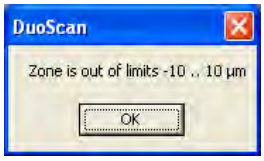
- Mapping Pixel – the scan area is set according to the pixel size specified in the Mapping Properties dialog window (see section 4.5.5, page 97). The "Width" and "Height" boxes will be inactive and greyed out, since the values are taken directly from the Mapping Properties dialog window. Note that if SWIFT™ ultra-fast Raman mapping is activated (see section 4.5.5, page 97), the "Width" value will be set to 0, because SWIFT™'s continuous scanning in the X direction does not require DuoScan™ scanning in the X (width) direction.

- Video Cursor – the scan area is set according to the dimensions and position of the Rectangular Mapping cursor (see section 5.19, page 187) on the video window. The "Width" and "Height" boxes will be inactive and greyed out, since the values are taken directly from the video Rectangular Mapping cursor.

Click **[Start]** to activate the DuoScan™ scanning mirrors.

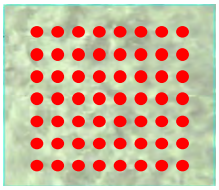
If the scan area has been set greater than the maximum allowable dimensions a warning message will be displayed. This shows the maximum negative and positive displacement possible for the selected objective. The area size set in the "Height" and "Width" boxes, or defined according to the map pixel, or defined by the rectangular video cursor, must not be greater than the total displacement. In the example shown right, the maximum displacement is -10 µm to +10 µm, meaning a total displacement of 20 µm.



The Raman measurement (spectrum or multidimensional spectral array) can now be set up and acquired in the normal way. Remember that the spectrum recorded will be an average spectrum from the scanned area.

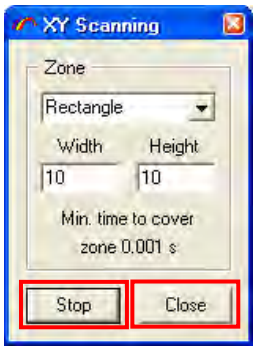
Click **[Stop]** to stop the DuoScan™ scanning mirrors.

4.5.10.1.2. Point Mode



When DuoScan™ is used in Point Mode, it is possible to move the laser spot to any position on the sample surface, within the maximum possible scan displacement. In this mode DuoScan™ can be used as a substitute to the standard motorized stage for point-by-point XY mapping (multidimensional spectral array acquisition) – this function is particularly useful when it is not possible to move the sample underneath the laser beam, or ultra-fine step size is required (down to DuoScan™'s minimum step size of 50 nm).

To use DuoScan™ in Point Mode the scanning mirrors must be static. To confirm this, open the DuoScan™ XY scanning dialog window by clicking on the DuoScan icon.



If the DuoScan™ mirrors are scanning, the **[STOP]** button will be active. Click on **[STOP]** to stop the DuoScan™ scanning, and then click **[CLOSE]** to close the dialog window.

Set the DuoScan™ module as the active XY stage using the 'switch stage' icon in the XYZ Coord section of the Control Panel (see section 9.10.2, page 237).

The laser spot position can now be controlled by inputting XYZ coordinates in the XYZ Coord section of the Control Panel (see section 9.10, page 236), or by acquiring a Raman XY map (multidimensional spectral array) in the standard way. In both cases, the laser spot is now moved across the sample using the DuoScan™ mirrors. The sample does not move.

4.6. Data Processing and Analysis Icons

4.6.1. Spectral ID Search

Launch the 'one click' Spectral ID database search for the active spectrum.

Clicking on this icon will initiate the following processes:

- Export the active spectrum in Grams .spc format
- Open Spectral ID
- Load the exported spectrum into Spectral ID
- Run a full spectrum matching search through the active databases
- Report the list of matching spectra, with match quality scores

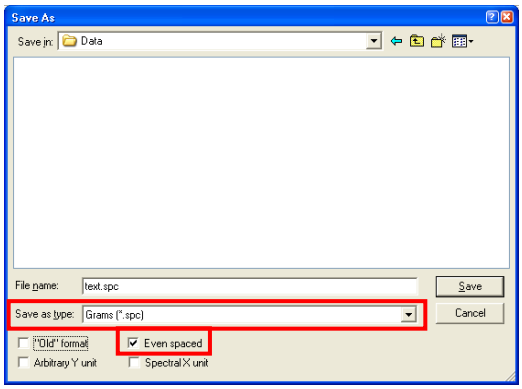
Please see the HORIBA Scientific manual "Using the Spectral ID Database Software with LabSpec 5" and full documentation provided with the Spectral ID database software for more information about Spectral ID and how it can be used and configured.

4.6.1.1. Configuring LabSpec 5 for Correct Spectral ID Searching

The export procedure for the 'one click' Spectral ID search is defined by the options set in the File > Save As dialog window for a Grams .spc file.

To ensure the 'one click' Spectral ID search performs correctly please complete the following set up procedure in LabSpec 5:

- Open a spectrum in any format
- Click on File > Save As
- Select "Grams (.spc)" from the "Save as Type" drop down box
- Tick the box for "Even spaced"
- Click **[Cancel]**



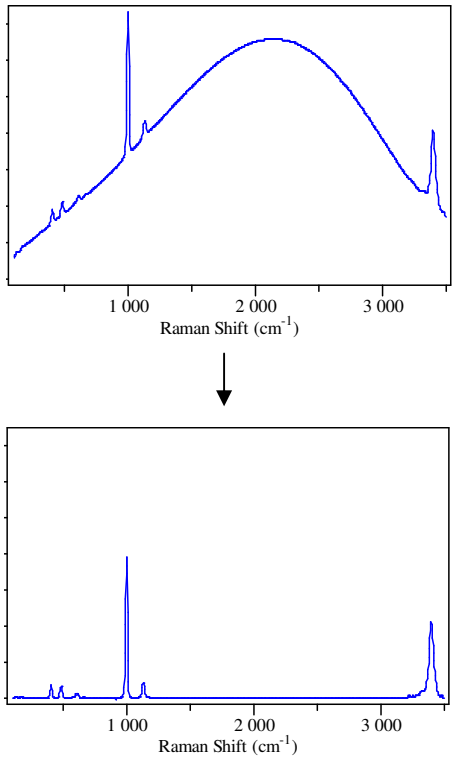
If the "Even spaced" box is not ticked, the Spectral ID search will not be performed correctly, and spurious results may be displayed.

4.6.2. Baseline Correction

Opens the Baseline dialog window.

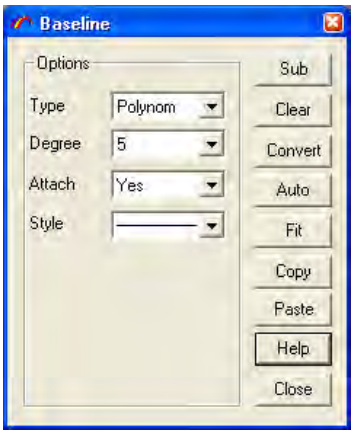
Baseline correction allows a high background in a spectrum to be subtracted, to yield a spectrum with a flat, zero baseline. The correction can be applied to a single spectrum or a multidimensional spectral array of spectra (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes).

The example shown right illustrates a spectrum before and after baseline correction.



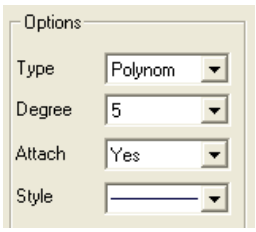
4.6.2.1. Baseline Dialog Window

The Baseline dialog window allows the baseline correction to be configured and performed.



Options

Set the parameters for the baseline. Please see section 4.6.2.1.1, page 117, for information about the individual parameters and their affect on the baseline correction.



Sub

Click on **[Sub]** to subtract the current baseline from the active spectrum.

Clear

Click on **[Clear]** to clear the current baseline from the active spectrum.

Convert

Click on **[Convert]** to convert the active spectrum to the displayed baseline curve. This function is useful to save a baseline curve in a standard spectrum file format.

Note that the active spectrum will be overwritten by the baseline curve. Make sure that the file is saved with a different name to ensure the original spectrum data is not permanently overwritten and lost.

Auto

Click on **[Auto]** to automatically fit a baseline to the active spectrum (based on the parameters set in the "Options" section) and subtract it.

Fit

Click on **[Fit]** to automatically fit a baseline to the active spectrum (based on the parameters set in the "Options" section). The best fit baseline curve will be displayed on the spectrum. It can be subtracted by clicking on **[Sub]**.

Copy

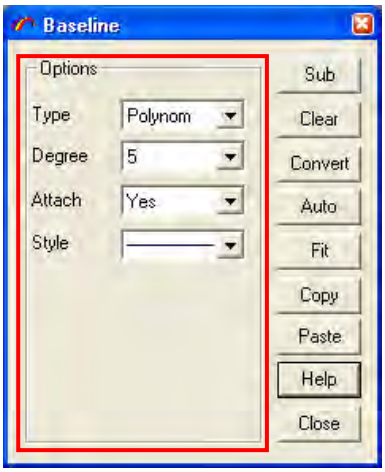
Click on **[Copy]** to copy the displayed baseline curve to the baseline clipboard.

