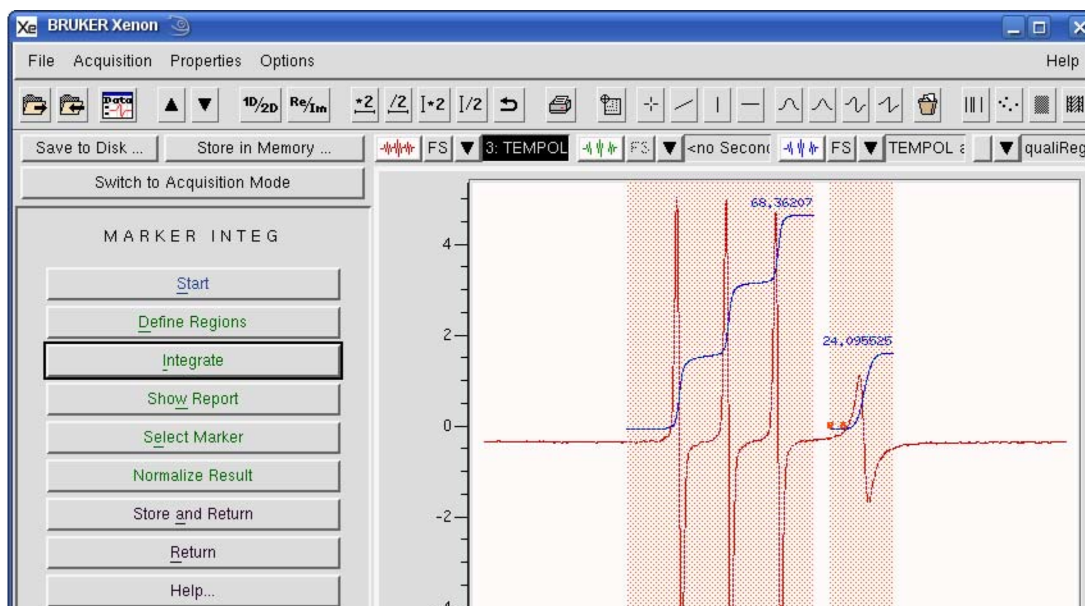

Figure 13-20 Integrating the EPR spectrum.

5. **View the integration results.** Click Show Report. A new window appears

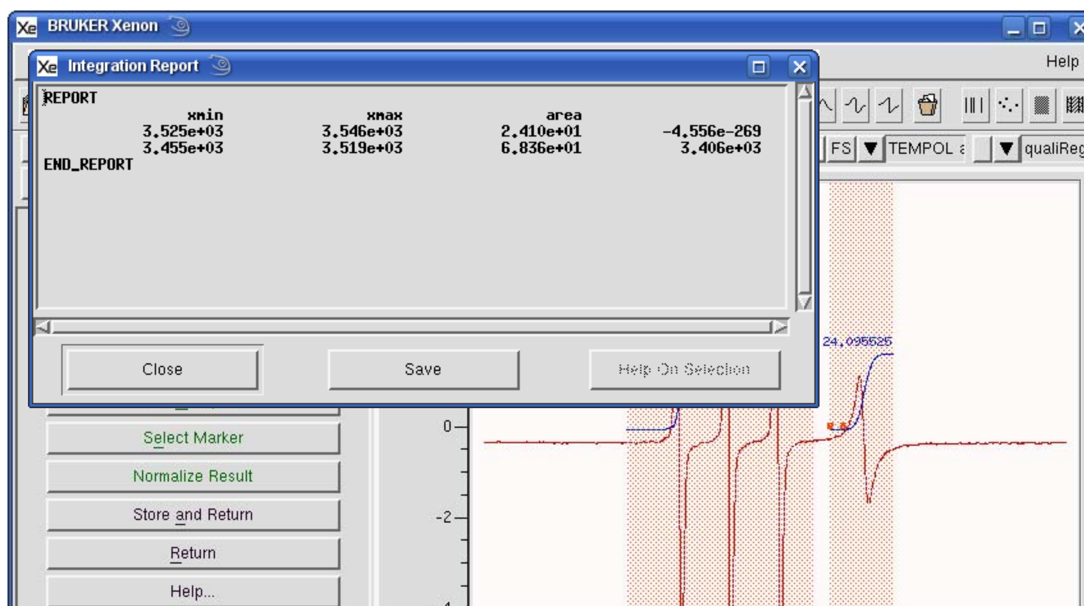

Figure 13-21 Viewing the integration results.

If you wish to save these values in a text file, click Save. A dialog box appears prompting you where to save the file.

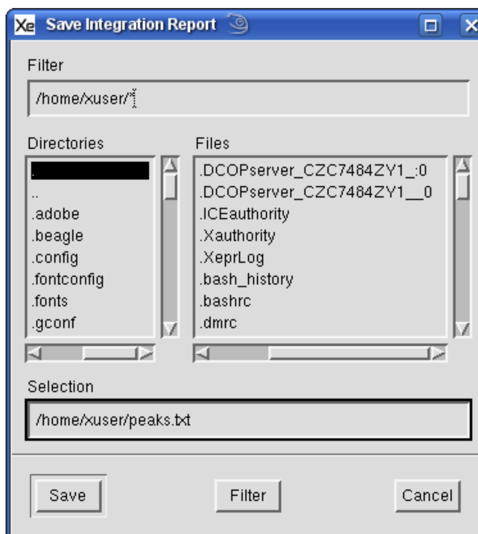

Figure 13-22 Saving the integration list.

6. **Select the marker.** Click Select Marker and the qualifiers disappear. Click and drag to select the range of the marker signal.

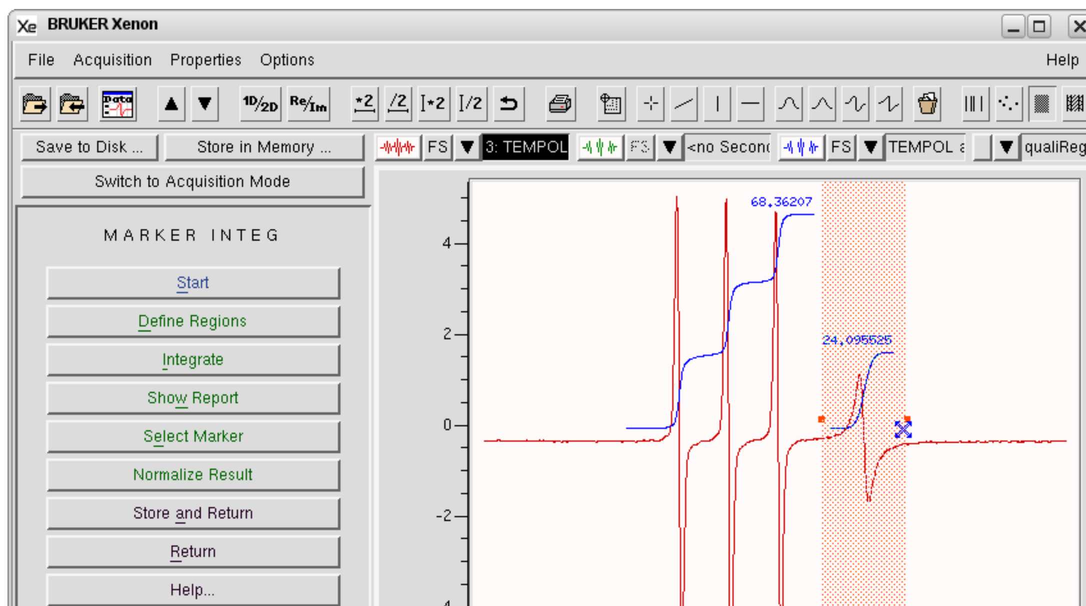

Figure 13-23 Viewing the integration results.

7. **Normalize the integral.** Click Normalize Result. The signal integral is then normalized by the marker integral. The marker integral is set to 100 and the signal integral is changed accordingly. If you were to click Show Report again, the normalized integration intensities are displayed.

Marker Peaks 13.4

The Marker Peaks sub-task compares the intensity determined via peak-picking of an unknown and known sample. Note that this operation is very similar to peak picking described in Section 5.2 and most of what is described there is also applicable here. It is assumed you are already in the Quantitative EPR task bar. The comparison is accomplished as follows:

1. **Load the unknown sample's EPR spectrum into the Primary dataset.**
2. **Click Marker Peaks.** A new taskbar appears.

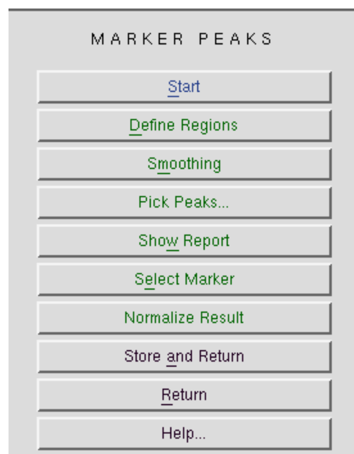

Figure 13-24 The Marker Peaks taskbar.

3. **Qualify the peak picking ranges.** Click Define Regions and then click and drag to select the desired peak picking ranges. Perform this for both the signal and marker.

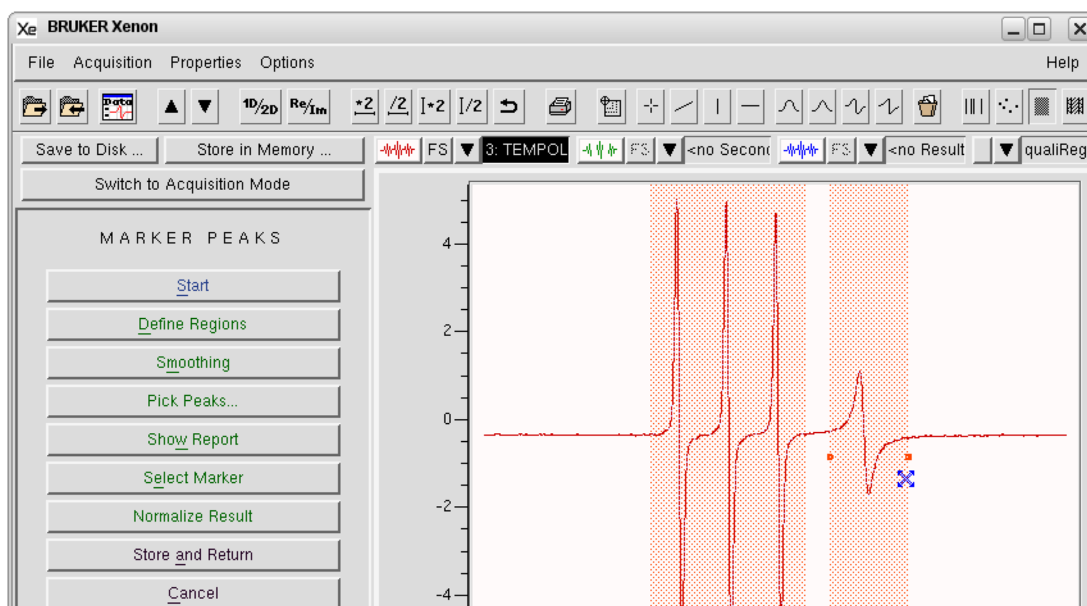

Figure 13-25 Qualifying the marker signal.

4. **Smooth the EPR signal.** Click Smoothing. A new window appears. This is described in Section 8.2. The purpose of smoothing is to minimize the scatter in peak picking results due to noise. After the marker has been satisfactorily smoothed, click OK and the result appears in the primary dataset. Note this an optional step.

5. **Peak pick the EPR spectrum.** Click Pick Peaks. The peaks picked appears in the result dataset.

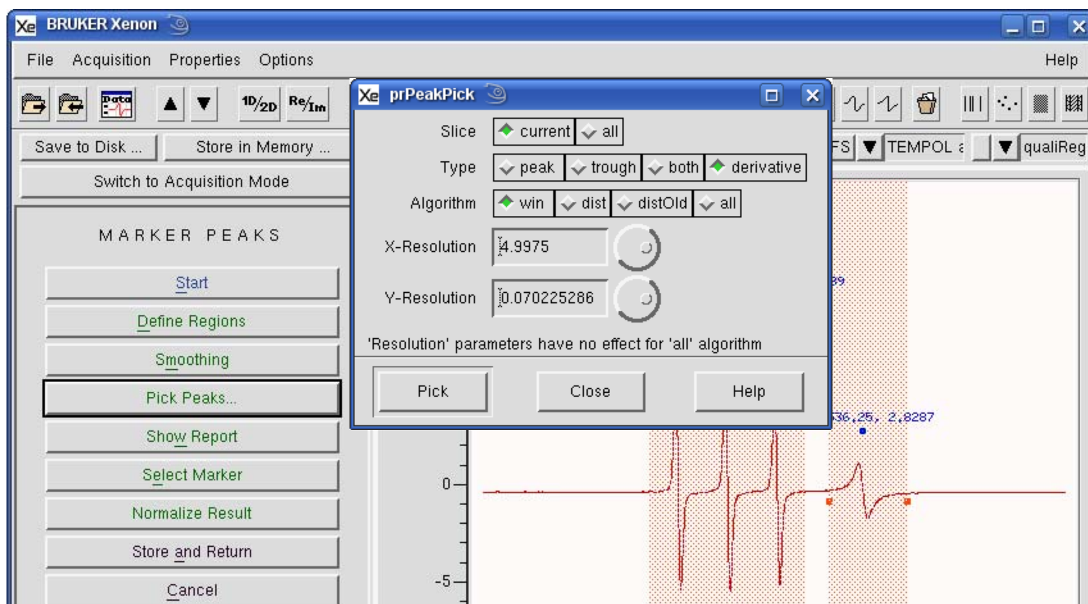

Figure 13-26 Peak picking the marker signal.

6. **View the peak picking results.** Click Show Report. A new window appears You can save the results in an ASCII file by simply clicking Save.

