Part XV

15 ElViS

Mnova 14 comes with a new ElViS plugin designed to aid in analysis of various optical spectroscopy data including ultraviolet and visible (UV/Vis), near and mid infrared (NIR/MIR), Raman, fluorescence, and other spectroscopic methods operating in the whole region of wavelength from 100 nm to 100 µm (100 000 – 100 cm^{-1}). Covered by the practical optical spectroscopy and actually beyond.

The name of ElViS (Electronic Vibrational Spectroscopic) abbreviates the main optical techniques and spectral ranges covered by the software. The product concept takes into account the main modern trends in optical spectroscopy, in particular, growing data volumes, multivariate quantitative analysis of spectra, distinct shift from laboratory to industrial analysis, development of multi-spectral and hyphenated techniques, and recent advances in optical sensing.

Practical application areas of Mnova/ElViS include:

- Spectroscopic analysis of various samples from pure substances to complex mixtures;
- Analysis of spectral peaks and features, their chemical interpretation;
- Data preparation and preprocessing for advanced multivariate analysis (chemometrics);
- Investigation and monitoring of chemical reactions and industrial process;
- Analysis of hyphenated data, such as HPLC-DAD and TGA-IR;
- Building spectral data banks; and
- Professional reporting of experimental data and results.

15.1 Formats supported

Here you can see a list of some of the main formats supported by Mnova ElViS:

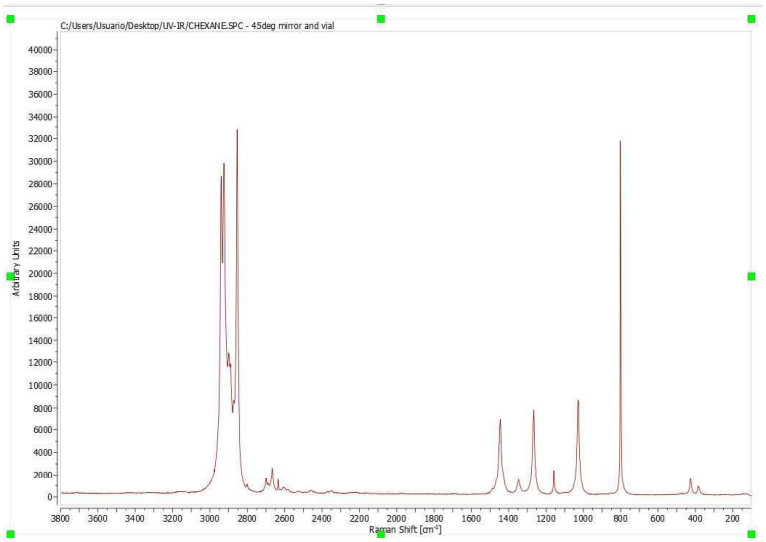
ASCII (.txt, .csv). This format must be loaded by following the menu 'File/Open' and selecting the ElViS CSV (*.csv; *.txt) converter from the scroll down menu. The CSV files could contain two XY columns (being the first column: wavenumber/wavelength/arbitrary) or three or more XYY columns in case of stacked plots.
JCAMP-DX (.jcamp, .dx, .jdx, .jcm)
OPUS (.0, .1,...)
Thermo Nicolet Omnic (.spa)
Thermo Galactic GRAMS (.spc)

Mnova will allow you to export your ElViS datasets as .csv, JCAMP-DX, .txt and .mnova and different image formats; by following the menu 'File/Save As'.

15.2 Fast Visual Guide to process routine datasets

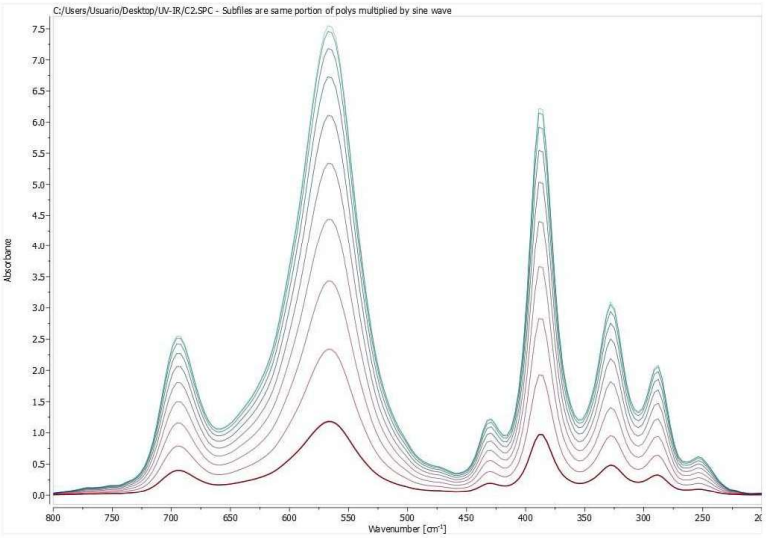
Go through the following procedure to process a routine ElViS spectrum (you can download the examples from here):

1. Drag and drop the file into Mnova to get your spectrum fully processed. (You can also follow the menu 'File/Open')

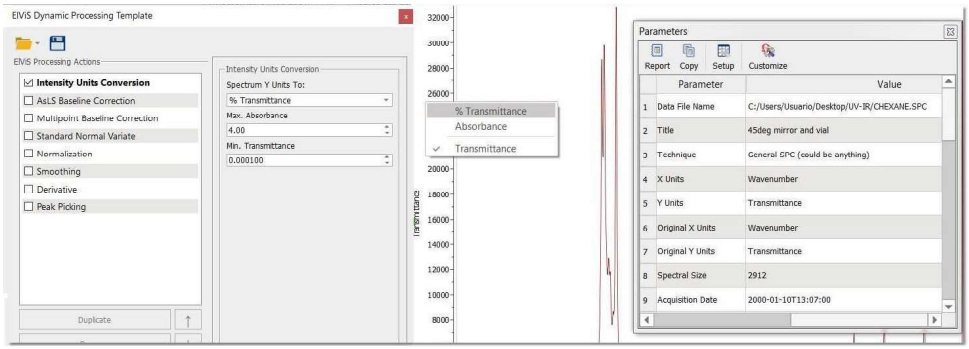


If you have several datasets, you can load of all them in your Mnova document, to generate a stacked plot (after having highlighted them on your Page Navigator and followed the menu 'Stack/Stack Items')

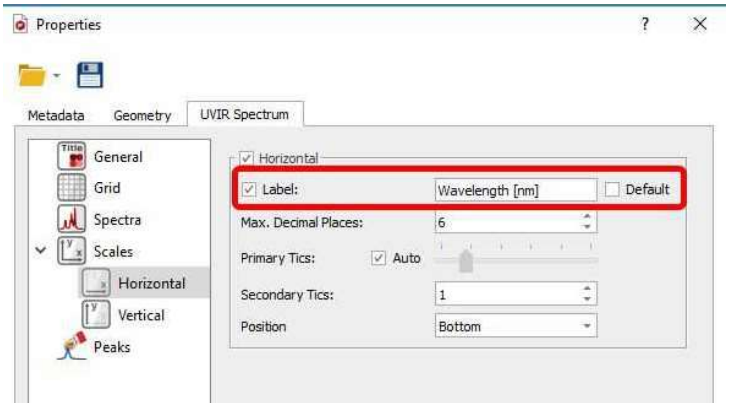
You can also load spectra acquired in arrayed mode:



2. You can convert the spectral X-scale into another units by right clicking on the scale and choosing another compatible unit type from the drop-down list. Similar operation is available for the Y-units (intensity). The intensity units can also be converted from the Processing Template. If the units were not recognized by the import function or were absent in the source data format, they appear as ‘Arbitrary units’ in spectra. You can reset ‘Arbitrary units’ (as well as any other wrong original units) from the 'Parameters Table' (which can be displayed by following the menu 'View/Parameters') Double click to ‘Original X units’ or ‘Original Y units’ field values and choose an appropriate unit type from the drop-down list of available unit types. (Note, this operation is generally discouraged, because it can lead to data visualization and analysis mistakes! Avoid its application unless you are confident).

