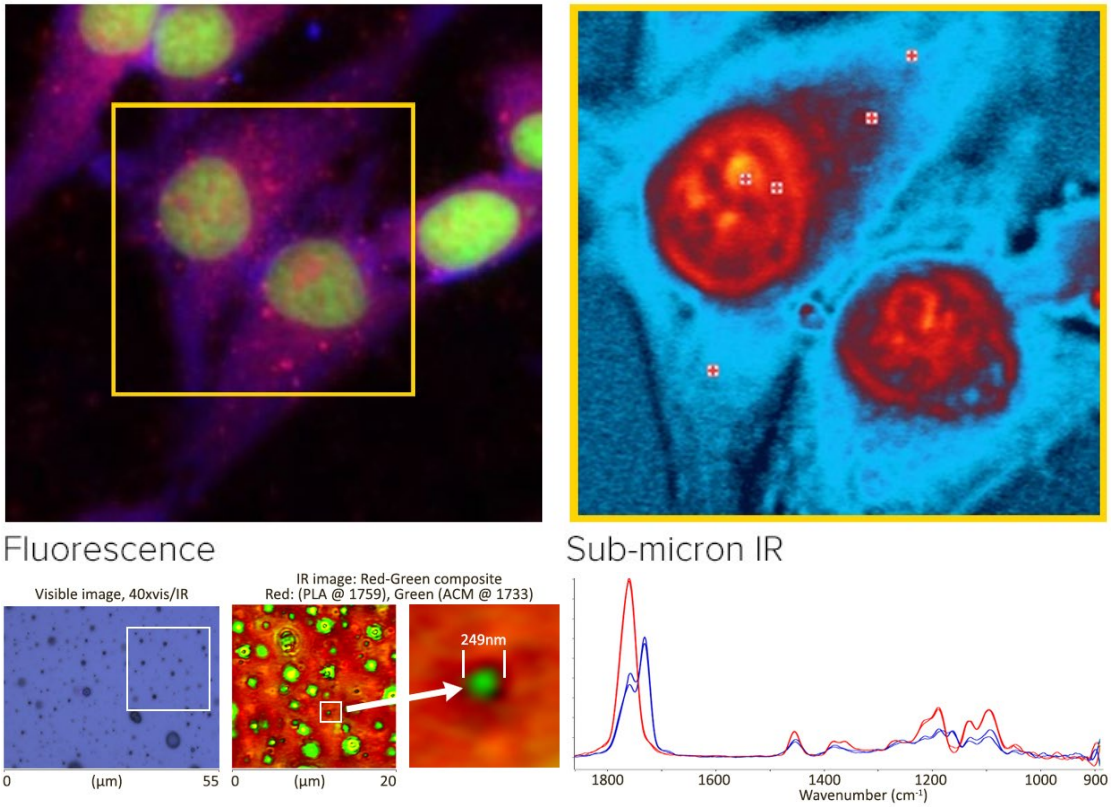
PHOTOTHERMAL
SPECTROSCOPY CORP

mIRage® *Software Manual*



Part #00-0037-02
Issued June 2023

Table of contents

Contents

Notices

Photothermal Spectroscopy Corp Office

For information on our latest products and more, see our web site at:

www.photothermal.com

Photothermal Spectroscopy Corp

325 Chapala St.

Santa Barbara, CA 93101

Email: support@photothermal.com

www.photothermal.com

Tel: (805) 730-3310

HELPLINE

For assistance with applications or instrument service and repairs, please call the Photothermal Spectroscopy Corp Help Desk at: (805) 845-6568 or email us at support@photothermal.com.

Feedback

Please send any comments, suggestions, feature requests, bug reports or any other feedback to support@photothermal.com.

Photothermal Spectroscopy Corp Patents and Trademarks

The mIRage® product line is covered by a large number of U.S. and international patents. For a current list, see www.photothermal.com/patents

mIRage® and FeatureFindIR™ are trademarks of Photothermal Spectroscopy Corp, 325 Chapala St., Santa Barbara, CA 93101

End User License Agreement

This copy of PTIR Studio ("the Software") and accompanying documentation is licensed and not sold. This Software is protected by copyright laws and treaties, as well as laws and treaties related to other forms of intellectual property. Photothermal Spectroscopy Corporation ("the Company") owns intellectual property rights in the Software. The Licensee's ("you" or "your") license to download, use, copy, or change the Software is subject to these rights and to all the terms and conditions of this End User License Agreement ("Agreement").

Acceptance

YOU ACCEPT AND AGREE TO BE BOUND BY THE TERMS OF THIS AGREEMENT BY DOWNLOADING THE SOFTWARE OR BY INSTALLING, USING, OR COPYING THE SOFTWARE. YOU MUST AGREE TO ALL OF THE TERMS OF THIS AGREEMENT BEFORE YOU WILL BE ALLOWED TO USE THE SOFTWARE. IF YOU DO NOT AGREE TO ALL OF THE TERMS OF THIS AGREEMENT, YOU MUST NOT INSTALL, USE, OR COPY THE SOFTWARE.

License Grant

This Agreement grants you a royalty-free, non-exclusive license to possess and to use a copy of the Software. You may install and use an unlimited number of copies of the Software on a shared computer or concurrently on different computers, and make multiple backup copies of the Software, solely for your business use. Copies of the Software may be distributed solely for your business use, as long as the Software is not sold or included in a product or package that intends to receive benefits through the inclusion of the Software.

Restrictions

You will not disassemble, decompile, or reverse engineer the Software, in whole or in part, except to the extent expressly permitted by law notwithstanding the limitation.
You may not transfer, loan, rent, lease or sell the Software or parts thereof either free of charge or against payment to any third party. If a transfer of the Software is unavoidable (e.g. by selling the product) this transfer can only be done by a representative officer of Photothermal Spectroscopy Corporation. You are obligated to inform the Company in time and in writing about the intended transfer.
Warranty Disclaimer
UNLESS OTHERWISE EXPLICITLY AGREED TO IN WRITING BY THE COMPANY, PHOTOTHERMAL SPECTROSCOPY CORPORATION MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED IN FACT OR IN LAW, INCLUDING, BUT NOT LIMITED TO, ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OTHER THAN AS SET FORTH IN THIS AGREEMENT OR IN THE LIMITED WARRANTY DOCUMENTS PROVIDED WITH PHOTOTHERMAL SPECTROSCOPY CORPORATION PRODUCTS.
Photothermal Spectroscopy Corporation makes no warranty that the Software will meet your requirements or operate under your specific conditions of use. Photothermal Spectroscopy Corporation makes no warranty that operation of the Software will be secure, error free, or free from interruption. YOU MUST DETERMINE WHETHER THE SOFTWARE SUFFICIENTLY MEETS YOUR REQUIREMENTS FOR SECURITY AND UNINTERRUPTABILITY. YOU BEAR SOLE RESPONSIBILITY AND ALL LIABILITY FOR ANY LOSS INCURRED DUE TO FAILURE OF THE SOFTWARE TO MEET YOUR REQUIREMENTS. PHOTOTHERMAL SPECTROSCOPY CORPORATION WILL NOT, UNDER ANY

CIRCUMSTANCES, BE RESPONSIBLE OR LIABLE FOR THE LOSS OF DATA ON ANY COMPUTER OR INFORMATION STORAGE DEVICE.
The Company shall not be liable for any special, incidental, indirect or consequential damage of any kind or other damages whatsoever (including, but not limited to damages for loss of business profits, business interruptions, or other pecuniary loss) arising out of or in connection with the use or non-use of the Software, even if the Company has been notified of the possibility of such damages.

Governing Law and General Provisions

This Agreement will be governed by the laws of the State of California, U.S.A., excluding the application of its conflicts of law rules. This Agreement will not be governed by the United Nations Convention on Contracts for the International Sale of Goods, the application of which is expressly excluded. If any part of this Agreement is found void and unenforceable, it will not affect the validity of the balance of the Agreement, which shall remain valid and enforceable according to its terms. You agree that the Program will not be shipped, transferred or exported into any country or used in any manner prohibited by the United States Export Administration Act or any other export laws, restrictions or regulations. This Agreement shall automatically terminate upon failure by you to comply with its terms. All copies of the Software under your control shall be destroyed upon termination. This Agreement may only be modified in writing signed by an authorized officer of Photothermal Spectroscopy Corporation.

Chapter 1

1. PTIR Studio Software Overview

PTIR Studio is the software used to control the mIRage and mIRage-LS microscopes and review/analyze acquired data. This manual is focused on tools used to filter, analyze, display and export acquired data. See the appropriate product manual for details on microscope operation.

1.1. Installing PTIR Studio

PTIR Studio will be installed by PSC on your mIRage or mIRage-LS system at the time of system installation. PSC will be in contact regarding periodic software updates. It is also possible to install PTIR Studio on separate computers for offline analysis and data processing. Contact PSC tech support to get a download link for PTIR Studio for offline analysis.

To install PTIR Studio for offline analysis, click on the setup.exe file.

IMPORTANT: Do not install a new version of PTIR Studio on a mIRage microscope system without specific guidance from PSC. Installation of new software versions may require special settings for your specific system configuration.

1.2. Starting PTIR Studio

Double-click the PTIR Studio icon 

to open the software.

1.3. PTIR Studio Software Overview

The interface has two windows: the Document window (usually on the left) and the Live window (usually on the right). The two windows are positioned independently on each of the two monitors. The Document window (Figure 1-1) is where acquired spectra and images will be displayed for analysis. The Live window (Figure 1-2) is used for live microscope video, sample navigation, focusing, real-time controls, settings and system monitoring. The Live window functions are described in the manual for your mIRage microscope system.

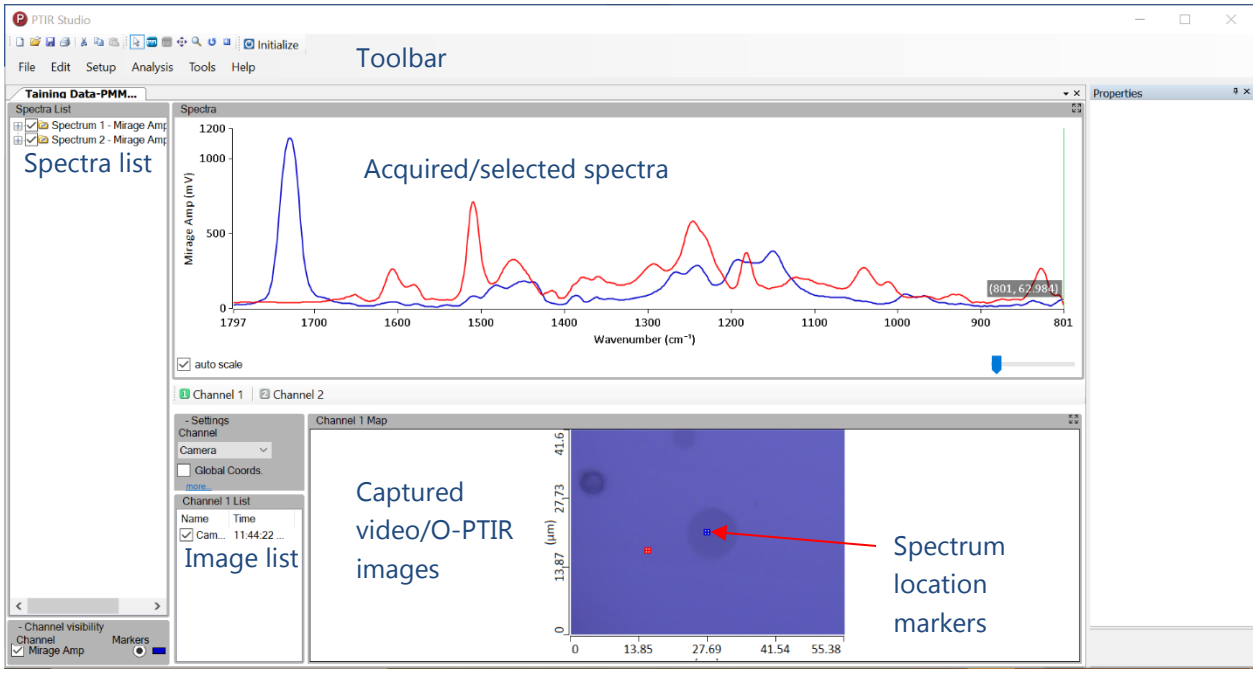


Figure 1-1. The Document window (Left monitor)

