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TM012 - Data processing and simple analysis WiRE™ 5

This document aims to show the WiRE™ 5 user how to process single spectra and perform basic analysis. The following methods are discussed:

- Baseline subtraction (processing)
- Arithmetic functions on data (processing)
- Smoothing (processing)
- Zapping (processing)
- Peak Pick (analysis)
- Curve-fitting (analysis)
- Integration (analysis)
- Direct classical least squares (DCLS) component analysis (analysis)

Baseline subtraction

Samples may exhibit Raman spectra with varying degrees of fluorescence or thermal background. Providing that there is sufficient Raman signal 'on top' of the sloping background, the baseline may be subtracted to yield a spectrum with a 'flat' baseline. In some cases, the measurement can be re-performed with an alternative excitation wavelength to more effectively remove the effects of fluorescence.

The following methods are available:

- **Intelligent fitting**
  – default intelligent automated option

- **Through fixed points**
  – user controls point positions (XY) and baseline type

- **Through chosen points on each spectrum**
  – user controls X point which the baseline travels through for each spectrum within the dataset

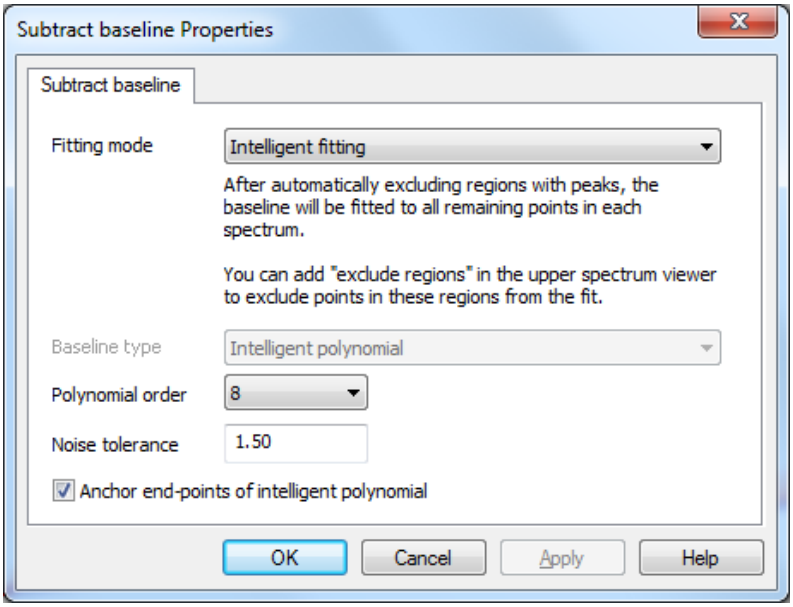
- **Through whole spectrum**
  – Automatic fitting with no in-built intelligence

With the spectrum open in a Viewer, select **Processing…Subtract Baseline**.

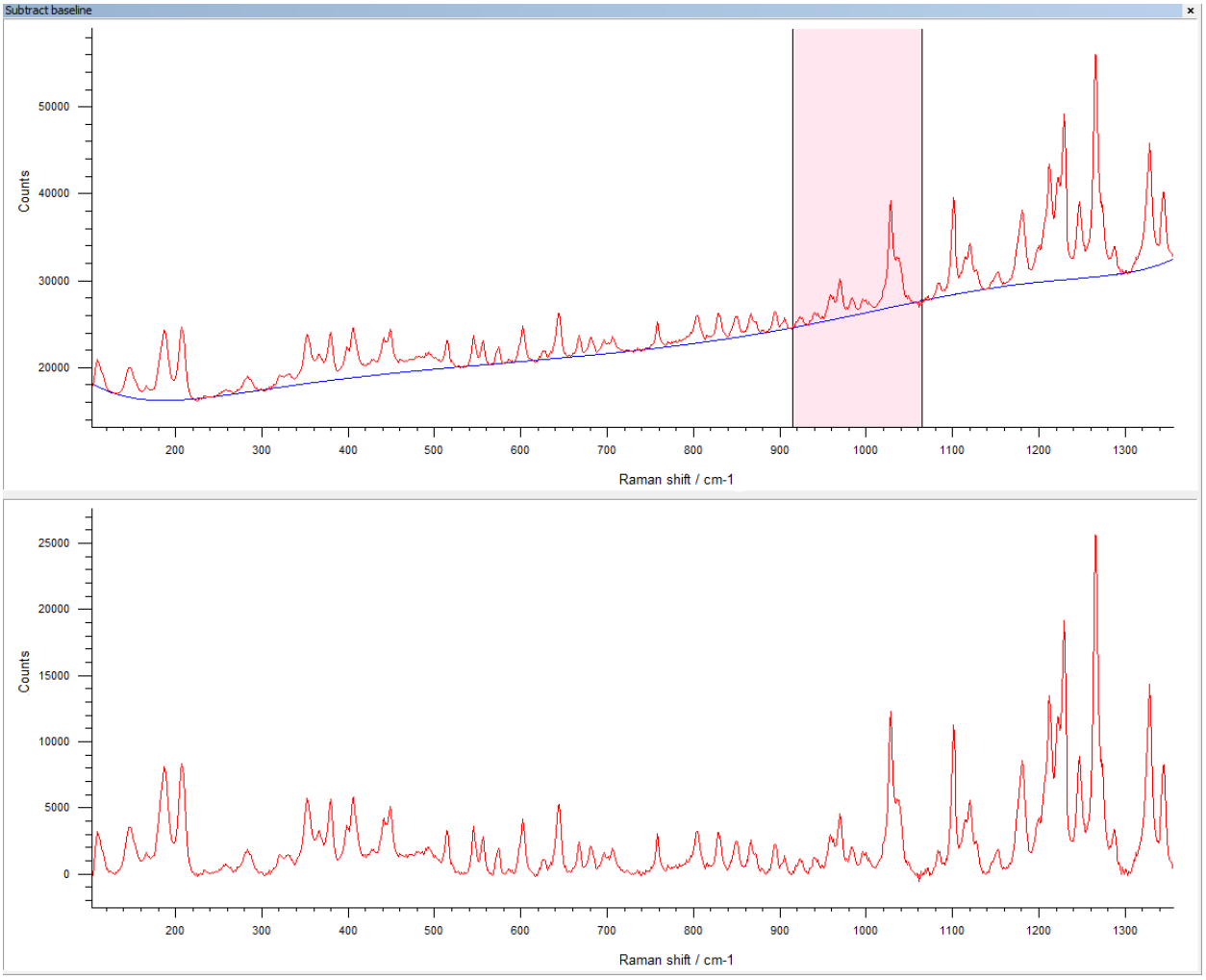
<u>Intelligent fitting</u>

A new Viewer opens with the spectrum in the top half and the result of the automatically applied baseline subtraction in the lower half. By default the 'Intelligent fitting' baseline is applied with a polynomial value of 11. This method is Renishaw patented and enables simple or complex backgrounds to be removed automatically.

Using a right click and selecting properties brings up the property page:



Here the polynomial order can be adjusted if a better fit is needed. The context menu also enables exclude regions to be added to the spectrum. Excluded regions do not contribute to the fitting of the baseline.