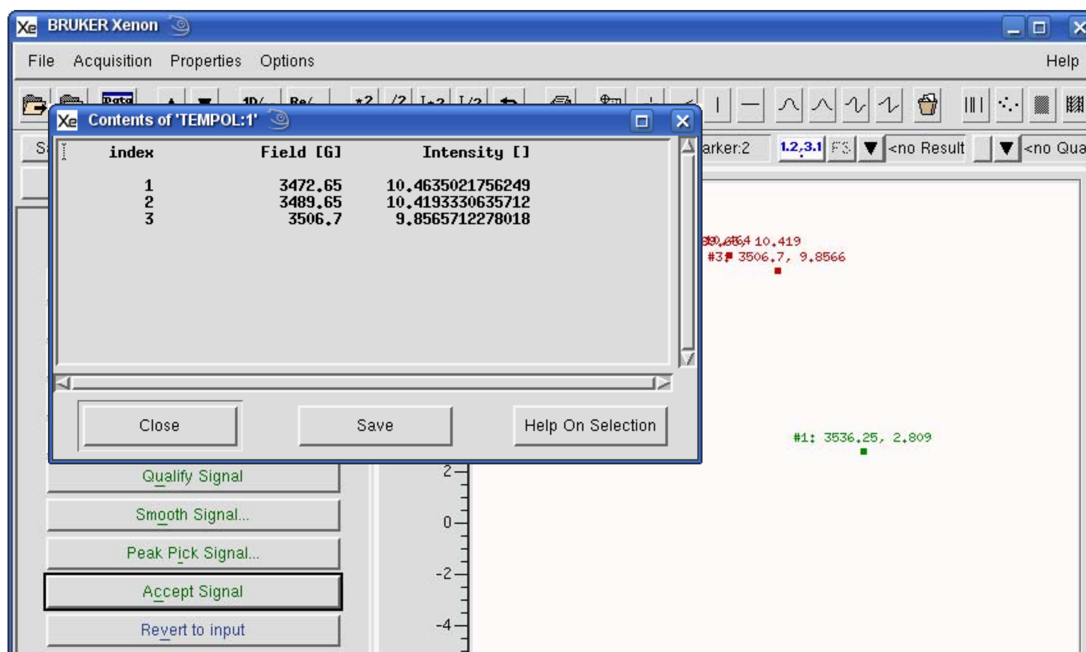

Figure 13-27 Viewing the peak picking results.

A new dialog box appears to prompt you for a filename and folder where results are to be saved. Click Save to continue.

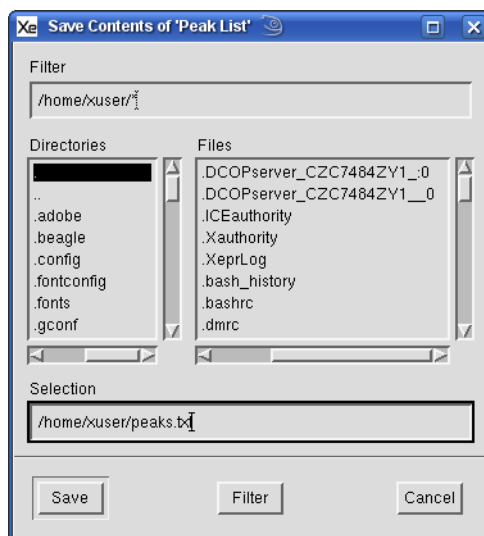

Figure 13-28 Saving the peak picking results.

7. **Select the marker.** Click Select Marker and the qualifiers disappear. Click and drag to select the range of the marker signal.

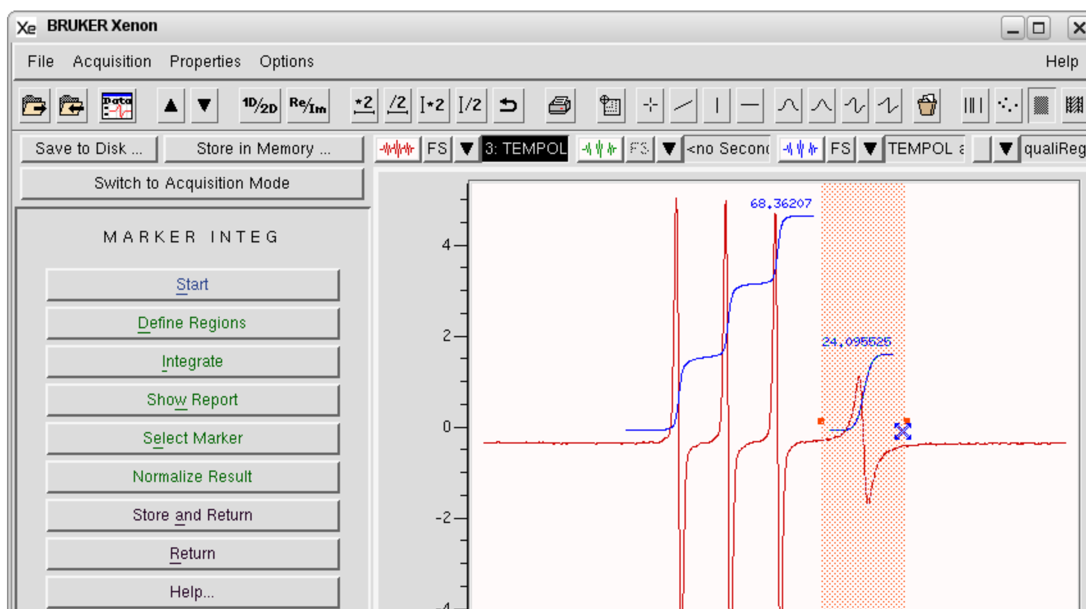

Figure 13-29 Selecting the marker signal.

8. **Normalize the peak picking results.** Click Normalize Result. The signal peak picking results are then normalized by the marker peak picking results. The marker peak is set to 100 and the signal peaks are changed accordingly. If you were to click Show Report again, the normalized peak picking intensities are displayed.

Absolute Number of Spins 13.5

The number of spins can be calculated from the double integral of the EPR signal without the use of a reference standard if we keep track of all experimental parameters, This is described in detail in Section 2.7.3 of the Xenon User's Guide.

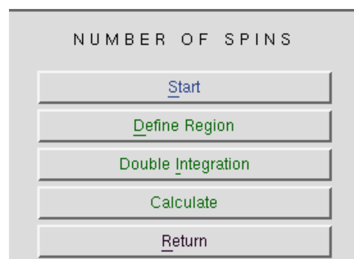

Figure 13-30 Performing a filtered derivative.

One requirement for measuring the Absolute Number of Spins is that you have measured the Q of the resonator as described in Section 5.4.2 of the Xenon User's Guide. It is important that the EPR spectrum is acquired with the microwave power set sufficiently low that the EPR signal is not saturated. Finally, it is best if the sample is centered in the resonator. For the ER 4119HS or ER 4122SHQE resonator, this is 62.5 mm from the top collet.

Note that this operation is very similar to double integration described in Section 6.2 and most of what is described there is also applicable here. Note that it is necessary to baseline correct the data as described in Chapter 4 before performing the integrations.

It is assumed you are already in the Quantitative EPR task bar and the spectrum is in the Primary dataset. Quantitative EPR performs the selected operation on the Primary dataset. If you have a Secondary dataset loaded, you can select it by clicking the dataset label to highlight it. (See Section Figure 2-3.) Then the operation is performed on the Secondary dataset. Here is how to perform a spin count:

1. **Load a dataset into the Primary or Secondary dataset.**
2. **Highlight the desired dataset.** Select the Primary or Secondary dataset for the operation.
3. **Start the Quantitative EPR task.** Click Quantitative EPR in the TASKS menu. A new task bar then appears.

Figure 13-31 The Quantitative EPR task.

4. **Start the Absolute Number of Spins task.** A new task bar appears.

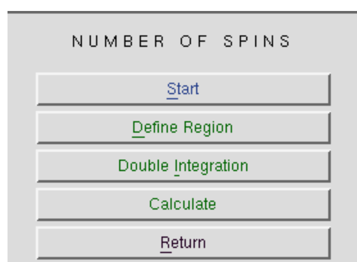

Figure 13-32 The Absolute Number of Spins task.

5. **Define the baseline and integration region.** Clicking on the Define Region button activates the Baseline qualifier definition mode. The mouse cursor changes into an x shape. Clicking and dragging the mouse cursor creates a Baseline qualifier consisting of four lines and a shaded area. The center region is the region to be integrated. The outer two regions are selected as baseline. A straight line is fitted to the baseline regions and this fitted line is then subtracted from the EPR data.

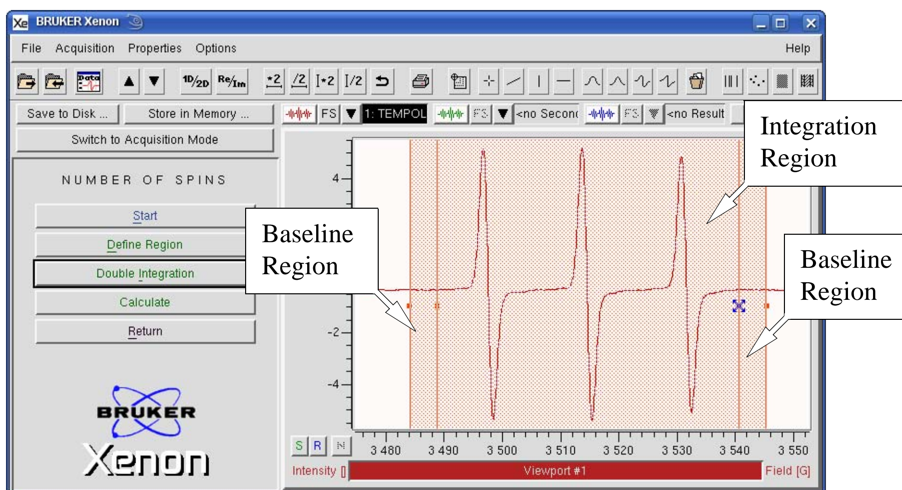

Figure 13-33 Defining the integration region.

Don't be worried if the region selection was not perfect. We now edit the regions. Click the Change Region button; now we can click and drag the lines to change their positions and thereby change the regions. The second and third lines control start and end points of the integration region. You may notice that the widths of the baseline regions do not change. The first line follows the second line and the fourth line follows the third line.

The baseline region widths are changed by clicking and dragging the first or fourth line. You may notice that both widths are linked. Chang-

ing the position of the first line changes the position of the fourth line and vice versa.

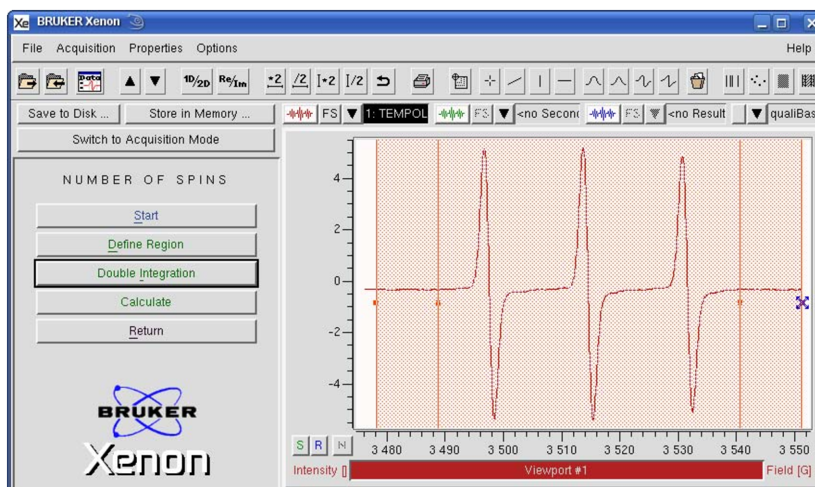

Figure 13-34 Defining the baseline regions.

It may take a few iterations to get what you want. Once you are satisfied, proceed to integration.

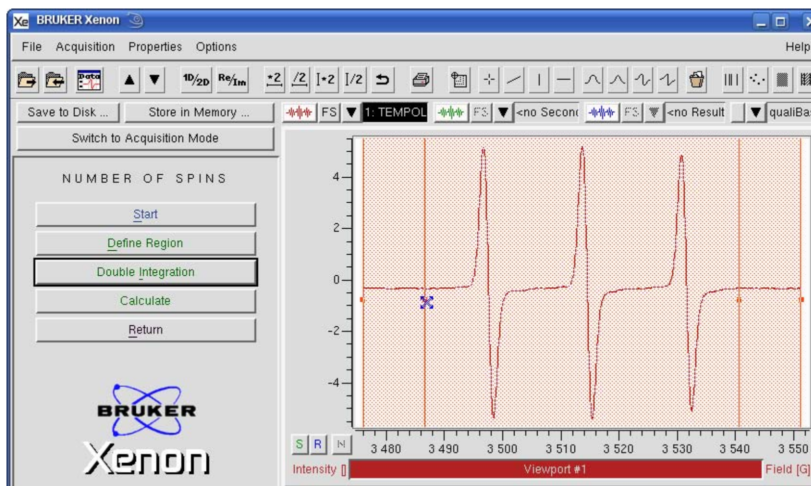

Figure 13-35 Well chosen integration and baseline regions.

6. **Double integrate the spectrum.** Click Double Integration. The region of the spectrum you selected is integrated twice and displayed in the Result dataset.

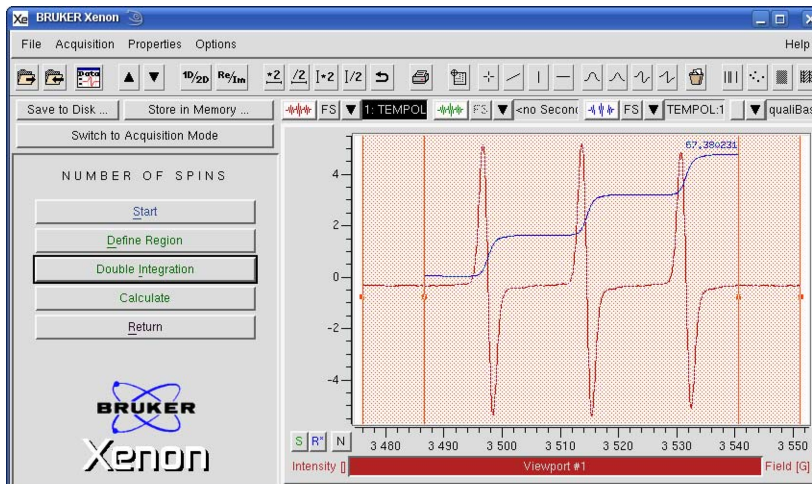

Figure 13-36 The double integral of the selected region.