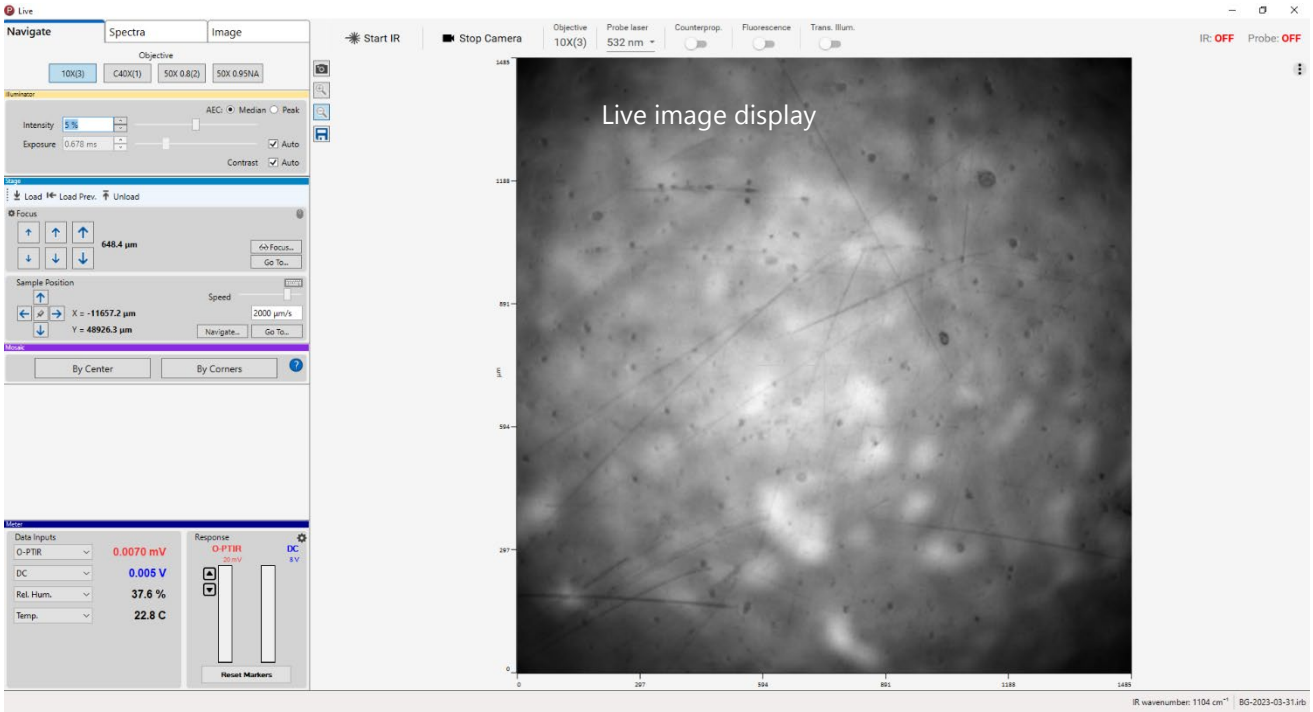


Figure 1-2. The Live window (right monitor), described in separate microscope manual

1.3.1. Document Window

The document window is comprised of several sections as indicated in the figure below.

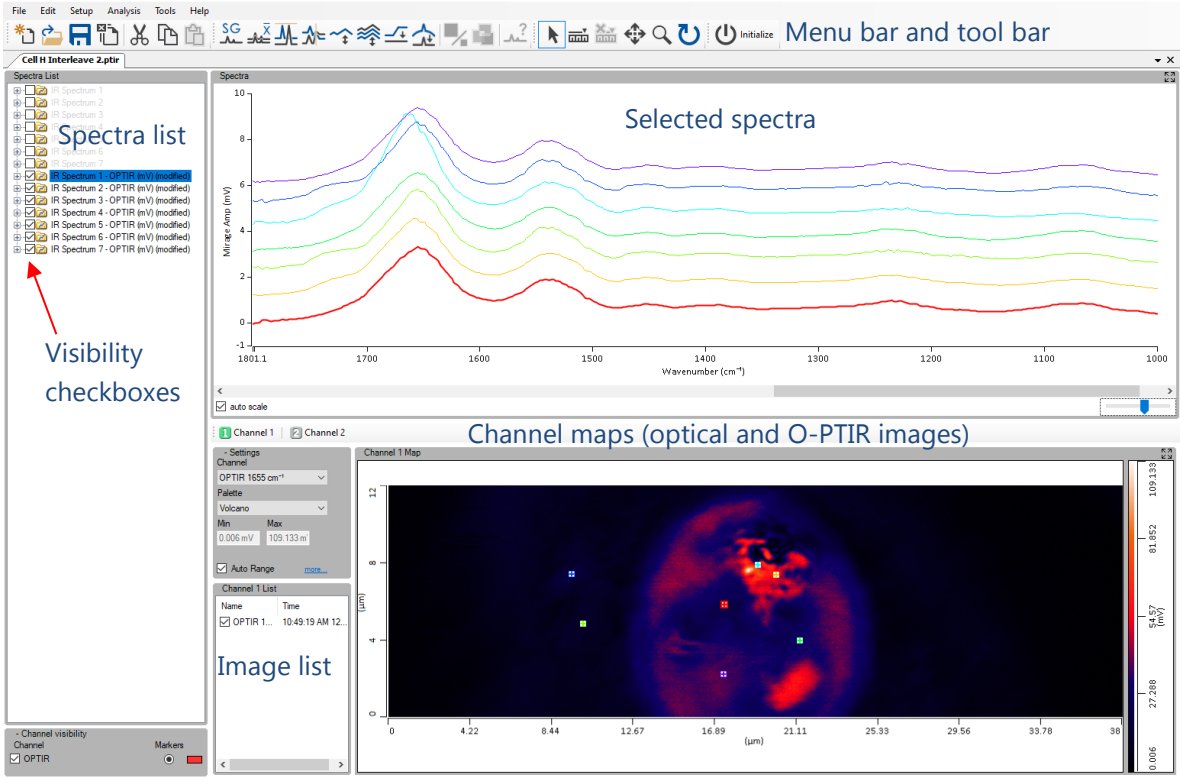


Figure 1-3. The Document Window

As O-PTIR and/or Raman spectra are acquired, the acquired spectra are plotted in the Spectra panel. The name of each spectrum appears as a folder in the list along the left edge of the window. Spectra which are checked in the list are displayed in the graph. Individual data channels of a spectrum can be shown or hid in the graph as desired using the list. To delete a spectrum from a document, select it in the list and press Delete on the keyboard or right-click and select delete.

Each document is a tab in the window. Multiple documents can be open simultaneously. Select File/Open or File/New to open or create a document. A document is closed by clicking the Close (X) button at the right edge of the window below the upper toolbar.

Right Click Commands for Spectra

Right clicking on one or more selected spectra opens a menu that supports copy, cut, paste, rename, delete as well as various functions controlling the selection and display of spectra.

Recolor—this command enables automatic recoloring of selected spectra. This is described in more detail in Sec. 2.13

Expand/Collapse—Spectra are displayed in a tree view where in some cases more than one data type can be listed with each spectral acquisition. Expand/Collapse commands show/hide the acquired signals for each spectrum. In most cases it is not necessary to use the Expand/Collapse commands.

Show/Hide—these commands show or hide selected spectra. In the case of the hide command, the user has the option of hiding either the selected or unselected spectra.

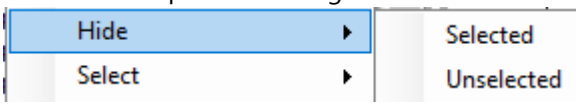


Figure 1-4. Hide options

Select—this command can be used to select certain spectra to display, copy/cut/delete, and/or select for analysis. This command will select either all spectra or just the currently displayed spectra. In general this command is most useful for selecting all spectra. To select a subset of spectra use the Shift or CTRL keys to select a range of spectra.

Figure 1-5. Select Options

Visibility—this command toggles the visibility setting for spectra between all spectra and selected spectra. This command is useful for displaying a smaller subset of spectra without having to manually uncheck lots of other spectra.

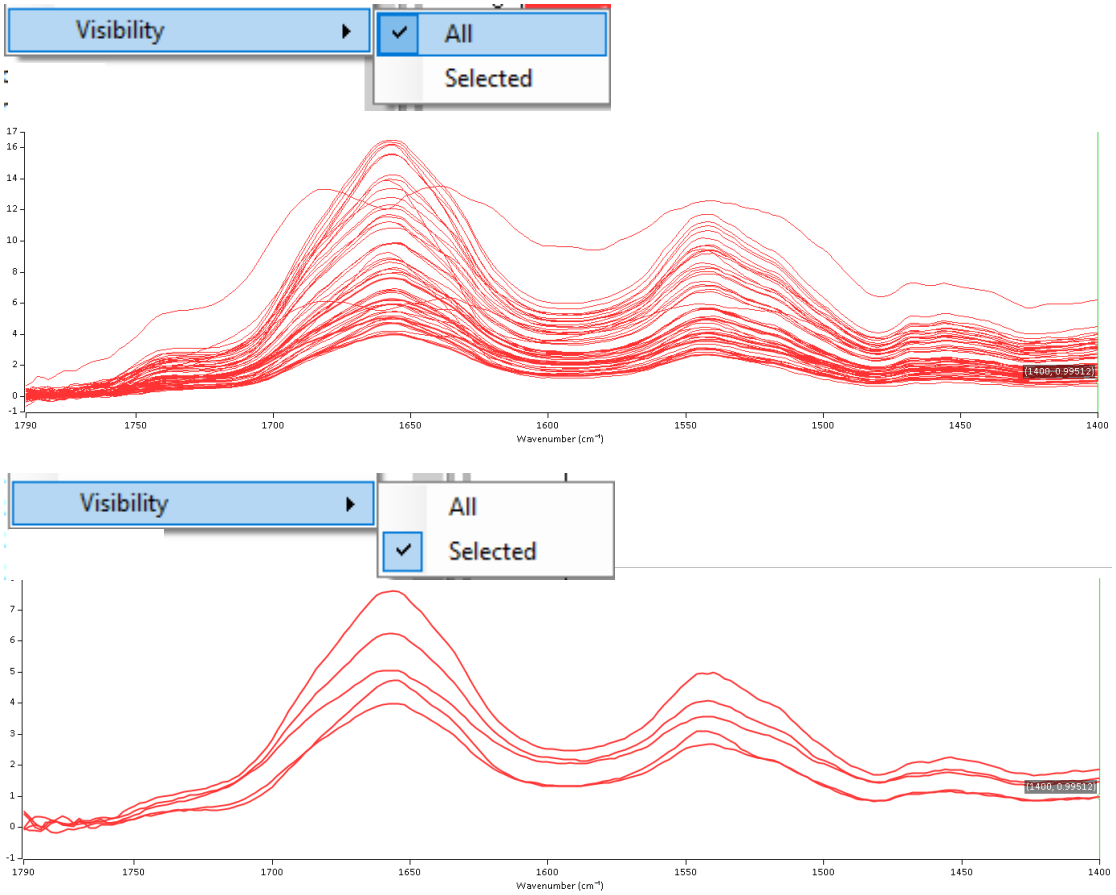


Figure 1-6. Spectra visibility options

Channel Image Map

The Image Map on the right of the window does not appear until an image is written to the document. The Image Map displays all the images of a certain channel (i.e. O-PTIR or DC) in a spatial map.

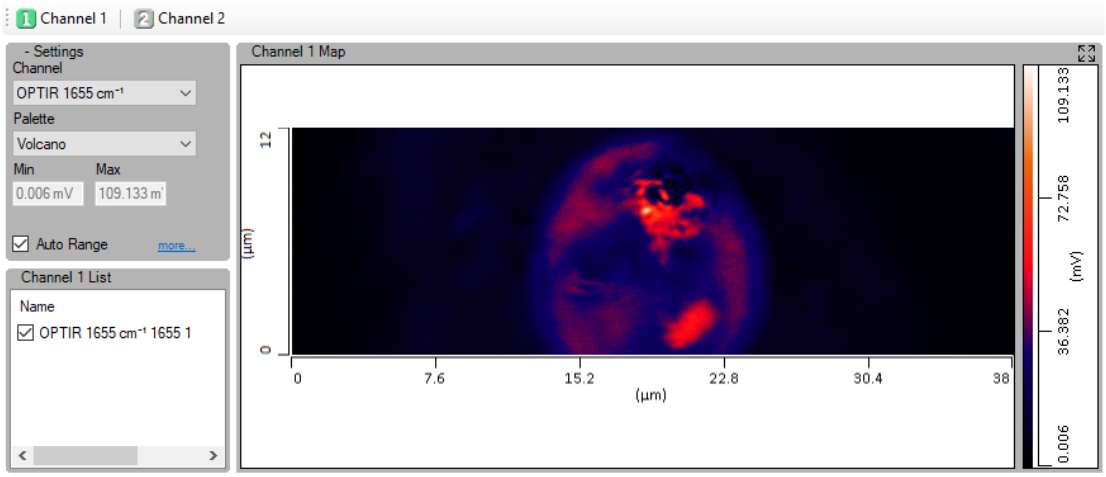


Figure 1-7. Channel Image Map

A list of the images is directly above the map. The top image in the list is the "top layer" of the map. For overlapping images, the image higher up in the list is displayed in the map. When a new image is captured, it is added at the top of the list. Click and drag on an image in the list to reorder it.