Paste

Click on **[Paste]** to paste a baseline curve from the baseline clipboard onto the active spectrum.

4.6.2.1.1. Baseline Correction Options

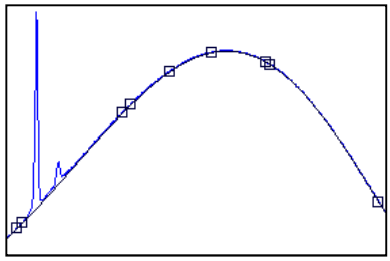
The Baseline options control the parameters of the baseline that will be used for baseline correction.



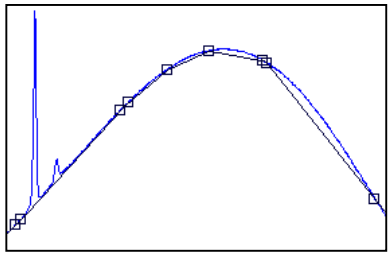
Type

Select the algorithm used to define the baseline curve.

- Polynom: fits a polynomial curve through the baseline points set on the spectrum. The degree of the polynomial is set in the "Degree" drop down box.



- Lines: fits a straight line between baseline points set on the spectrum.



Degree

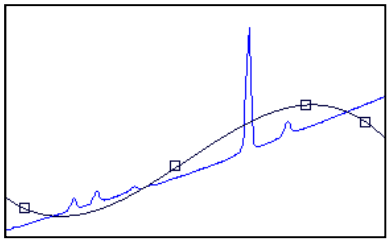
Select the degree of polynomial equation used to create the baseline curve – this option is only used when the baseline "Type" is set to "Polynom".

In general the higher the degree the more adaptable the baseline curve will be to strangely shaped, non-uniform backgrounds.

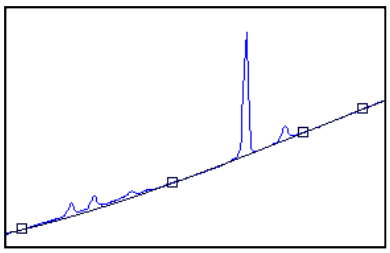
Attach

Select the "Attach" mode for manual definition of baseline points used to define the baseline curve.

- No: baseline points are set at the exact intensity and spectral position (cm^{-1} or nm) specified by the user.



- Yes: baseline points are forced to sit on the spectrum, at the spectral position (cm^{-1} or nm) specified by the user.



The "Attach" mode is particularly important when defining a baseline to be used for correction of multidimensional spectral array data. In this case, "Attach" should be set to "Yes" to ensure that the baseline points will adapt to the varying intensities of the spectra within the multidimensional spectral array.

Style

Set the display style of the baseline curve. The colour, width and line style of the baseline curve can be set using the "Style" drop down box.

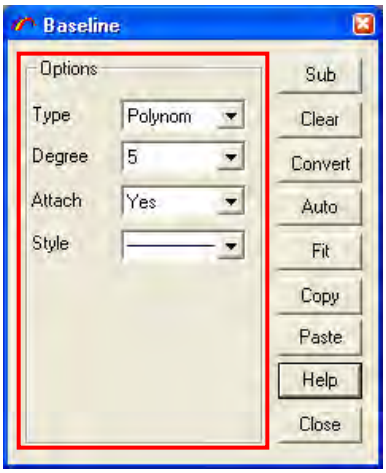
4.6.2.2. Setting a Baseline for Baseline Correction of a Single Spectrum

Select the spectrum which is to be baseline corrected.

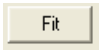
Click on the Baseline icon to open the Baseline dialog window.

Select the baseline parameters within the “Options” section. See section 4.6.2.1.1, page 117, for full details about the Baseline Correction Options.

The options can be modified at any point during the process of creating the baseline – the displayed baseline will immediately update.



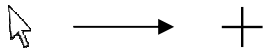
Click on **[Fit]** to fit a baseline curve to the spectrum.



Baseline points can be manually inserted, adjusted and removed by using the “Add baseline points” icon (see section 5.14, page 180) and “Remove baseline points” icon (see section 5.15, page 181) in the Graphical Manipulation Toolbar.

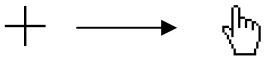
- Click on the “Add baseline points” icon.

The cursor will change from the mouse cursor to the Add Baseline Points cursor.



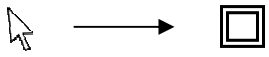
Left click on the spectrum to add a baseline point to the displayed baseline curve. If there is no baseline curve on the spectrum the first left mouse click in this mode will create the baseline.

Hover the cursor over an existing baseline point. When the cursor changes from the Add Baseline Points cursor to the Adjust Baseline Points cursor, left click to drag the baseline point to a new position.



- Click on the “Remove baseline points” icon.

Hover the cursor over an existing baseline point. When the cursor changes from the mouse cursor to the Remove Baseline Points cursor, left click to delete



the baseline point from the baseline curve.

The entire baseline can be cleared by clicking on **[Clear]**.

When the baseline curve is completed click **[Sub]** to subtract it from the spectrum.

4.6.2.3. Setting a Baseline for Baseline Correction of a Multidimensional Spectral Array

The procedure as outlined above (section 4.6.2.2, page 118) should be followed, but the baseline curve should be created on the SpIm (spectral image) window.

In general it is useful to have the “Attach” mode set to “Yes” when applying a baseline to the SpIm window, since this ensures the baseline points can adapt to the varying intensities typical in a multidimensional spectral array.

Individual spectra within a multidimensional spectral array can be baseline corrected by applying the correction to the individual spectrum displayed in the Point window.

When the baseline has been subtracted, ensure the corrected spectrum is re-inserted into the spectral array by clicking on the ‘blue arrow’ icon displayed in the point window, or click **[Correct]** in the Map Analysis dialog window (see section 4.6.9, page 147). This must be done before the map/profile cursor is moved.

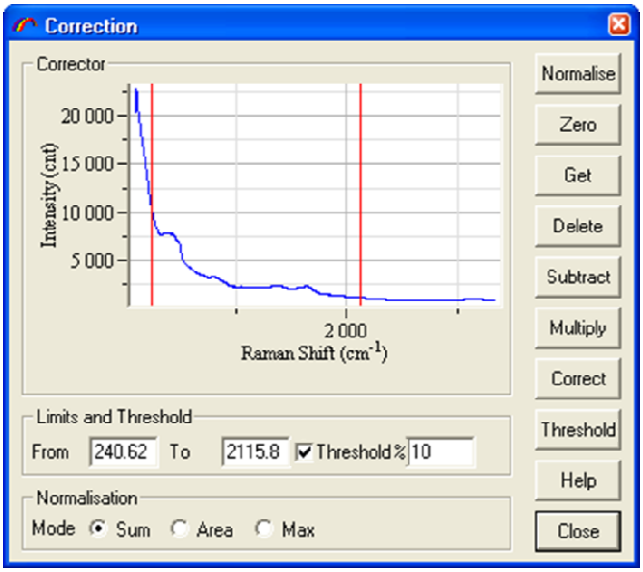
4.6.3. Spectral Normalization and Correction

Opens the Correction dialog window.

The correction functions allow spectra to be normalized and zeroed, and for a substrate/contaminant spectrum to be automatically subtracted.

4.6.3.1. Correction Dialog Window

The Correction dialog window allows the user to apply spectral normalization and correction to both single spectra, and multidimensional spectral arrays.

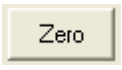


Normalize

Click on **[Normalize]** to normalize each spectrum so that the total area, sum or maximum signal of the spectrum is 100. If the "Limits" tick box is ticked, the normalization will only be applied within the displayed limits. In this case, the spectrum will be normalized so that the area, sum, or maximum signal within the limits is 100. The limits can be adjusted by typing values in the "Limits" boxes, or adjusting the cursor positions in the "Corrector" window.

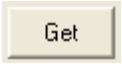
The normalization mode can be selected from the "Normalization" section of the dialog window.

Zero



Click on **[Zero]** to automatically subtract a constant intensity value from the active spectrum or multidimensional spectral array, so that the lowest intensity pixel is at zero. When applied to a multidimensional spectral array, each spectrum in the array is zeroed independently.

Get



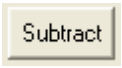
Click on **[Get]** to load the active spectrum in the main LabSpec 5 graphical user interface (GUI) into the "Corrector" window.

Delete

Click on **[Delete]** to clear the "Corrector" window, and remove the corrector spectrum displayed there.

Subtract

Click on **[Subtract]** to subtract the corrector spectrum (displayed in the "Corrector" window) from the active spectrum or multidimensional spectral array.



Multiply

Click on **[Multiply]** to multiply the active spectrum or multidimensional spectral array by the corrector spectrum (displayed in the "Corrector" window).

Correct

Click on **[Correct]** to correct the active spectrum or multidimensional spectral array for the contribution of the corrector spectrum (displayed in the "Corrector" window). The intensity of the corrector spectrum will be automatically adjusted before subtraction to best fit the spectrum.

If the "Limits" tick box is ticked the corrector intensity adjustment will be calculated only within the displayed limits. The limits can be adjusted by typing values in the "Limits" boxes, or adjusting the cursor positions in the "Corrector" window.