7. **Calculate the number of spins.** Click Calculate. A new window appears with some parameters to enter. Enter the appropriate parameter values.

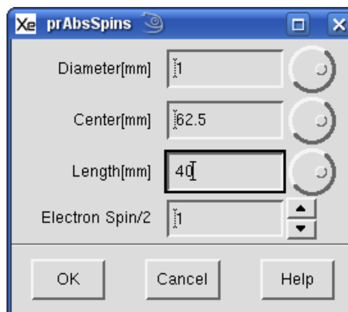

Figure 13-37 The Absolute Spins window.

Parameter	Definition
Diameter[mm]	Diameter of the sample (Note that this is inner and not the outer diameter of the sample tube.)
Center[mm]	Distance from the center of the sample to the top collet. For the ER 4119HS resonator and a properly centered sample, this is 62.5 mm
Length[mm]	Sample length (Not the sample tube length)
Electron Spin/2	Spin of the species. *I.e.* 1 corresponds to S = 1/2

Table 13-1 Absolute Spins parameter definitions.

8. **Click OK.** A new window appears with the results of the calculation. The results are displayed in three different units, spins/mm^3, molarity, and absolute number of spins. When the window first appears, not all entries are visible; you need to use the slider bar or stretch the window wider in order to view the last two values.

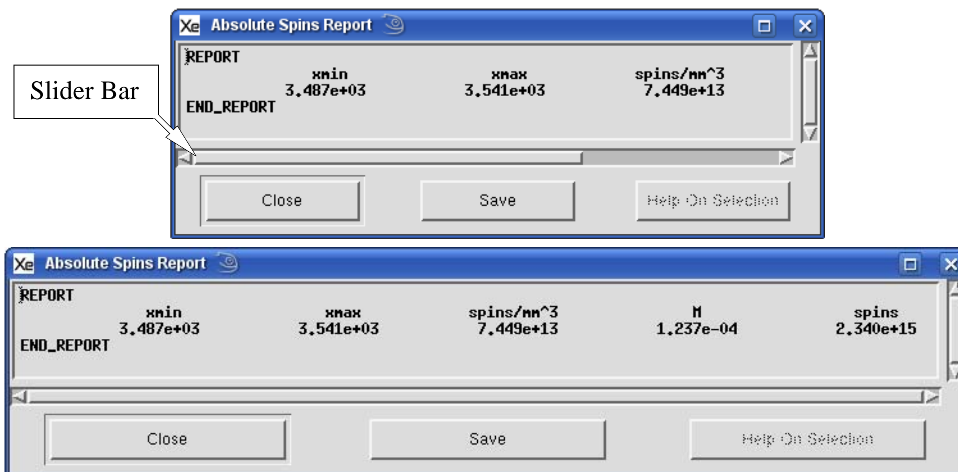

Figure 13-38 The results of the Absolute Spins calculation.

If you wish to save these values in a text file, click Save. A dialog box appears prompting you where to save the file.

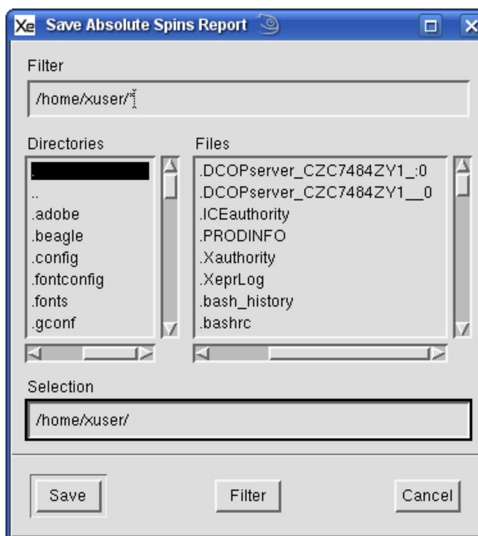

Figure 13-39 Saving the concentration list.

SpinFit 14

Spectral titration often can yield good results when disentangling spectra. Another approach is to simulate the two or more species and using least-squares analysis and optimization. SpinFit offers you this capability. SpinFit is a simulation task that simulates multi-species isotropic EPR spectra. It can also optimizes spin hamiltonian parameters, lineshapes, linewidths, and amplitudes using experimental data. Simulated spectra with their simulation parameters can be saved on the hard disk as library spectra for future use. There is a standard library including many spin adducts in the ../xenonFiles/Data/sharedData/SpinFitSimulations. The other nice feature in SpinFit is that the simulated spectra can be analyzed using Absolute Number of Spins to quantitate concentrations. The SpinFit sub-task is invoked by clicking the SpinFit button.

SpinFit Operations 14.1

Load From Dataset 14.1.1

Simulation parameters are stored with datasets. You can load these parameters from a previous simulation that is resident in memory by clicking Load>From Dataset. A new window appears.

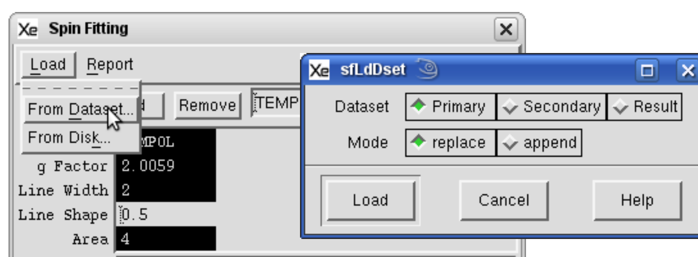

Figure 14-1 Loading parameters from a dataset.

Primary Secondary Result Clicking any of these buttons selects the dataset to be loaded. The selected datasets button is green.

replace When selected (green), the present simulation parameters are deleted and replaced by the loaded dataset after the Load button is clicked.

append When selected (green), the present simulation parameters are retained and the loaded dataset is appended to the list of radicals after the Load button is clicked.

Load This button loads the simulation parameters from the selected dataset. If the dataset does not have any simulation parameters, you will receive an error message.

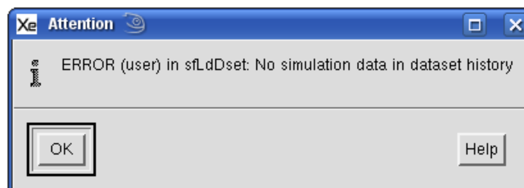

Figure 14-2 Error message when loading from a dataset without simulation parameters.

Load From Disk 14.1.2

Simulation parameters are stored with datasets. You can load these parameters from a previous simulation that is stored on the hard disk by clicking Load>From Disk. A new window appears.

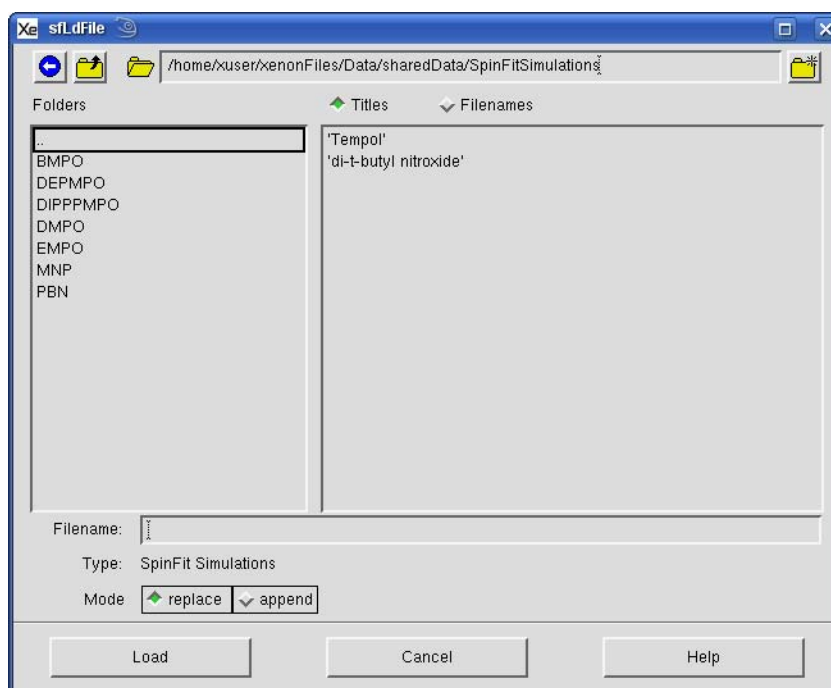

Figure 14-3 Loading a dataset from the hard disk.

There is a folder, /home/<username> /xenonFiles/sharedData/SpinFitSimulations (where <username> refers to the current user's name) that contains many example spectral simulations. Datasets are selected by clicking the desired file.

replace When selected (green), the present simulation parameters are deleted and replaced by the loaded dataset after the Load button is clicked.

append When selected (green), the present simulation parameters are retained and the loaded dataset is appended to the list of radicals after the Load button is clicked.

Load This button loads the simulation parameters from the selected dataset. If the dataset does not have any simulation parameters, you will receive an error message.

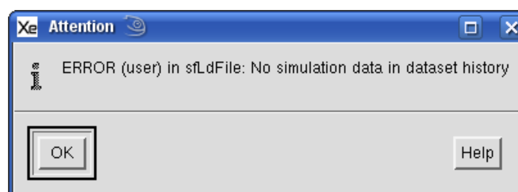

Figure 14-4 Error message when loading from a dataset without simulation parameters.

Report Parameters 14.1.3

You can view the results of fitting by clicking Report>Parameters. A new window appears displaying the fitting results. The results can be saved as a text file by clicking Save.

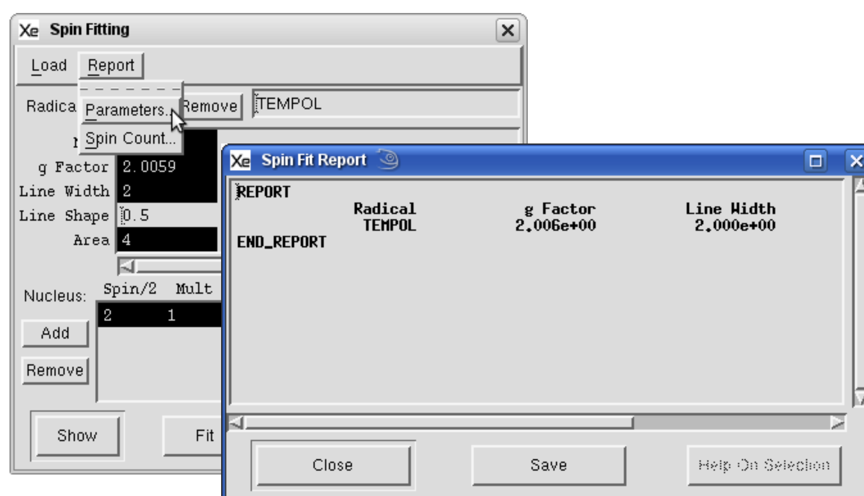

Figure 14-5 Viewing the SpinFit results.

A new window appears. Type in the Selection field the name of the text file. Then click Save.

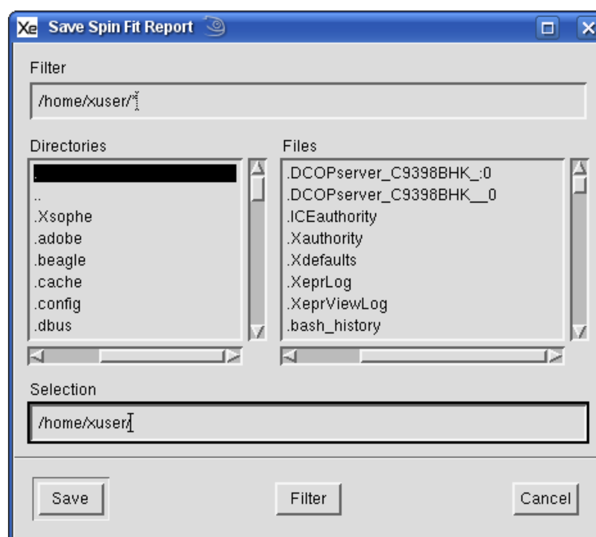

Figure 14-6 Saving SpinFit parameters as a text file.

Report SpinCount 14.1.4

The resulting simulated spectrum can be used in Absolute Number of Spins to calculate the concentrations of the individual species in the sample. A new appears. This operation is described in Section 13.5.

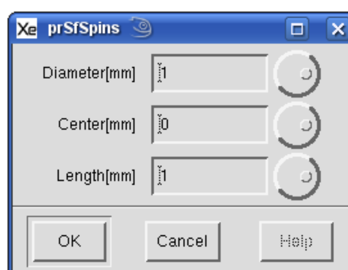

Figure 14-7 Invoking SpinCount for quantitation.

Add/Remove Radical 14.1.5

Add Clicking Add adds a new radical species to the simulation. To the right is a window in which you can type in a description of the radical. This may contain spaces and punctuation. Default parameters are added for this radical in the species table. If you click Add again, more radicals are entered into the species table.

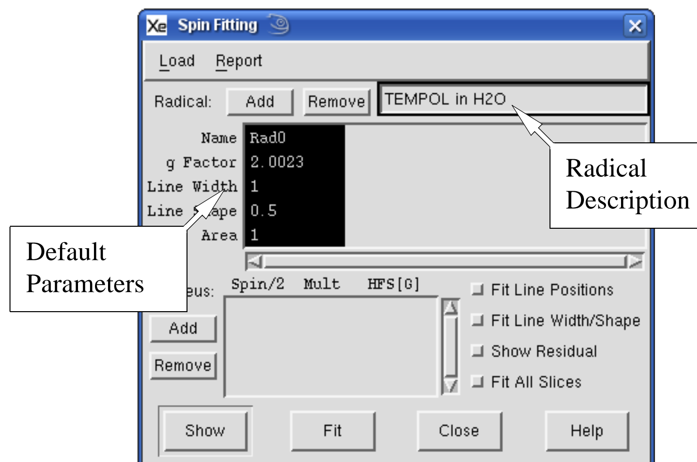

Figure 14-8 Adding a radical.