Global Coords. – By default, the lower left corner of the map is set at (0, 0). When Global Coordinates are used the true x,y scanner positions are displayed.

Measurement Toolbar

Figure 1-8. The Measurement Toolbar on the Document Window

The buttons put the cursor into different modes which are used on the Spectra Graph and the Image Map.


Pointer – Click and drag a vertical cursor on the Spectra.


Ruler – Draw a measurement line on the Image Map.


Clear Ruler –Erase all rulers from the Image Map

Pan – Click and drag on the Spectra Graph or Image Map to move the field of view around. (Or hold the Ctrl key while dragging cursor)

Zoom In – Draw a box on the Spectra Graph or Image Map to zoom in on that data. (Or press the Shift key while drawing a box on the graph).

Reset Zoom and Pan – Reset the Spectra Graph and Image Map to their full views, clearing any zooms or pans.

1.4. Selecting spectra and images

Spectra are selected for analysis by clicking on the spectrum name(s) in the spectra list or by clicking on a spectrum trace in the Spectra graph panel. Images are selected by clicking on the image name in the image channel list. Multiple spectra/images can be selected when appropriate using the shift control buttons.

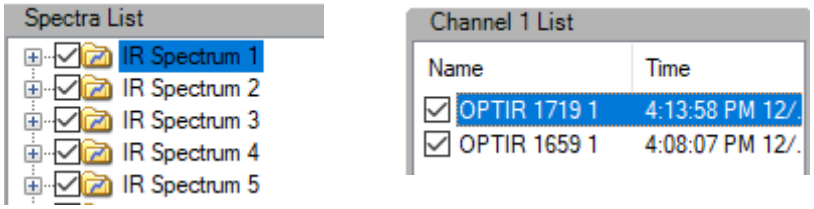


Figure 1-6: Selecting data for analysis. Left shows a spectrum selected; right shows an image selected.

1.5. Exporting data

To export data, select File/Export. Data can be exported in the formats shown in the figure below.

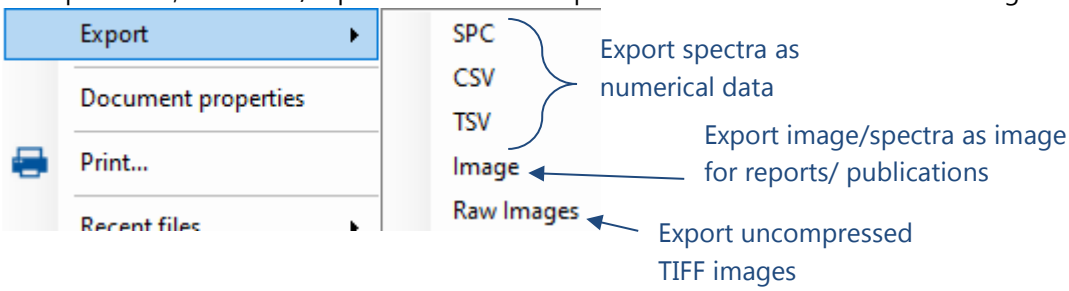


Figure 1-9. Available export formats

1.5.1. Exporting data in numerical format

To export spectra or image data in a numerical format, select the spectra or images you wish to export and then click on File/Export and then choose one of the supported export formats, SPC, CSV or TSV.

Export Options

There are several options to customize export for spectrum data. The Export Options dialog has a checkbox to include column headers. There are three options to specify which data to include in the export:
Same as document - exports only the spectra and data channels **currently displayed** in the graph (i.e. the export excludes spectra that have been hidden.)
All data - exports all the spectra (with all their data channels) in the document.
Selection only - exports only the spectra highlighted in the list.

Additional choices include the option to export channel headers, include the IR power background, and whether to export the channels in row or column format. Not all options are available for every export format.

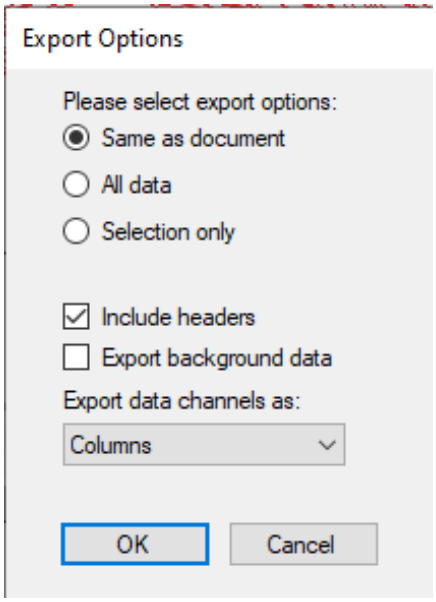


Figure 1-7: The Export Options menu for spectra CSV and TSV export.

SPC Export

The SPC file format is a commonly used file format for spectroscopic data. Use this export option if you are analyzing O-PTIR and/or Raman spectra in a third-party software package that prefers SPC data format. SPC export is only applicable to spectra, not images.

CSV/TSV Export

These functions export IR/Raman spectra or IR/camera images into a text file where each value is separated by either a comma (CSV) or a tab (TSV). The text file can be used to import the spectra or image into other programs such as Excel.
The type of data, image or spectrum, to be exported is determined by which kind of data is currently selected (highlighted within their respective lists). Choose the data before opening the export function. To export image data, click the desired channel in the Channel Map image list. To export spectra data, click its name in the list. Use shift-click and ctrl-click to highlight multiple spectra.

1.5.2. Export to Image

Export to Image creates a graphics file of the Image Map and/or the Spectra graph. There are several lay out and sizing options set in the "Export to Image" preview window.

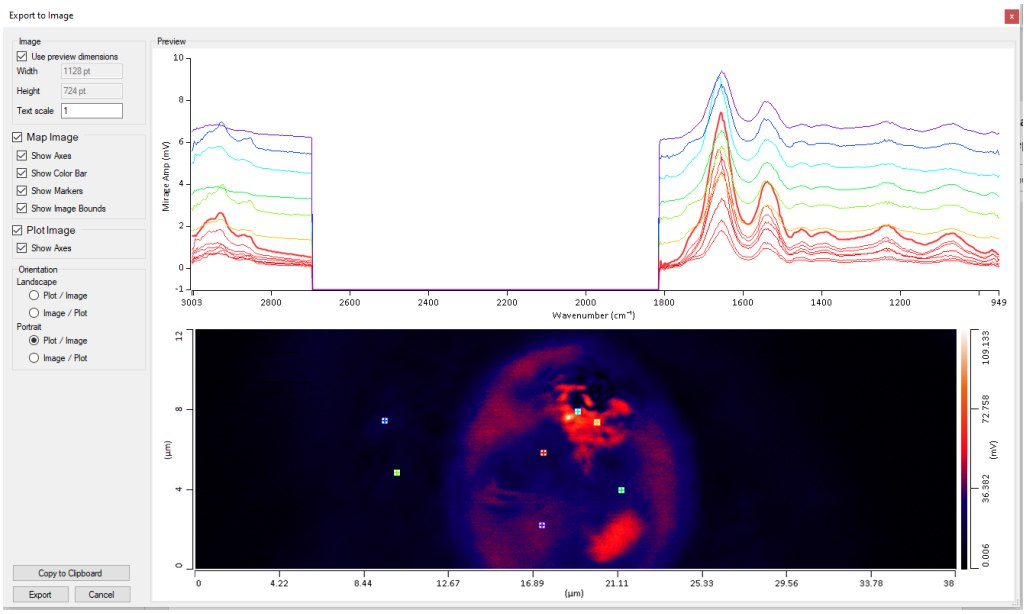


Figure 1-8: The Export to Image preview window.

1.5.3. Spectra/image metadata

Measurement settings for each image/spectrum are automatically saved with each acquisition. To view the metadata, select a spectrum/image and then go to File/Document properties. A Properties box will open to the right of the displayed spectrum/image containing all settings used. There is also a Notes field where additional sample/measurement details can be entered manually.

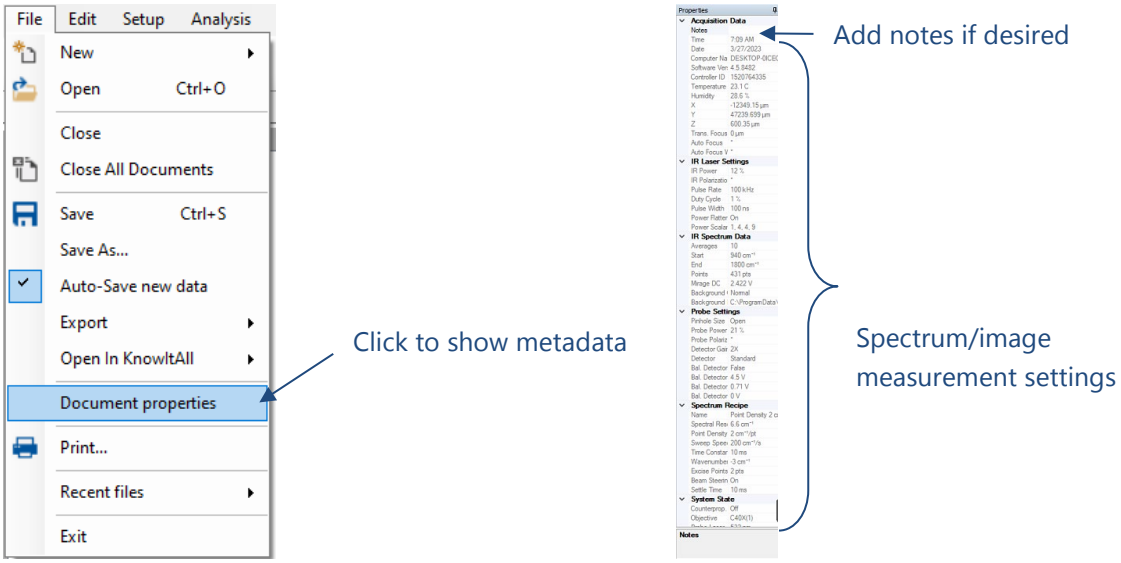


Figure 1-10. Image/spectrum metadata available under document properties

When a single data channel of one of the spectra is highlighted in the list, the Properties menu displays information that includes how that data is displayed on the graph. All the graph related properties are editable. Select the property you want to edit and then click on the drop-down arrow to see the available options (except for Point Size which is edited directly). 'Color' and 'Style' set the characteristics of the line drawing of the data. The 'Point' properties are the display options for the data points themselves, which are not shown by default (Point style = None).

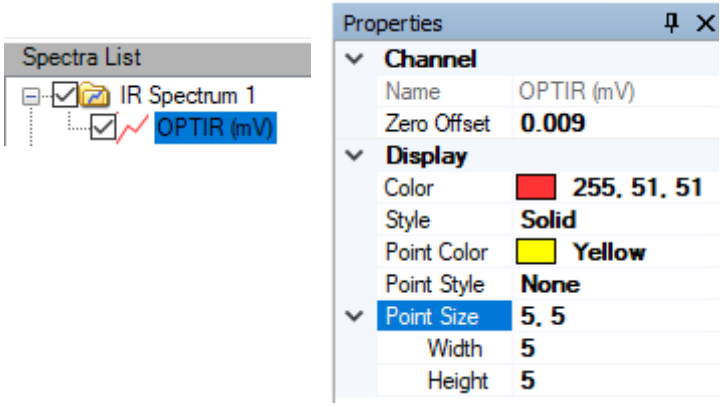


Figure 1-11: The Properties menu when an individual data channel is highlighted within a spectrum.