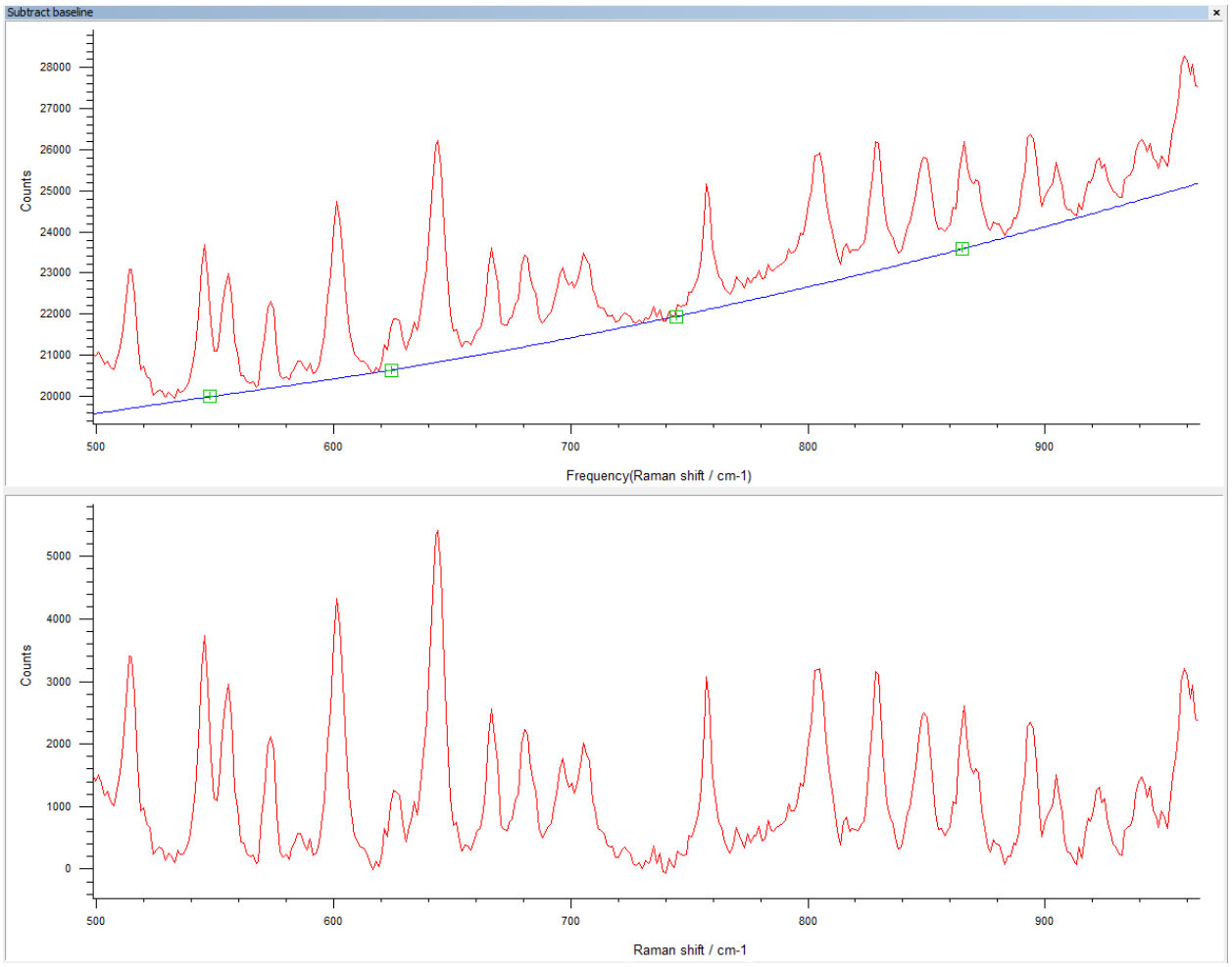
Intelligent fitting can be applied to single spectra and multifiles (e.g. mapping dataset). The baseline will automatically ‘fit’ each spectrum within the multifile.

Through fixed points

Selecting ‘Through fixed points’ as the fitting mode enables the user to manually specify points in XY to determine the baseline shape.

The user can choose between ‘polynomial’ (and the order) and ‘cubic spline’ options. Cubic spline is only available if 2 points are added (4 total points). This method can be applied to single spectra or multifiles, but the baseline is fixed and will not ‘fit’ to different spectra within multifiles.

It is useful to zoom in, by left-clicking and dragging in either window, and adjusting the points added in the top window by moving them with the mouse.

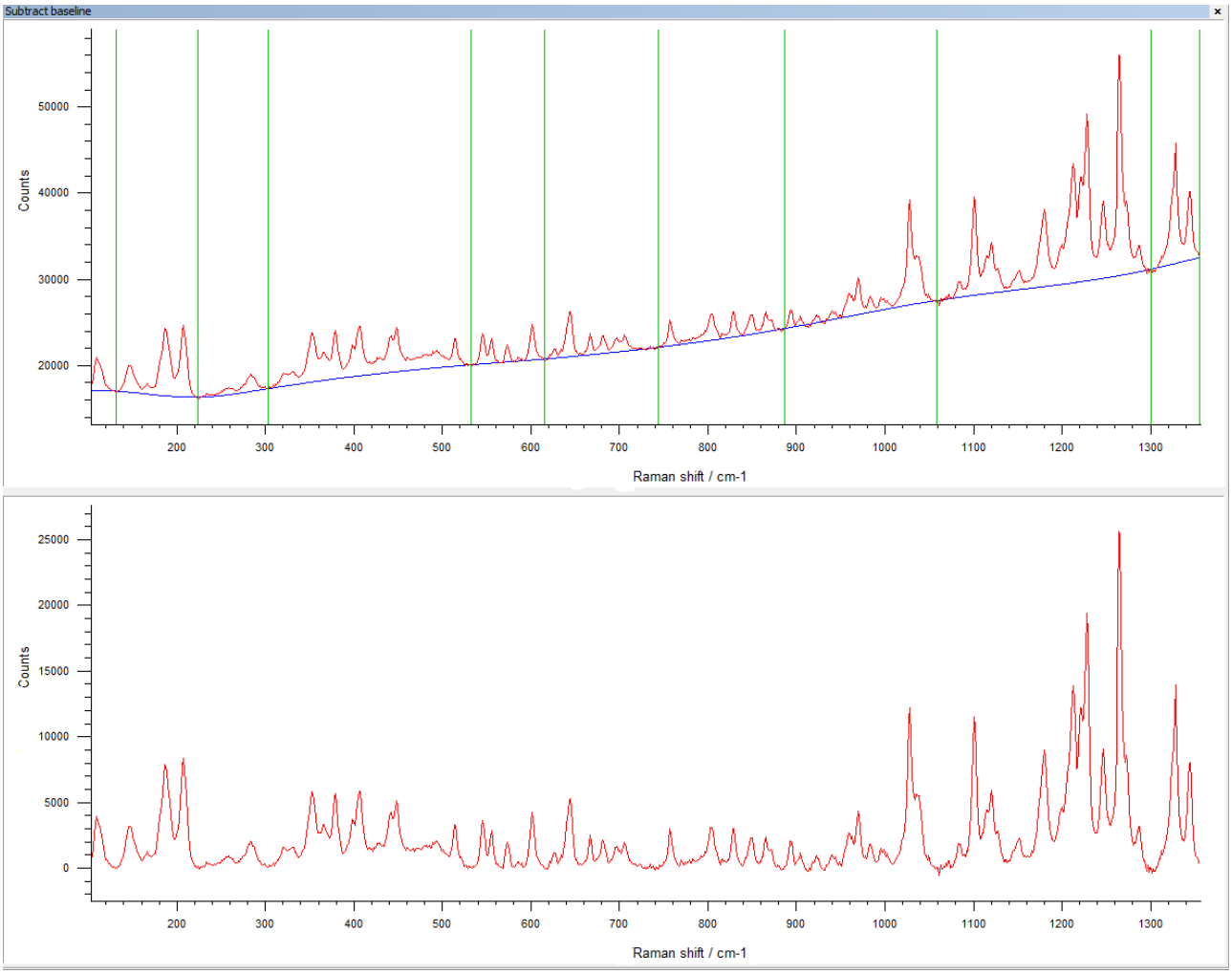


Through chosen points on each spectrum

Selecting ‘Through chosen points on each spectrum’ as the fitting mode enables the user to manually add points (vertical lines) to the spectrum which are fixed to the data.

The user can choose between ‘polynomial’ (and the order) and ‘cubic spline’ options. Cubic spline is only available if 2 points are added (4 total points).

This method can be applied to single spectra or multifiles. When applying to a multifile, common X positions where no Raman bands are present should be found. The baseline will optimise based on the X position for each spectrum within the dataset.