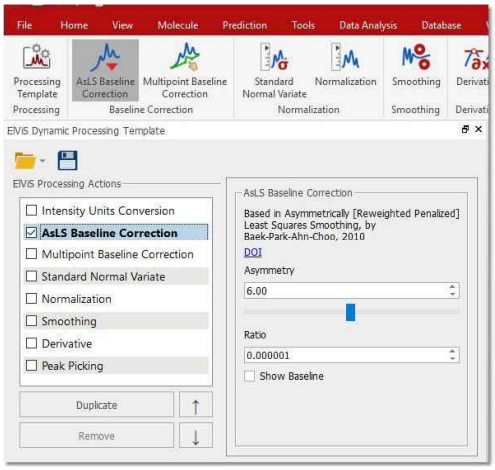


Besides, independently on the current and original X- and Y-units, you can define any custom labels on the respective axis using the Properties dialogue:



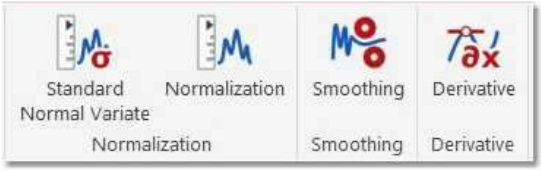
3. You can zoom in by using the applicable button from the View ribbon (or the shortcut: Z). With active 'Zoom In' mode press Z to switch between horizontal, vertical and rectangular zoom. From that ribbon, you will find different zoom modes and a tool to create expansions (shortcut: E):

4. Click on the 'AsLS Baseline Correction' icon to correct the baseline of your spectrum automatically. The Dynamic Processing Template panel will appear to allow you to change the ALS baseline correction parameters:



A manual multipoint baseline correction mode can also be selected, when fine tuning of the corrected baseline is necessary.

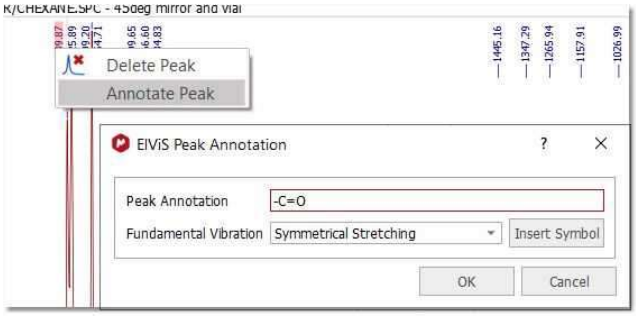
5. If spectrum processing is needed, to improve the data to subsequent analysis,you can apply a normalization, smoothing or derivative from the main toolbar (or from the 'Dynamic Processing Template'):



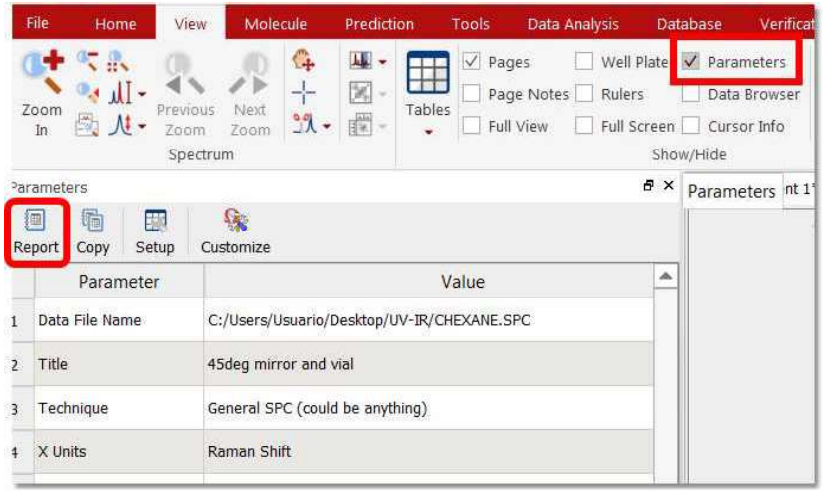
6. Clicking on the Automatic Peak Picking button will label the peak in a spectrum on screen in accordance with the options.

Manual Peak Picking can also be applied by selecting the 'Manual Threshold mode' and clicking and dragging over the desired region of interest to label the peaks within it. The 'Peak by peak' mode also allows setting or removing a label in an arbitrary position one by one to select unresolved peaks.

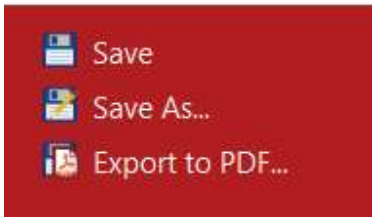
Right clicking on any peak label will allow you to annotate it:



7. To paste the Parameters Table on the spectral window, just follow the menu: 'View/Parameters' and click on the **'Report'** icon.



8. Finally, you will be able to save, print or export the document to PDF by clicking on a respective icon under the 'file ribbon' (or on the toolbar):

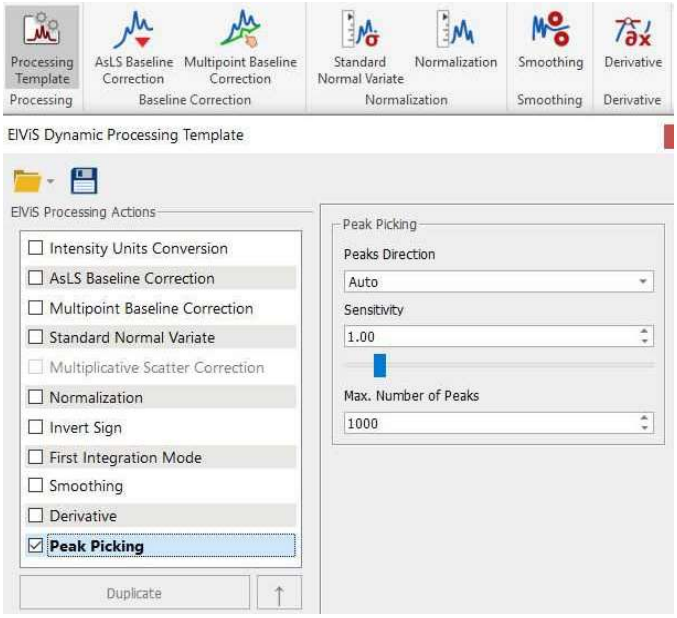


You can also export your datasets as ASCII (*.txt or *.csv), JCAMP-DX or any image format by selecting 'Save As'.

15.3 Basics Processing

Our aim in developing Mnova has been to make the opening, processing, handling, analysis, saving and printing of data simpler than it has ever been before, so that even the novice can enjoy the software and obtain excellent results from the very start.

All the processing features can be applied directly from the ribbon or from the Dynamic Processing Template:



The main idea behind the template is to apply the tools and their combinations “on the fly”, to remember favorite processing patterns and quickly apply them, also automatically to a new opened data.

15.3.1 Baseline Correction

Spectra suffering from high background intensity may need baseline correction to improve their quality prior to analysis.