The graphing properties of a single data channel can be applied to other data channels in the Spectra list. In the list, right-click the data channel with the desired formatting and select "Copy format". Now select the channels to be formatted. Right-click and select "Paste format".

When a single data channel of one of the Image Maps is highlighted in the 'Image Map Images' list, the Properties menu displays information about that acquired image.

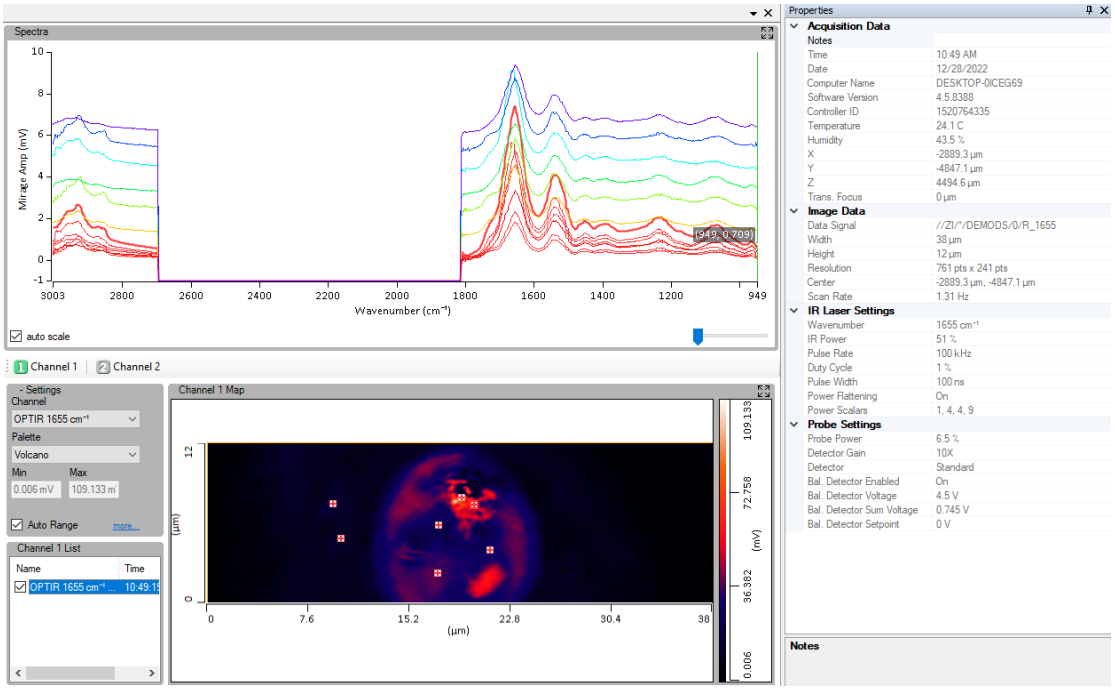


Figure 1-12: The Properties box showing information (metadata) about an acquired image.

1.6. Edit Menu

The Edit menu has the standard cut/copy/paste and delete functions that behave similar to normal Windows functions, except that they can only be used to cut/copy/paste images and/or spectra between documents in PTIR Studio. To export an image and/or spectrum into another application, use the Export function on the File menu (or where available using the right mouse click).

1.7. Help Menu

The Help/About command displays the version of software and a link to email Photothermal Spectroscopy Corp' support as well as the option to open system and software documentation.

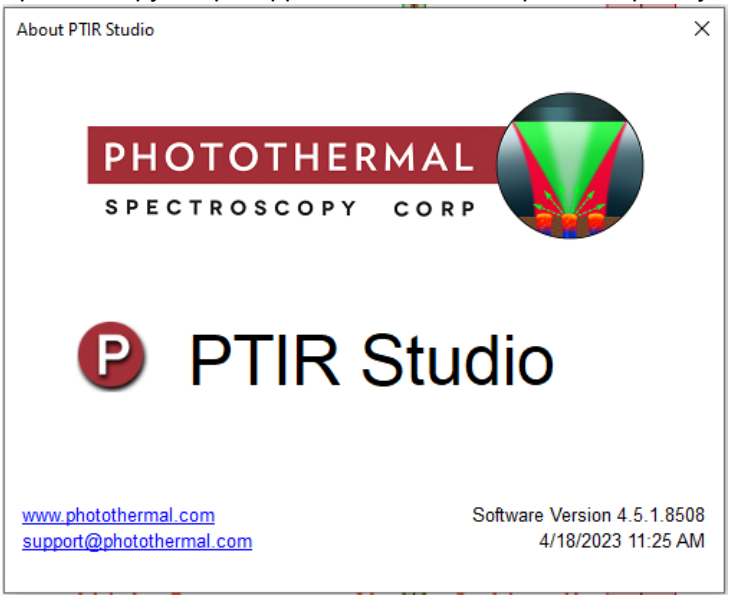
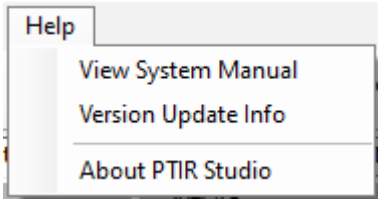


Figure 1-11. The Help/About window

1.8. Version Update Info

Help Version Update Info will display a summary of new features implemented in the software since the last release.



Chapter 2

2. Spectrum Modify and Analysis Functions

There are various analysis functions that modify or measure and analyze O-PTIR and Raman data.

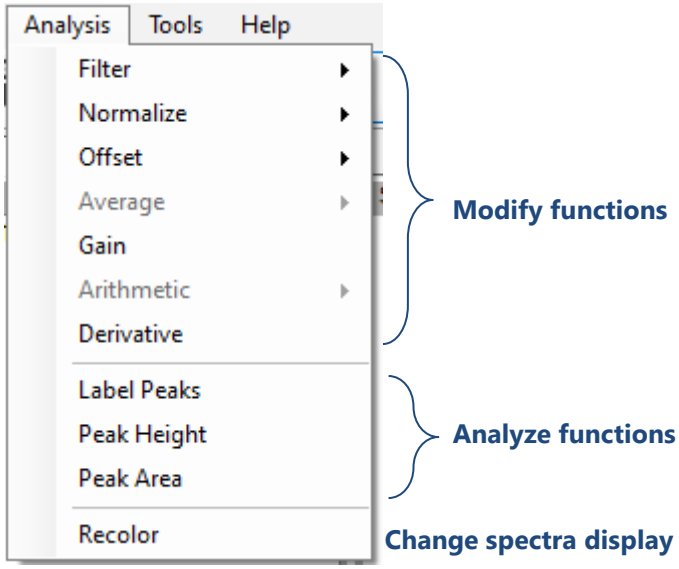


Figure 2-1. Spectrum modify, analysis, and display functions

To access these functions first select the data to be analyzed by clicking on it in the Spectra list at the left of the document and then applicable modify/analysis functions will be available under the Analysis selection on the menu bar.

2.1. General guidelines about modify functions

The functions that are listed in Figure 2-1 as "Modify functions" operate on the data in a way to change the data. Here are some guidelines that apply to use all filter functions (Figure 2-2).

1) Always check the Make a copy check box to avoid overwriting original raw data.
2) Change the Output Color in the dropdown menu at top right of the modify function to be able to easily distinguish input and output spectra.
3) If desired toggle the Show display options to show the Input & Output, Input Only or Output Only.
4) If you wish to save an image of the modified spectra, click the Image save button
5) To load the modified data into the current document, click on Accept. If you are not satisfied with the results, click on Cancel.

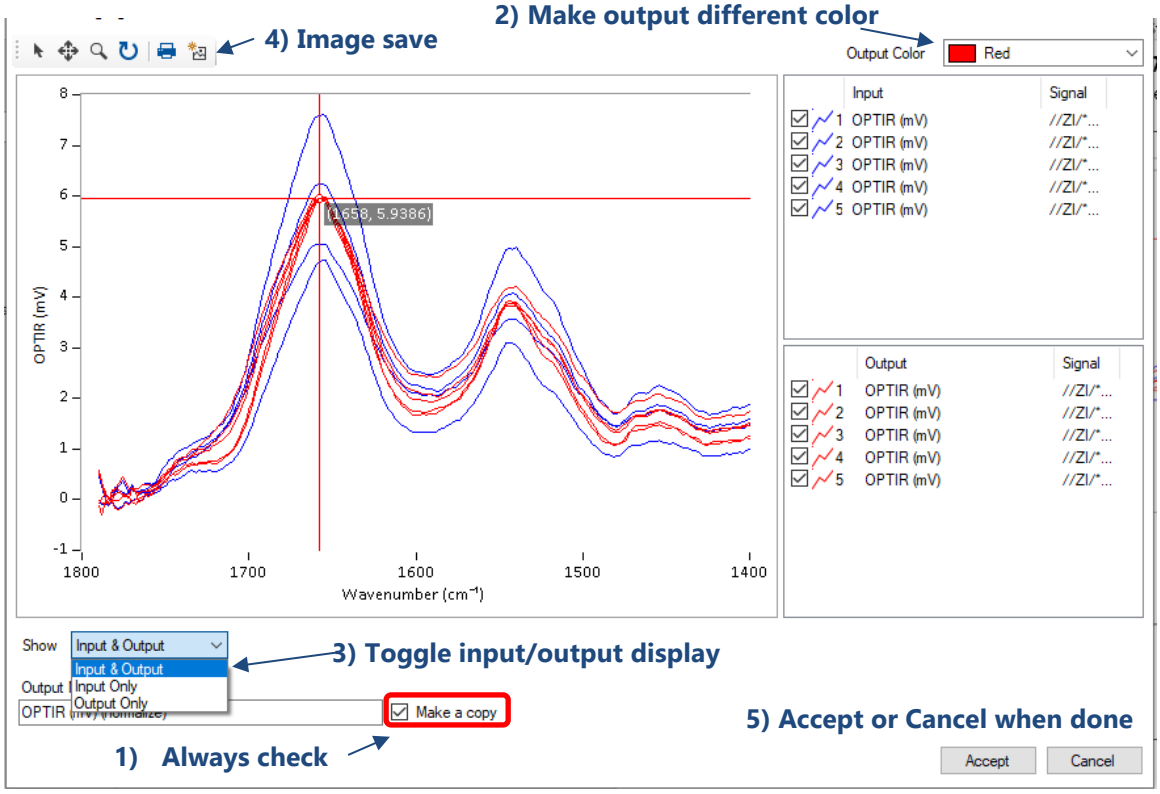


Figure 2-2. General guidelines about filter functions

The remainder of this chapter documents how to use the various modify and analysis functions.

2.2. Filter Functions

Filter functions are used to modify the spectral data (e.g. by smoothing) or to change the display format of selected spectra. Some filter functions are available via icons on the toolbar. When available, the associated toolbar icon for each filter function is shown next to the filter name heading in the sections below.

Figure 2-3. Example filter toolbar icons

All filter functions are available under the Analysis/Filter menu choice as shown below.

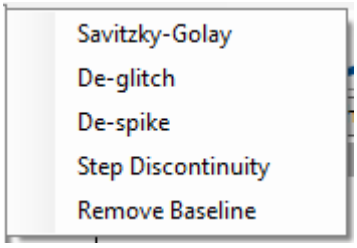


Figure 2-4. Spectrum filter functions

Each filter function is described in more detail below.

2.2.1. Savitzky-Golay

This function applies a Savitzky-Golay filter to one or more spectra for the purpose of smoothing the data while preserving peak positions. A least squares polynomial fit is made for each successive data point using its adjacent data points. The Polynomial Order and the number of adjacent Side Points used in the fits are specified by the user. Good starting values to smooth out small noise without shifting IR absorption peaks are Polynomial Order = 3 and Side Points = 5. Increase the polynomial order and/or Side Points to increase the strength of the filtering. Always keep Make a Copy selected to avoid overwriting your original data.