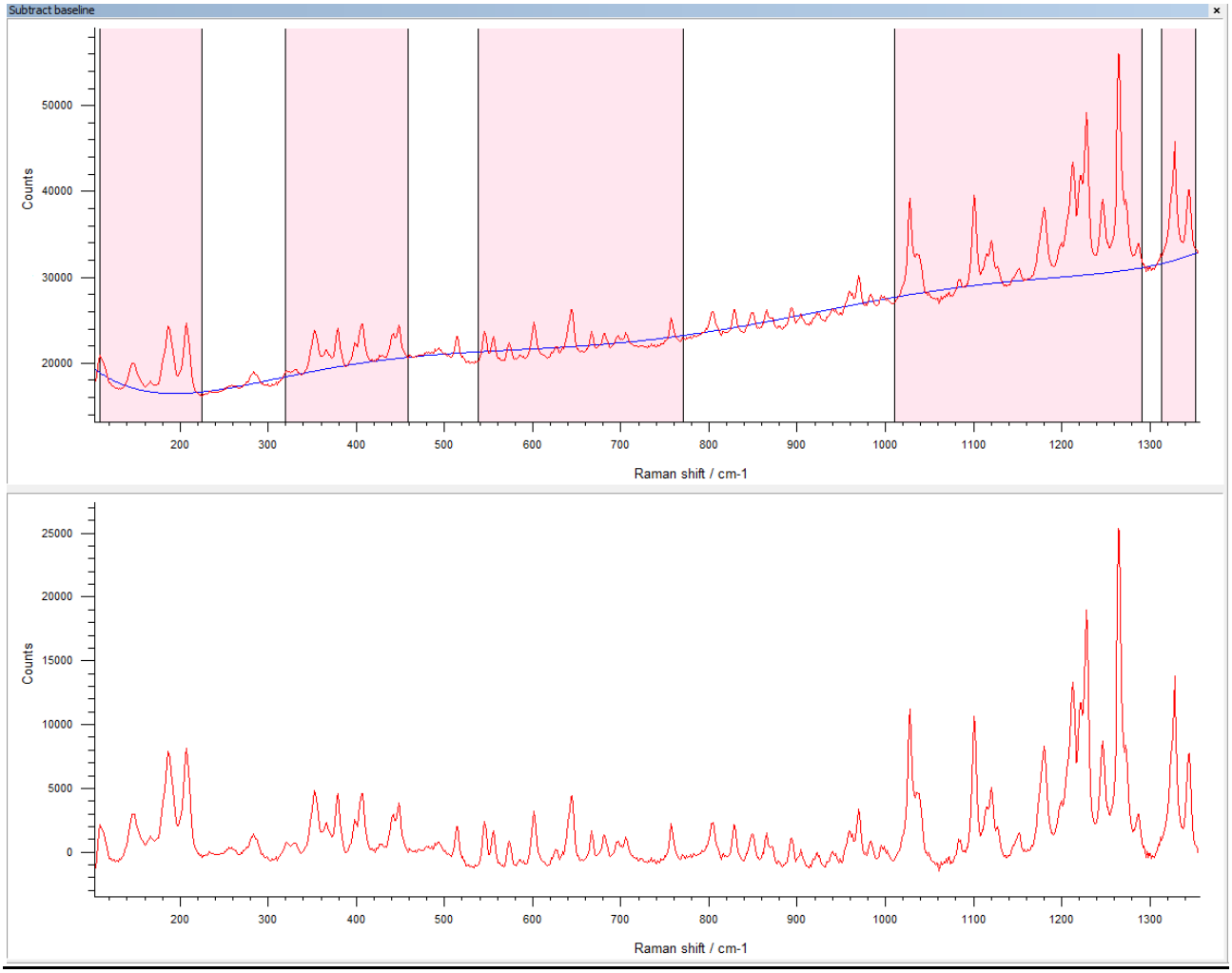


Through whole spectrum

Selecting ‘Through whole spectrum’ automatically fits a defined polynomial order through the entire spectrum.

The context menu enables exclude regions to be added to the spectrum. Excluded regions do not contribute to the fitting of the baseline.

This method can be applied to single spectra or multifiles, and is a less intelligent equivalent to the recommended intelligent fitting option.



Accepting a correction

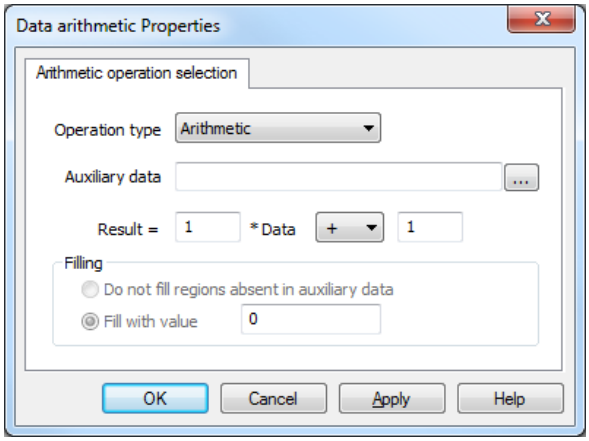
When you are satisfied with the correction, either select **Accept** from the context menu or close the window, upon which, there will be a prompt asking if you want to keep the correction.

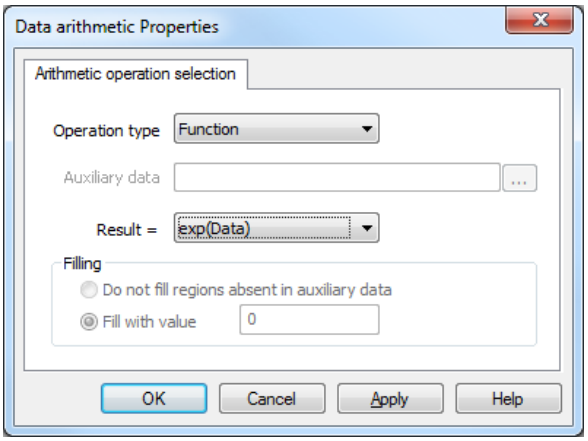
To save the change to your file use the **File…Save** or **Save as** option from WiRE.

Arithmetic functions on data

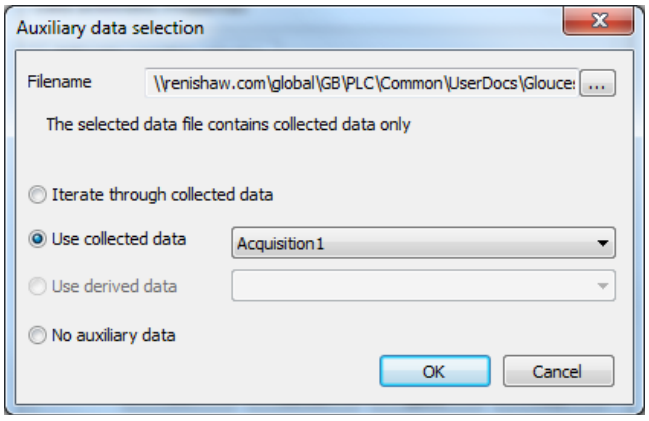
A variety of mathematical operations can be performed on single data files. For example, you can multiply a spectrum by a user defined factor, add files together or subtract one file from another. It can be an effective method of subtracting a background spectrum or filter ripple profile.

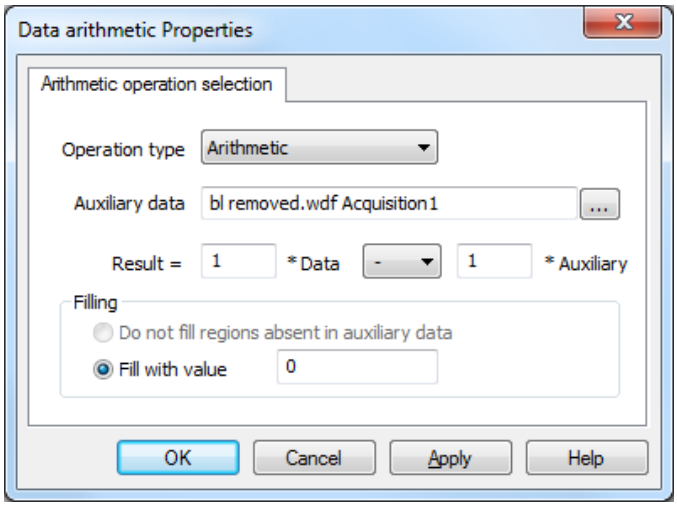
With a file open, select **Processing…Data Arithmetic**. A new viewer will open, initially split into two separate areas showing the sample spectrum in the top half and a blank spectrum in the bottom half. The 'Data arithmetic Properties' dialogue box allows the user to perform either an 'Arithmetic' or a 'Function' operation on their sample spectrum. The default option is to perform an arithmetic operation using 'No auxiliary data'. Performing such an operation and clicking **Apply** will display the resulting spectrum in the bottom half of the data arithmetic viewer. Choosing 'Function' from the drop down list in the 'Operation type' field allows the user to transform their sample spectrum using certain functions.