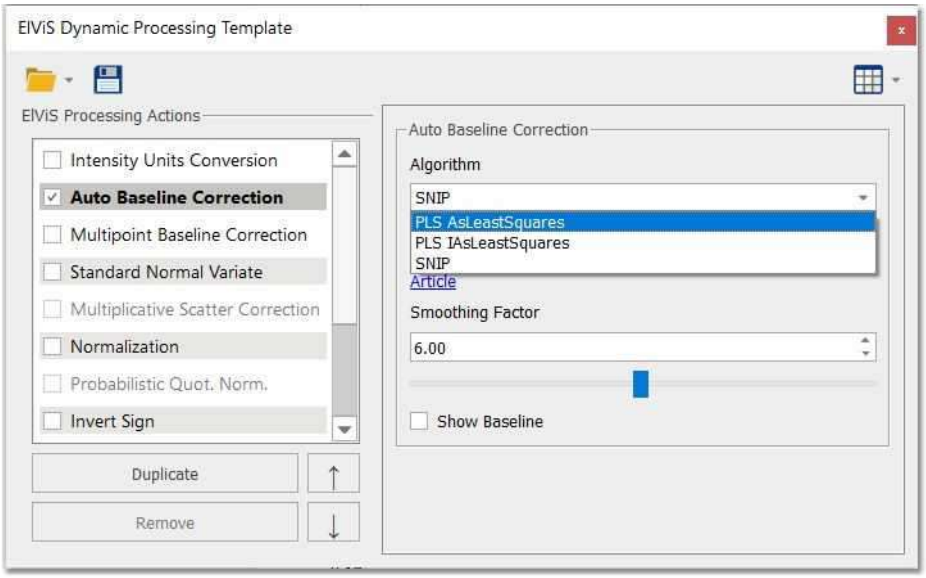
The UVIR plugin of Mnova offers two algorithms that can handle even very complex baselines:

- Asymmetric Least Squares (AsLS) Baseline Correction
- Multipoint Baseline Correction

Asymmetric Least Squares Baseline Correction:[1,2] Combines a smoother with asymmetric weighting of deviations from the smooth trend to get an effective baseline estimator.

There are two algorithm parameters helping to optimize the results; “AsLS Logλ” (from 0.1 to 12; being 6 the default value) and “AsLS asymmetry” (0.000001 - 0.999999; being 0.000001 the default value).

[1] *P.H. Eilers, H.F. Boelens, Leiden University Medical Centre Report 1, 5,* ***2005.***
[2] *Journal of The Institute of Electronics and Information Engineers Vol.53, NO.3,* ***2016.***



In the IAsLeast Squares algorithm, the parameters Asymmetry and Ratio have similar influence in the construction of the baseline, though the Ratio tends to have a less critical influence (i.e. in the number of needed internal iterations for convergence). For further information about his algorithm, please check the reference below:

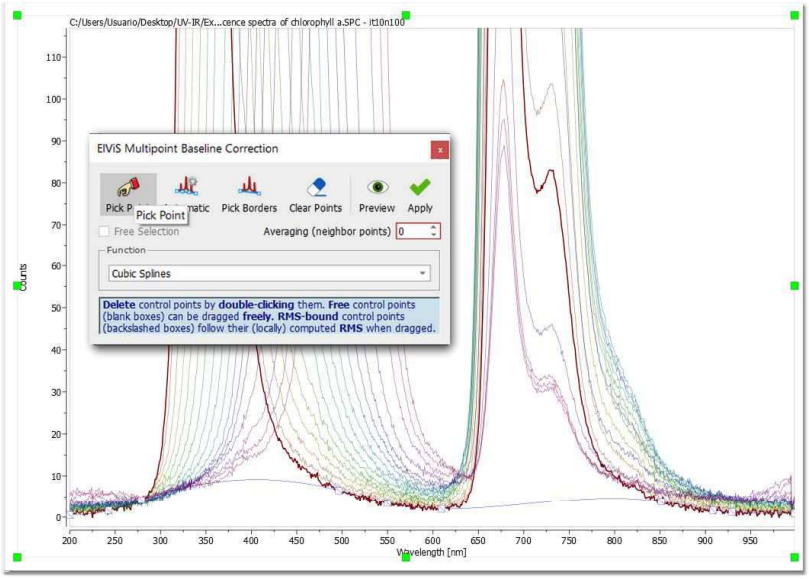
https://doi.org/10.1364/AO.404863

For further information about SNIP algorithm, please check this article:

https://doi.org/10.1016/S0168-9002(97)01023-1

Multipoint Baseline Correction: This method provides a way of modeling the baseline by selecting a well-distributed set of points that fall on the baseline and then interpolating between those points to complete the model.

Multipoint series baseline correction is a very useful algorithm of spectral (pre)processing that eliminates intensive spectral backgrounds, such as a fluorescence signal in Raman spectra, in a simple and efficient way. Although the algorithm can be applied to single spectra, its main value is the possibility of processing spectral series with essentially different individual baselines using a set common baseline points.



After having clicked on the 'Multipoint Baseline Correction button'; you will get a dialog box which will allow you to put down points along the baseline (by clicking on the 'Pick Point' button) to help the program to find the correct coefficients for the baseline correction equation that it will subtract from the spectrum. If you want to remove any undesired point, just double click on it (click and drag if you want to change the location of the point).

Let´s see the functionality of each button of the "Multipoint Baseline Correction' dialog box:

'Pick Baseline points': Allows the user to pick the points. To remove any selected point, just double click on it.

'Automatic': Automatically add points for the baseline correction.

'Pick Borders': Allows the user to easily pick both the first and the latest points, if they need a correction.

'Clear Points': Click on this button to delete all the selected points (restart your work).

'Preview': To see a preview of the baseline correction prior to apply the changes.

'Apply': To apply the correction.

'Free Selection': Check this box to be able to pick points anywhere (otherwise their vertical positions are strictly defined by the baseline curve).

'RMS Calculation span (points): This option takes spectrum noise into account at the baseline construction. The value of 1 means that any picked point is picked exactly on the spectral curve. Otherwise, the point will be set into a vertical position corresponding to a virtually smoothed spectrum. The virtual smoothing is performed by simple averaging of points within a window where the selected point stays in the centrum and the present option indicates the number of neighbors from each side to be included into the averaging window. Therefore, the full window width is 2**n*+1 (*n* is the option value). For example, if you set this option to 3, the ordinates of each selected baseline point will be calculated as an average of ordinates of the spectral points within a window of 7 points with the selected point in the middle.

'Function': from this drop-down menu, we can select the baseline construction function on the basis of selected points:

Linear Segments: Simply connects the points with lines.

Cubic Splines: Uses the method of splines to connect the points.

Witthaker: Fits the baseline points using the Witthaker Smoother (the points do not generally belong to the resulting baseline!).

15.3.2 Normalization

Normalization is a re-scaling of the spectral intensity that may be necessary to compensate for various experimental effects for better presentation, comparison, and quantitative or qualitative analysis of the data.