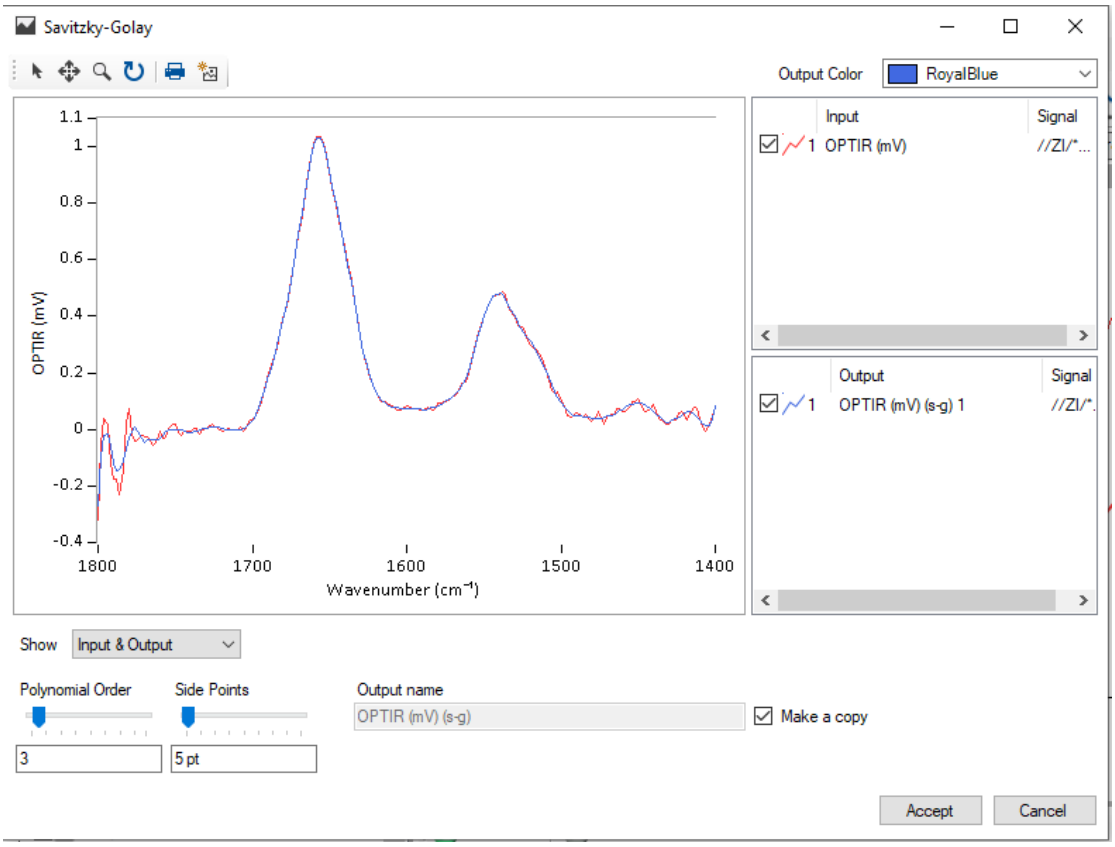


Figure 2-5. Savitzy-Golay smoothing function

2.2.2. De-glitch

The De-Glitch function is used to remove and interpolate over spurious regions of data. This function is rarely used and generally it is desirable and achievable to adjust acquisition settings to avoid data glitches that would require this filter. If used, it identifies regions of data where there are high excursions from surrounding data and replaces the identified glitch data with interpolated data from surrounding data points. This filter should be used with caution as it can also change the shape/amplitude of real absorption peaks if used improperly.

To use this filter function, click on a spectrum and then select Analysis/Filter/De-glitch. Adjust the Intensity slider to adjust the width window used to detect data glitches. Inclusion or exclusion zones can be selected to specifically include or exclude data regions from glitch removal.

2.2.3. De-spike

The De-spike tool is used to remove isolated spikes in the data. To use this filter function, click on a spectrum and then choose Analysis/Filter/De-spike. Adjust the Threshold value until the data spike is eliminated. Note that this feature should be used with caution as well as it can alter or eliminate real data peaks if misused.

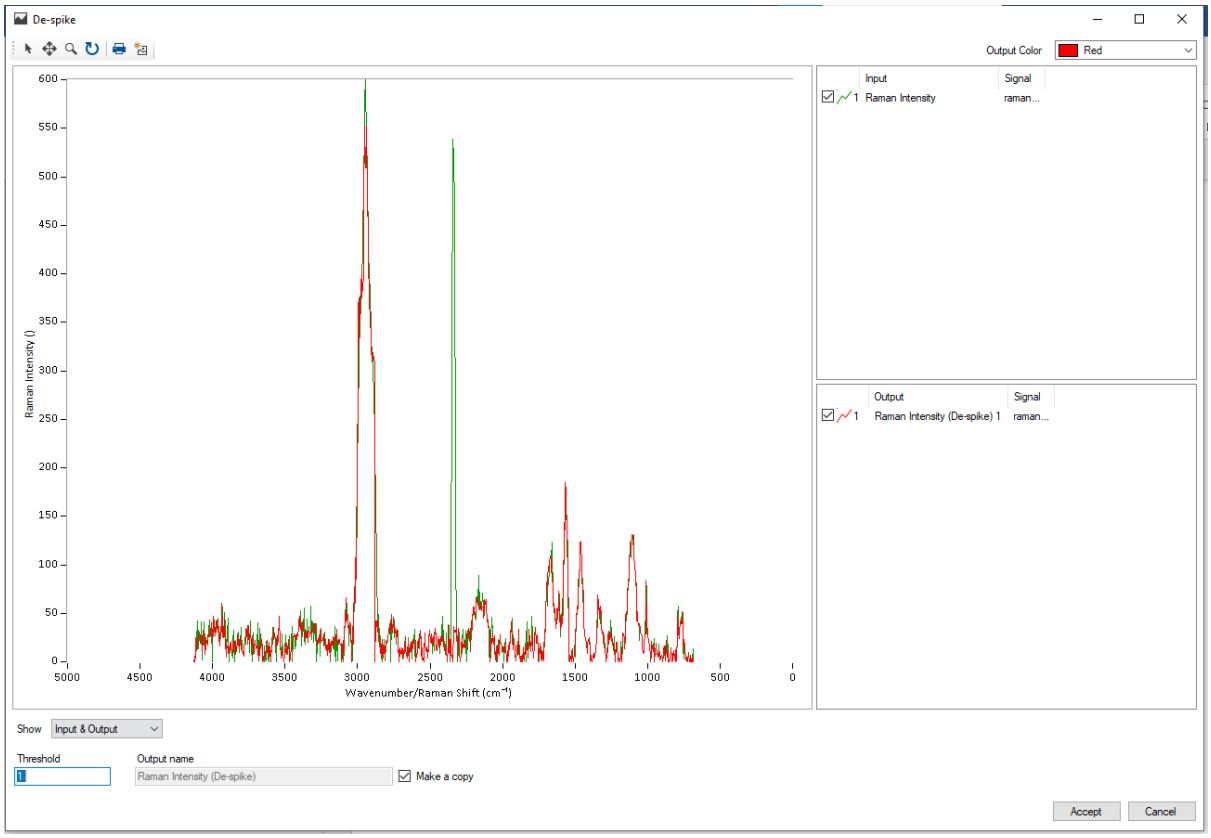


Figure 2-6. De-spike filter function

2.2.4. Step Discontinuity

The Step Discontinuity filter is used to remove level shifts in spectra especially associated with transitions across different stages of a QCL. In some cases a focus difference between the different QCL chips can cause different O-PTIR sensitivity for different QCL stages, especially if the background used for the spectrum is not recent or the IR/probe beam focus is different than for the background measurement. A step discontinuity can be removed by rescaling a portion of the spectrum to make the spectrum continuous at a selected wavenumber. To use the Step Discontinuity Filter, take the following steps:

1) Highlight an spectrum in the spectra list.
2) Select Analysis/Filter/Step Discontinuity on the Document Window menu bar.
3) Click on Add to add a cursor
4) Slide the cursor to overlap the discontinuity
5) Make sure that Make a Copy is checked (to avoid overwriting original data)
6) If satisfied with the results, click on Accept. If not click on Cancel.

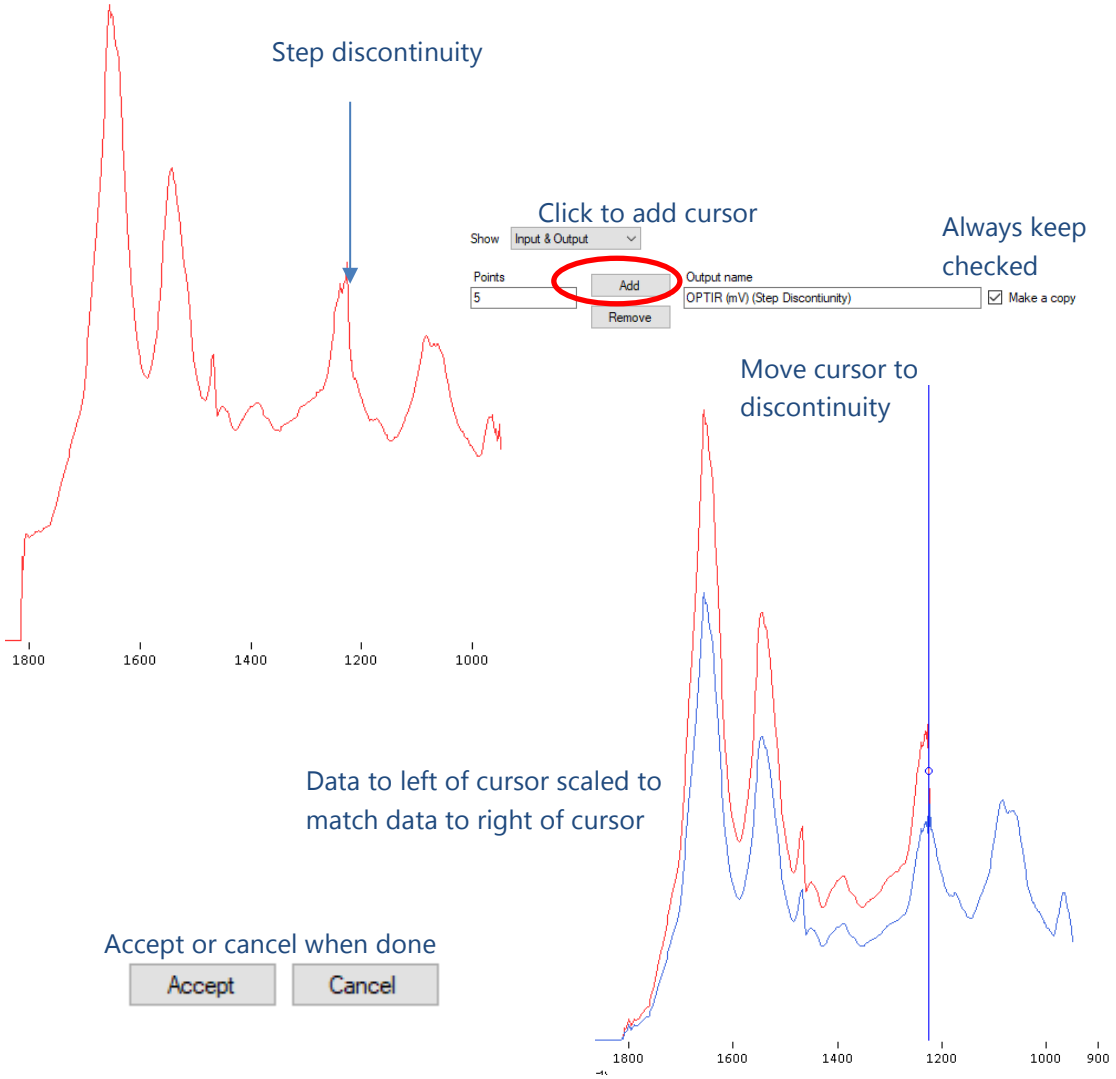


Figure 2-7. Step Discontinuity filter tool

2.2.5. Remove Baseline

To use this filter, click on a spectrum in the Spectra List and then select Analysis/Filter/Remove Baseline. With the Strength parameter set to 1, the Remove Baseline subtracts any DC offset and linear slope from the selected spectrum. Increasing the Strength will remove higher order features.