Remove A radical can be selected by clicking its parameters. The radical entry will then be highlighted (black background). If you click Remove, that entry is deleted from the species table.

Name This is the name of the radical and is used to identify the radical in the parameter report. Clicking in the Name field enables you to edit the Name. It can be up to 10 characters long. This may contain spaces and punctuation. The default for the first radical is Rad0 with each additionally added radical having an incremented number appended to its end.

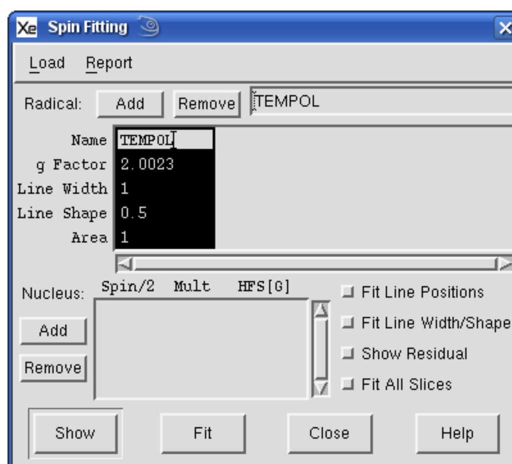

Figure 14-9 Editing a radical's Name.

g Factor The default value is the free electron value of 2.0023. Clicking in the g Factor field enables you to edit its value. Up to five places after the decimal point can be displayed.

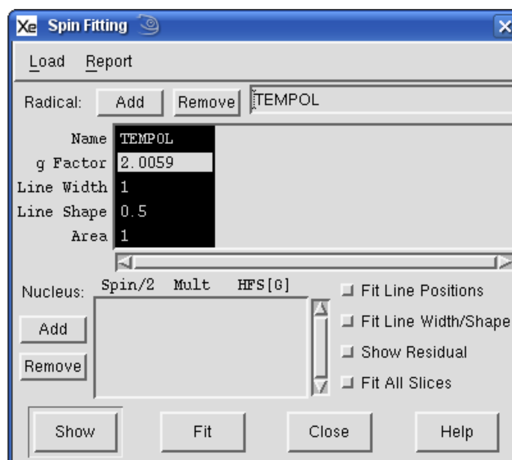

Figure 14-10 Editing a radical's g Factor.

Line Width This is the peak-to-peak linewidth as described in Section 7.3. Clicking in the Line Width field allows you to edit the value. The entry can be up to ten characters long.

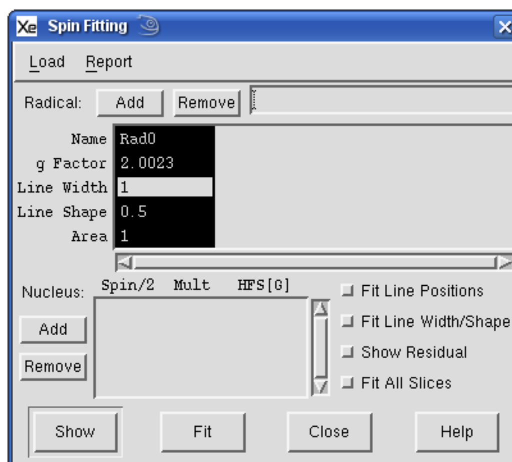

Figure 14-11 Editing a radical's Line Width.

Line Shape This indicates the amount of Gaussian or Lorentzian character in the lineshape. The lineshape is assumed to have the following form:

$$\begin{aligned} y = \text{amplitude} \cdot \{ &\text{Line Shape} \cdot \text{Gaussian}(x\ \text{offset, width}) + \\ &(1 - \text{Line Shape}) \cdot \text{Lorentzian}(x\ \text{offset, width}) \} \end{aligned} \qquad [14\text{-}1]$$

0 corresponds to a purely Lorentzian shape and 1 corresponds to a purely Gaussian shape. Clicking in the Line Width field allows you to edit the value. The entry can be up to ten characters long.

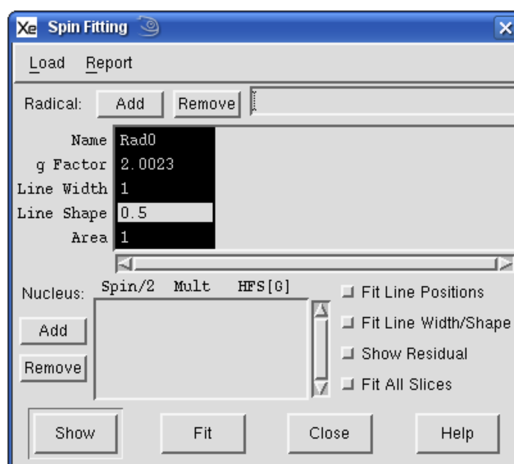

Figure 14-12 Editing a radical's Line Width.

Area This is the integrated intensity (double integral) for the selected species. Clicking in the Area field allows you to edit the value. The entry can be up to ten characters long.

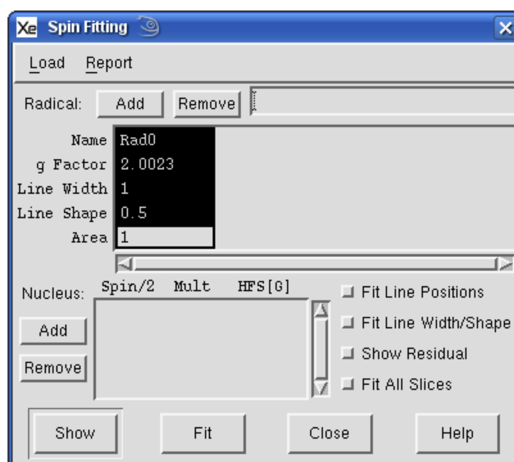

Figure 14-13 Editing a radical's Area.

Add/Remove Nucleus 14.1.6

Once a series of radicals has been defined. information regarding the nuclear hyperfine interactions need to be entered.

Add Nucleus Clicking on Add Nucleus adds a new nucleus to the radical that is currently selected. Default parameters are added to the nucleus entry in the nucleus table. If you click Add again, more nuclei are entered into the nucleus table.

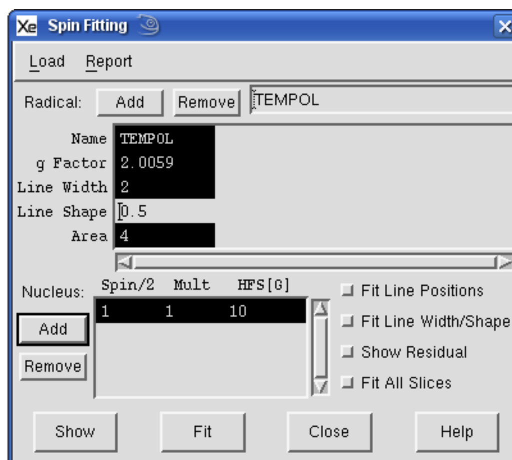

Figure 14-14 Adding a nucleus to a radical.

Spin/2 This is the spin of the nucleus multiplied by two. For example for a spin of 1/2, the value should be 1. Clicking in the Spin/2 field allows you to edit the value. Two small arrows appear to the right of the field. Clicking the up arrow increases the value and clicking the down arrow decreases the value. The values are constrained to be integers.

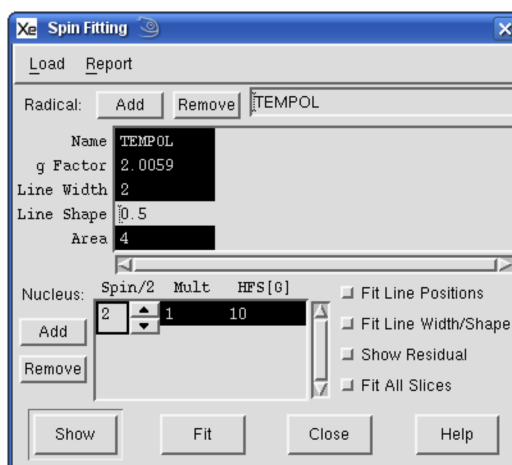

Figure 14-15 Editing nuclear spin.

Mult This is the number of equivalent nuclei. Clicking in the Mult field allows you to edit the value. Two small arrows appear to the right of the field. Clicking the up arrow increases the value and clicking the down arrow decreases the value. The values are constrained to be integers.

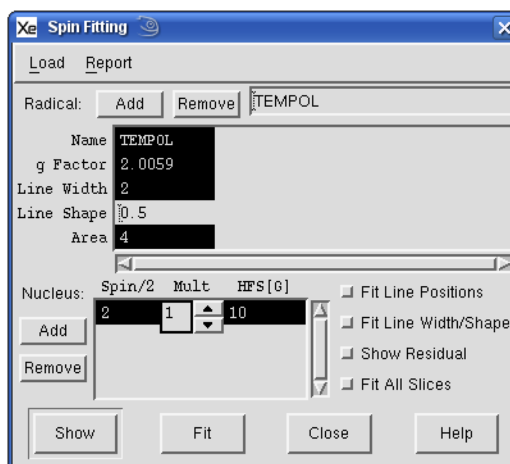

Figure 14-16 Editing number of equivalent nuclei.

HFS This is the hyperfine splitting in units of Gauss. Clicking in the Area field allows you to edit the value. The entry can be up to eight characters long.

Fit Line Positions Clicking the Fit Line Position button toggles it on and off. It is enabled when green. When enabled, the g Factor and HFS parameters are fitted to the experimental data in the primary or secondary dataset.

Fit Line Width/Shape Clicking the Fit Line Width/Shape button toggles it on and off. It is enabled when green. When enabled, the Line Width and Line Shape parameters are fitted to the experimental data in the primary or secondary dataset.

Show Residual Clicking the Show Residual button toggles it on and off. It is enabled when green. When enabled, the difference between the experimental and simulated data is displayed in the result dataset. Otherwise the simulated spectrum is displayed.

Fit All Slices Clicking the Fit All Slices button toggles it on and off. It is enabled when green. When enabled, the parameters are fitted to the experimental data in the primary or secondary dataset for all slices of a 2D dataset. Otherwise only the current slices is fitted.

Show 14.1.7

When the Show button is clicked, the current simulation parameters are used to simulate a spectrum and it is displayed in the result dataset. A value of 9.8 GHz is used for the microwave frequency. The center field is chosen to center the simulated spectrum. The sweep width is chosen to contain the complete simulated spectrum. The result appears in the result dataset.

Fit 14.1.8

When the Fit button is clicked, the simulation parameters are least-squares fitted to the experimental data in either the primary or secondary dataset. The dataset is selected by clicking the dataset label. The result appears in the result dataset.

How to Simulate a spectrum 14.2

EPR spectra may be simulated with SpinFit. The results then appear in the result dataset. The simulation parameters are stored with the dataset, so the new data can be used to fit mixtures of multiple species in unknown samples. The simulated dataset can be saved to disk for future use, allowing you to build up a library of EPR spectra and simulation parameters.

1. **Start the SpinFit task.** Click SpinFit in the TASKS menu. (See Figure 3-1.) A new window then appears.

2. **Add a species.** Click the Radical Add button. A new species is created with default parameters. The simulated spectrum which is a single line appears in the result dataset as a blue trace. The field to the right of button is for entering a comment or description for the species. Click the area to the right of Name and enter a name for the species.

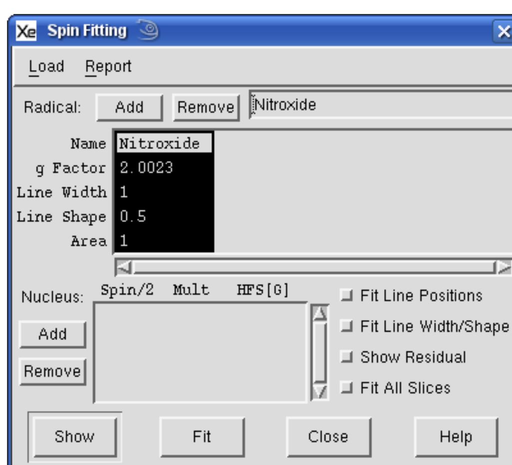

Figure 14-17 Adding a new species.

3. **Enter a g-Factor.** Click the area next to g-Factor and enter a value.

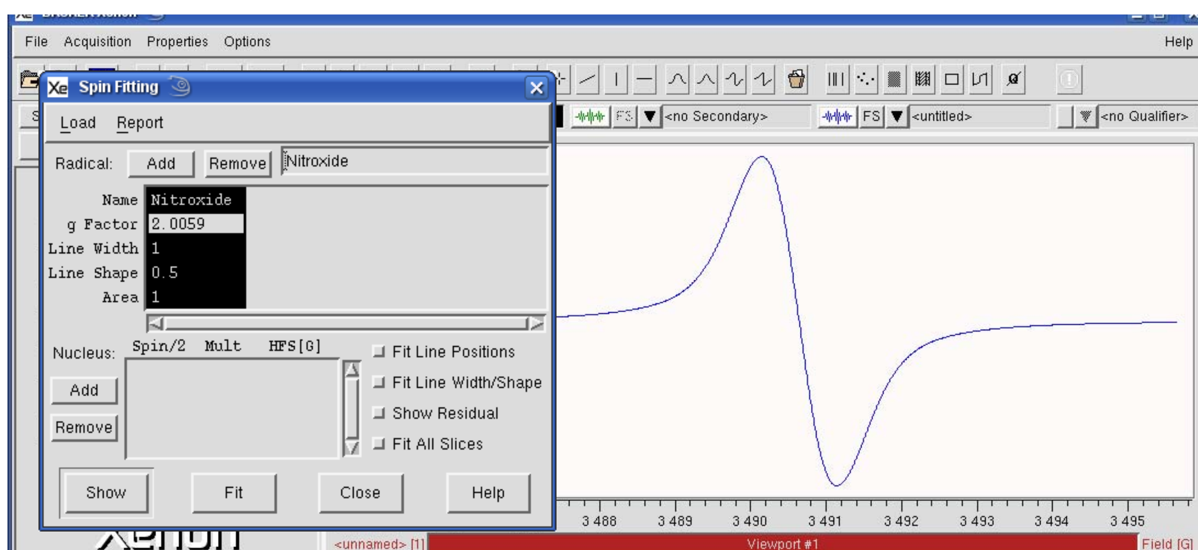

Figure 14-18 Entering a g-Factor.