Mnova ElViS offers two different algorithms of spectrum re-scaling:

- Standard Normal Variate
- Normalization

Standard Normal Variate

Standard normal variate (SNV) is a popular transformation that treats each spectrum as a vector (*S*) in accordance with the following equation:

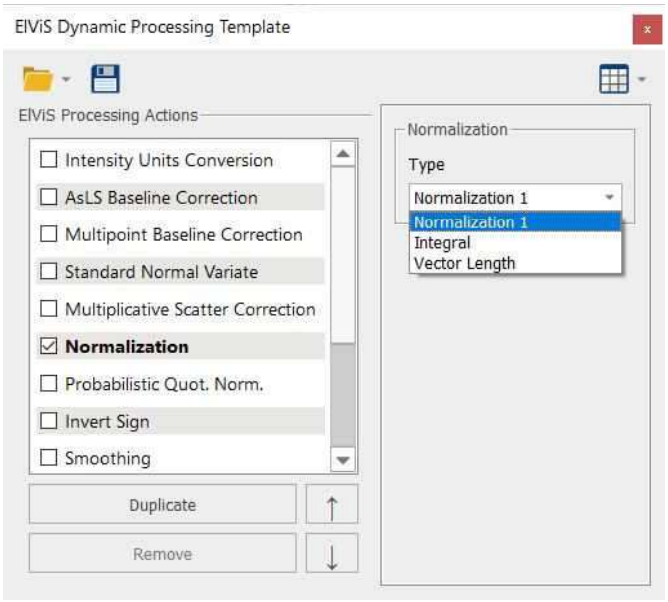
$$\tilde{S}_i(t) := (S_i(t) - \bar{S}_i)/\sqrt{\mathrm{var}(S_i)}$$

SNV is particularly useful when the sample volume (physical or virtual, as for on-line analysis) is not stable and may change from measurement to measurement. It is also applied to eliminate the so-called „scatter effect“ often observed in Vis/NIR spectra of solid and powder materials obtained in diffuse reflectance mode. SNV-correction is typically applied to spectral series prior to quantitative analysis.

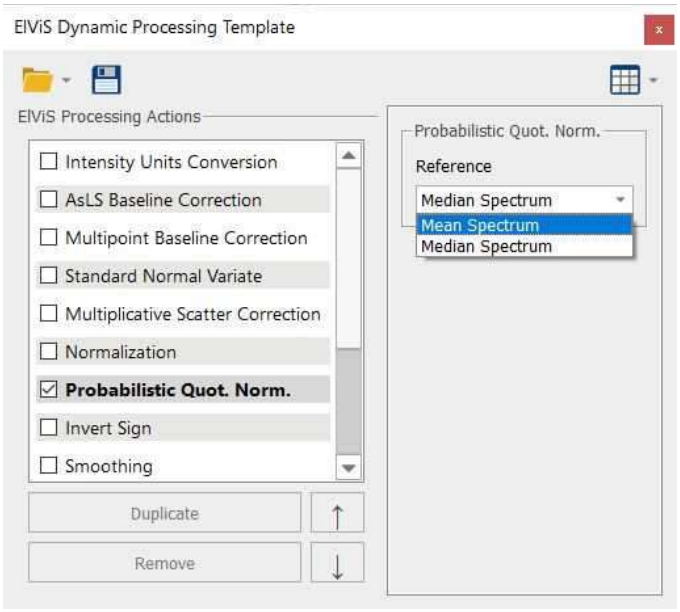
Normalization

Simple normalization to 1 is a standard (pre)processing tool for UVIR spectral type. It is typically applied in small series to make spectra acquired at different conditions better suitable for comparison, peak interpretation and qualitative analysis. In databases all spectra are typically normalized to the unit intensity.

You can also apply spectrum wise integral and vector length normalizations by selecting the appropriate option from the Processing template scroll down menu:



For Raman datasets, you can also apply a spectrum set dependent probabilistic quotient normalizaton (PQN). The reference spectrum computed internally can be chosen to be the mean or median spectrum of those selected (from the stacked items table). For further information about it, check this paper: Anal. Chem. 2006, 78, 4281-4290.



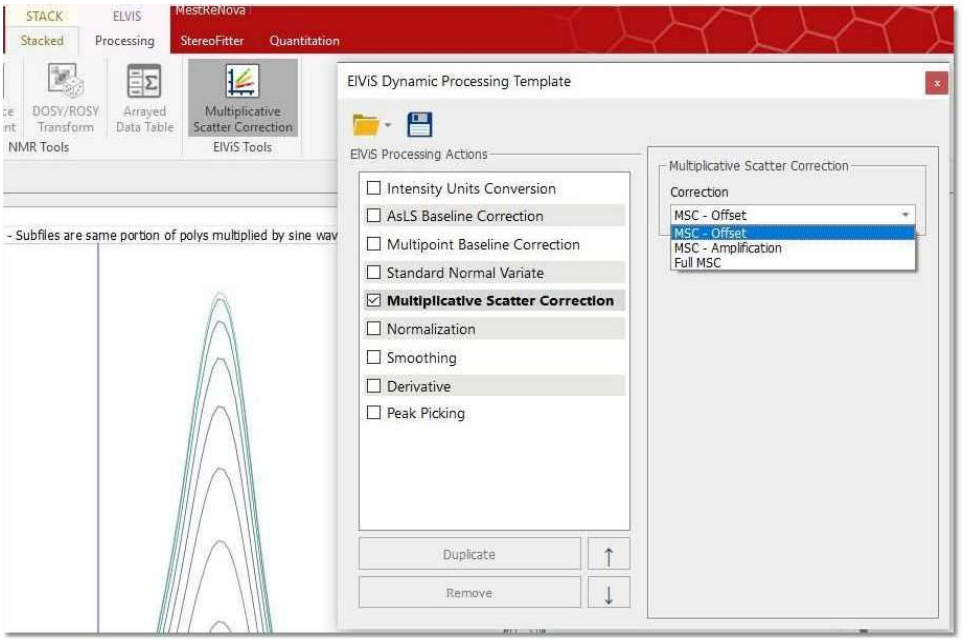
15.3.3 Multiplicative Scatter Correction

The multiplicative scatter correction is a good method in NIR spectroscopy to reduce the noise and background effects which cause baseline shifting and tilting in stacked plots.

The MSC function replaces every element in the original X-matrix according to one of the equations below:

Common offset	$M_{new}(i,k) = M(i,k) - A(i)$
Common amplification	$M_{new}(i,k) = \frac{M(i,k)}{B(i)}$
Full MSC	$M_{new}(i,k) = \frac{M(i,k) - A(i)}{B(i)}$

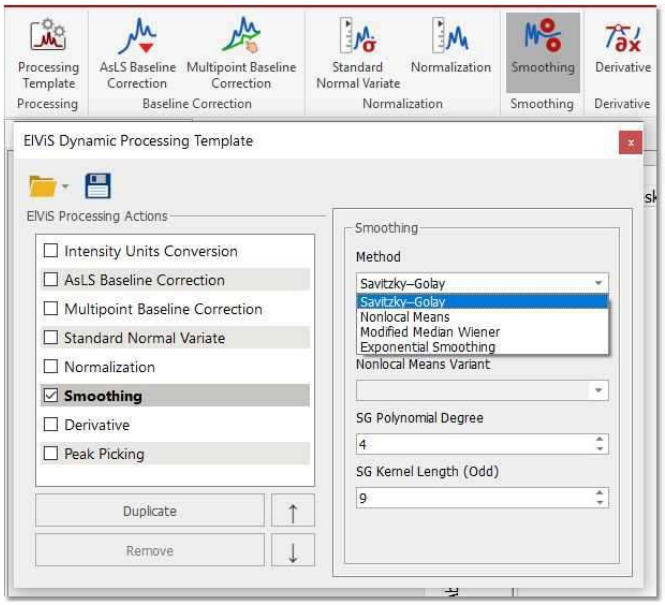
You can select any of the methods above from the Dynamic Processing template:



15.3.4 Smoothing

The signal to noise ratio (S/N) of a spectrum can be enhanced by smoothing (or filtering) technique. The noise contains high-frequency (compared to the spectral features) fluctuations of the signal. Smoothing applies a low-pass filter to the spectral data to remove the noise while having the informative spectral signals preserved.

Mnova ElViS incorporates some of the most used signal smoothing algorithms.