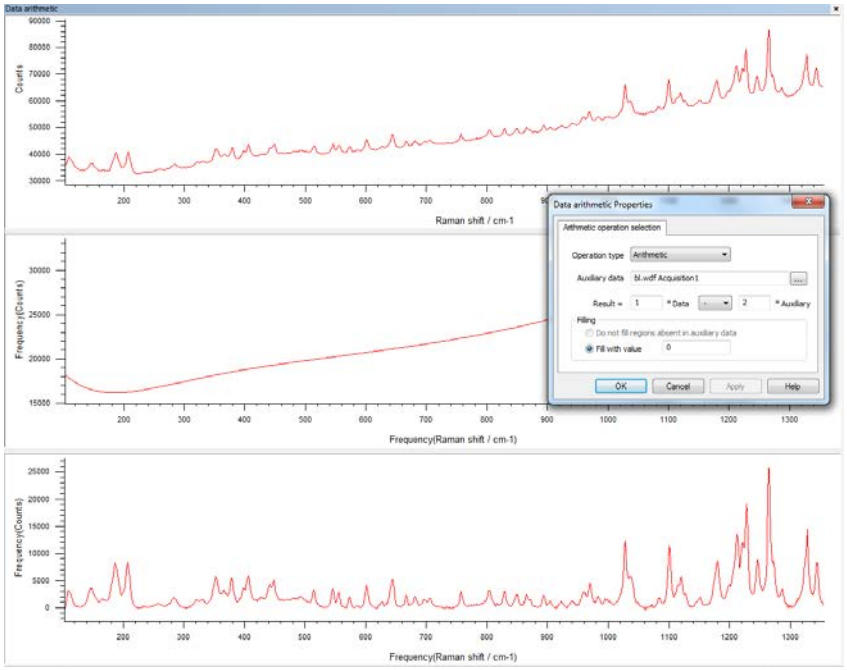


The 'Auxiliary data' field is available only when performing an arithmetic operation. Clicking the  button next to this field opens the 'Auxiliary data selection' dialogue box, which allows the user to navigate to the desired auxiliary data, i.e. the data file they would like to add/subtract/multiply/etc. their sample spectrum by. Selecting the 'Use collected data' option and clicking **OK** sets the desired arithmetic operation. Clicking **Apply** will split the data arithmetic viewer window into three sections. The upper and lower sections will continue to display the sample spectrum and resulting spectrum, respectively, and the additional middle section will display the auxiliary data.





It can be useful to multiply either the sample file or the auxiliary file by a factor so that the Y axes are comparable. In the example below, 1* the sample file and 2* the background correction file have been used to remove the baseline. **Accept** the change either from the context menu or by closing the window.



The options available within the auxiliary data selection dialogue box will differ depending upon the type of data selected as the sample spectrum and the auxiliary data:

- Where the sample spectrum is a single spectrum and the auxiliary data is a map file, the user will be forced to choose a single acquisition from the auxiliary map file to apply to the sample spectrum.

- Where the sample data is a map file and the auxiliary data is a single spectrum, the data arithmetic operation will apply the single auxiliary spectrum to optional acquisitions within the sample map file. Note that upon accepting the data arithmetic operation, the user will have the option to accept for 'All datasets' or the 'Current dataset' only.

- Selecting two maps of different sizes (i.e. each map contains a different number of acquisitions) as the sample data and the auxiliary data will force the user to choose a single acquisition from the auxiliary data map file to be applied to every acquisition within the sample map file. Note this is the case regardless of which of the two map files is larger.

- Selecting equally sized map files as the sample and auxiliary data will allow the user to choose a single acquisition from the auxiliary data to apply to every acquisition within the sample map file. Alternatively, the user can choose to 'Iterate through collected data', in which case each acquisition in the auxiliary map file will be applied to each corresponding acquisition in the sample map file, i.e. auxiliary acquisition no. 1 will be applied to sample acquisition no. 1, auxiliary acquisition no. 2 to sample acquisition no. 2, no. 3 to no. 3, etc.

Image arithmetic

A special case of arithmetic is performing operations on Raman image data, i.e. images created from mapping measurements.

Ratio images can be generated from the 'Map generation' option (see TM014). More complex image arithmetic is performed using the data arithmetic option.

The initial image is loaded into the viewer (data tab of the **Navigator...derived data…..right click….. load dataset**). Under **Processing…Data arithmetic**, select auxiliary data (image) by browsing for the mapping measurement wdf file, then selecting the image from the drop down in 'Use derived data'. Use the value boxes to adjust the Data and Auxiliary scaling.

The format (image or surface) and LUTs of each image (initial, auxiliary and result) can be adjusted from the context menu (**View…View mode** and**…LUT control**). Accept or reject the result image.

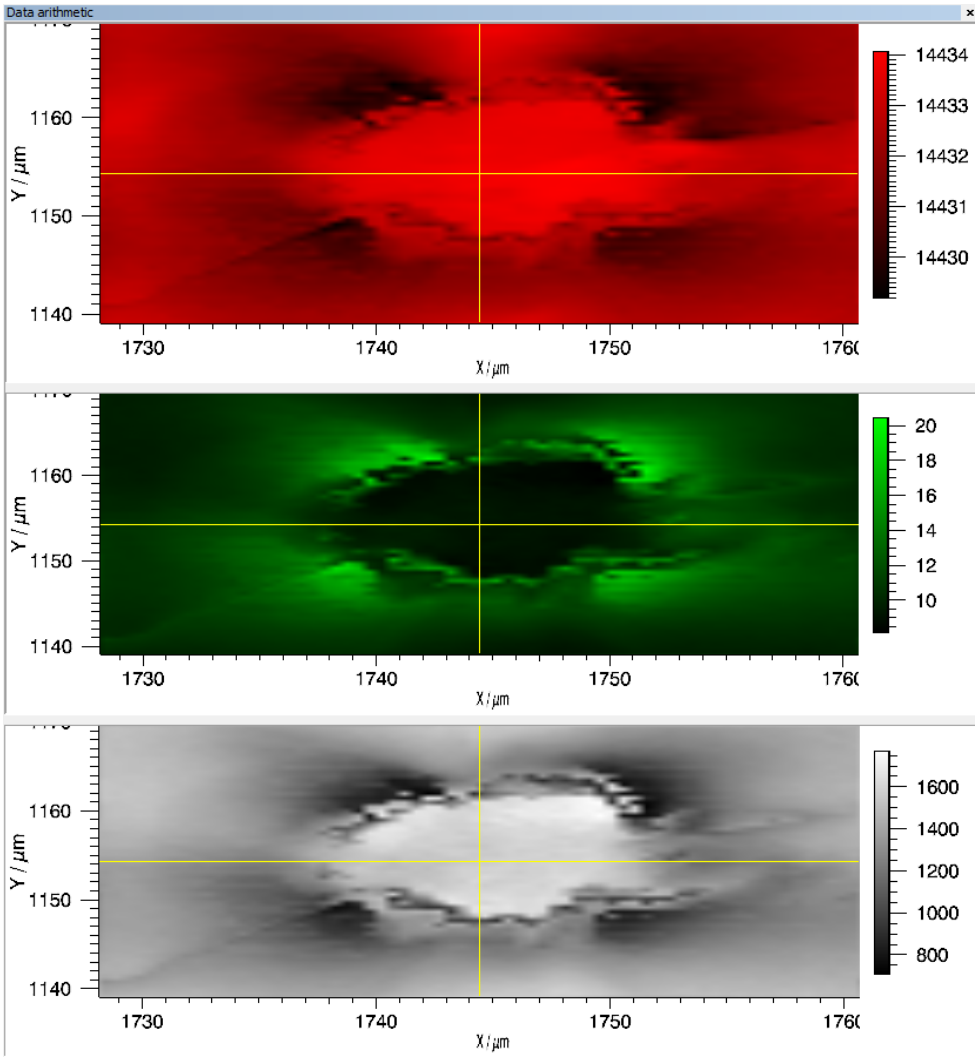


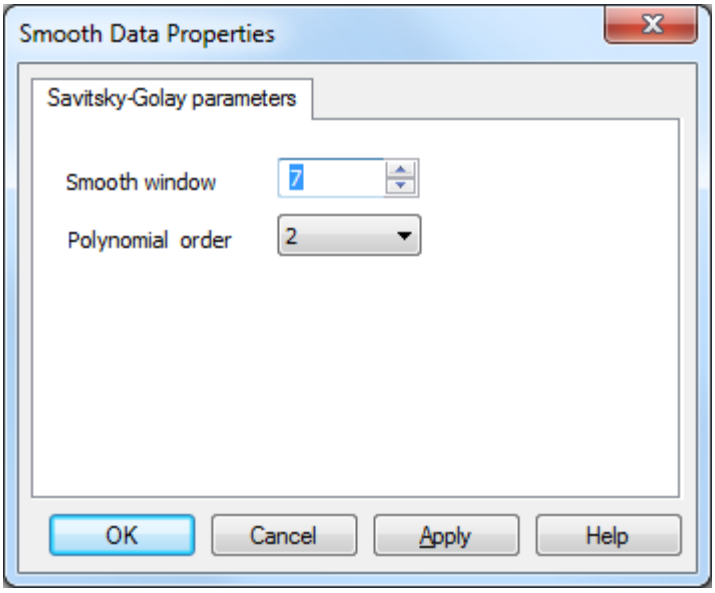
Image 1

Auxiliary image

Result image (Image 1 divided by auxiliary)