4. **Enter the Line Width and Line Shape.** Click the Line Width entry and enter a value. In this case its value is 1.4 G. Click the Line Shape entry and enter a value. In this case its value is 0.

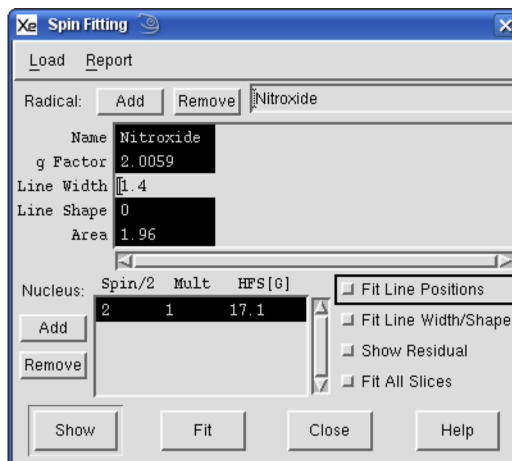

Figure 14-19 Entering a Line Width.

5. **Enter the HFS.** Click Add under the Nucleus heading. A new entry for the nucleus is then created with default parameters. First enter the spin of the nucleus. Click the spin entry. Up and down arrows appear to the right of the entry. The entry is the spin divided by two. Therefore it is 1 for I=1/2, 2 for I=1, *etc*., where I is the nuclear spin. Click the up or down arrows in order to select the desired nuclear spin. In this case it is I=1 for ^{14}N. Next enter the Mult. (multiplicity) which is the number of identical spins. Click the Mult. entry. Up and down arrows appear to the right of the entry. Click the up or down arrows in order to select the desired number of nuclei. In this case we have a single nitrogen and its value is 1. Finally enter the HFS (Hyperfine Splitting). Click its entry and enter the HFS value. In this case it is 17.1 G. Continue adding nuclei until all the nuclei for the species have been entered.

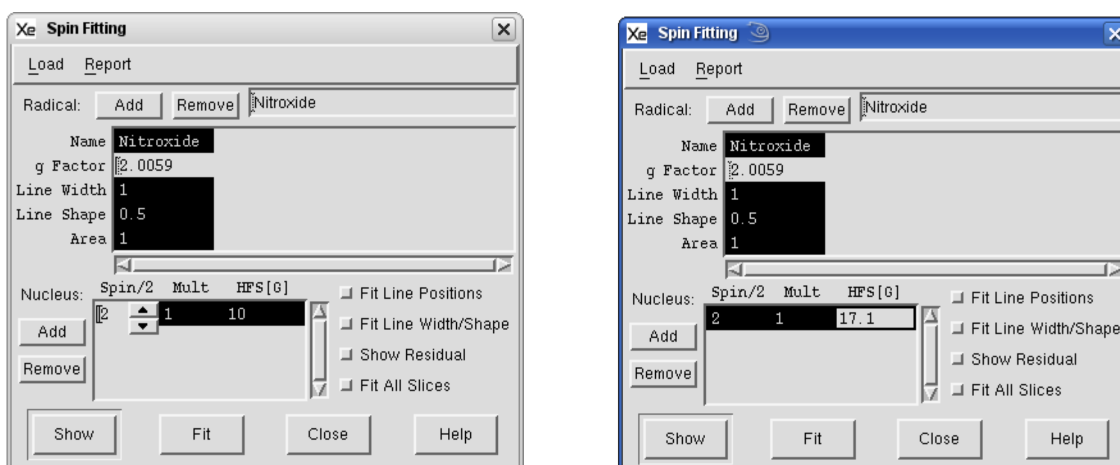

Figure 14-20 Entering the hyperfine parameters for a species.

As the nuclei are added, the result simulation appears in the Result dataset as a blue trace.

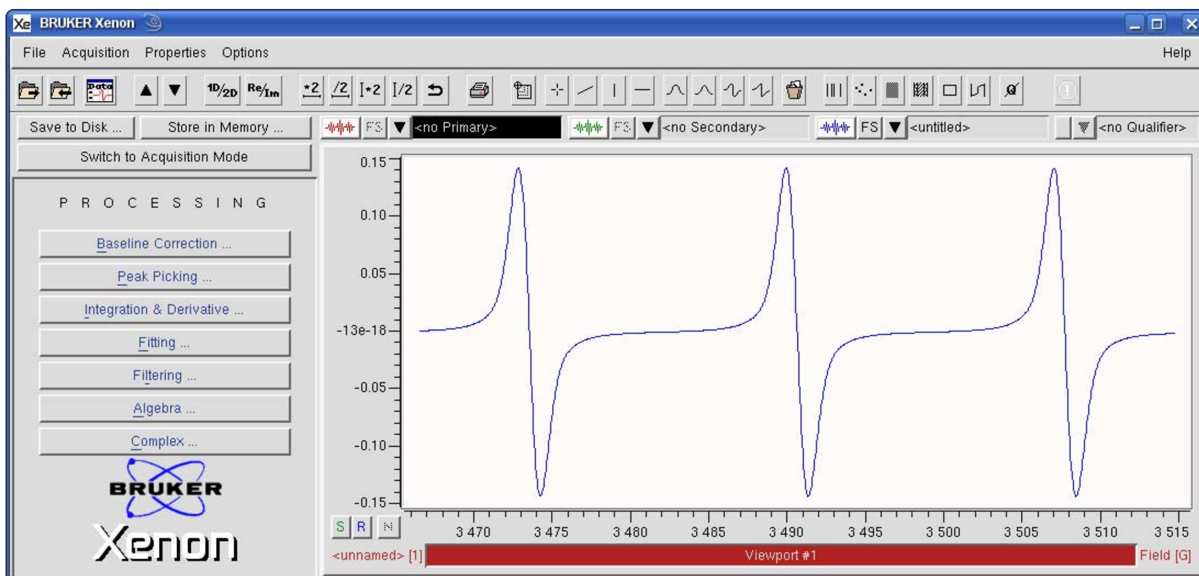

Figure 14-21 A simulated spectrum reflecting both the g-Factor and HFS.

6. **Save the result.** Click on the properties menu of the Result dataset. Select Save on Disk from the drop-down menu. All the simulation parameters for the species and the simulated spectrum are saved with the dataset. By organizing and saving the simulated spectra in folders you can create your own libraries for collections of different paramagnetic species.

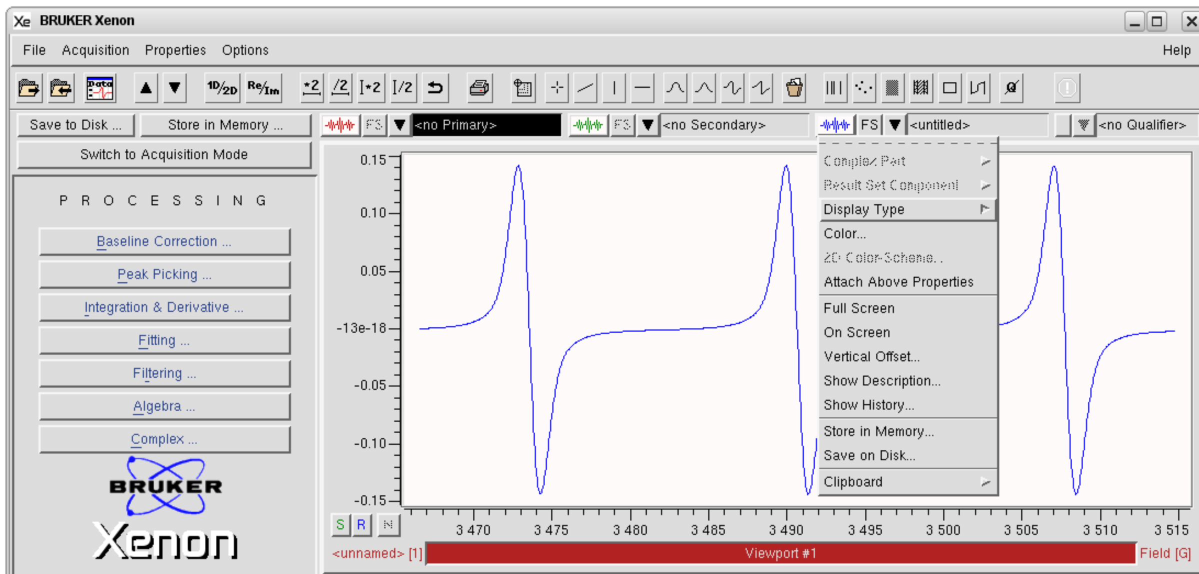

Figure 14-22 Saving the simulation result.

How to Fit Spin Hamiltonian Parameters to Data 14.3

Often radical species are identified via their spin hamiltonian parameters such as the g-factor or hyperfine splittings. SpinFit can be used to obtain these parameters from the experimental EPR spectrum.

The steps in this section can be followed to learn how to estimate simulation parameters. There are further explanations and hints in Section 8.3.5 of the Xenon User's Guide.

1. **Load the spectrum.** Load the experimental EPR spectrum into the Primary dataset. In this example we are using a single species nitroxide spectrum.

2. **Start the SpinFit task.** Click SpinFit in the TASKS menu. (See Figure 3-1.) A new window then appears.

3. **Add a species.** Click the Radical Add button. A new species is created with default parameters. The simulated spectrum which is a single line appears in the result dataset as the blue trace. The field to the right of button is for entering a comment or description for the species. Click the area to the right of Name and enter a name for the species.

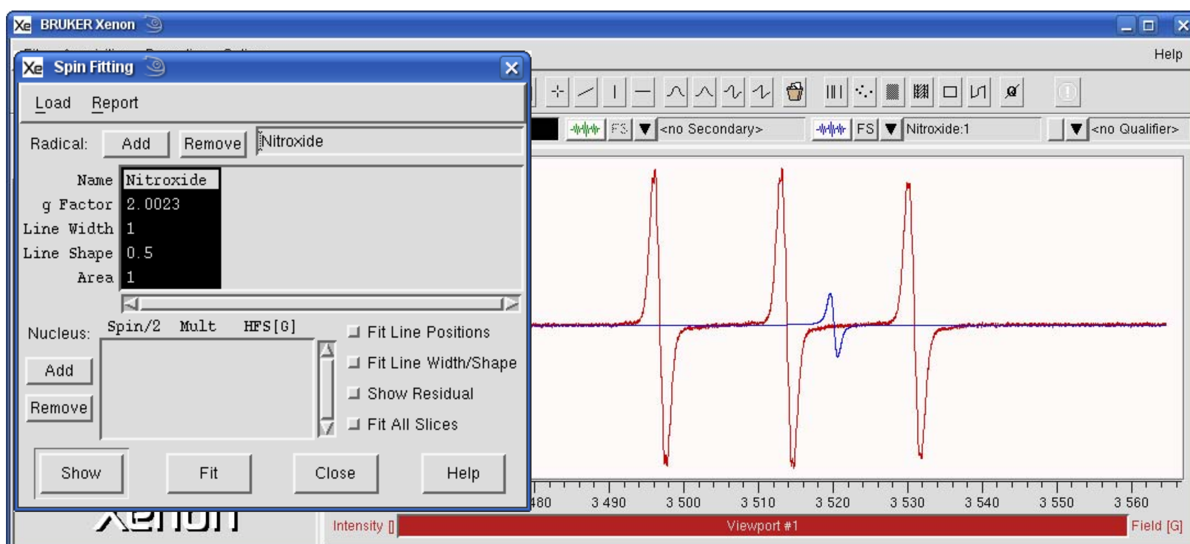

Figure 14-23 Adding a new species.

4. **Enter a g-Factor.** The g-Factor can be estimated by clicking the center of the spectrum (not necessarily corresponding to the center of the field sweep) and reading the g-Factor displayed in the viewport selector bar. (See Section 2.4.2.) Click the area next to g-Factor and enter this value.

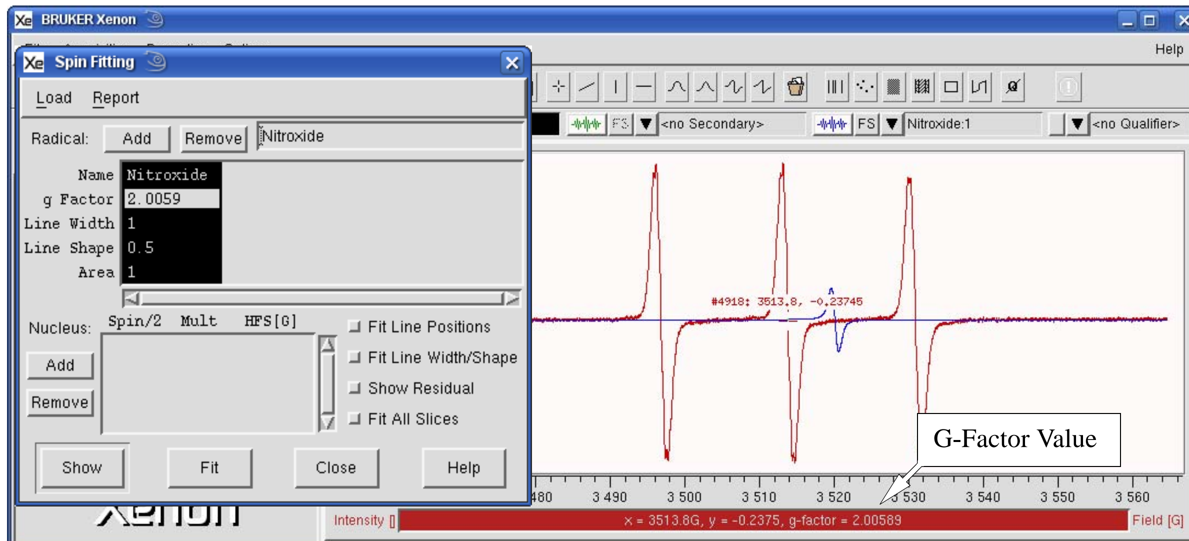

Figure 14-24 Estimating the g-Factor using the cursor readout.