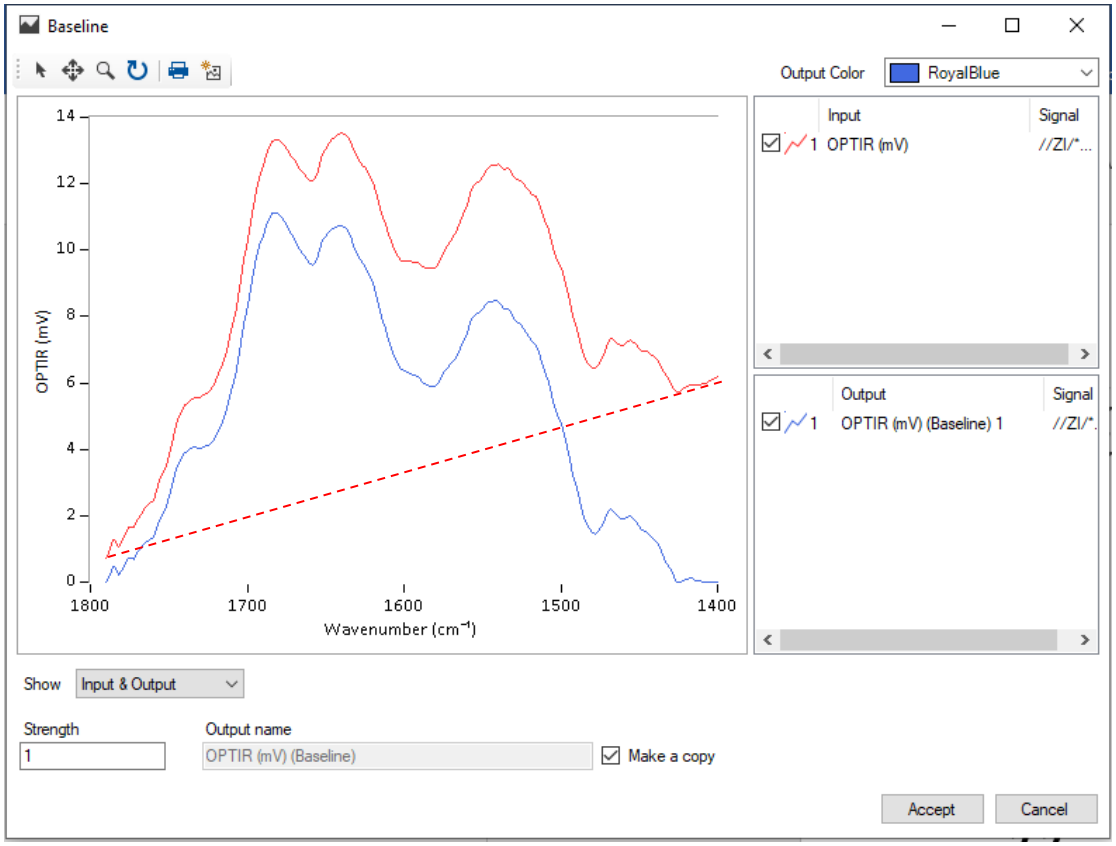


Figure 2-8. Remove Baseline filter tool

2.3. Normalize

The Normalize functions are used to adjust the relative scale for a selection of spectra. There are two choices for Normalize – Constant and Converge. Normalize-Constant is used to normalize the maximum of all spectra to a common value (usually 1), whereas Normalize-Converge is used to normalized all spectra to the same value at a selected wavenumber.

2.3.1. Normalize/Constant

This function is used to take one or a number of data sets and scale and offset them such that the minimum value in the plot is set to 0 and the maximum value in the plot is set to a value determined by the user. As with the above modify function, the user needs to set a value that the data will be normalized to, the output name and the color, and then choose whether to overwrite the data or make a copy.

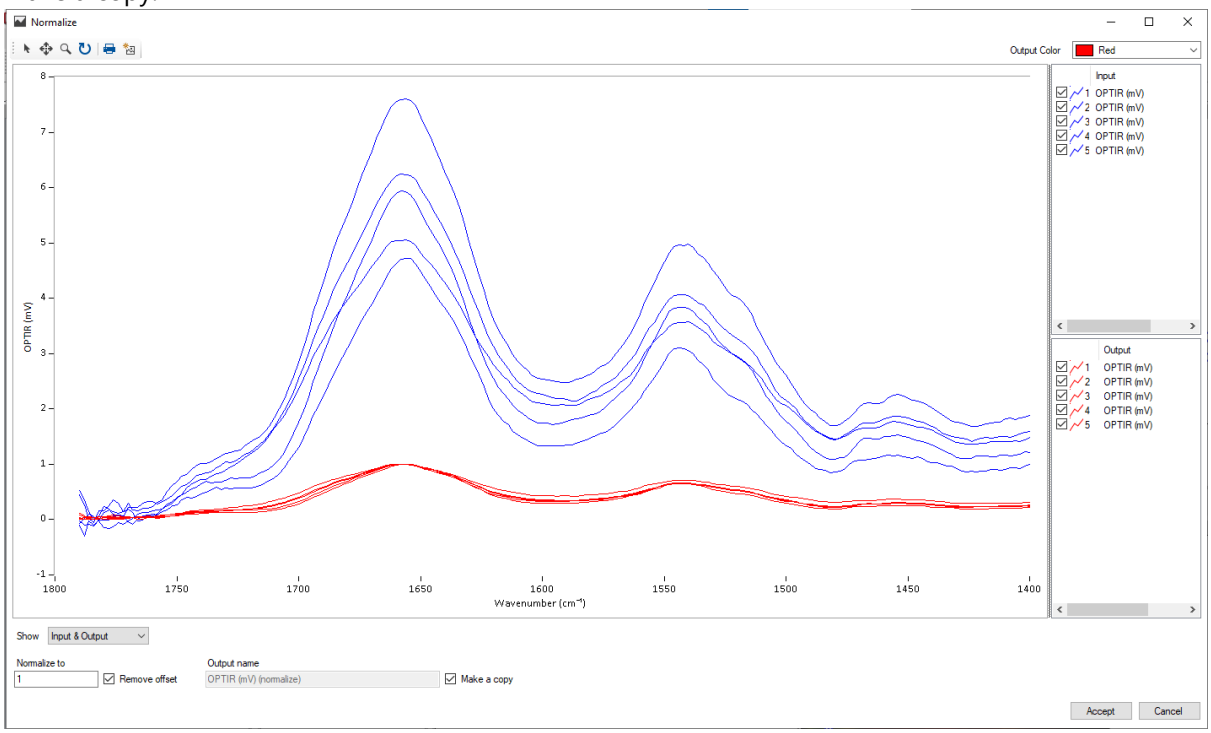


Figure 2-9. Normalize-Constant filter function

2.3.2. Normalize/Converge

Normalize/Converge is very similar except that the user selects a position along the x axis, and the plots are scaled so that their corresponding y values are equal at that position. The x position is selected by dragging a vertical cursor to the desired location. For O-PTIR and Raman spectra normalizing may be used in some cases to compensate for effects on the amplitude due to variations in sample thickness or mechanical properties. It is generally most useful to normalize spectra at a specific wavenumber, so Normalize/Converge is often the appropriate choice.

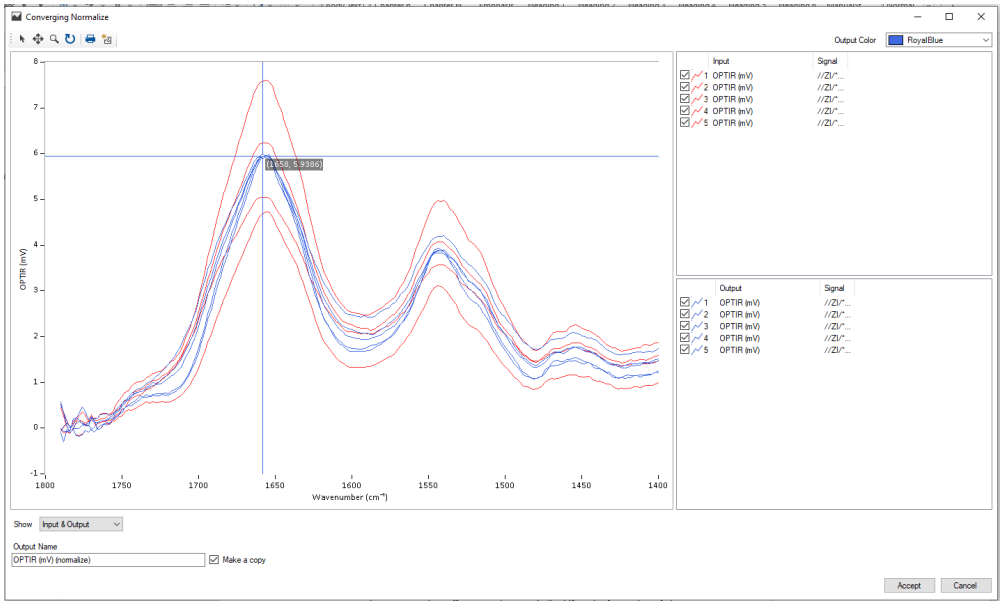


Figure 2-10. Normalize/Converge

2.4. Offset

The Offset function has three capabilities. It can be used to offset one or more data sets by a constant value by using the Offset/Constant selection. All of the plots will be offset by the same amount. The user can select Offset/Cascade to vertically shift each of a number of plots by a fixed amount at a selected horizontal value. Cascade is generally used for display purposes, to make multiple data sets easier to view and qualitatively compare.

2.4.1. Offset-Cascade 

Applies a user selectable offset between each spectrum. Used to enhance spectra visibility.

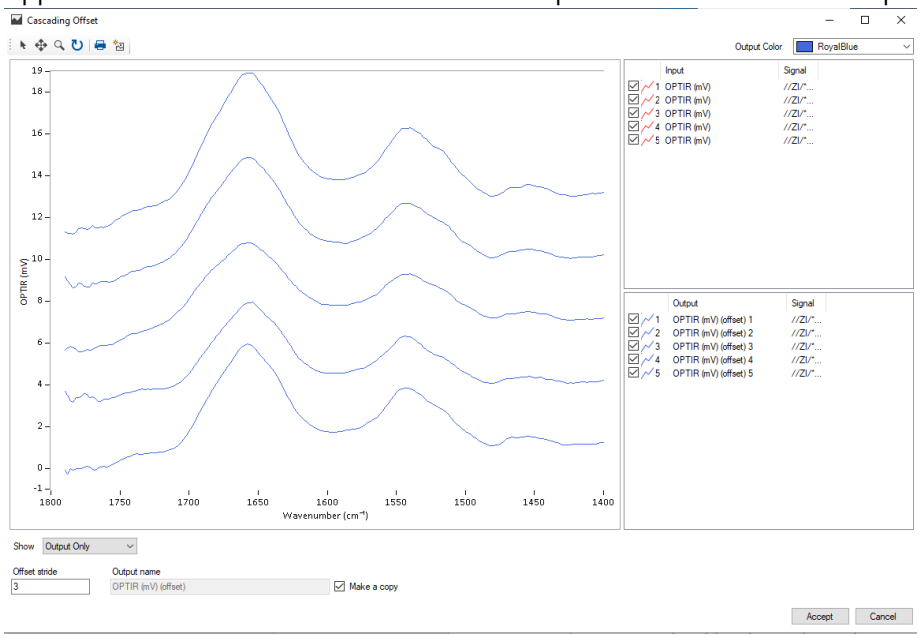


Figure 2-11. Offset Cascade

2.4.2. Offset-Converge

This function converges the spectra to have a common offset at a selected wavenumber. To use this function, click to select a range of spectra, then select Analysis/Offset/Converge from the menu bar. Move the cursor to the wavenumber where you would like to have the spectra have a common offset. Click Accept when done.

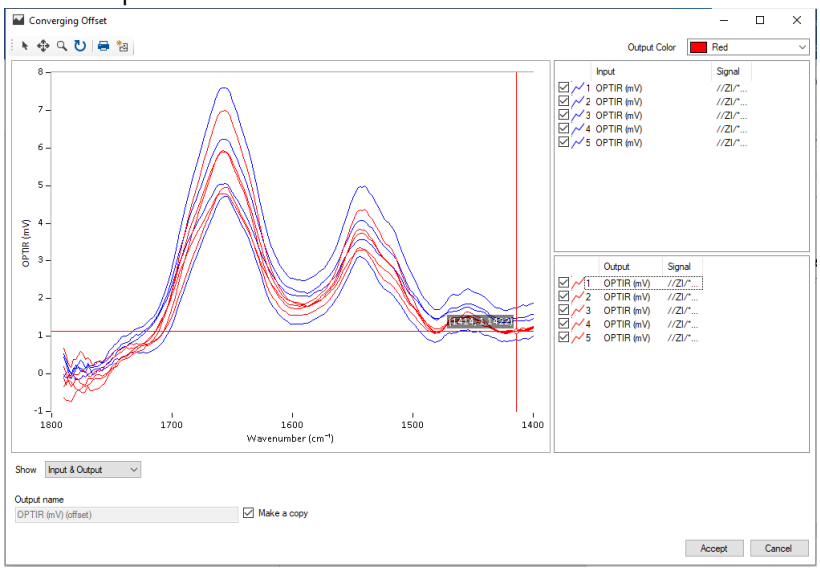


Figure 2-12. Offset-Converge filter function.