Smoothing

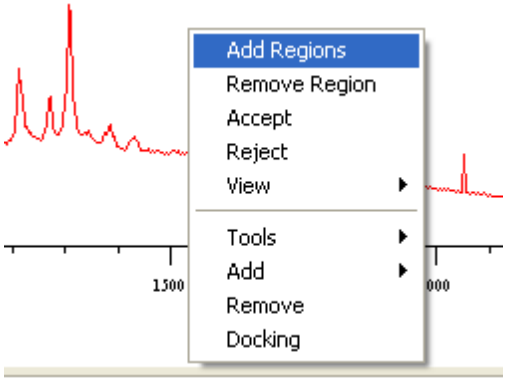
It can be useful to smooth data. This operation has the effect of improving the signal to noise ratio but must be used with caution as it degrades the spectral resolution. Smoothing is no substitute for performing a better measurement, i.e. using longer acquisition times or more accumulations. When using SynchroScan, the binning function can be used (again, with caution) to gain a better signal to noise ratio. To perform smoothing, with the file you wish to smooth open, select **Processing…Smooth**. A new window will open with the sample spectrum at the top and the result spectrum below. This data will be smoothed. To increase or change the degree of smoothing, select **Properties** from the context menu to see the **Smooth Properties** window.



The application uses a Savitsky-Golay algorithm. Use the 'Smooth Window' and 'Polynomial Order' functions to change the degree of smoothing. Pressing 'Apply' performs the change and 'OK' completes the operation. You can use the zoom function to see more closely the effect of the smoothing. You will be asked if you want to accept the resulting smoothed spectrum.

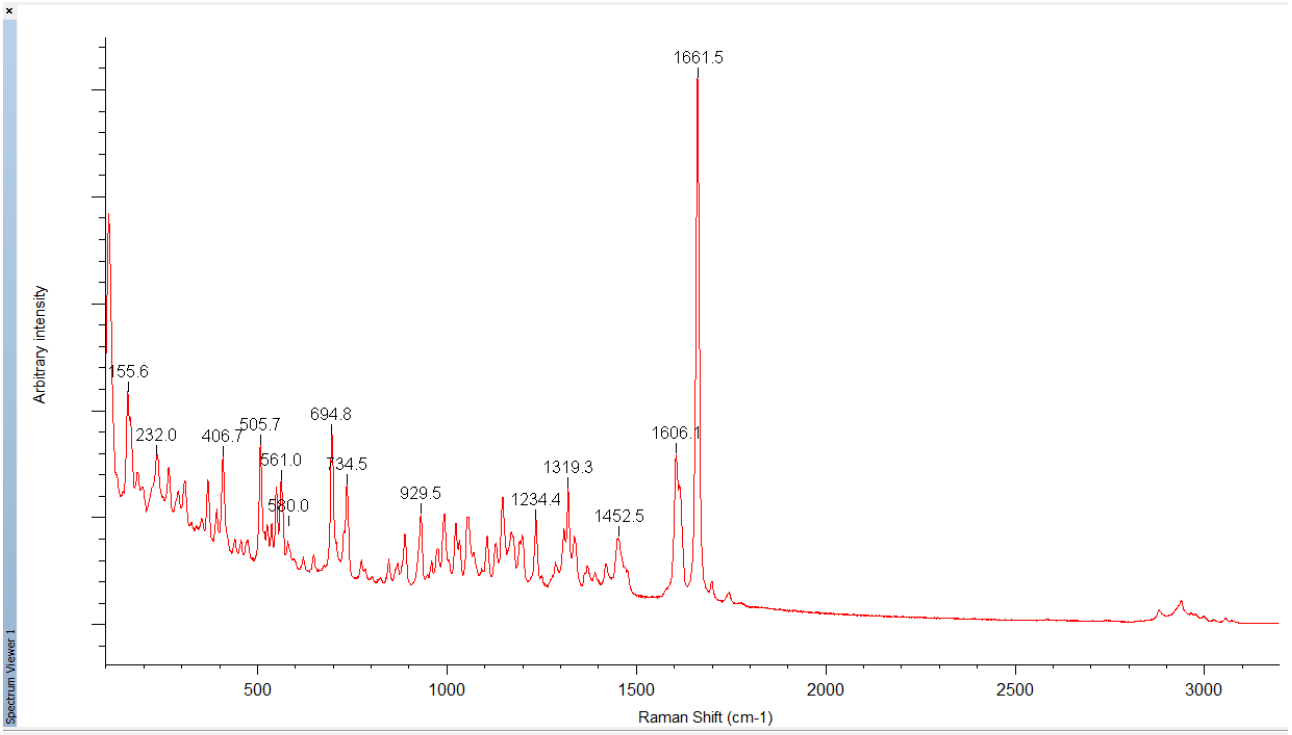
Zap

Stray bands can be removed from the spectrum using the Zap function. Generally, these will be cosmic ray features or other spurious lines. Ideally, the measurement would be re-performed but you may decide that zapping is acceptable. To remove a band on an open spectrum, select **Processing…Zap**. A new viewer will open with the sample spectrum at the top and the result spectrum below. The upper spectrum has a zap region between two vertical black lines. Grab each vertical bounding line in turn and adjust the position of the zap region so it just encloses the band to remove. Then use the zoom function to isolate the band to zap out. Notice that the result spectrum updates to show the effect of the zap. Additional zap regions can be added from the context menu.



Peak Pick

Peak pick is a quick and simple method to label band positions on a spectrum and enable these to be printed out together. To initiate peak picking select Analysis > Peak pick, or click the Peak pick button on the Analysis toolbar.



Peak pick

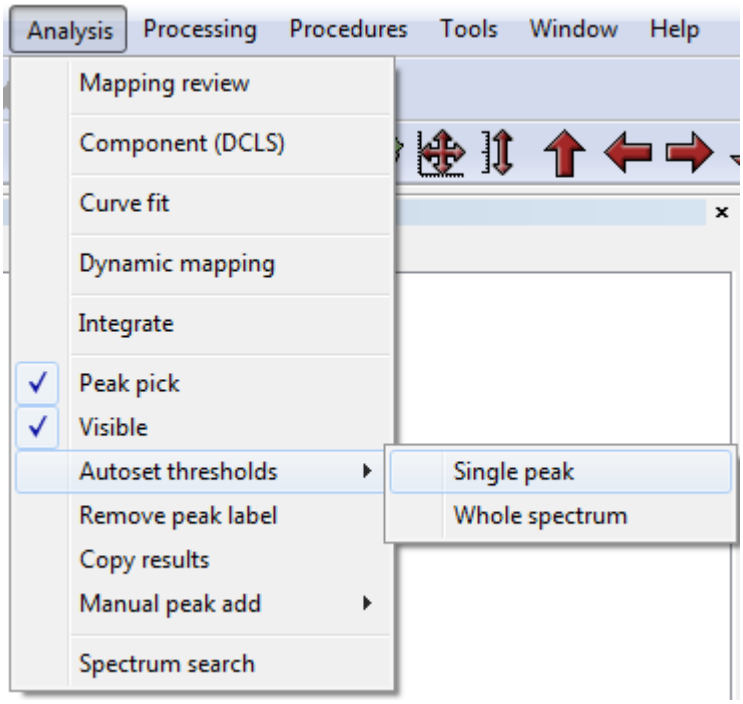
Peak no.	Centre	Height	Width	Area	Absolute inten...	Low edge	High edge
1	155.638	10250.2	15.6173	1.77017e+006	21800.1	134.509	202.972
2	232.033	4266.84	13.1133	991115	15815.9	203.797	244.215
3	406.69	6914.77	7.37158	901089	15758.5	386.916	436.408
4	505.722	9853.63	7.19457	769548	16845	479.301	527.968
5	560.986	6768.61	9.40512	1.32467e+006	13499	528.793	622.002
6	579.968	4724.68	18.9718	207027	9156.73	623.652	642.624
7	694.777	12250.9	8.78093	672090	17786.9	667.369	716.036
8	734.483	7926.38	8.80111	536554	13144.9	716.861	761.404
9	929.515	6242.84	9.71824	429559	10337.8	908.229	951.947

Note that it may be necessary to maximise the window containing the active spectrum in order to see the peak results table window, depending on where it is currently docked.