The simulated spectrum is now a line approximately at the center of the experimental EPR spectrum.

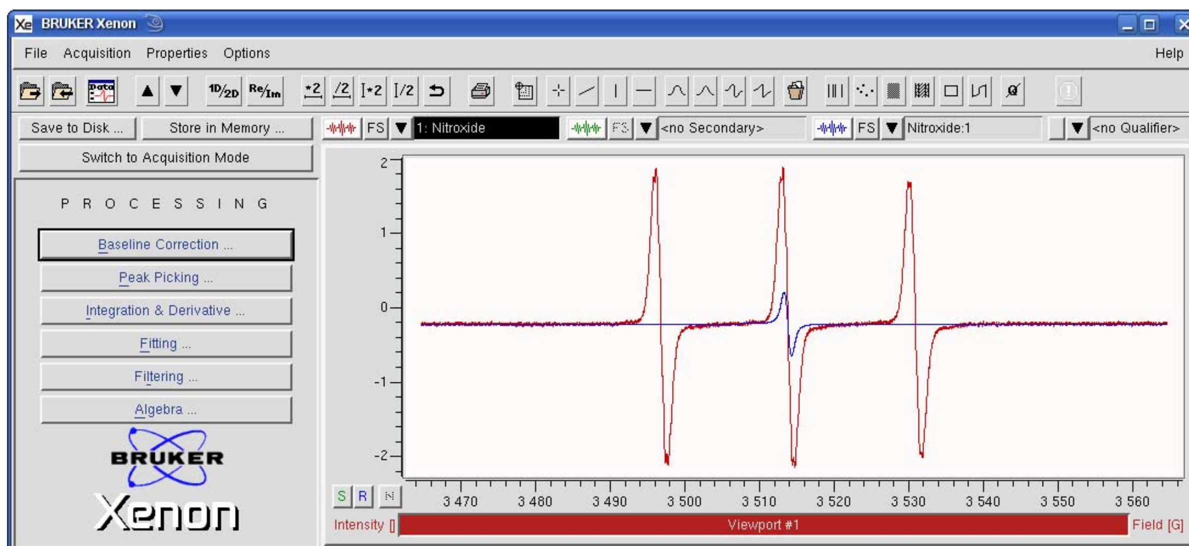

Figure 14-25 The simulated spectrum should consist of a single line centered approximately in the experimental spectrum if the g-Factor is approximately correct.

5. **Enter the HFS.** First use the distance tool to measure the splittings of the hyperfine lines due to one of the nuclei. (See Section 4.4.3.) Its value is displayed in the viewport selector bar. Look for lines with constant splittings and regular patterns and measure the splittings to obtain the hyperfine splitting in Gauss. Refer to Section 2.1.3 of the Xenon User's Guide for hints.

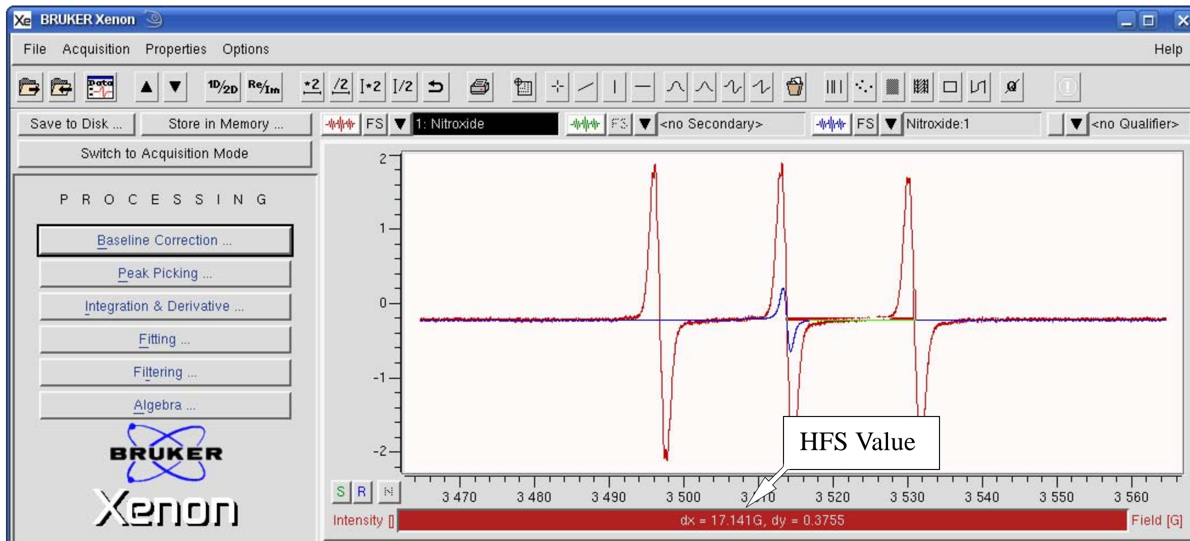

Figure 14-26 Estimating the HFS using the distance tool.

Once the approximate HFS values have been measured the data needs to be entered for the nuclei. Click Add under the Nucleus heading. A new entry for the nucleus is then created with default parameters. First enter the spin of the nucleus. Click the spin entry. Up and down arrows appear to the right of the entry. The entry is the spin divided by two. Therefore it is 1 for I=1/2, 2 for I=1, *etc*., where I is the nuclear spin. Click the up or down arrows in order to select the desired nuclear spin. In this case it is I=1 for ^{14}N. Next enter the Mult. (multiplicity) which is the number of identical spins. Click the Mult. entry. Up and down arrows appear to the right of the entry. Click the up or down arrows in order to select the desired number of nuclei. In this case we have a single nitrogen and its value is 1. Finally enter the HFS that we measured earlier in this step. Click its entry and enter the HFS value. In this case it is 17.1 G. Continue adding nuclei until all the nuclei for the species have been entered.

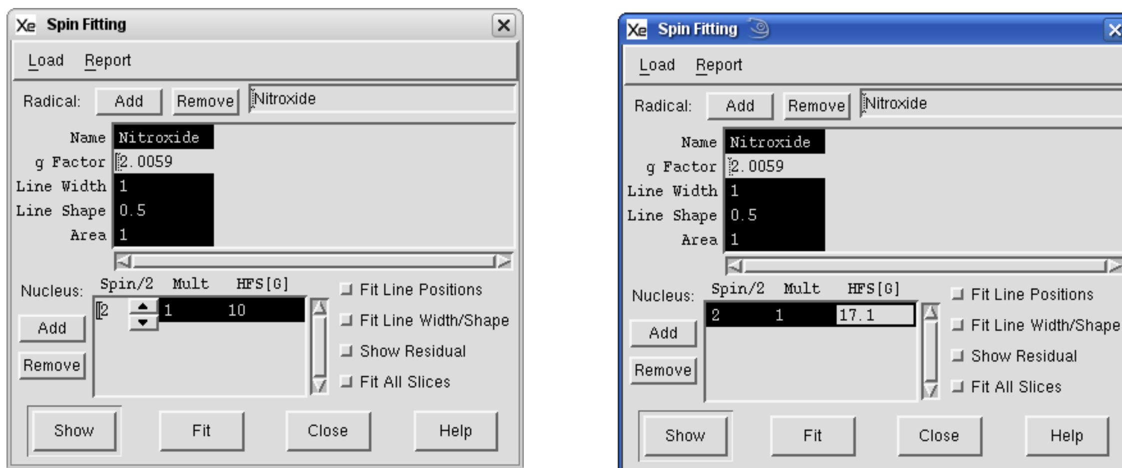

Figure 14-27 Entering the hyperfine parameters for a species.

As the nuclei are added, the result simulation appears in the Result dataset as a blue trace.

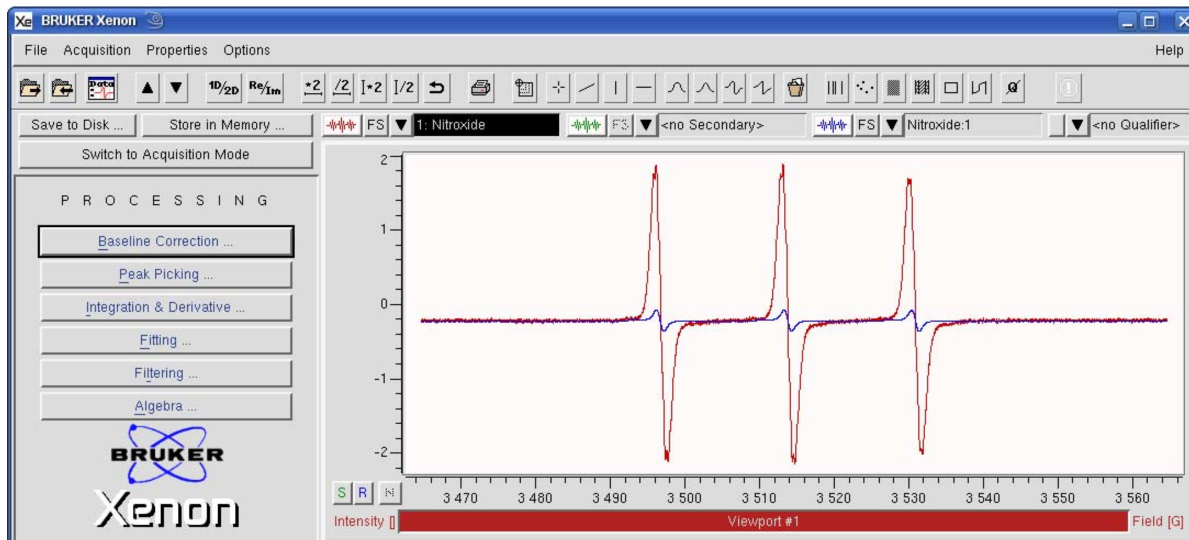

Figure 14-28 A simulated spectrum reflecting both the approximate g-Factor and HFS. The line positions in the experimental and simulated spectra should line approximately up.

6. **Enter the Line Width.** Use the distance tool to measure the peak-to-peak linewidth of the EPR lines. (See Section 2.4.3.) Its value is displayed in the viewport selector bar. Click the Line Width entry and enter the measured value. In this case its value is 1.4 G.

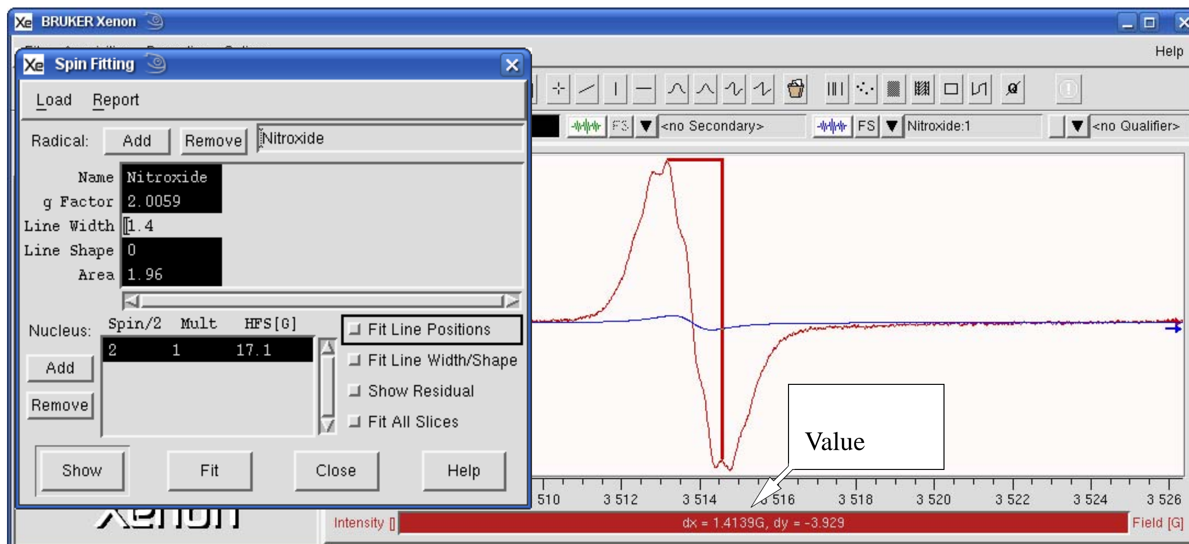

Figure 14-29 Estimating the peak-to-peak linewidth with the distance tool.

7. **Fit the spectrum.** Enable Fit Line Positions and Fit /Shape. Click Fit and all the parameters are optimized via least-squares analysis to minimize the difference between the simulated and experimental spectrum. The optimized simulated spectrum appears in the Result dataset as a blue trace.

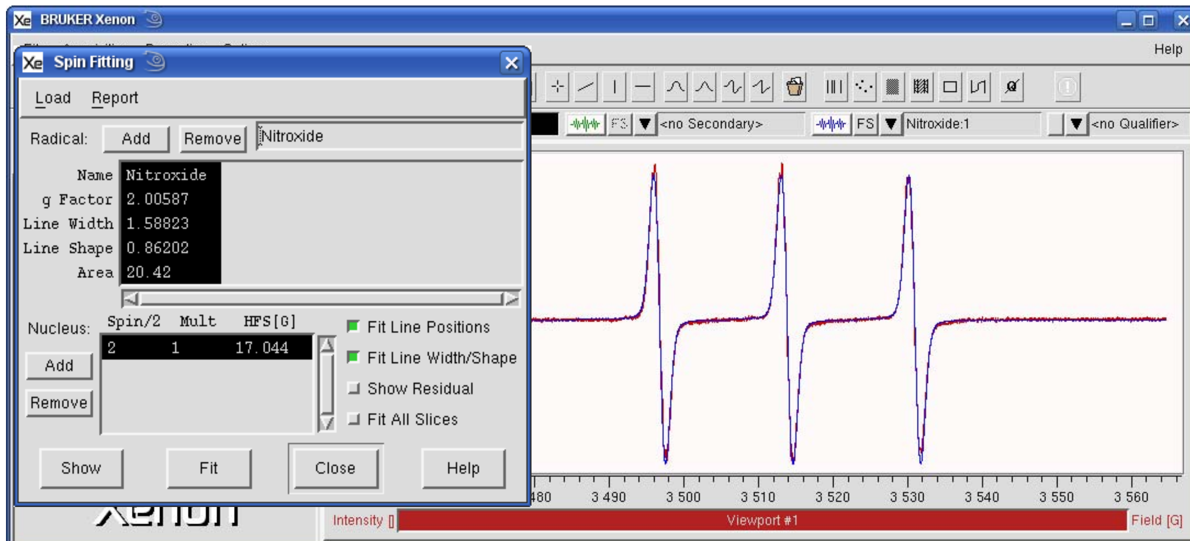

Figure 14-30 Optimizing the simulation parameters.