This function is useful if a substrate, diluent or contaminant spectrum needs to be removed from a sample spectrum.

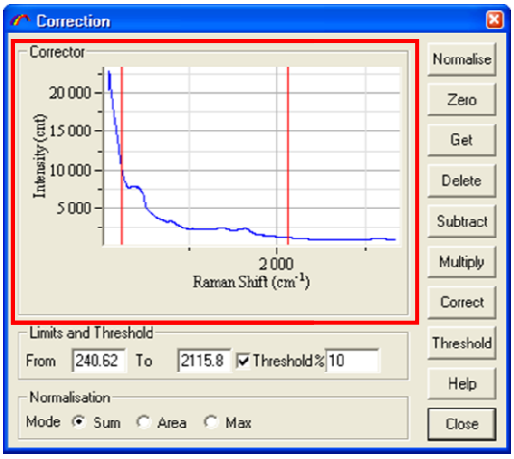
Threshold

Click on **[Threshold]** to threshold the active multidimensional spectral array. The threshold function will be applied to any spectra within the array which has a maximum intensity (relative to the most intensity spectrum in the array) less than the displayed threshold value set in the "Limits and Threshold" section.

The threshold function will convert the spectrum to have zero intensity throughout its spectral range.

Corrector Window

The current corrector spectrum will be displayed in the Corrector window. Click on **[Get]** to load the active spectrum in the main LabSpec 5 graphical user interface (GUI) into the "Corrector" window. Click on **[Delete]** to clear the "Corrector" window, and remove the corrector spectrum displayed there.

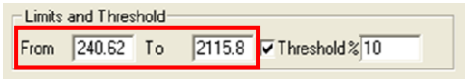


The spectrum in the Corrector window can be manipulated using the standard Graphical Manipulation Toolbar icons – see section 5, page 164.

If the cursors are not visible in the Corrector window click on the Center Cursors icon in the Icon bar.

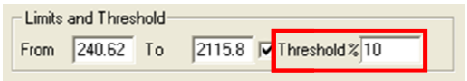
Limits and Threshold

Set the limits for Normalize and Correct functions ticking the "Limits" tick box, and typing the "From" (minimum) and "To" (maximum) limit values.



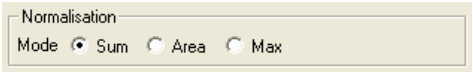
The limits can also be set by adjusting the position of the cursors in the Corrector window. As the cursors are moved the "From" and "To" values shown in the "Limits and Threshold" section are continuously updated.

Set the threshold value by typing in the desired level (in percent, %) in the "Threshold %" box.



Normalization

Select the normalization mode for the Normalize function.



- {Sum} – normalize so that the sum of the spectrum intensity is 100
- {Area} – normalize so that the area of the spectrum is 100
- {Max} – normalize to the maximum intensity point in the spectrum.

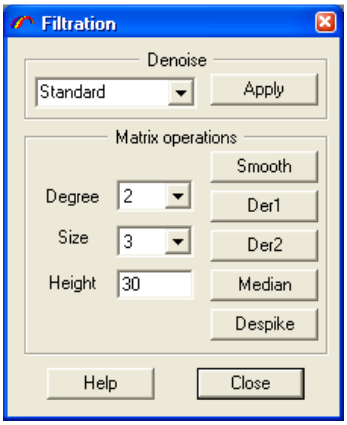
4.6.4. Smoothing

Opens the Filtration dialog window.

The smoothing or filtration functions allow spectra to be smoothed, converted to first and second derivative functions, or despiked. Typically these functions allow spectral quality to be improved after acquisition.

4.6.4.1. Filtration Dialog Window

The Filtration dialog window allows the smoothing and processing functions to be configured and performed on both single spectra, and multidimensional spectral arrays.

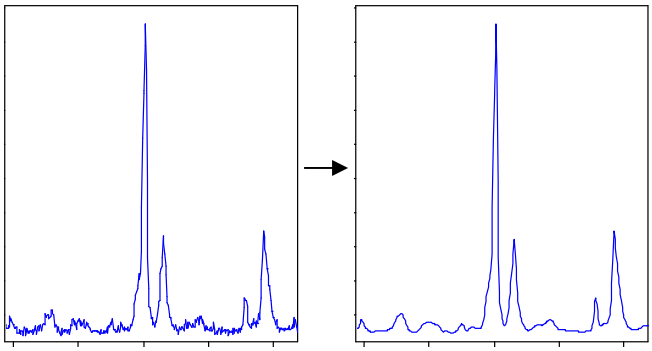


4.6.4.1.1. Denoise

The Denoise function is a unique noise reduction algorithm which can be used to significantly enhance spectrum quality without losing subtle spectral information.

Standard smoothing functions can result in loss of peak shape and position, and subtle features (such as weak shoulders on a strong band) can be lost. The Denoise function ensures that all this important information is retained, whilst still reducing noise in the spectrum.

The spectra shown right illustrate the effect of the Denoise function.



Two main Denoise algorithms are available from the "Denoise" drop down box:

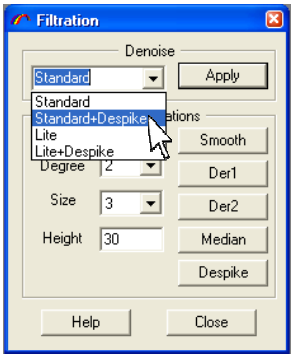
- Standard: recommended for spectra with signal to noise ≥ 20
- Lite: recommended for very noisy spectra with signal to noise ≤ 20

In addition, both algorithms can be used with an integrated Despike function to remove random spikes (also known as cosmic rays). See also section 3.5.4.7, page 37, for more information about other spike filter options in LabSpec 5.

Note that the Denoise function can be automatically applied to all acquired data through the Acquisition > Options dialog window – see section 3.5.4.15, page 46. If a spectrum has had the Denoise function automatically applied through Acquisition > Options, it cannot have the function applied again through the Filtration dialog window.

Using the Denoise Function

Select the desired Denoise algorithm from the "Denoise" drop down box.



Click **[Apply]** to apply the Denoise algorithm to the active spectrum.

4.6.4.1.2. Matrix Operations

The functions described below require the "Degree", "Size", and "Height" values to be set in the drop down boxes, as described in the text.

Smooth

Click on **[Smooth]** to apply a Savitsky-Golay smoothing function to the active spectrum.

Savitsky-Golay smoothing fits a polynomial function of a specified "Degree" through a range ("Size") of adjacent pixels, and replaces those pixels with the polynomial curve. The window where this operation is applied is moved across the entire spectrum. The "Degree" and "Size" must be set to an appropriate level.

Typically the smaller the "Degree" and the larger "Size" the more significant the smoothing. Note that in some cases smoothing can remove or alter

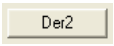
features in a spectrum. Smoothing should be used with care.

Der1

Click on **[Der1]** to convert the active spectrum to its first derivative function. At each pixel position the derivative is calculated using a defined range ("Size") of pixels either side of it.

Der2

Click on **[Der2]** to convert the active spectrum to its second derivative function. At each pixel position the second derivative is calculated using a defined range ("Size") of pixels either side of it.



Median

Click on **[Median]** to apply a non-linear median smoothing function to the active spectrum.

Median smoothing replaces a spectrum pixel intensity value by the median of intensity values within a defined range ("Size") either side of it. This replacement process is repeated for all pixels in the spectrum.

Typically the larger the "Size" the more significant the median smoothing. Note that in some cases median smoothing can remove or alter features in a spectrum. Median smoothing should be used with care.

Despike

Click on **[Despike]** to remove random spikes (also known as cosmic rays) from a spectrum.

A spike is calculated as a pixel which has an intensity greater than the average spectrum intensity + "Height".

The Despike function removes the spike and replaces it with a weighted average of the surrounding pixels.

Note that in some cases the Despike function can remove or alter features in a spectrum, particularly when the spectrum is comprised of very sharp peaks. Despike should be used with care.

Please also see section 3.5.4.7, page 37, for more information about other spike filter options in LabSpec 5.

4.6.5. Fourier Transform

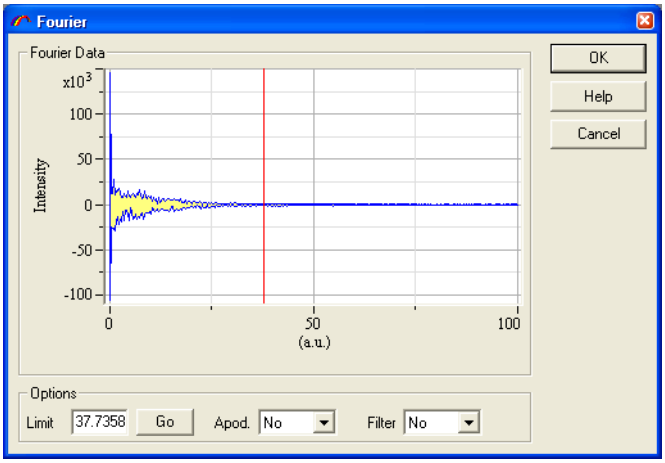

Opens the Fourier Transform dialog window.