Peak Pick detects peaks for the active spectrum of the active spectrum viewer *using the current threshold settings* and displays the results. It also adds a peak results table to the current window, which gives details for the picked peaks, which can include some or all of the following information.

- Centre
- Height
- Width
- Area
- Absolute intensity
- Low edge
- High edge

Peaks are automatically labelled on selection of the Peak pick option from the Analysis menu.

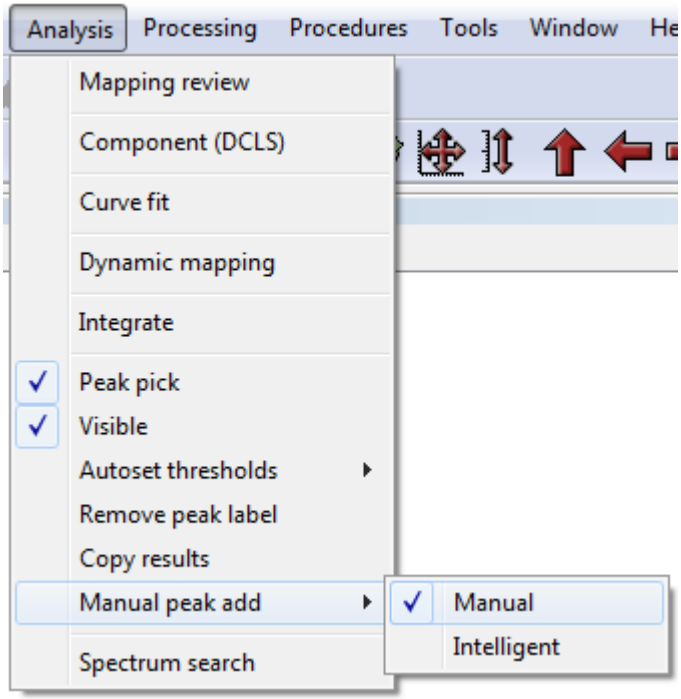


If the peaks are not suitably labelled the following methods can be used to add or remove the labels:

1. Use the Autoset thresholds > Whole spectrum option. This sets thresholds so that a limited number of the best-defined peaks will be found, and then performs peak picking. The maximum number of peaks can be set on the Automatic Thresholding tab.

2. Use Autoset thresholds > Single peak option. Zoom-in on a single peak (including some baseline either side of the peak) and then select this option. This function sets thresholds to locate all peaks in the spectrum that are as well defined (or better defined) than the displayed peak. Peak picking is then performed.

3. Manual peak addition is performed by using a double left mouse click on, or close to, the peak to be labelled. Use Manual peak add > Intelligent to enable the software to locate and label the closest peak within 5 falling points either side of the maximum. Complete manual control can be achieved by using Manual peak add > Manual. A double click on the spectrum will add a peak label exactly where the mouse cursor is located when in this setting.



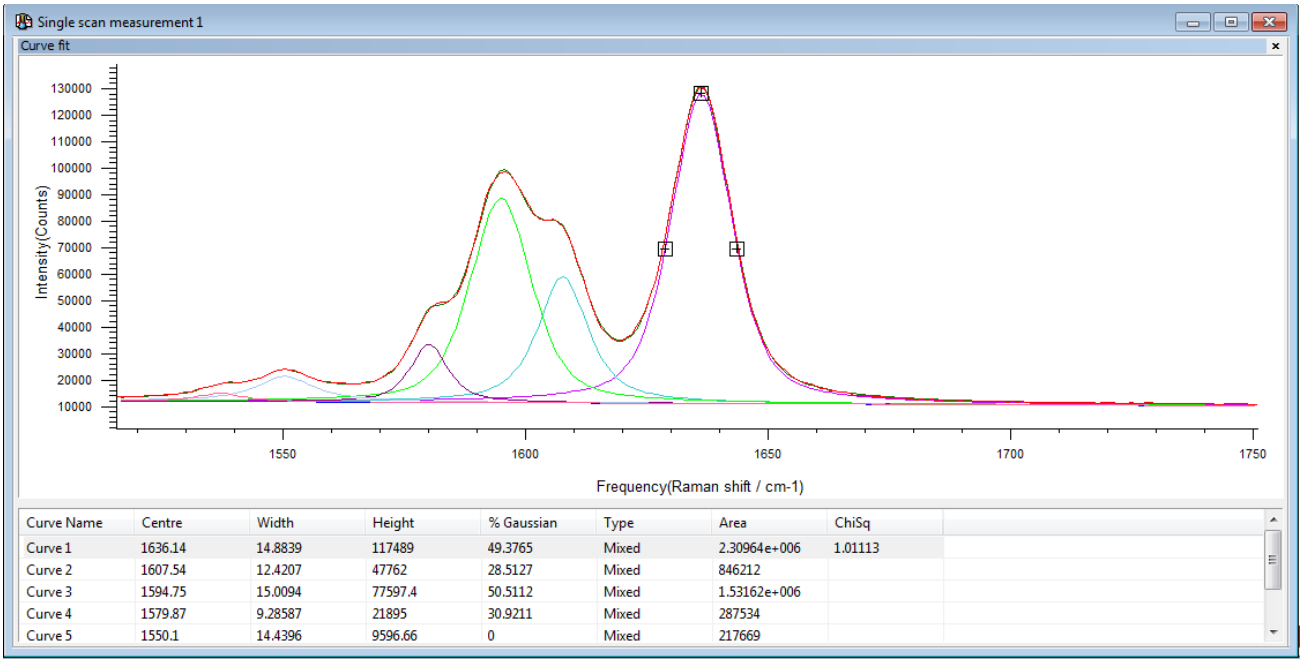
4. Manual peak removal is performed by right clicking on the relevant peak label in the peak pick table and selecting ‘Remove peak label’.

The peak result table may be copied to the Windows clipboard by selecting the Copy results option from its context menu (shown by right-clicking it). From here it may be pasted into e.g. spreadsheet or word-processing programs.

Curve-fitting

Curve-fitting calculates highly accurate values for simple, single bands but also for complex band systems where there may be two, three or more bands that overlap. Curve-fitting can produce a *.wxc file that can be saved and applied later to a spectrum or set of mapped data.

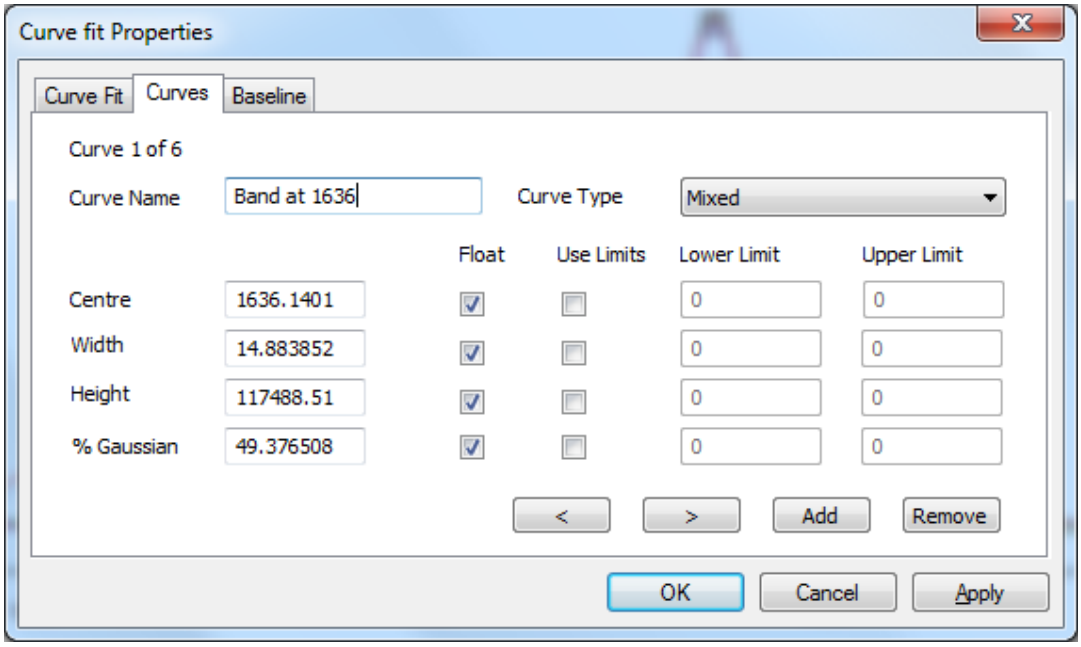
To fit a curve to a band or series of bands, select **Analysis…Curve fit** to open the Curve fit window, zoom in to a region that contains the band and some baseline data either side. A baseline may be added automatically between the end points of the spectrum. This can be used, or removed via the context menu. Use the mouse to position the approximate centre of the band. Click to add the band and repeat for the centres of other bands if part of a system of bands. You may need to use the context menu and select ‘Add Curves’ if the curve symbol does not appear with the cursor. Pressing ‘Remove curve’ from the context menu will remove the last node you added.