8. **Save the result.** Make sure that the Show Residual option is not selected. Click on the properties menu of the Result dataset. Select Save on Disk from the drop-down menu. All the simulation parameters for the species and the simulated spectrum are saved with the dataset. This saved spectrum can be used for simulating and Spin Counting as shown in Section 14.4. By organizing and saving the simulated spectra in folders you can create your own libraries for collections of different paramagnetic species.

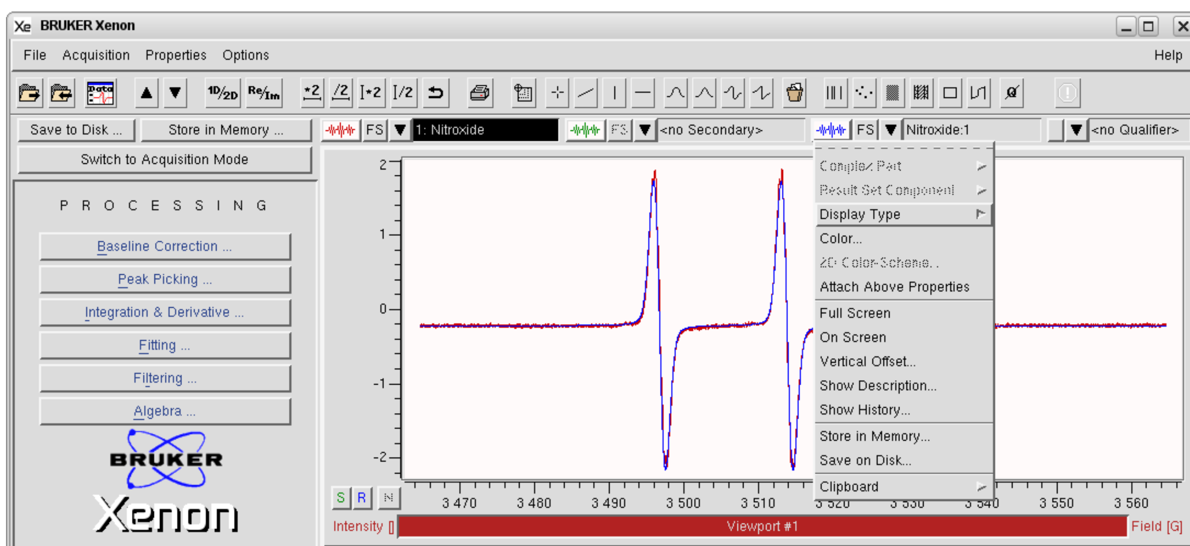

Figure 14-31 Saving the simulation result.

9. **View the fitting results.** You can view the results of fitting by clicking Report>Parameters. A new window appears displaying the fitting results. The results can be saved as a text file by clicking Save.

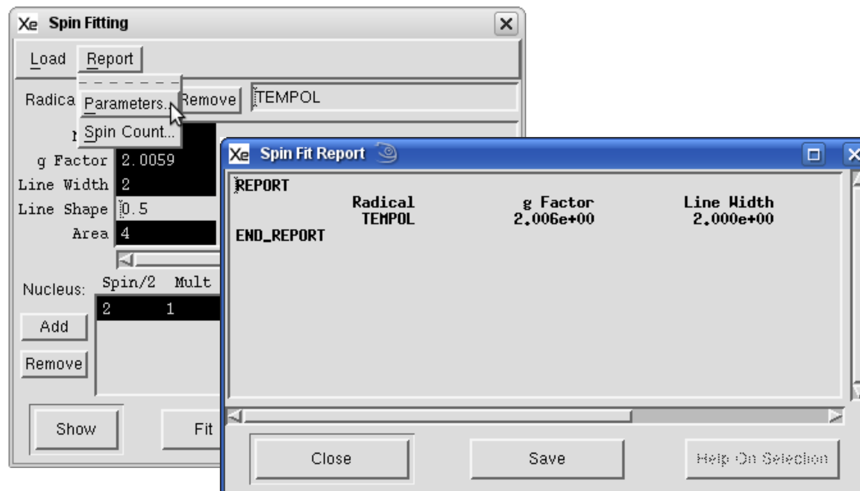

Figure 14-32 Viewing the SpinFit results.

A new dialog box appears to prompt you for a filename and folder where results are to be saved. Click Save to continue.

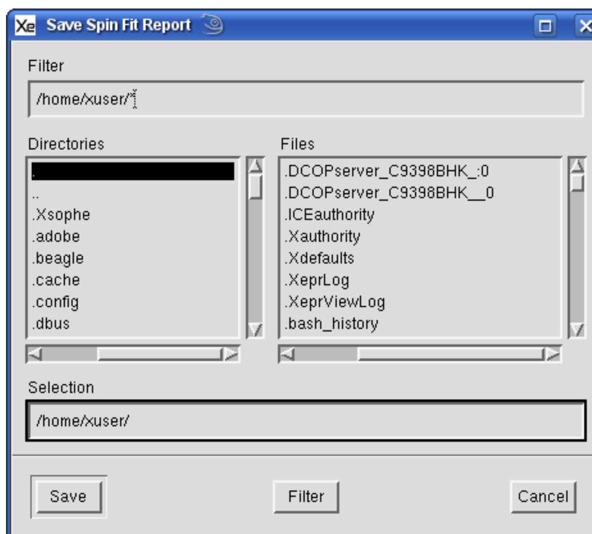

Figure 14-33 Saving the SpinFit results.

SpinFit Using a Spectral Library 14.4

SpinFit can use previously simulated spectra for a single species that are stored on the disk of the computer as a starting point for fitting simulations of spectra with multiple species. A collection of previously simulated spectra stored in a folder is called a spectral library. Section 14.2 shows how to create spectra for your own personal spectral library. There is a standard library including many spin adducts in the ../xenonFiles/Data/sharedData/SpinFitSimulations.

1. **Load the spectrum.** Load the EPR spectrum into the Primary dataset. In this example we are using one slice from the example given in Section 8.3 of the Xenon User's Guide. There are two species, a superoxide anion and acyl adduct.

2. **Start the SpinFit task.** Click SpinFit in the TASKS menu. (See Figure 3-1.) A new window then appears.

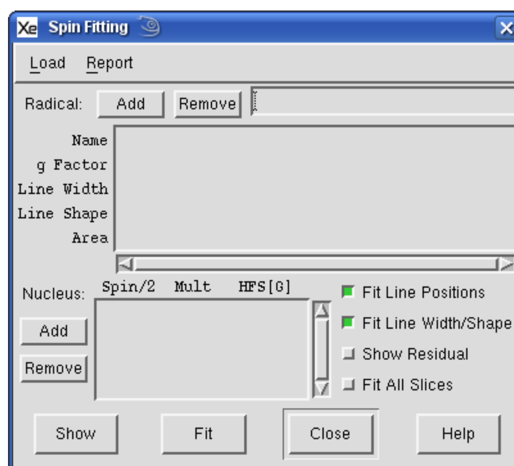

Figure 14-34 The SpinFit window.

3. **Load spectra from the a spectral library on the disk.** Click Load>From Disk. A new window appears for loading the reference datasets for simulating the spectrum with multiple species. We wish to simulate at least two species here, so the append option needs to be selected by clicking its radio button. Otherwise the previous spectra is replaced. Navigate to the folder with the previously simulated spectra (spectral library). Click the desired spectrum followed by Load. Continue until all the needed spectra are loaded. In this case it is spectra of superoxide and acyl adducts of DMPO. When finished, click Cancel. The g-Factor and HFS (HyperFine Splittings) as well as the lineshape/linewidth parameters are loaded for the species. (See Figure 14-36.)

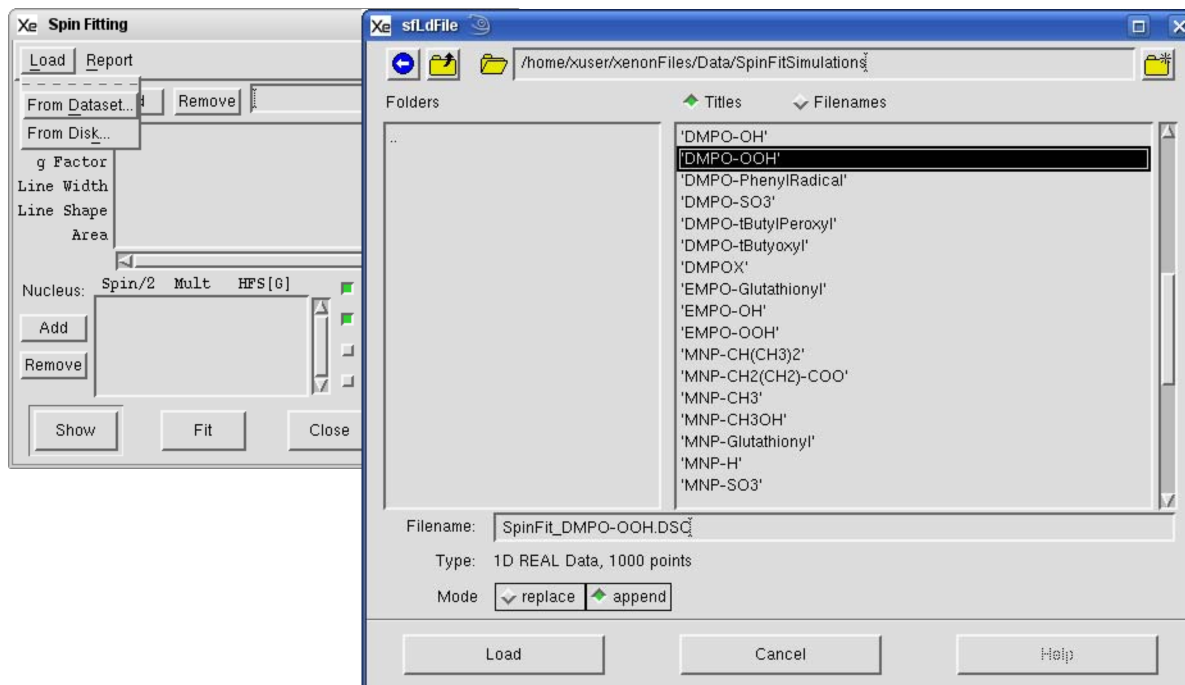

Figure 14-35 Loading previously simulated spectra from a spectral library on the disk.

4. **Fit the species parameters to the spectrum.** There are several different parameters that are fit. When Fit Line Positions is selected the g-Factor and HFS are fitted. When Fit Line Width/Shape is selected, the linewidth and the Gaussian/Lorentzian contribution is fitted. In this example, both are fitted simultaneously. The Area (integrated intensity of the species) of each species is also fitted. This value reflects the concentration of the species. The simulated spectrum appears in the Result dataset.

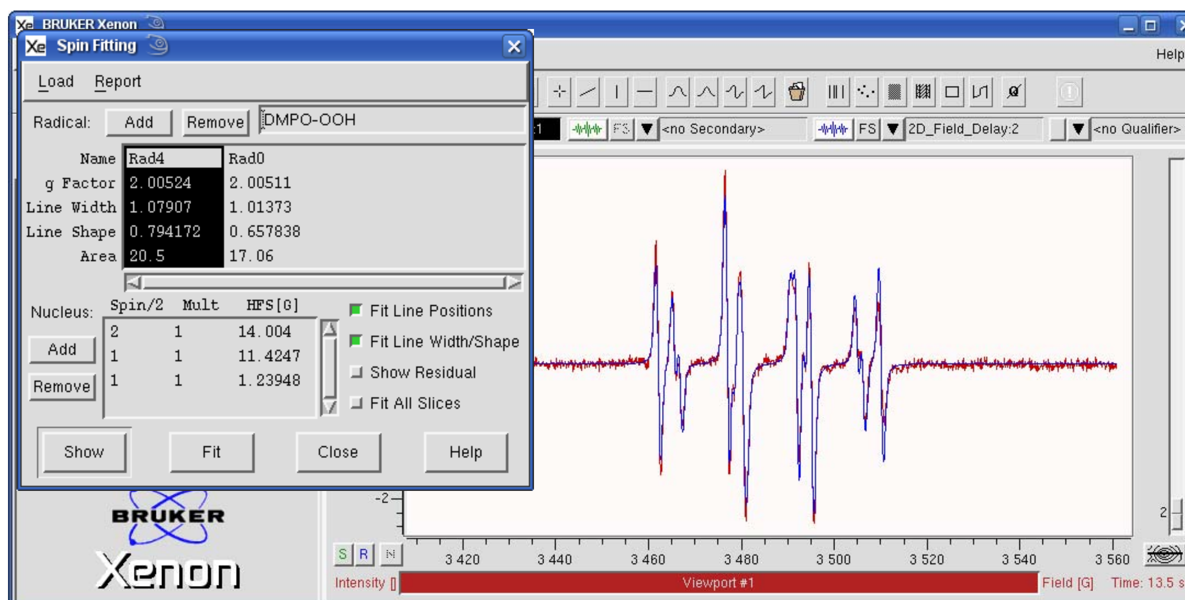

Figure 14-36 Fitting the parameters of the individual species. The result simulation is shown in blue.

Instead of viewing the simulated spectrum, clicking the Show Residual button displays the difference between the experimental and simulated spectra.