The Fourier Transform function allows smoothing of a spectrum based on direct Fourier data transformation, applying filter and apodization functions. The spectrum is converted into its real and imaginary Fourier functions, which essentially represents the spectrum as a combination of wave patterns of varying frequency. Smoothing can be applied by removing high frequency contribution (corresponding to noise) and leaving medium and low frequency contribution (corresponding to Raman peaks).

The Fourier Transform smoothing function can be performed on both single spectra, and multidimensional spectral arrays.

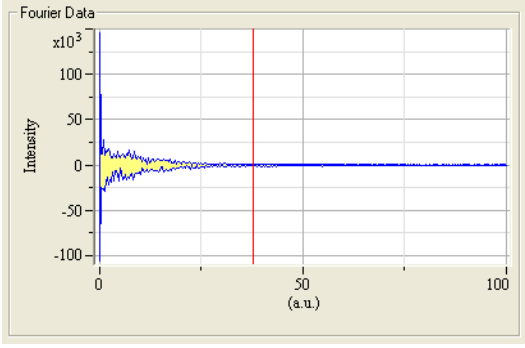
4.6.5.1. Fourier Transform Dialog Window

The Fourier Transform dialog window displays the Fourier functions of the active spectrum, and provides Apodization and Filter options for the transformation.



Fourier Data

The Fourier Data window displays the real and imaginary Fourier functions of the active spectrum.



The red cursor can be used to set the high frequency limit – frequencies above the limit position will be removed from the spectrum.

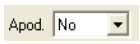
Typically the lower the cursor position the more smoothing is applied. At position 0 the spectrum is fully smoothed (to a flat line). At position 100 the spectrum is fully unsmoothed.

Limit

The Limit allows manual control of the "limit" cursor displayed in the Fourier Data window. Type in the required cursor position value (ranging between 0 and 100) and click on **[Go]**.

Apod.

Select the type of apodization to be used in the Fourier transformation from the "Apod." drop down box. The apodization reaches zero at the Limit position.

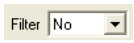


Four modes are available:

- No: no apodization
- Line: linear apodization function
- Sqrt: parabolic apodization function
- Cos: cosine apodization function

Filter

Select whether a filter will be used for the Fourier transformation, using the "Filter" drop down box.



Two modes are available:

- No: no filter
- Traffic: traffic filter

OK

Click **[OK]** to permanently apply the smoothing to the active spectrum, and close the Fourier Transform dialog window.

Cancel

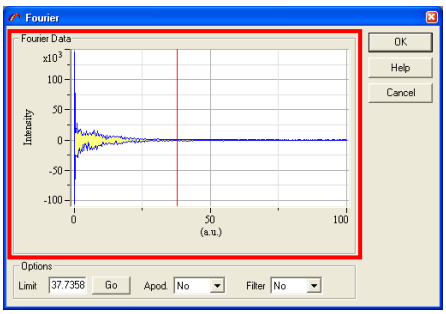
Click **[Cancel]** to close the Fourier Transform without applying the smoothing. The original spectrum will be left unchanged.

4.6.5.2. Using the Fourier Tranform Function to Smooth a Spectrum

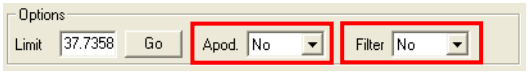
Select a spectrum to be smoothed.

Open the Fourier Transform dialog window by clicking on the Fourier Transform icon in the Icon Bar.

The real and imaginary Fourier functions of the active spectrum are displayed in the Fourier Data window.

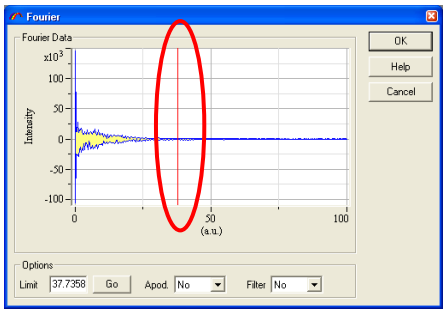


Select the Apodization and Filter functions to be used from the "Apod." and "Filter" drop down boxes.

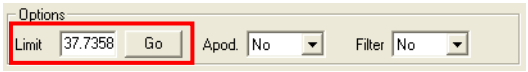


Set the limit for high frequency contribution which will be removed from the spectrum. The spectrum is continuously updated allowing the degree of smoothing to be monitored. The limit can be set in two ways:

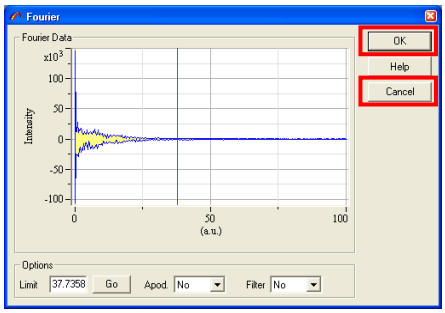
- Click and drag the red cursor in the Fourier Data window.



- Type in the required limit into the "Limit" box, and click **[Go]**.



When the desired smoothing is achieved, click **[OK]** to permanently apply the smoothing to the active spectrum and close the Fourier Transform dialog window. Alternatively click **[Cancel]** to close the dialog window without applying any smoothing to the spectrum. The spectrum will be left unchanged.



4.6.6. Math

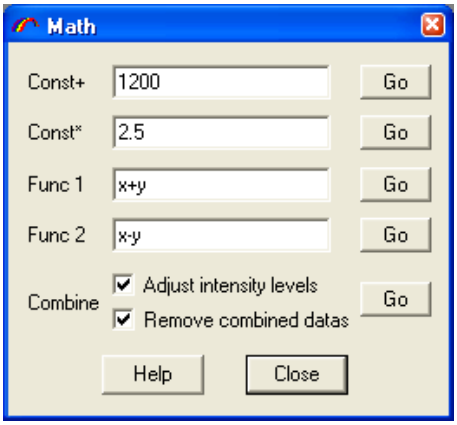
Opens the Math dialog window.

Arithmetic functions can be applied to the intensity values of an active spectrum. Additionally, the Extended Range spectral acquisition "Combine data" function can be applied post-acquisition through the Math dialog window.

The Math functions can be performed on both single spectra, and multidimensional spectral arrays.

4.6.6.1. Math Dialog Window

The Math dialog window contains text input boxes so that arithmetic functions can be created, and then applied to the active data.

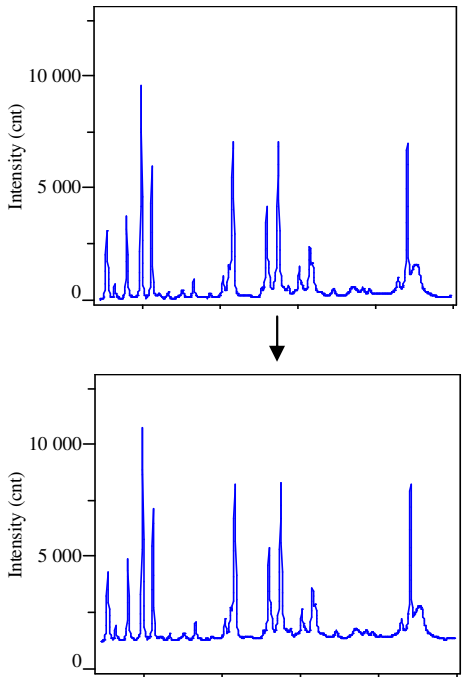


Const+

The "Const+" function adds a constant intensity value to all pixels in the active spectrum.

Type in the desired constant, and click on the adjacent **[Go]** button to apply the function to the active spectrum. A positive constant will be added to the spectrum intensity values; a negative constant will be subtracted from the spectrum intensity values.

The example right shows the result of applying "Const+"=1200 to a spectrum.



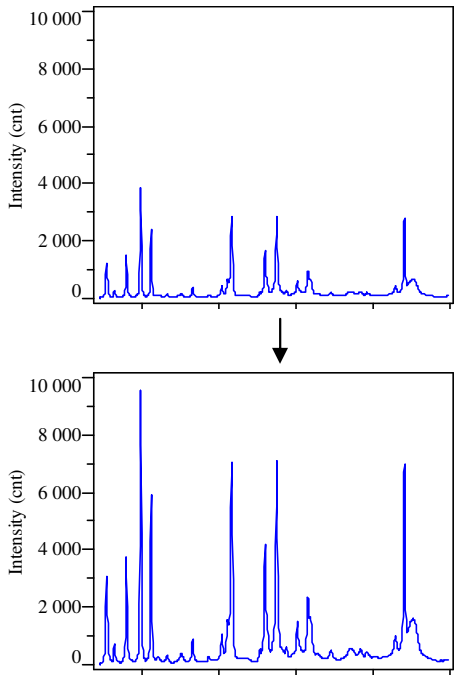
The "Const+" function can also be applied using the "Add Constant" icon in the Graphical Manipulation toolbar – see section 5.8, page 170.

Const*

The "Const*" function multiplies all pixel intensity values in the active spectrum by a constant value.

Type in the desired constant, and click on the adjacent **[Go]** button to apply the function to the active spectrum. A constant greater than zero will increase the spectrum intensity; whilst a constant less than zero will decrease the spectrum intensity.

The example right shows the result of applying "Const*"=2.5 to a spectrum.



The "Const*" function can also be applied using the "Multiply by Constant" icon in the Graphical Manipulation toolbar – see section 5.9, page 171.