You can select a desired smoothing algorithm from the Method drop-down list: Nonlocal Means, Modified Median Wiener, Exponential Smoothing and Savitzky-Golay.

'Savitzky-Golay algorithm': This approach performs a least squares fit of a set of consecutive data points to a polynomial and takes the calculated central point of the fitted polynomial curve as the new smoothed data point.

Savitzky and Golay has shown that a set of integers (A_{-n}, $A_{-(n-1)}$ …, A_{n-1}, A_n) could be derived and used as weighting coefficients to carry out the smoothing operation. The use of these weighting coefficients, known as convolution integers, turns out to be exactly equivalent to fitting the data to a polynomial, as just described, and it is computationally more effective and much faster. Therefore, the smoothed data point $(y_k)_s$ by the Savitzky-Golay algorithm is given by the following equation:

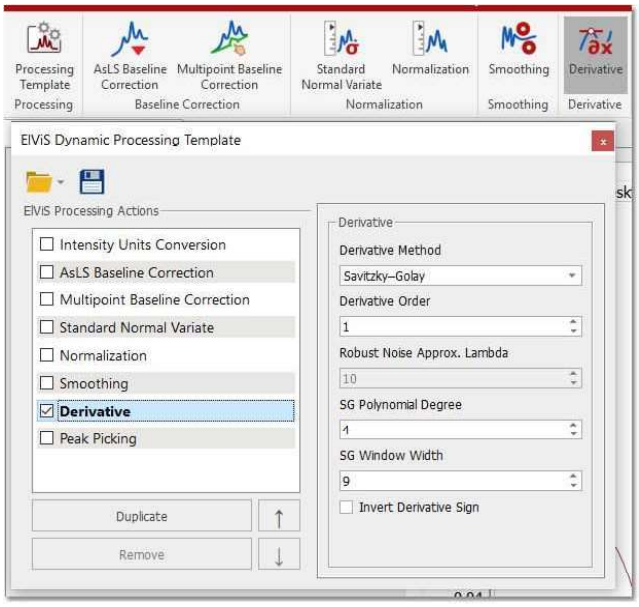
$$(y_k)_s = \frac{\sum_{i=-n}^{n} A_i y_{k+i}}{\sum_{i=-n}^{n} A_i}$$

Sets of convolution integers, instead of the smoothed signal, can be used to obtain directly, instead, its 1st, 2nd, …, mth order derivative, therefore the Savitzky-Golay algorithm is very useful for calculation of the derivatives of noisy signals consisting of discrete and equidistant points.

15.3.5 Derivative

Differentiation of spectral data is a useful tool to enhanse the data information content. First or second derivatives are typically used to improve the interpretation by revealing unresolved peaks or to correct spectra for a baseline (offset or slope, respectively) prior to quantitative or qualitative analysis.

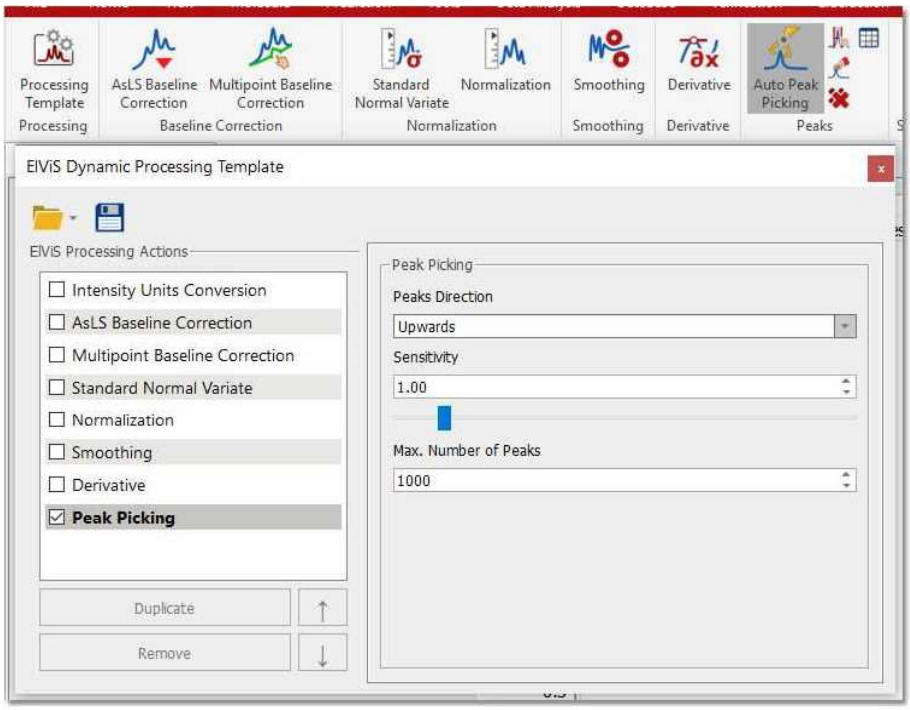
The Derivative tool is available under the ElViS processing ribbon and in the dynamic processing template:



Mnova ElViS offers three algorithms for the calculation of spectral derivatives: Savitzky-Golay, Robust Noise and Centered differences.

15.3.6 Peak Picking

Mnova ElViS implements a series of peak picking algorithms which can be accessed from the Processing Ribbon and from the Dynamic processing template:



From the ribbon, you will be allowed you to use **Automatic**, **Manual** (or Manual Threshold), **Peak by Peak** methods of Peak Picking, to **Delete all peaks in visible range** and to display the peaks table.

NOTE

You can annotate and delete peaks by right clicking on the peak label.

Automatic Peak Picking

The Automatic Peak Picking option sets labels all spectral peaks in accordance with the selected options. It can be launched by simply clicking on the **'Auto Peak Picking'** icon on the toolbar.

Manual Threshold

The Manual Peak Picking allows the user to set an area of the spectrum where the software will carry out the Peak Picking, by simply left clicking and dragging the mouse over the area.

Peak by Peak

The Peak by Peak option will allow you to select peaks to be labeled (unlabeled) one by one by hovering the mouse over a peak of interest and clicking on it. The procedure is very simple: just point to the peak you want to pick so that it is highlighted and then left click on the mouse.

To pick a peak at an arbitrary position (e.g. for a non-resolved peak), press <Shift> and click.

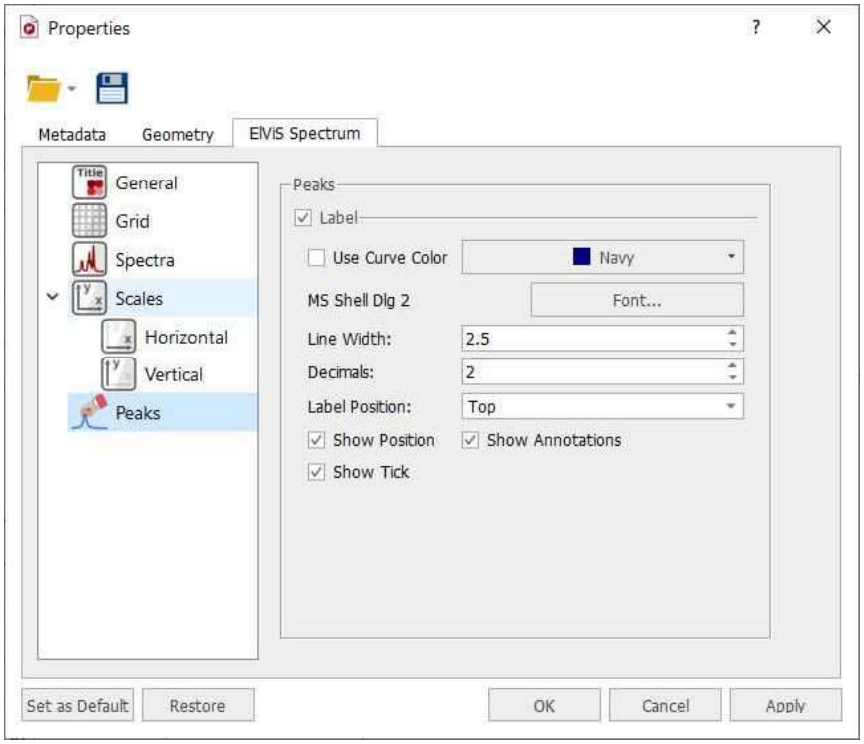
From the Dynamic Processing Template, you can select the 'peaks direction' (upwards, downwards or both), the algorithm sensitivity (to avoid picking the noise) and the maximum number of peaks.