Select ‘Start Fit’ from the context menu to fit the added curves to the data. The algorithm will perform many iterations until the best fit has been achieved.

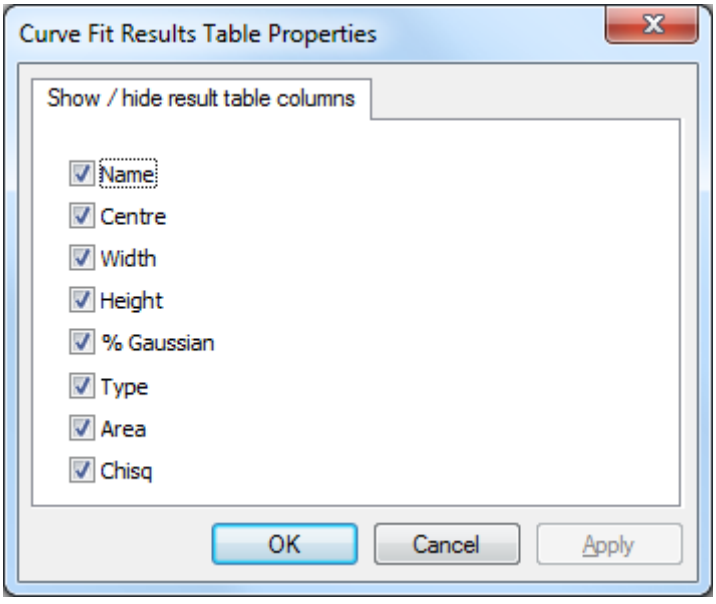
You can save the curve fit file as a *.wxc from the context menu (**Curve parameters….Save curves**). To reapply this saved curve ‘template’, perhaps to a similar sample, start the curve fit application and use the context menu (**Curve parameters…Load curves**) and then ‘Start fit’.

You can modify or make changes to the curve fit using the Curve Fit Properties window from the context menu **Properties**. This provides greater control over the fitting process instead of the automatic parameters that are usually used. For example, you can choose to fix a band centre instead of letting it ‘float’ during the curve fit, or apply limits to parameters. This can be useful for complex band shapes. Curves can also be named, different types of baseline can be used or the curve type can be defined. Use the ‘Curve Fit’, ‘Curves’ and ‘Baseline’ tabs to adjust the curve fit.



The context menu allows the truncation of the fitted region on zooming (Fit viewed region). It is generally beneficial to have this ticked. If this is not active then the baseline form and height will be somewhat dependent upon other bands that are present throughout the whole spectral range. It may be necessary to re-apply the baseline on zooming, as its original position will be persisted.

A curve-fitting procedure produces a table of data; the columns are selected from the context menu of the table (**Show/hide columns**). The table lists the various parameters for each of the curves. The data in the table can be copied and pasted into a spreadsheet package, for example (context menu, **Copy results**).

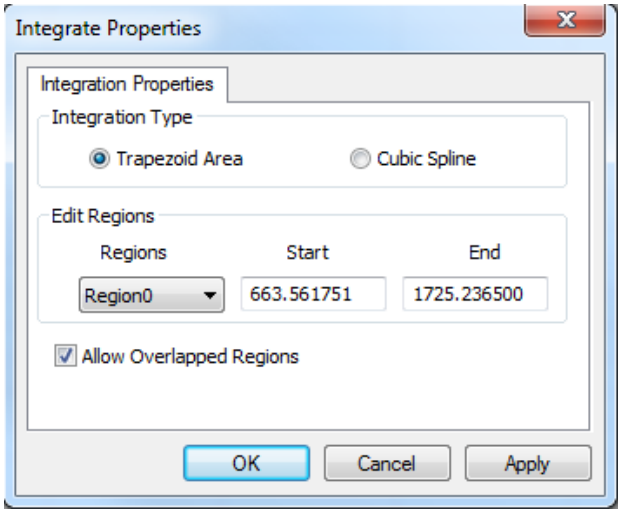


The fitted curves, baseline and result curve (sum of the fitted curves and baseline, if used) can all be saved and reloaded as ‘spectra’. Once the curve fit has completed, select **Save curve data** from the context menu and save to a location. This saves a multifile that can be opened like a spectrum in WiRE. Use the Data tab in the Navigator and expand the branches to show the Collected data. Highlight each ‘acquisition’ and right click to show ‘Load dataset’. To save the curves, result, or baseline as a separate ‘spectrum’ or trace, highlight the trace in the View tab of the navigator and select ‘Save spectrum as’ from the context menu.

Integration

The integration option provides a method where the total area under the spectrum can be determined.

The left and right vertical bars determine the region which is being analysed within the spectrum. The properties are selected by using a right click on the spectrum. These enable the exact start and end position to be defined and the type of integration (Trapezoid or cubic spline) to be selected.



Trapezoid calculates the area between adjacent points using a trapezium drawn between the points and the x-axis.

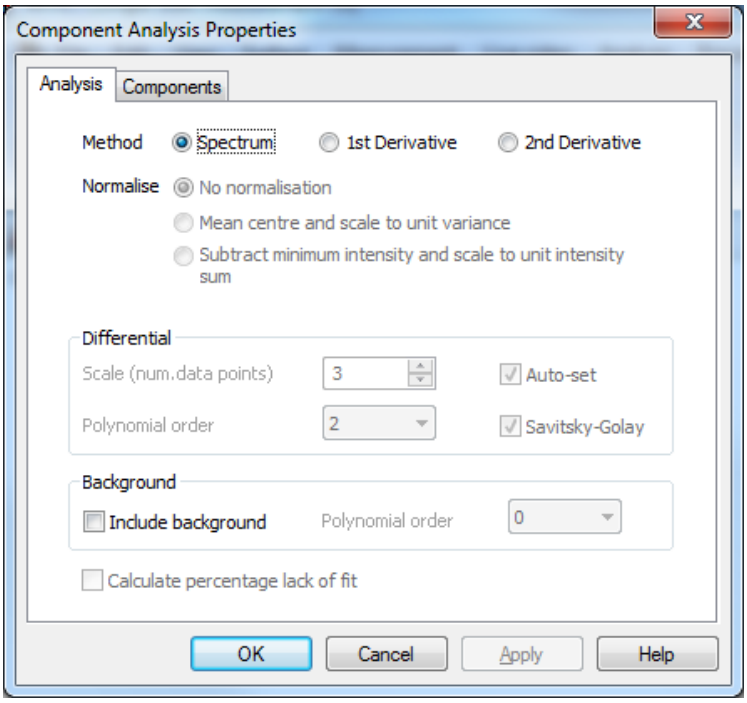
Cubic spline approximates the spectrum with local cubic polynomial models and uses integration to get an estimate for the area between adjacent points.

In each case the result is the sum of areas across all pairs of points in the region.

Direct classical least squares (DCLS) component analysis

The ‘Component (DCLS)’ method can be applied to a single spectrum to determine quantitative information (i.e. fraction estimates) regarding its constituent properties. This method should be applied where reference spectra are available for all components within the mixture. Fraction estimates are obtained for each reference spectrum. If only a limited number of reference spectra are available, DCLS can be used to gain useful but potentially inaccurate quantitative information.