Func 1 and Func 2

The "Func 1" and "Func 2" sections allow arithmetic functions to be created by the user, and applied to the active spectrum.

The terminology used for these functions are as follows:

- **x** and **y** refer to the intensity values of spectra which are open in LabSpec 5. **x** is the active data file, and **y** is the other data file open in LabSpec 5. In the event that there is more than one spectrum which could be used for **y** a message box will ask for the **y** spectrum to be chosen from a list.
- **a**, **b** and **c** refer to the values of the first, second and third axes of the active data file.
- Standard arithmetic functions are also possible, including **+**, **-**, *, /, **^**, **exp**, **log**, **sin**, **asin**, **cos**, **acos**, **tan**, **atan**, **abs**, **sqrt** etc.

As an example, if "Func 1" = **x** + **y**, **x** refers to the intensity value at each pixel of the active spectrum, and **y** refers to the intensity value at each pixel of another open spectrum. Assume the two spectra have values as follows (where **a** represents the spectral axis):

a →	1	2	3	4	5	6	7	8	...
x →	10	12	15	25	22	13	10	7	...
y →	1	2	3	2	1	1	4	3	...

applying the function **x** + **y** to this data will result in the following values:

a →	1	2	3	4	5	6	7	8	...
x →	**11**	**14**	**18**	**27**	**23**	**14**	**14**	**10**	**...**
y →	1	2	3	2	1	1	4	3	...

As another example, if "Func 1" = **x** + 2***a**, **x** refers to the intensity value of each pixel of the active spectrum, and **a** refers to the first axis (i.e., the spectral axis, with units Raman shift, cm^{-1}) value of each pixel. Assume the data has the following values:

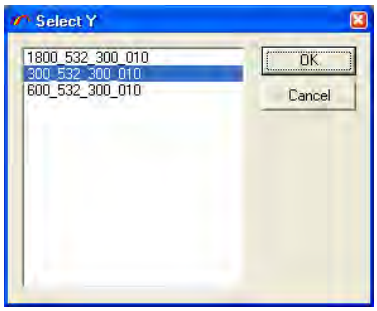
a →	1	2	3	4	5	6	7	8	...
x →	10	12	15	25	22	13	10	7	...

applying the function **x** + 2***a** to this data will result in the following values:

a →	1	2	3	4	5	6	7	8	...
x →	**12**	**16**	**21**	**33**	**32**	**25**	**24**	**23**	**...**

Type in the desired arithmetic function for either "Func 1" or "Func 2", select the appropriate active spectrum (corresponding to **x** in the function), and click on the adjacent **[Go]** button.

If there are multiple options for the spectrum **y**, a "Select Y" message box will ask for the desired spectrum to be selected. Click on the desired spectrum and then click **[OK]** to complete the arithmetic procedure.

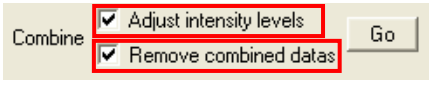


Combine

The "Combine" function allows individual spectral windows in an Extended Range spectrum acquisition to be glued together to yield a single spectrum. This process can also be applied automatically during an Extended Range acquisition (see section 3.5.6, page 49).

Equally, the "Combine" function can be used to create an average spectrum from all open spectra.

Set the options as desired:

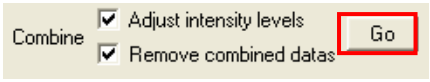


- Adjust intensity levels: if this box is ticked the baselines of the individual spectral windows will be adjusted prior to gluing,

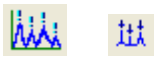
to yield a seamless final spectrum. See section 3.5.6.3, page 53, for full information about this mode.

- Remove combined datas: if this box is ticked the individual spectra or spectral windows will be deleted after combination, leaving only the single combined spectrum on screen.

Click on the adjacent **[Go]** button to apply the "Combine" process.



4.6.7. Peak Searching and Fitting



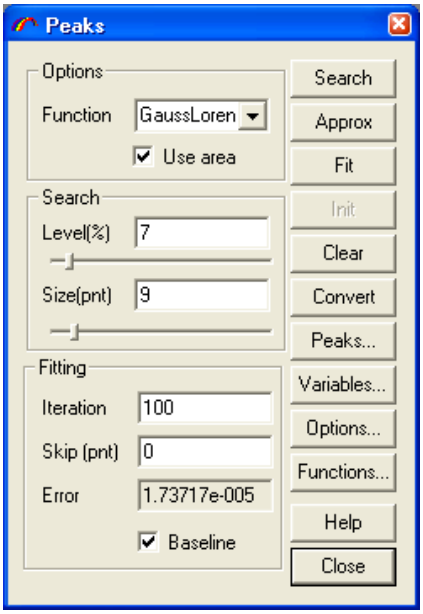
Opens the Peaks dialog window.

The Peak Searching and Fitting module allows peaks in a spectrum to be automatically labelled by their position, and full peak fitting can be carried out to fully characterise peak parameters such as position, amplitude, full width at half maximum height (FWHM) and area. Overlapping peaks can be fully deconvoluted through the peak fitting routine.

The Peak Searching and Fitting functions can be performed on both single spectra, and multidimensional spectral arrays.

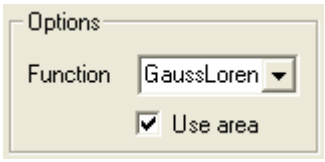
4.6.7.1. Peaks Dialog Window

The Peaks dialog window allows the peak searching, labelling and fitting processes to be configured and applied, and displays the peak parameters after fitting.



Peak Options

Select the appropriate function to be used for peak fitting from the "Function" drop down box. Three default functions are provided:



- Gaussian
- Lorentzian
- Mixed Gaussian-Lorentzian

Other functions can be defined by clicking on **[Functions...]** in the Peaks dialog window – see 4.6.7.5, page 144, for full information.

If "Use area" is ticked the peak fitting routines will also calculate the area of the peak(s). When "Use area" is unticked only the default peak parameters will be calculated. These are peak position, amplitude (i.e., maximum height), full width at half maximum height (FWHM) and (for mixed Gaussian-Lorentzian functions) the degree of Gaussian contribution.

Search Options

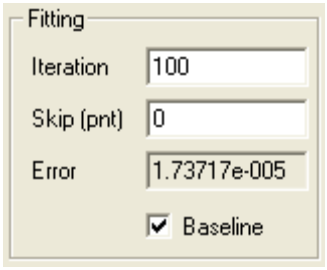
Set the parameters used for automatic peak searching and identification. The search routine locates local intensity maxima, and assigns these as peaks.

A local maximum must be greater than a certain percentage of the maximum intensity in the whole spectrum. The "Level (%)" parameter defines this percentage of maximum spectral intensity. Typically, as the "Level (%)" is increased, only the most intense peaks will be identified. If low intensity peaks need to be identified "Level (%)" should be reduced.

A local maximum is assumed to exist within a finite number of adjacent data points. The "Size (pnt)" parameter defines this number. Typically, as the "Size (pnt)" is increased, only peaks which are widely separated will be identified. If close lying peaks need to be identified "Size (pnt)" should be reduced.

The "Level (%)" and "Size (pnt)" values can be set by typing a value in the appropriate box, or using the appropriate scroll bar. The peak labelling displayed on the active spectrum will update continuously, so that the result can be monitored.

Fitting Options



Set the parameters used for the peak fitting routine. This routine uses a Levenberg-Marquardt non-linear peak fit algorithm, and iteratively adjusts all peak parameters to minimise the standard error.

The maximum number of iterations can be set by typing an appropriate number in the "Iteration" box. Typically the larger the iteration number the more accurate the final fit result will be, but the longer the process will take.

The algorithm can be set to miss out data points within the spectrum, in order to speed up the process. "Skip (pnt)" is used to define how many points are missed.

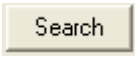
- "Skip (pnt)" = 0, all data points are used for the fitting.
- "Skip (pnt)" = 1, every second data point is used for the fitting routine.
- "Skip (pnt)" = 2, every third data point is used for the fitting routine.

Typically as "Skip (pnt)" is increased the fit results will be less accurate, but the process will be faster.

The "Error" box displays the Standard Error between the fit result and the raw data. The smaller the Standard Error the more accurate the fit result.

If the "Baseline" box is ticked the peak fitting routine will additionally fit the specified baseline. The baseline must be specified first, using the Baseline dialog window – see section 4.6.2.1, page 115.

Search



Click on **[Search]** to start the automatic peak searching and identification routine.

Adjust the "Size (pnt)" and "Level (%)" to control the searching and identification procedure – see above for more information about these parameters.

Approx

Click on **[Approx]** to run the peak approximation routine, which can be used to estimate the initial peak parameters prior to fitting. Only the peak position and width parameters are adjusted.

The peak approximation is a useful function to assist in complex peak fitting procedures. Running the peak approximation routine prior to full fitting ensures that the starting parameters are realistic and close to their true values. This reduces the possibility of the peak fitting routine locating an incorrect solution.

Fit

Click on **[Fit]** to run the peak fitting routine, which can be used to calculate peak position, amplitude, full width at half maximum height (FWHM), Gaussian contribution and area.