Sensitivity: it is basically a kind of smoothing parameter. For example, in noisy spectra, large values of this parameter will smooth the spectrum in the background so that less peaks will be detected.

The ElViS Peaks table will be displayed just by clicking on this button of the ribbon 

15.3.6.1 Peak Picking Properties

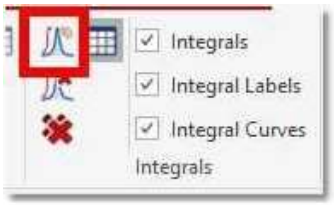
You can modify the properties of the **Peak Picking** by following the menu 'Home/Properties/Peak Picking' or by double clicking the left mouse (or pressing the right mouse) button on the spectrum display and selecting *Properties/Peak Picking* from the *pop-up* menu.



From here, the user can display or hide the peaks labels and the Position, Annotations and Ticks by checking (or unchecking) the applicable check box. You can select the color, the font, the number of decimal figures to be shown on screen and the position of the 'peak labels'.

15.3.7 Integration

Mnova ElViS implements a manual integration feature which can be applied over one single spectrum or to a stacked plot. You can use the 'Manual Integration' button from the ribbon (or the shortcut: I) and next click and drag over the region of interest:



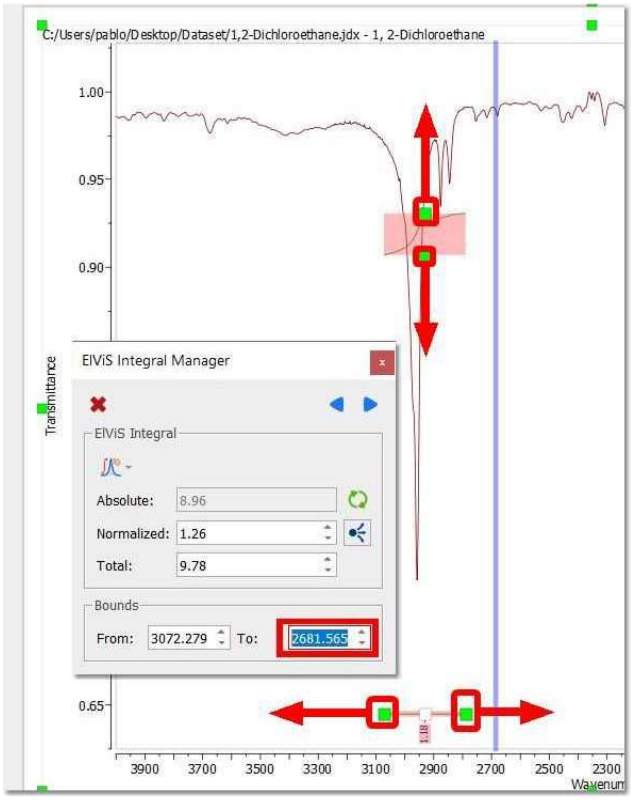
You can use the check boxes of the ribbon to hide/display the integrals, labels and curves.

The first integral will be normalized to 1.0. All following integrals will be referenced relative to the first integral.

All the integral curves are mouse-sensitive and they respond to usual mouse operations. If you want to move up or down all the integrals, just click and drag (with the left mouse button) over anyone of the integrals (notice that hovering the mouse over the integral will highlight it in red). If you keep pressed the SHIFT key at the same time, the height of the integral curves will be changed.

The same effect will be obtained if you hover the mouse over integral curve, click on one of the green squares and drag the mouse up or down.

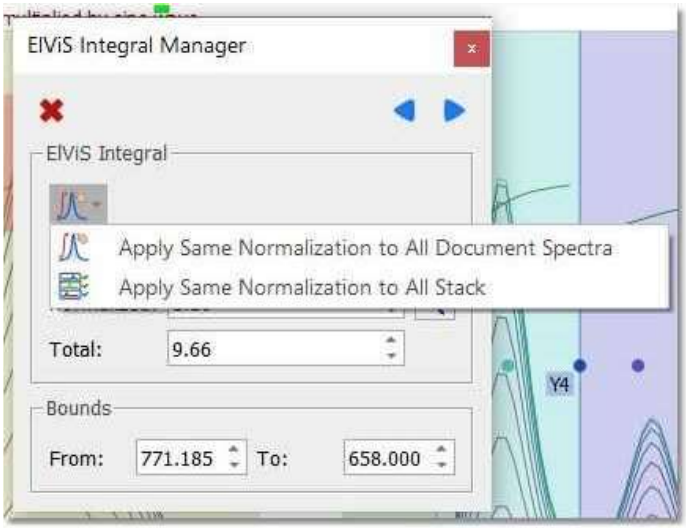
If you want to change the range of the integral, do the same with the integral label (notice that once you hover the mouse over the green squares of the integral curve or label, the mouse pointer turns to vertical or horizontal arrows, respectively). The integral regions can also be resized the 'Integral manager' which can be accessed by double clicking on the integral curve:



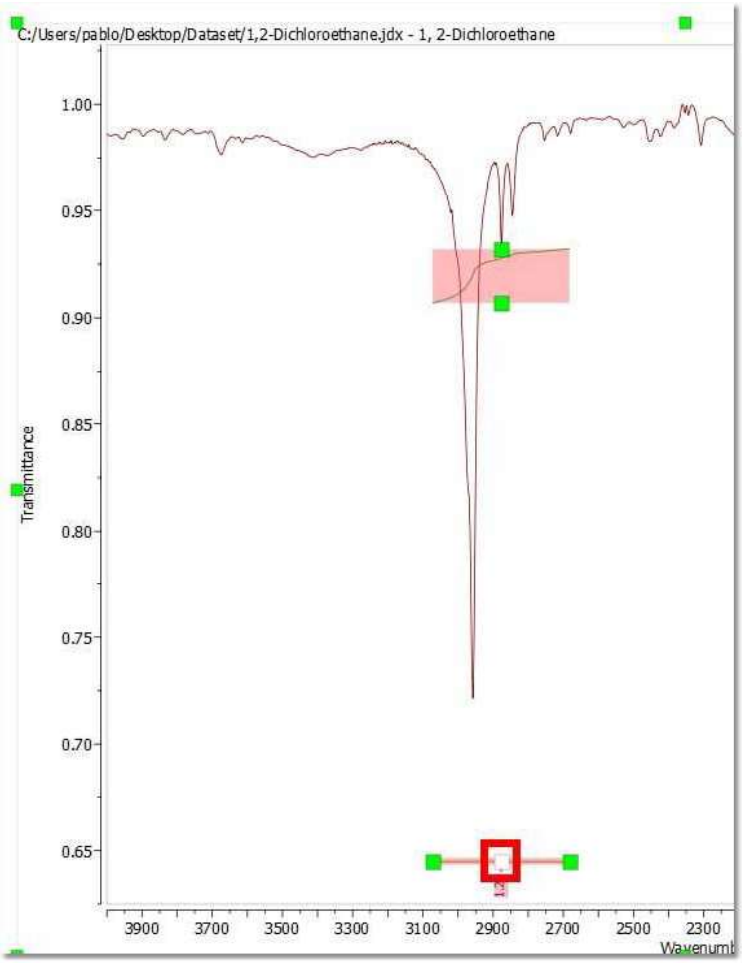
Bear in mind that you are able to navigate over the integrals by using the **'Previous or Next'** icons. You can delete an integral one by one by clicking on the 'Delete' button.