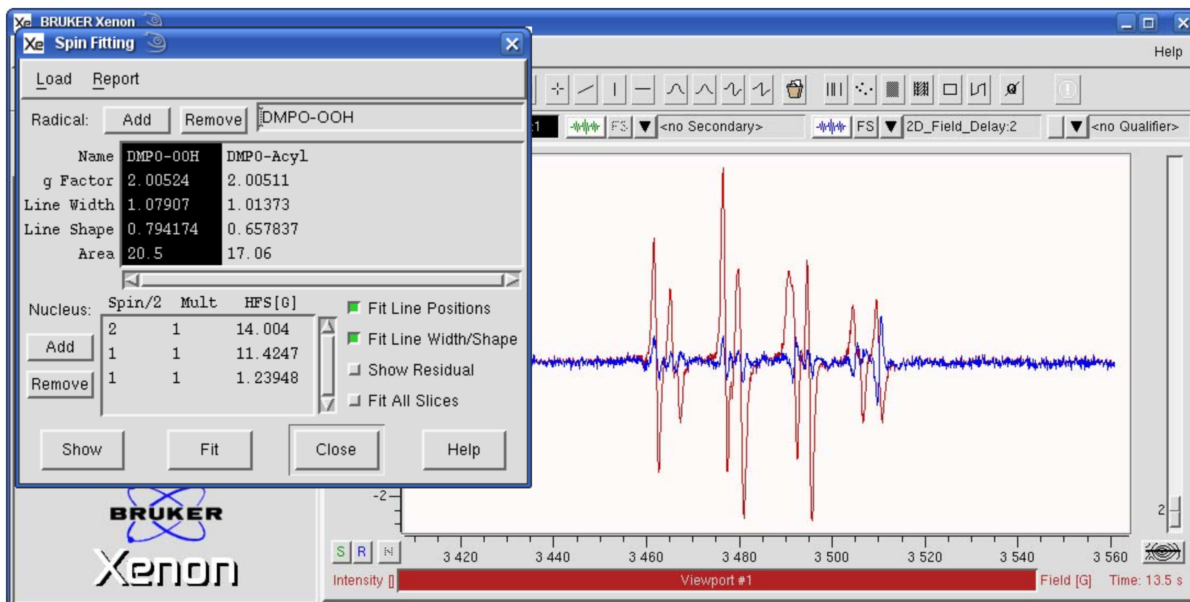

Figure 14-37 Fitting the parameters of the individual species. The residual is shown in blue. The increasing values of the residual at higher fields is caused by the decay of the species.

5. **Store or save the result.** Make sure that the Show Residual option is not selected. Click on the properties menu of the Result dataset. Select either Store in Memory or Save on Disk from the drop-down menu. All the simulation parameters for both species and the simulated spectra are stored or saved with the dataset.

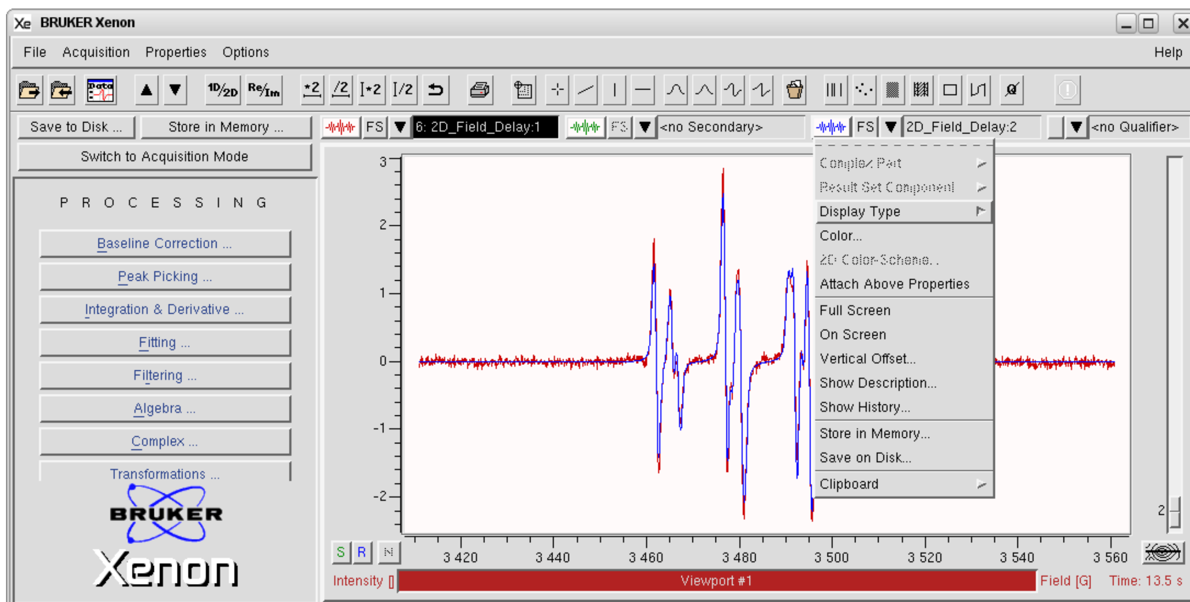

Figure 14-38 Saving or storing the simulation result.

6. **View the results.** Click Report>Parameters. A new window appears displaying the results of the fit.

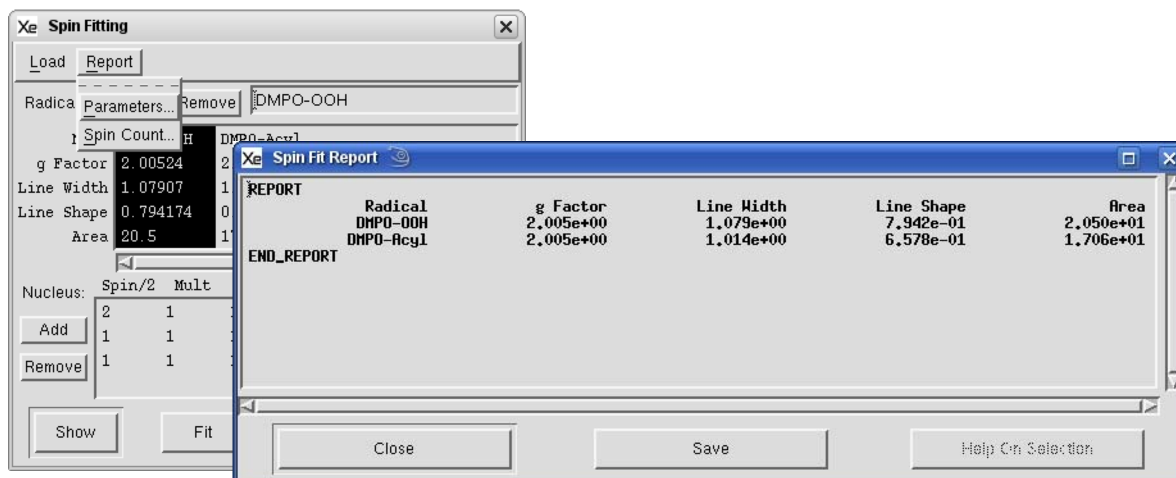

Figure 14-39 The display of the results of the fit.

A new dialog box appears to prompt you for a filename and folder where results are to be saved. Click Save to continue.

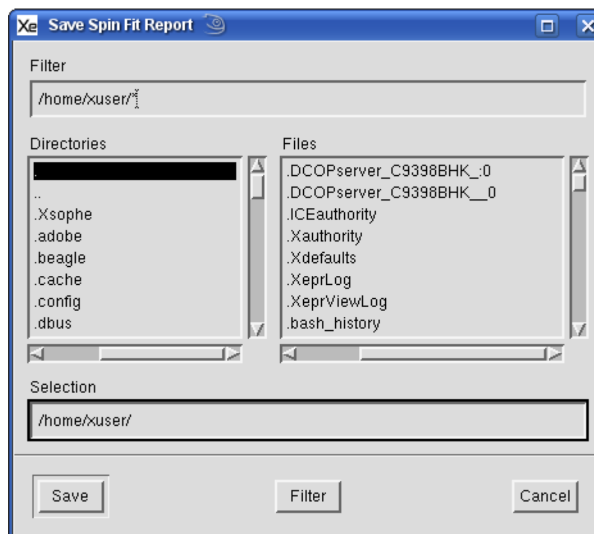

Figure 14-40 Saving the SpinFit results.

SpinFit Using Spectra in Memory 14.5

In the previous section, we loaded simulated spectra from a spectral library on the disk. We may also use simulated spectra that are in memory as well. Select Load>From Dataset. and a new window appears. This works similarly to Load>From Disk. A new window appears with which you can add species for the simulation. Dataset specifies which dataset is to be added. Clicking Load adds the specified dataset (Primary, Secondary, or Result) to the simulation. Continue until all the needed spectra are loaded. If you need more than three species, load the additional datasets into the Primary, Secondary, or Result datasets and continue. When finished, click Cancel.

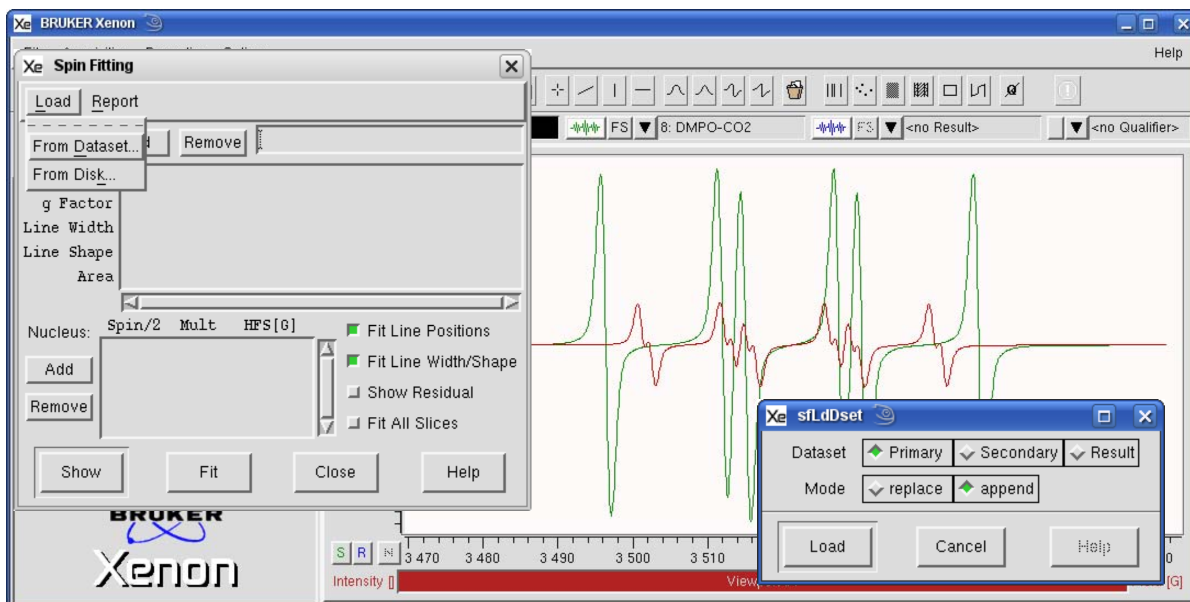

Figure 14-41 Loading previously simulated spectra from memory.

Spin Counting with SpinFit 14.6

After simulations and fitting have been performed, the simulated spectra can also be used for quantitation. This procedure works similarly to what is described in Section 13.5 except the integral of the simulated spectrum is used for the quantitation. Therefore no baseline correction is required.

1. **Perform Spin Counting.** Click Report>Spin Count. A new window appears requesting sample information. Click OK and a new window appears displaying the concentrations and number of spins of the individual species.

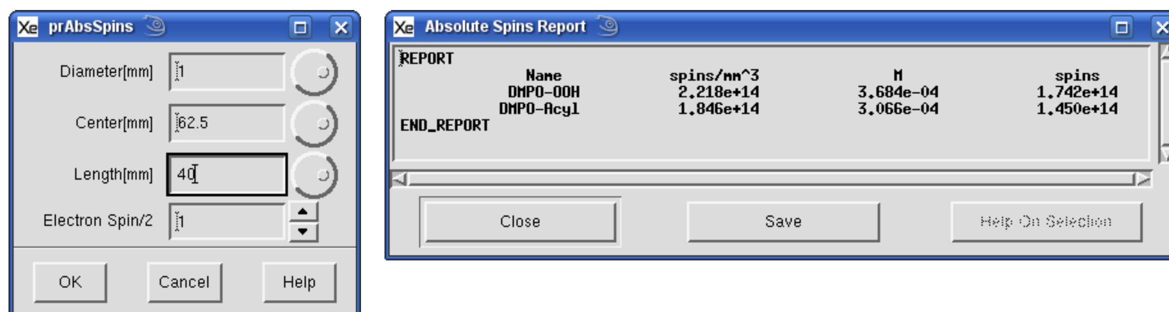

Figure 14-42 The display of concentration and number of spins calculated from the simulation.

Fitting 2D Datasets 14.7

SpinFit also works with 2D datasets in which the second axis can be time or some other parameter.

1. **Fitting 2D datasets.** Simply click Fit All Slices and the fit is performed on each of the slices. Refer to the example in Section 8.3 of the Xenon User's Guide for further details.

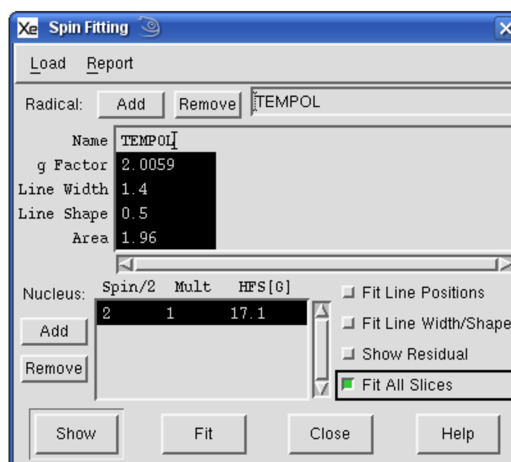

Figure 14-43 Caption text.

Simulating and fitting many slices can require quite a bit of time. One means of reducing the calculation time is to identify the species that are present in a few of the slices of the 2D dataset. Then disable Fit Line Positions and Fit Line Width/Shape. Then when you fit the 2D dataset, only the Area of each of the species in each slice is fitted, thereby saving a considerable amount of computation time.