The peak fitting routine can only be used if peaks have been located in the spectrum. Peaks can be located automatically using the **[Search]** button, or manually by using the "Add peak" icon in the Graphical Manipulation toolbar (see section 5.10, page 172).

Init

Click on **[Init]** to restore peak parameters in the SpIm window of a multidimensional spectral array to the initial values before the peak approximation **[Approx]** or fitting **[Fit]** routines were run.

Clear

Click on **[Clear]** to clear all peaks from the active spectrum.

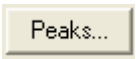
Convert

Click on **[Convert]** to convert the active spectrum to the sum of the displayed peaks. This function is useful to save a theoretical peak fit solution in a standard spectrum file format.

Note that the active spectrum will be overwritten by the sum of the displayed peaks. Make sure that the file is saved with a different name to ensure the original spectrum data is not permanently overwritten and lost.

Peaks...

Click on **[Peaks...]** to open the Peak Parameters dialog window, to view and manually set peak position, amplitude, full width at half maximum height (FWHM), Gaussian contribution and area values for all peaks labelled on a spectrum.



See section 4.6.7.2, page 138, for more information.

Variables...

Click on **[Variables...]** to open the Peak Variables dialog window, to view and set initial values and maximum/minimum values for variables within the peak fitting routine.

See section 4.6.7.3, page 140 for more information.

Options...

Click on **[Options...]** to open the Peak Options dialog window, to view and set display options for the peak labelling and fitting.

See section 4.6.7.4, page 143, for more information.

Functions...

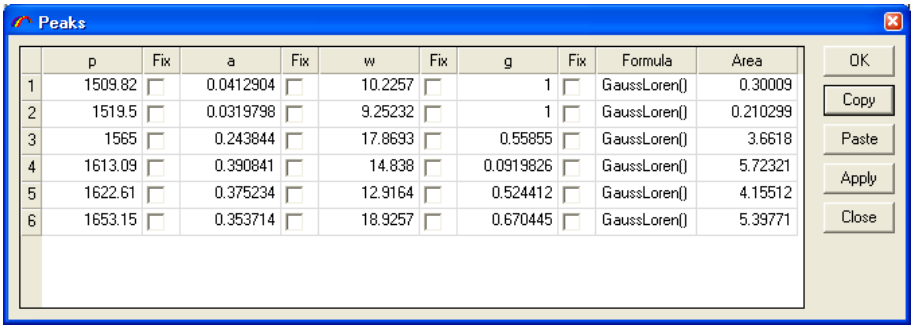
Click on **[Functions...]** to open the Peak Functions dialog window, to view and create user defined peak shape functions.

See page 4.6.7.5, page 144, for more information.

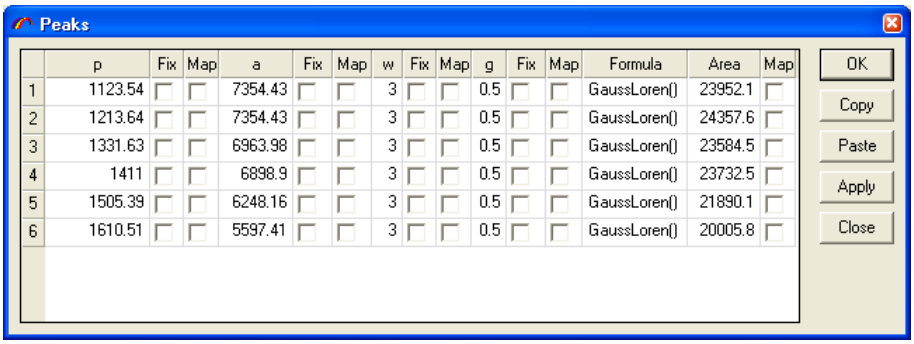
4.6.7.2. Peak Parameters Dialog Window

The Peak Parameters dialog window displays peak position, amplitude, full width at half maximum height (FWHM), Gaussian contribution and area for all peaks labelled on a spectrum. The parameters can be manually adjusted and fixed so that the parameter is not varied during the fitting routine.

The Peak Parameters dialog window for individual spectra (e.g., Spectrum window, or Point window of a multidimensional spectral array) has the following appearance:



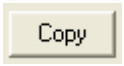
The Peak Parameters dialog window for multidimensional spectral arrays (e.g., the SpIm window of a multidimensional spectral array) has the following appearance:



Each row displays the parameters for a single peak:

- **p** – peak position, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).
- **a** – peak amplitude, in units as displayed on the spectrum's Y axis, typically counts (cnt), or counts per second (cnt/s).
- **w** – peak full width at half maximum height (FWHM) in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).
- **g** – Gaussian contribution in a mixed Gaussian-Lorentzian function. The value of Gaussian contribution varies from 0 (no Gaussian contribution, fully Lorentzian) through to 1 (fully Gaussian). The **g** column is only displayed when a mixed Gaussian-Lorentzian function is selected for peak fitting in the main Peaks dialog window (see section 4.6.7.1, page 134).
- **Formula** – the function used for the peak fitting, as selected in the main Peaks dialog window (see section 4.6.7.1, page 134). "Gauss()" = Gaussian, "Loren()" = Lorentzian, "GaussLoren()" = mixed Gaussian-Lorentzian.
- **Area** – the area of the peak, in area units based on the units displayed on the spectrum's X and Y axes. The **Area** column is only displayed if the "Use area" box is ticked in the main Peaks dialog window (see section 4.6.7.1, page 134).
- **Fix** - the "Fix" tick boxes to the right of each of the **p**, **a**, **w** and **g** parameters allows a parameter to be fixed. A fixed parameter will not be varied during the peak fitting routine. When a box is ticked the parameter is fixed. When a box is unticked the parameter will be varied during the fitting routine.
- **Map** – the "Map" tick boxes (which are only displayed for the SpIm window of a multidimensional spectral array) to the right of each of the **p**, **a**, **w**, **g** and **Area** parameters allows a profile or map image to be generated based on the parameter. For example, it is possible to create an image based on peak position, illustrating how the peak position varies across the map area. To display a profile/image based on a peak parameter tick the appropriate box and click **[Apply]**. A new map profile/image will be created. To close the map profile/image, untick the box and click **[Apply]**.

Copy



Click on **[Copy]** to copy the parameters displayed in the Peak Parameters dialog window. Parameters which have been copied can be pasted into other programmes (such as Microsoft Office) or into the Peak Parameters dialog window.

This function can be used to copy peak parameters from one spectrum and paste them to another spectrum (using **[Paste]**).

Paste

Click on **[Paste]** to paste parameters in the Peak Parameters dialog window.

This function can be used to paste peak parameters copied (using **[Copy]**) from one spectrum to another.

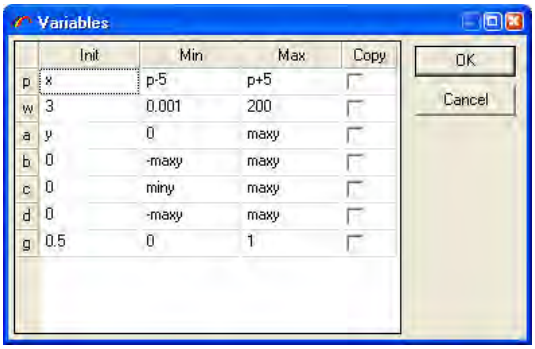
Apply

Click on **[Apply]** to update the peak(s) displayed on the spectrum according to parameters in the Peak Parameters dialog window.

This button must be used when parameters are manually adjusted in the Peak Parameters dialog window. The peaks displayed on the spectrum will not reflect the new parameters until **[Apply]** is clicked.

4.6.7.3. Peak Variables Dialog Window

The Variables dialog window displays the initial parameters used in the peak fitting procedure, and the minimum and maximum values they can take during the fitting procedure. In most cases the default values are suitable for general peak fitting routines, but in specific cases the initial parameters and their minimum and maximum values can be manually adjusted as required.



The Variables dialog shows the initial ("init") values, and minimum ("Min") / maximum ("Max") values for each parameter:

- **p** – peak position, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm). In the example shown above, the initial value of **p** is the X axis position ("x") at which the peak is initially located or positioned, and the position can be varied from **p**-5 to **p**+5 during the fitting procedure.
- **w** – peak full width at half maximum height, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm). In the example shown above, the initial value of **w** is 3, and width can be varied from 0.001 to 200 during the fitting procedure.
- **a** – peak amplitude, in units as displayed on the spectrum's Y axis, typically counts (cnt), or counts per second (cnt/s). In the example shown above, the initial value of **a** is the Y axis position ("y") at which the peak is initially located or positioned, and the amplitude can be varied from 0 to the maximum Y axis value ("maxy") during the fitting procedure.

- **b**, **c**, **d** – parameters within the Gaussian/Lorentzian equations. In the example shown above, the initial values of **b**, **c** and **d** are 0, and they can be varied from either the inverse of the maximum Y axis value ("-maxy") or the minimum Y axis value ("miny"), to the maximum Y axis value ("maxy") during the fitting procedure.
- **g** – Gaussian contribution in a mixed Gaussian-Lorentzian function. In the example shown above, the initial value of **g** is 0.5, and the Gaussian contribution can be varied from 0 (no Gaussian contribution, fully Lorentzian) through to 1 (fully Gaussian) during the fitting procedure.