2.4.3. Offset-Constant

This filter function adds a constant user selected offset to each spectrum. To use this function, click to select one or more spectra of spectra, then select Analysis/Offset/Constant from the menu bar. Enter a non-zero number into the Offset box to offset the selected spectra. Click Accept when done. If you are using this to offset spectra manually for clarity, you will want to hide the original (non-offset) data in the Document Window.

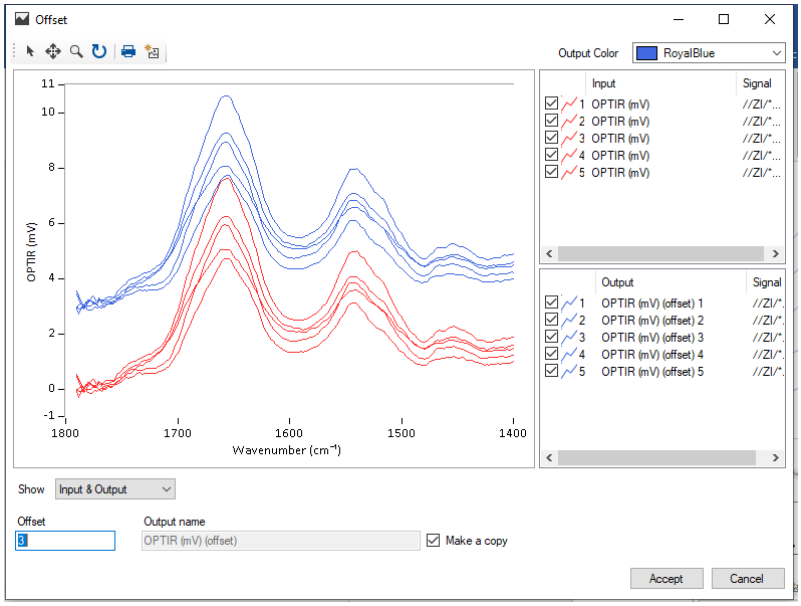


Figure 2-13. Offset-Constant filter function

2.5. Average

The Average function enables averaging of two or more spectra using one of the approaches shown below.

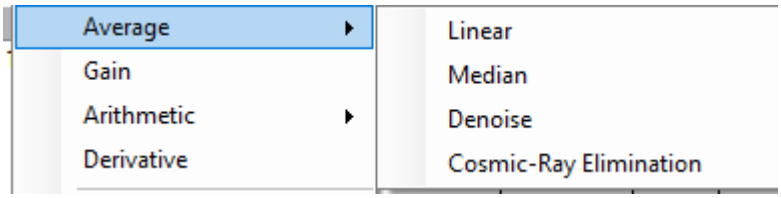


Figure 2-14. Spectrum averaging options

2.5.1. Average-Linear

The Average-Linear function is the traditional average, i.e. the sum of all spectra divided by the number of spectra.

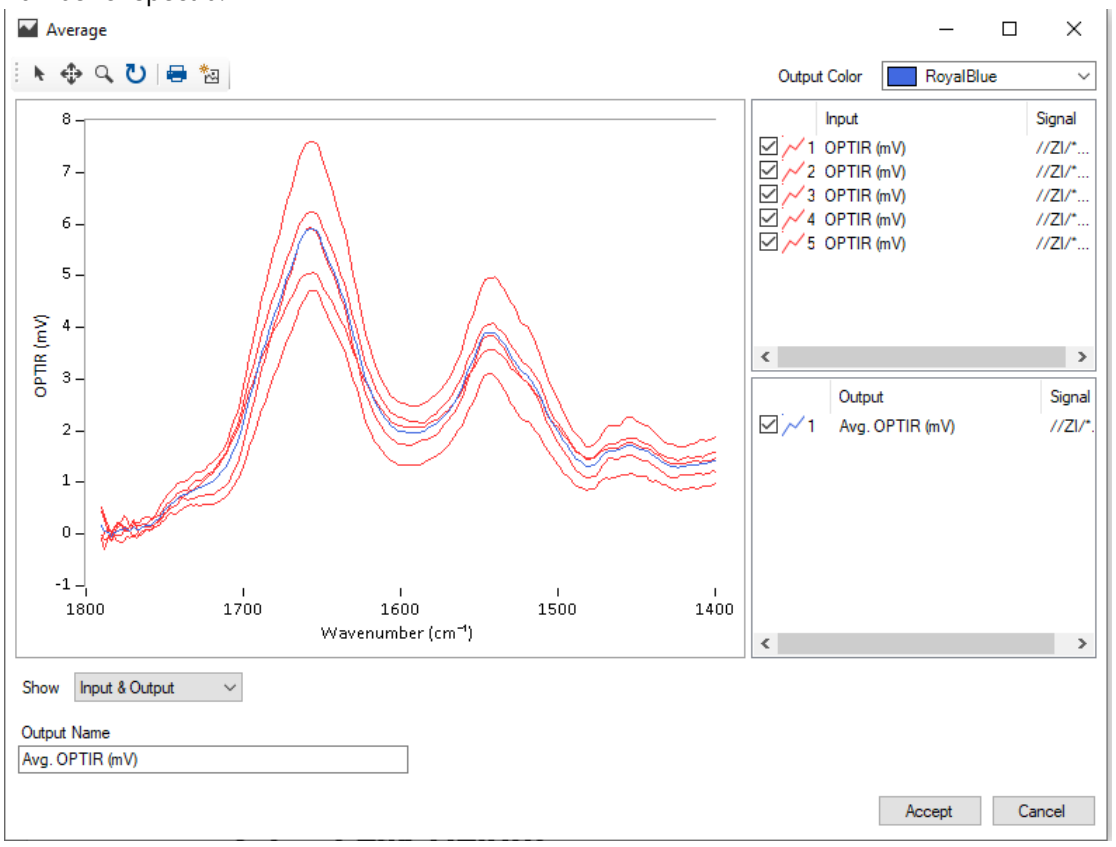


Figure 2-15. Average-Linear function

2.5.2. Average-Median

The Average-Median filter calculates the median value for selected spectra for each wavenumber. That is, for each wavenumber, it chooses the middle value of all the selected spectra. The Average-Median filter can be useful in averaging spectra with larger variability as it tends to reduce the impact of outlying data points.

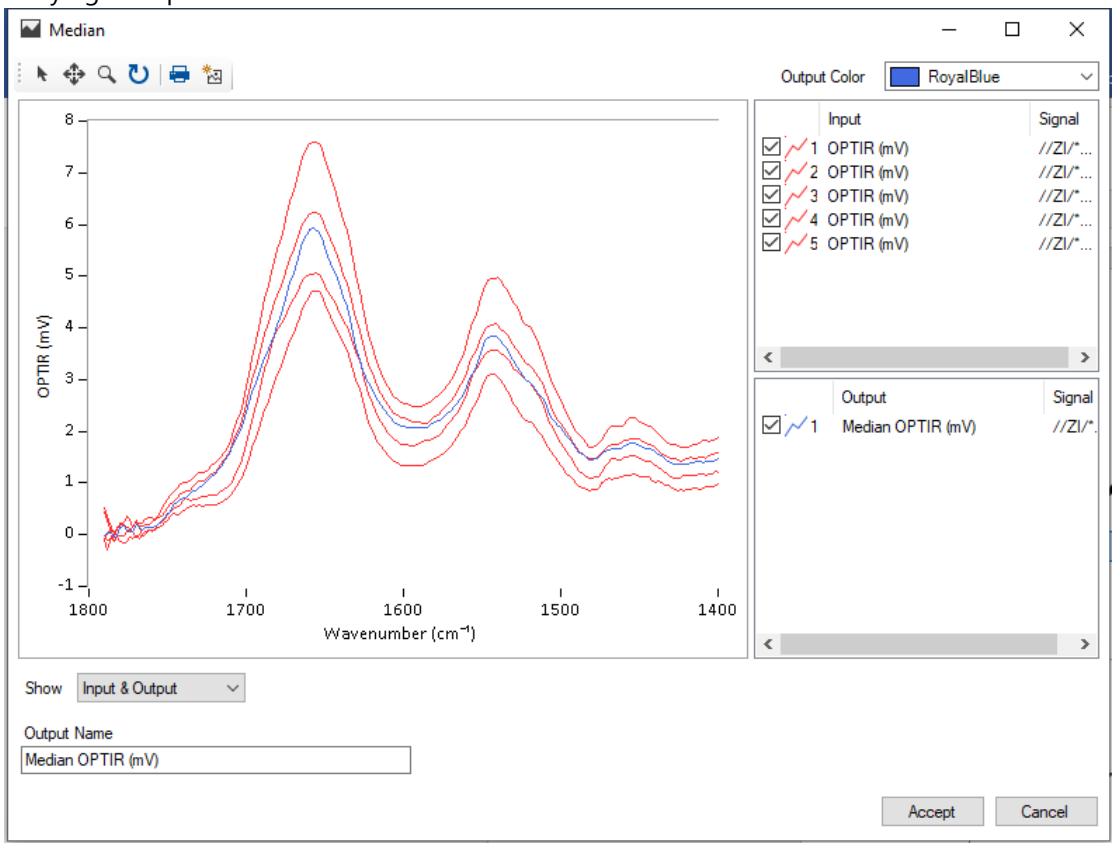


Figure 2-16. Average-Median function.

2.5.3. Average-Denoise

The Average-Denoise function uses a minimum noise fraction approach to discriminate signal from noise in a collection of spectra. The spectrum is deconvoluted into a series of wavelets and corresponding wavelet amplitudes. A signal to noise ratio (SNR) is calculated for each of the wavelet amplitudes. A high SNR indicates that a specific wavelet/wavelet amplitude is part of the signal whereas a low SNR indicates the wavelet comprises noise. The wavelet amplitudes are sorted by SNR into a so called "scree plot" of decreasing SNR. A cursor is used to set the dividing line between signal and noise. Then the spectrum is recalculated using only the wavelets and wavelet amplitudes to the left of the cursor. This approach can provide dramatically better results than simple averaging because it maintains the features that are common between spectra and rejects those that are different (the noise).

To use the Denoise function, select spectra to average and select Analysis/Filter/Denoise on the menu bar. Then slide the scree plot cursor left and right while monitoring the resulting spectral quality and residual. Generally, the best results will occur when the cursor is positioned at a knee in the scree plot.

Be careful not to position the cursor too far to the left or wavelet artifact spikes will appear in the filtered data.

Note this same functionality is available on the Live Window by checking the “Intelligent Co-averaging” option.

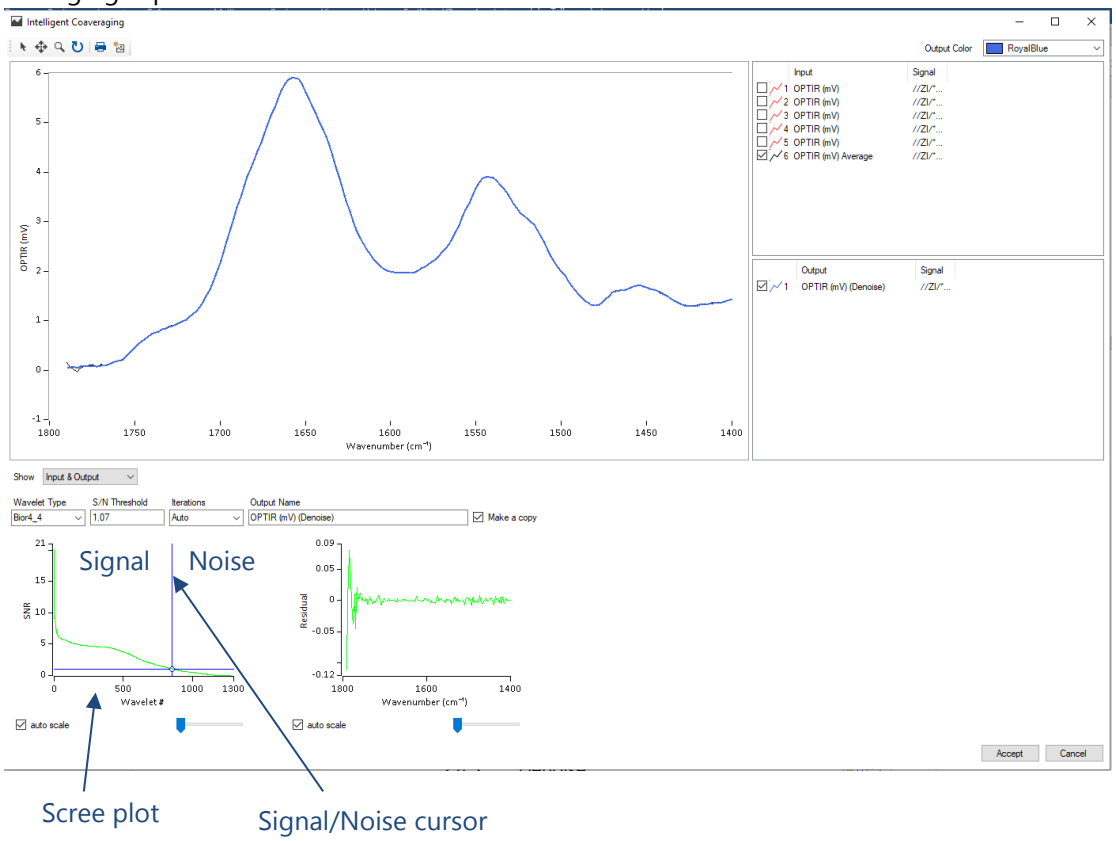


Figure 2-17. Average-Denoise function.