If you want to normalize the integrals, just overwrite the desired value on the 'Normalized box' and press OK, all integrals will update with reference to the chosen one.

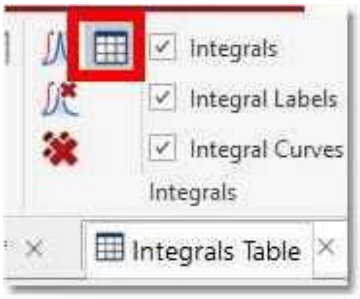
You can apply the same integral normalization to all the spectra (in the current document) or to all the traces of a stack plot by selecting the applicable option, as you can see below.



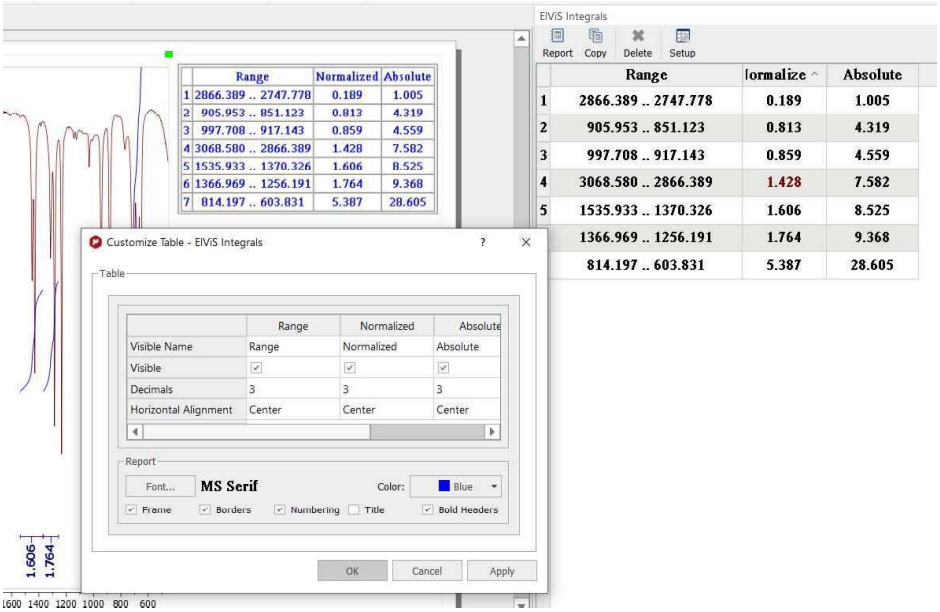
Integral regions can be split by clicking (or clicking and dragging) on the 'red square' of the integral segment:



All the integrals will be stored in the integrals table which can be displayed by clicking on the applicable button of the ribbon (or by following the menu 'View/Tables/ElViS/ElViS Integrals'):



The information displayed in the table can be customized by clicking on the 'Setup' button. As usual, you can report and copy to clipboard the table:



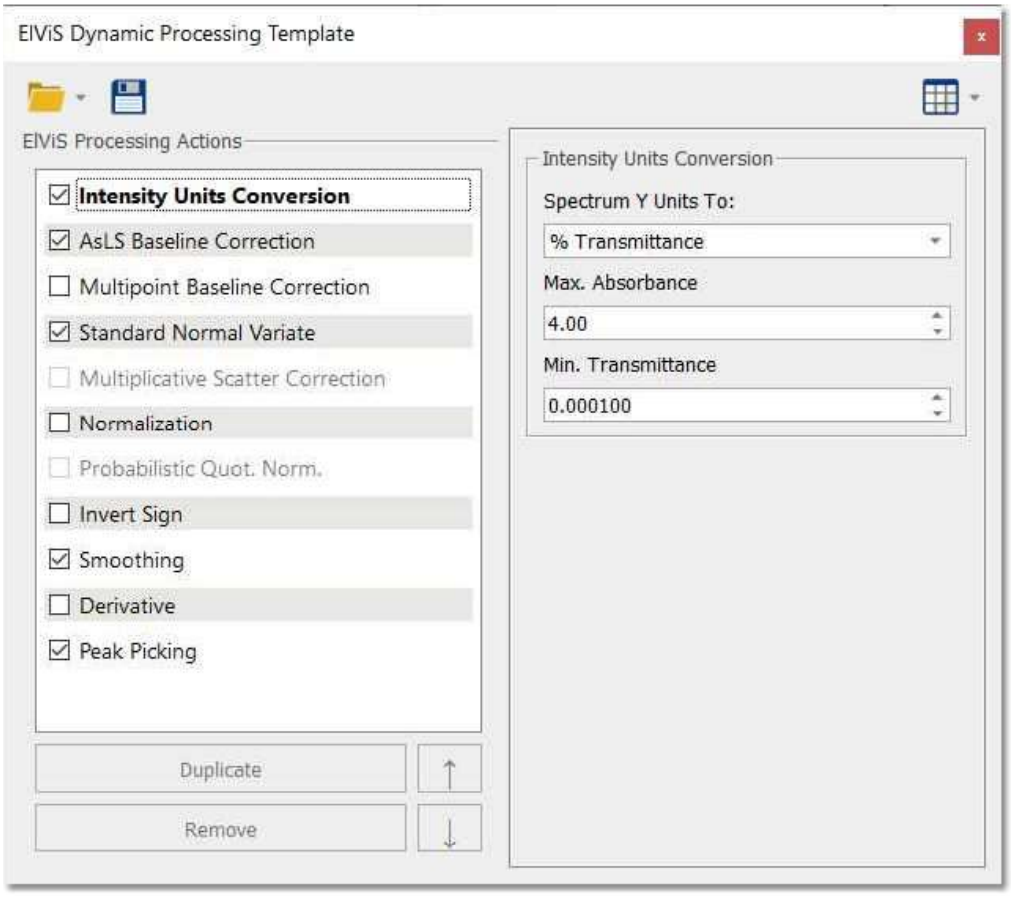
15.3.8 Processing Templates

Mnova includes a powerful and flexible feature which allows the user to easily automate the full processing of ElViS data sets. This is ideal for the batch processing of spectra of the same type, and can also incorporate analysis operations.