To perform DCLS on a single spectrum, select **Analysis...Component (DCLS)** to open the Component (DCLS) window.

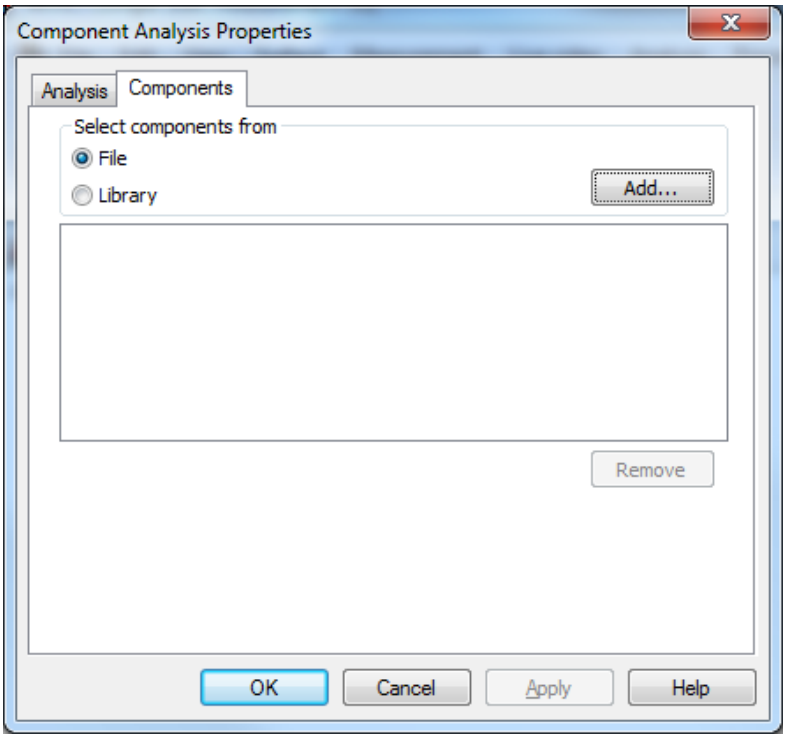


Select the method type. Choose Spectrum for a direct comparison. Using derivative spectra can be useful if the data contain background offsets and / or slopes. The 1st Derivative option is suitable for removing backgrounds with a consistent shape but variable offset. The 2nd Derivative option is suitable for spectra where the background changes dynamically for the same component (e.g. fluorescence quenching).

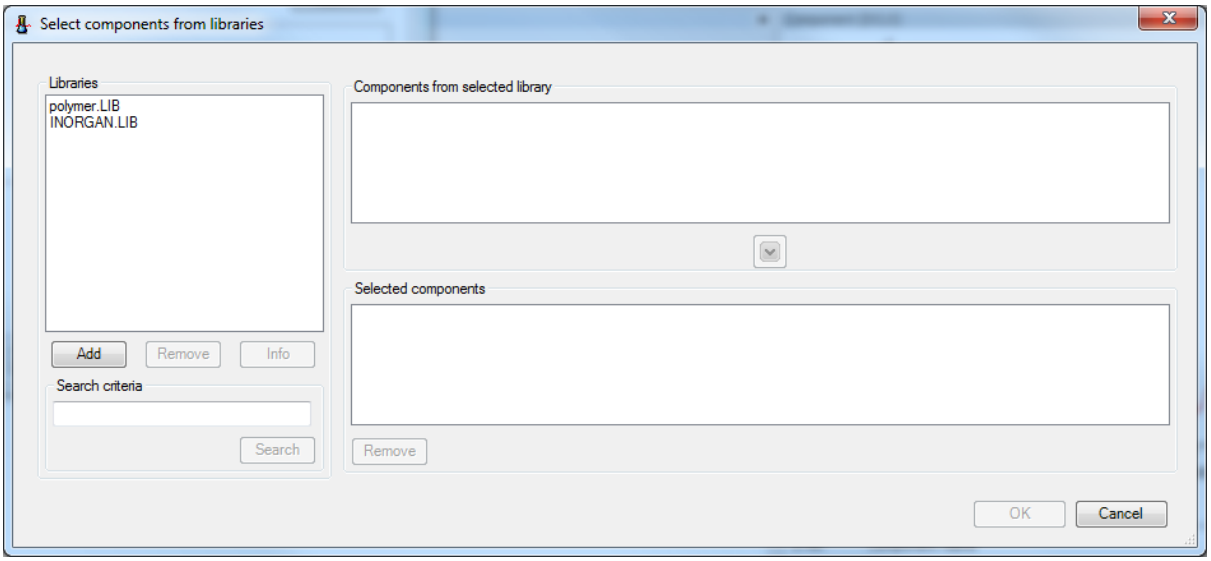
If the Spectrum method is selected the Include background option is made available to the user. This is useful to reduce the skewing of the DCLS analysis by the contribution of any background variation. The Differential options are available only where one of the Derivative methods is selected. The Normalise options are not available to the user during component analysis of a single spectrum.

Select the ‘Components’ tab and add reference spectra. Reference spectra can be added by:

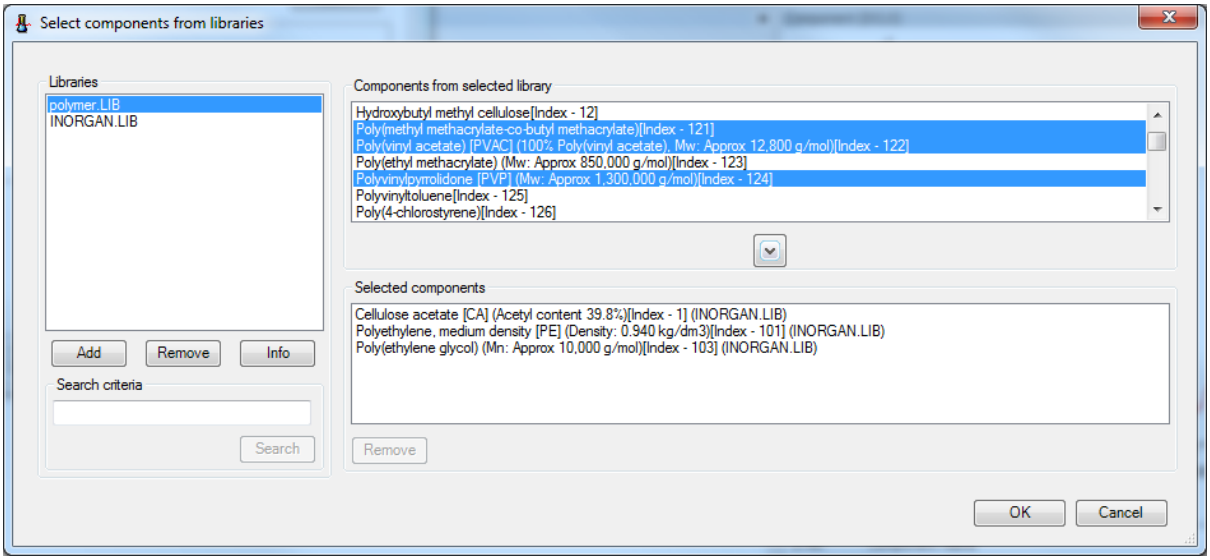
- Selecting ‘File’ and navigating to the file containing your reference spectra,
- Loaded in from an existing library
- A combination of reference spectra and library spectra



To add a component from a library, the user must initially add the desired library or libraries to the ‘Libraries’ section of the ‘Select components from libraries’ window (opened by selecting **Library...Add...**). Once loaded, WiRE will remember the library unless it is removed from the ‘Libraries’ section.



Highlighting a library allows the user to remove, view information on, or search for key words within the selected library. The ‘Components from selected library’ section lists all reference spectra within the selected library. This list can be reduced by entering key words into the ‘Search criteria’ section. To add component(s) from the library, click on the desired reference spectra so that they are highlighted. Click the down arrow to add the reference spectra to the ‘Selected components’ section. Components can be removed from this section by clicking to highlight and selecting ‘Remove’.



Clicking ‘OK’ adds the selected reference spectra to the components list. Clicking ‘OK’ again displays the spectrum being analysed along with the spectra of all components in a plot in the ‘Component (DCLS)’ window. The spectrum number, component name and fraction estimation value of each reference spectrum are listed in a table below the plot. To identify spectra and corresponding components, the Show dataset axis labels must be selected from the context menu.

See module TM015 for information regarding component (DCLS) analysis on multi-file data.