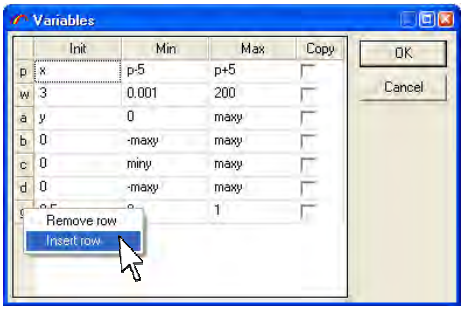
If "Copy" is ticked for a parameter the "min" and "max" values will be displayed individually for each peak in the Peak Parameters dialog window. This allows "min" and "max" parameters to be set individually for each peak. Note that the "Copy" box must be ticked before the peaks are automatically located using **[Search]** or manually located using the "Add peak" icon in the Graphical Manipulation toolbar (see section 5.10, page 172).

4.6.7.3.1. Setting the Init, Min and Max Values in the Variables Dialog Window

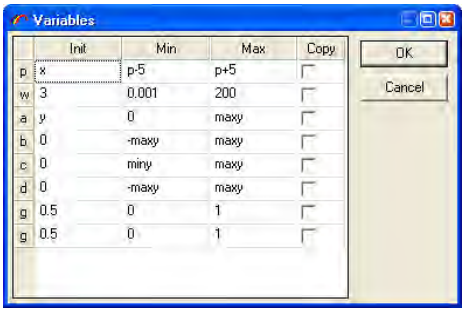
Left click in the desired parameter "init", "min" or "max" box and insert the desired value. Add information for each parameter as required.

4.6.7.3.2. Adding Parameters to the Variables Dialog Window

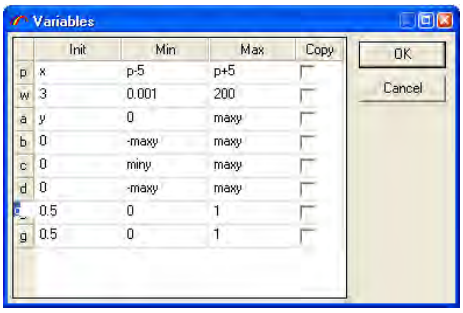
Additional categories can be added by right clicking on one of the parameter name boxes and selecting "Insert row".



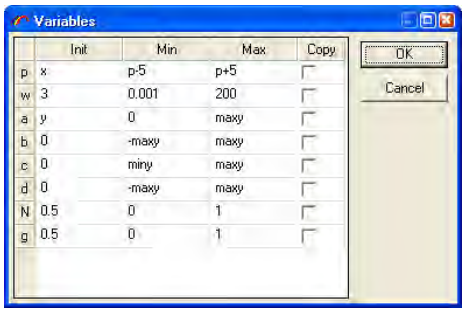
A new row will be inserted above the selected position, and will display the same name and "init", "min" and "max" values as the original parameter.



Double click on the parameter name box to allow the name to be edited. Type in the desired category name.



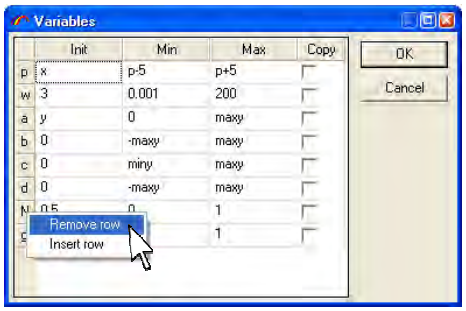
Click on any other parameter name box to register the new name.



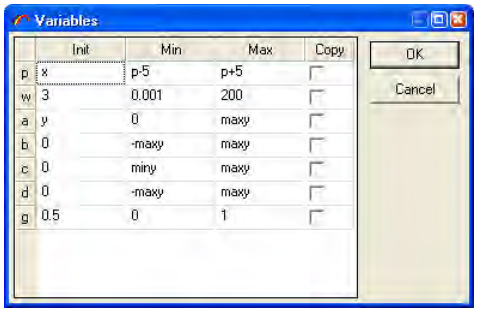
The name given to the new parameter must exactly match the name used in definition of functions in the Peak Functions dalog window (see section 4.6.7.5, page 144). The “init”, “min” and “max” values must be set to appropriate values.

4.6.7.3.3. Deleting Parameters from the Variables Dialog Window

Parameters can be deleted by right clicking on the parameter name boxes which is to be deleted and selecting “Remove row”.

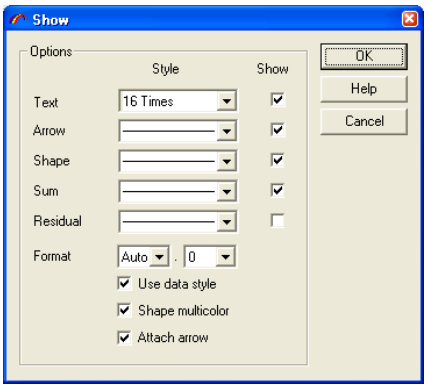


The parameter will be deleted from the Variables dialog window.



4.6.7.4. Peak Options Dialog Window

The Peak Options dialog window allows control of the display options for the peak labelling and fitting.



The following peak labelling and fitting display components can be controlled:

Text

Click on the Text "Style" drop down box to set the font, font style, font size and font color used to display the peak position on the spectrum. If "Show" is ticked the peak position will be displayed on the spectrum. Note that if "Use data style" is ticked the colour will be set to match the spectrum color. If "Use data style" is unticked the colour will be set according to the selection made in the "Style" drop down box.

Arrow

Click on the Arrow "Style" drop down box to set the colour, width and line style for the arrow marker indicating the peak position. If "Show" is ticked the arrow marker will be displayed on the spectrum. Note that if "Use data style" is ticked the colour will be set to match the spectrum color. If "Use data style" is unticked the colour will be set according to the selection made in the "Style" drop down box.

Shape

Click on the Shape "Style" drop down box to set the colour, width and line style for the individual peak shape(s) displayed on the spectrum. If "Show" is ticked the peak shape(s) will be displayed on the spectrum. Note that if "Shape multicolor" is ticked the colour will be automatically selected from a default palette; in this case, when multiple shapes are displayed on a single spectrum each shape will be a different color. If "Shape multicolor" is unticked the colour will be set according to the selection made in the "Style" drop down box; in this case, when multiple shapes are displayed on a single spectrum each shape will be the same colour.

Sum

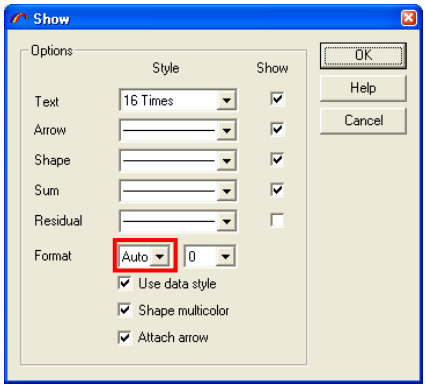
Click on the Sum "Style" drop down box to set the colour, width and line style for the sum spectrum (i.e., the combination spectrum created by summing all the peak shapes displayed). If "Show" is ticked the sum spectrum will be displayed on the spectrum.

Residual

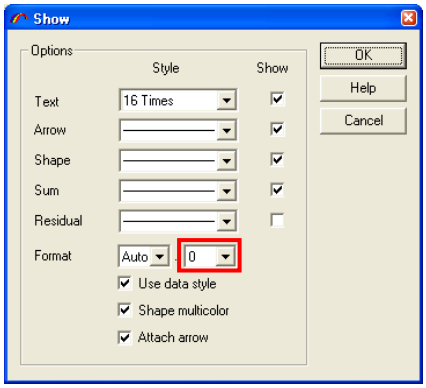
Click on the Residual "Style" drop down box to set the colour, width and line style for the residual spectrum (i.e., the difference between the sum spectrum and the raw data). If "Show" is ticked the residual spectrum will be displayed on the spectrum.

Format

Click on the Format left hand "Style" drop down box to set the number of display characters for the peak position value.



Click on the Format right hand "Style" drop down box to set the number of decimal places to be displayed for the peak position value.



Use data style

When "Use data style" is ticked the display color of the peak label text and arrow marker will be set to match the spectrum color. If "Use data style" is unticked the color will be set according to the selection made in the respective "Style" drop down box.

Shape multicolor

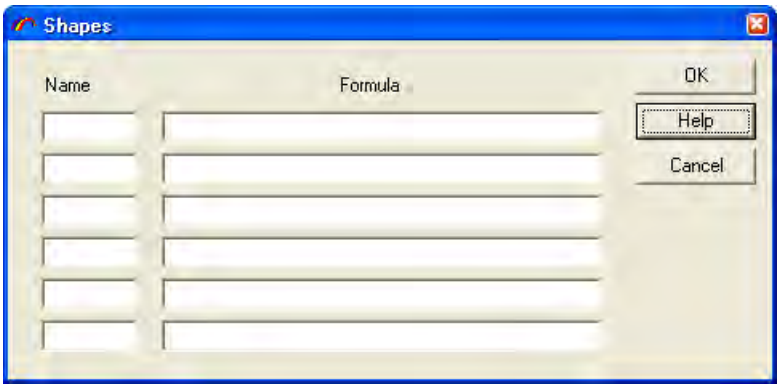
If "Shape multicolor" is ticked the individual peak shape display colour will be automatically selected from a default palette; in this case, when multiple shapes are displayed on a single spectrum each shape will be a different color. If "Shape multicolor" is unticked the colour will be set according to the selection made in the "Style" drop down box; in this case, when multiple shapes are displayed on a single spectrum each shape will be the same colour.