For example, let's say you have acquired 20 datasets which you want to process using the same processing operations. The procedure is:

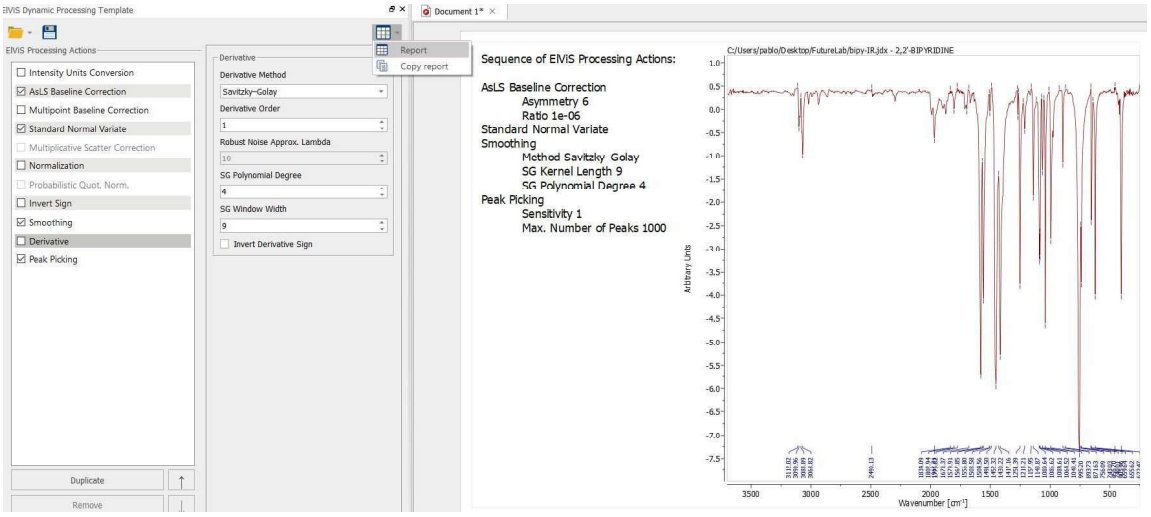
1. Load one spectrum and process it as desired.

2. Go to ***Processing/Processing Template***. The following dialog appears:



This dialog includes all the main processing features implemented in Mnova ElViS. From here, you can also change the units of your spectrum (Transmittance, Absorbance). You can choose the required options by selecting the applicable check boxes.

Clicking on the Report button will paste in your spectrum, the sequence of the processing features that you have applied:



3. Once you are happy with the result, save the script file to a Mnova Processing file (*.mnp) by clicking on the save button in the dialog box.

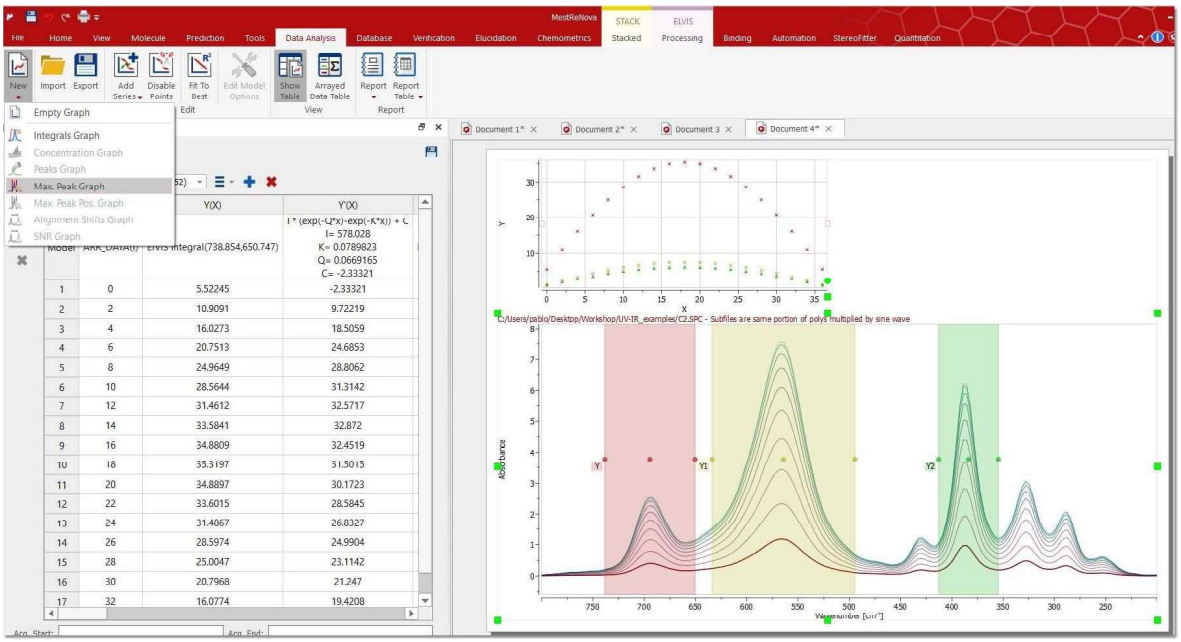
4. You can now apply this processing script file to any other spectrum/spectra by selecting the spectra you wish to process following this procedure (one or several spectra), and then opening the previously saved file by using the open command in the Processing Template dialog box.

A few helpful tips ...

1. If you want to apply a processing script to all the spectra opened in one document, first select all the spectra in all the pages.
2. Next you can simply go to ***Processing/Recent Processing Templates*** and select the appropriate processing file script.
3. Remember that only spectra acquired using the same conditions should be processed using a processing script file.

15.3.9 Data Analysis

You can use the Data Analysis feature with the ElViS module, just by loading your stack plot and following the menu 'Data analysis' to select the desired action from the 'New Graph' scroll down menu:

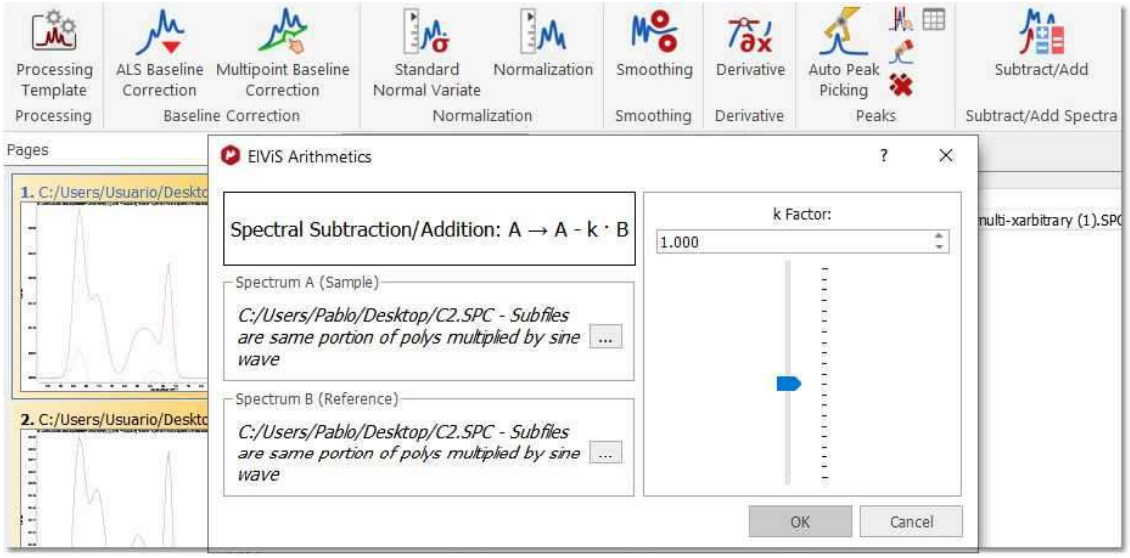


For further information about this feature, check this chapter.

15.3.10 Arithmetics

The ElViS arithmetics feature is available from the ElViS Processing tab ribbon (Subtract/Add button) or from the context menu (by right clicking on your ElViS spectrum).

When selected, a dialog is displayed with the ability to select the Sample Spectrum A (which can be a single spectrum or a stacked plot) and the reference B (which must be a single spectrum) and to adjust the factor *k* (coefficient). You can also select both pages from the page navigator before having clicked on the 'Subtract/Add' button and the Sample and Reference datasets will appear in the dialog:



Please bear in mind that both spectra must have the same number of points and units.

When working with a stack for Spectrum A (Sample), the subtraction will be applied to all the traces unless you have selected some spectra from the 'Stacked Items' Table.

NOTE

Use negative k to add a spectrum to the Sample one.