Attach arrow

If "Attach arrow" is ticked the peak arrow marker will be positioned immediately above the peak. If "Attach arrow" is unticked the peak arrow marker will be positioned at the top of the spectrum window.

4.6.7.5. Peak Functions Dialog Window

The Peak Functions dialog window allows custom peak fitting formulae to be defined, so that shapes other than the default Gaussian, Lorentzian and mixed Gaussian-Lorentzian can be used. For example, with the Peak Functions dialog window peak shapes such as Voigt or asymmetric Gaussian can be used.



To define a custom peak shape formula type the shape name in a "Name" text box, and input the formula in the "Formula" text box. The name will be displayed in the main "Functions" drop down box in the main Peaks dialog window.

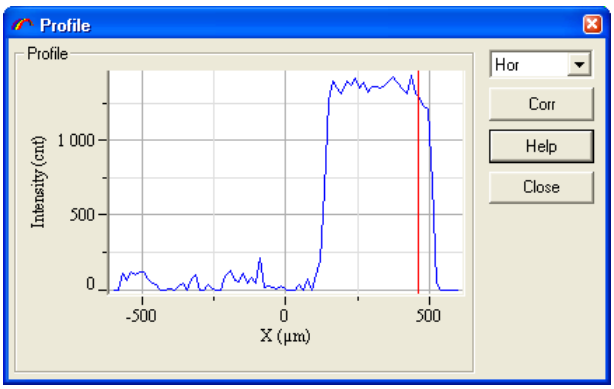
Any parameter can be used, but it must also be defined as a parameter in the Peak Variables dialog window (see section 4.6.7.3, page 140). Note that certain parameters are predefined:

- **p** – peak position, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).
- **a** – peak amplitude, in units as displayed on the spectrum's Y axis, typically counts (cnt), or counts per second (cnt/s).
- **w** – peak full width at half maximum height (FWHM) in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).

4.6.8. Profile

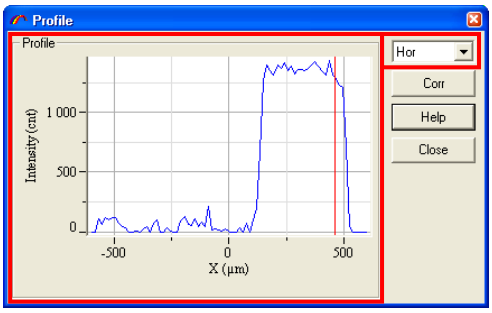
Opens the Profile dialog window.

The Profile dialog window displays an intensity profile across an image (such as optical image, or two dimensional Raman mapped image).



Profile

The "Profile" window displays the image intensity profile at the cursor position. The scales for the X and Y axes are taken directly from the image itself. The profile is created in either a horizontal (X axis) or vertical (Y axis) direction in the image, depending on the selection made in the drop down box:



- Hor – horizontal (X axis)
- Ver – vertical (Y axis)

Corr

Click on **[Corr]** to modify the image based on manipulation made to the profile. For example, if the profile displayed in the Profile dialog window is smoothed, clicking on **[Corr]** will apply the same smoothing function to the entire image in the horizontal or vertical dimensions (according to the selection of "Hor" or "Ver" in the drop down box).

4.6.8.1. Displaying an Intensity Profile from an Image

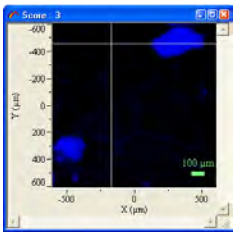
To display an intensity profile from an image (such as optical image, or two dimensional Raman mapped image) open the image file.

In the case of a two dimensional Raman mapped image click on the image window (either "Map" or "Score"). Select the component from which the profile is to be created, using the tags in the right hand Data bar (see section 6.3, page 198).

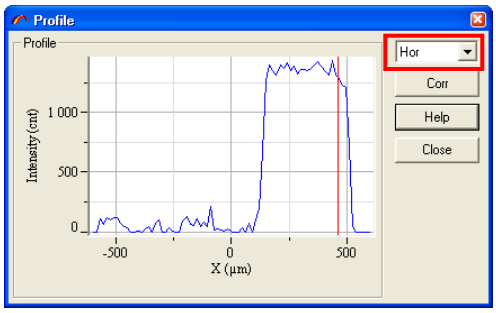
Ensure the cursor mode in the image window is set to "Cross" (right click and select "Cursor", and then choose "Cross" from the Style drop down box).

Open the Profile dialog window by clicking on the Profile icon in the Icon bar.

Position the cursor at the point of the image from where the profile is to be created.



Select “Hor” or “Ver” from the drop down box in the Profile dialog window to create an intensity profile in the horizontal (X axis) direction or vertical (Y axis) direction respectively.



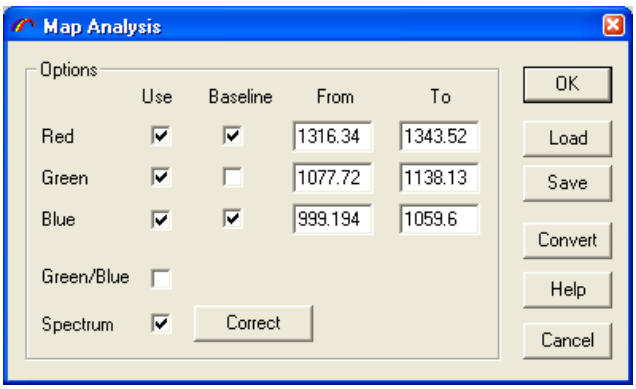
The profile is displayed in the Profile dialog window. Standard data processing functions (such as smoothing, peak fitting or baselining) and copy/paste functions can be used with this profile.

Click **[Close]** to close the Profile dialog window.

4.6.9. Map Analysis

Opens the Map Analysis dialog window.

The Map Analysis dialog window displays the positions and settings for the “Red”, “Green” and “Blue” cursors which are used to create intensity profiles and images from multidimensional spectral arrays (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes). The positions and settings can also be manually configured in this dialog window.



Use

If the “Use” box is ticked for a set of cursors (“Red”, “Green” or “Blue”) then a profile or image will be generated displaying the average intensity between the two cursors. The cursor positions are set by clicking the “Red”, “Green” or “Blue” cursor icons in the left hand Graphical Manipulation bar (see section 5.2, page 165) and dragging the cursors to the desired positions on either the “SpIm” or “Point” windows, or by typing in desired values into the “From” and “To” boxes in the Map Analysis dialog window.

Note that if “Use” is unticked for all three cursors and “Green/Blue” is also unticked, the “Map” window will not be displayed. To redisplay the “Map” window make sure that at least one of the “Use” boxes are ticked, or that the “Green/Blue” box is ticked.

Baseline
If the "Baseline" box is ticked for a set of cursors ("Red", "Green" or "Blue") then the cursor region is first baselined before calculation of the average intensity between the cursors. This mode is useful to ensure that the image created truly reflects peak intensity and not general background intensity (perhaps from fluorescence or photoluminescence).

From and ***To***
Displays the beginning ("From") and end ("To") spectral positions for a set of cursors ("Red", "Green" or "Blue"). These can be manually adjusted by typing in desired values and clicking **[OK]**.

Green/Blue
If the "Green/Blue" box is ticked an additional profile/image is displayed, showing the ratio of average intensities of the "Green" and "Blue" cursors (i.e., [$Intensity_{GREEN}$] / [$Intensity_{BLUE}$]). This display is useful to visualize a change in peak ratios within a multidimensional spectral array.

To remove the "Green/Blue" intensity profile/image untick the box.

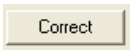
Spectrum
If the "Spectrum" box is ticked the "Point" window is displayed, showing the spectrum at the current cursor position.

Note that if the "Spectrum" box is unticked, the "Point" window will not be displayed. To redisplay the "Point" window tick the "Spectrum" box.

In the event that the "Point" window is accidentally deleted within the main LabSpec 5 graphical user interface (GUI), use the following procedure to re-display the window:

- open the Map Analysis dialog window
- untick the "Spectrum" box
- click **[OK]**
- re-open the Map Analysis dialog window
- tick the "Spectrum" box
- click **[OK]**
- the Point window is now displayed in the main LabSpec 5 graphical user interface (GUI)

Correct
Click on **[Correct]** to update the multidimensional spectral array with the "Point" spectrum after it has been processed/modified in some way (for example, smoothing or baselining).



The "Point" window only displays data held within the multidimensional spectral array, so if the spectrum in the "Point" window is modified it is necessary to update the actual data in the array by clicking on **[Correct]**. If this is not done, when the cursor on the profile/image is moved the modifications will be lost – the next time the spectrum is displayed in the "Point" window it will return to its original form.