2.5.4. Cosmic-Ray Elimination

This function can be used to eliminate the impacts of cosmic rays on Raman spectra that occur when cosmic rays strike the Raman spectrometer camera during acquisition. There is an option on the Live Window to suppress cosmic ray artifacts in the Raman settings panel. But if Raman spectra have been collected with this feature disabled, the Cosmic-Ray Elimination tool can be used as a post processing step to remove cosmic ray induced glitches in the data. Note that at least two Raman spectra from the same location are required for use of this filter as the filter rejects spikes that only occur in one spectrum and not in the other(s).

To use this tool, select at least two Raman spectra and then choose Analysis/Average/Cosmic-Ray Elimination from the menu bar. Adjust the Threshold value until sufficient suppression of any cosmic ray spikes is achieved. Click Accept or Cancel when done. Alternately use the De-spike filter tool (Sec. 2.2.3) which can be performed on a single spectrum.

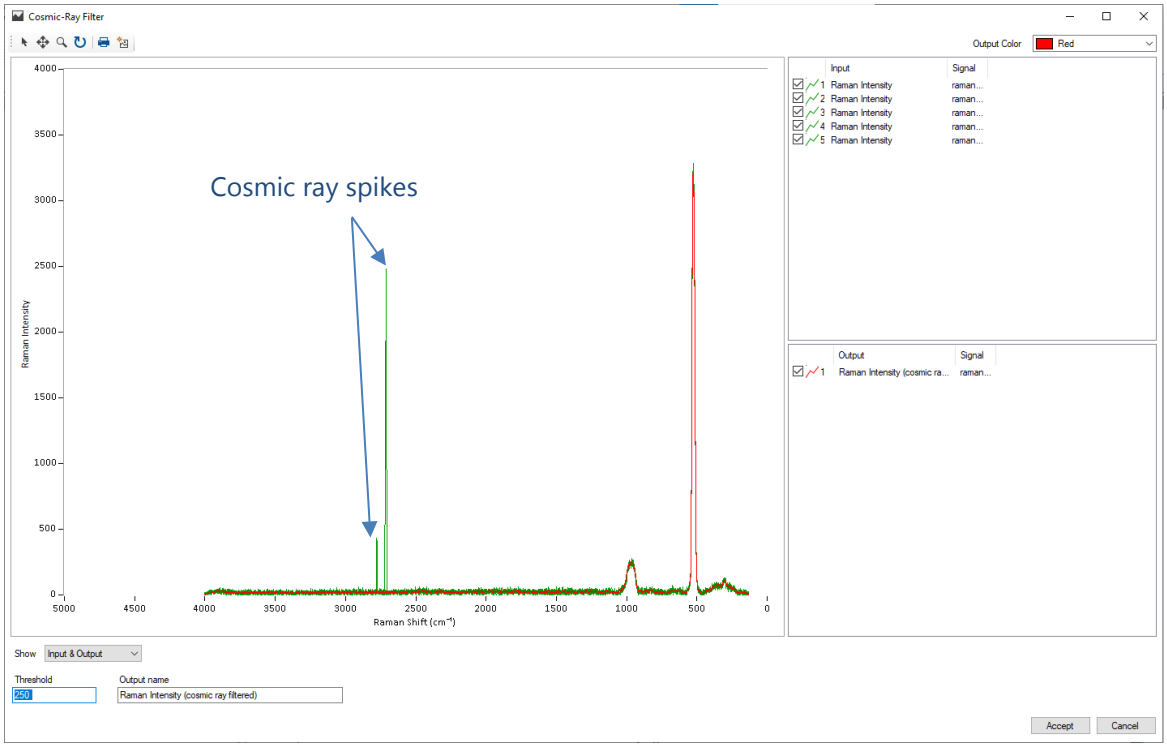


Figure 2-18. Average-Cosmic Ray Elimination filter function

2.6. Gain

The Gain function can be used to multiply one or a number of plots by a constant value. The user needs to input the gain value, output name, and output color. Additionally the user can decide to overwrite the original data or make a new plot of the data with the gain applied by checking the "Make a copy" checkbox.

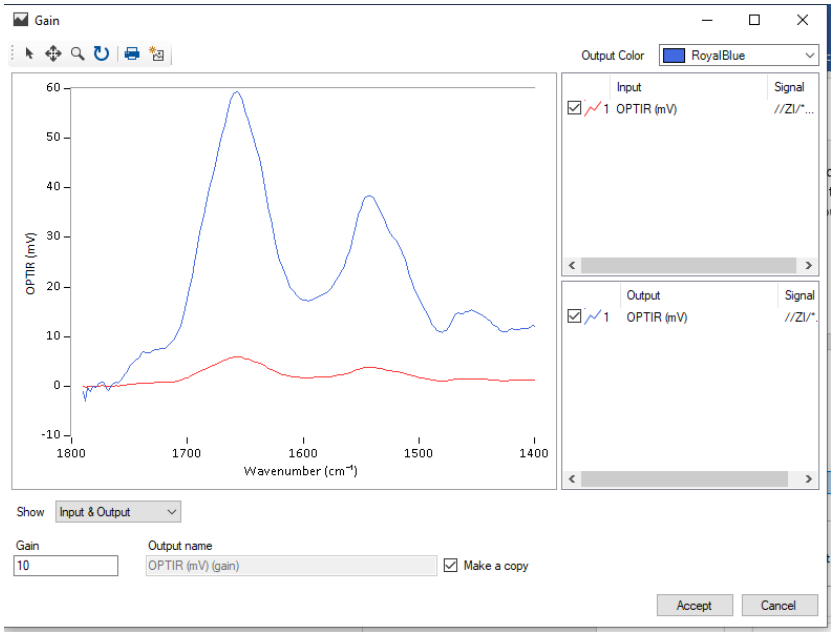


Figure 2-19. Gain function.

2.7. Arithmetic

This function performs basic arithmetic operations (addition, subtraction, multiplication, and division) to the selected data sets. Add and Multiply can be performed on two or more sets of data. Subtract and Divide work only with two data sets at a time. The data sets must be the same signal type to perform any of the arithmetic functions. If the data does not cover the same range the output will be truncated to the common range of data.

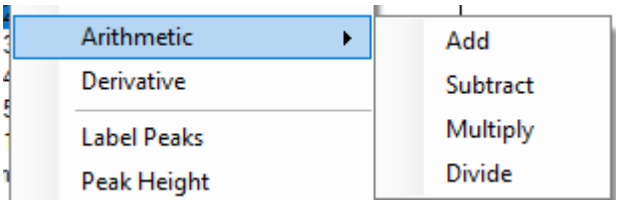


Figure 2-20. Arithmetic function options

To use this function, click on the spectra you want to operate on then select Analysis/Arithmetic from the menu bar.

2.8. Subtract

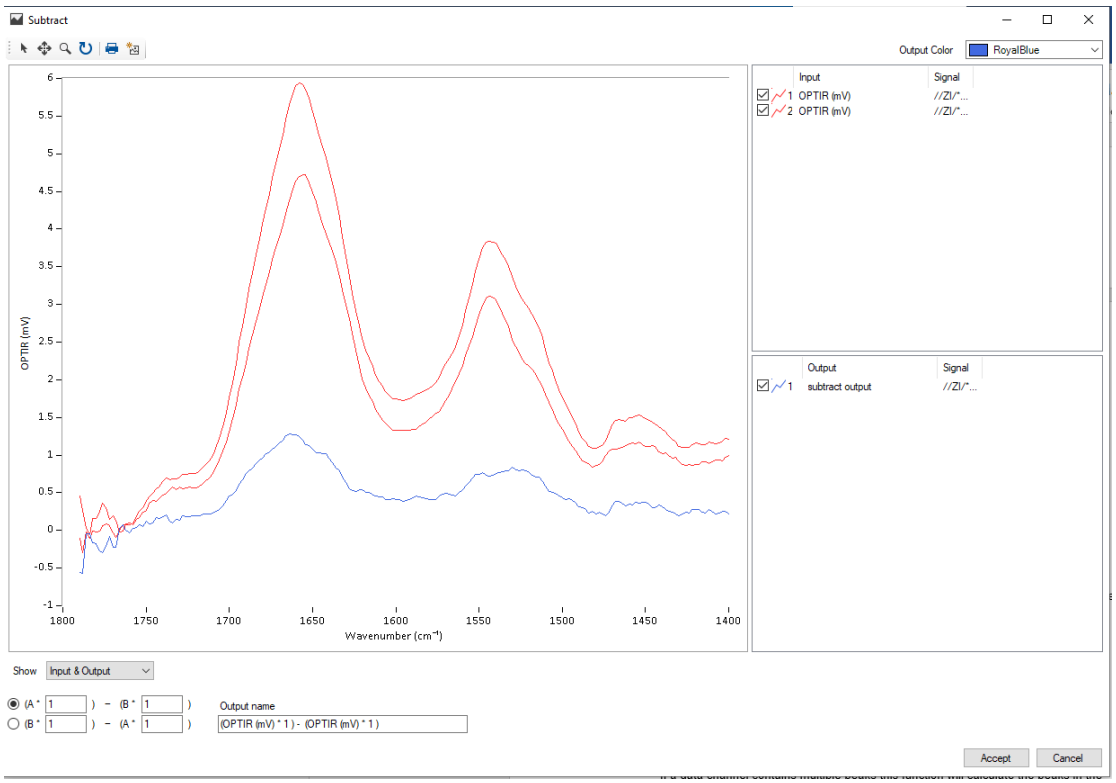


Figure 2-21. The Arithmetic/Subtract View

Shown above is the subtract view as an example. The Add, Multiply and Divide views are similar in terms of their layout and functionality. For the subtract function, the user can select whether to subtract the first set of data from the second or the inverse by clicking on the top or bottom button in the lower left corner of the view. The user can also select the color of the output in the upper right

and the name of the output in the lower middle. Once the output is correct, the user can click on the Accept button to return to the main view and add the output to the current document. The Reset and Preview buttons can be used to remove and recalculate the output plots if desired for scaling issues. The Subtract function can be especially useful for creating difference spectra where one of the spectra is a mixed spectrum and another spectrum is for a pure component. An example would be calculating a difference spectrum for a defect on a substrate that has its own background IR absorption background. Note that it may be desirable to scale or normalize one or more of the spectra before subtraction. See Normalize (Sec. 2.3) and Gain (2.6) functions for details.

2.9. Derivative

This function calculates the derivative of a data channel. To use this function, click on a spectrum and then select Analysis/Derivative from the menu bar. Choose the order (1st or 2nd derivative) from the drop-down menu. The user specifies an averaging width over which the spectrum is smoothed before the derivative calculation. Alternatively, a Savitzky-Golay filter (Sec. 2.2.1) can be used before applying the Derivative filter. Since derivatives are sensitive to noise, this function works best with high SNR spectra or with spectra that have been otherwise averaged or smoothed. To visualize the derivative more clearly, change the Show setting to Output only as shown in Figure 2-23.

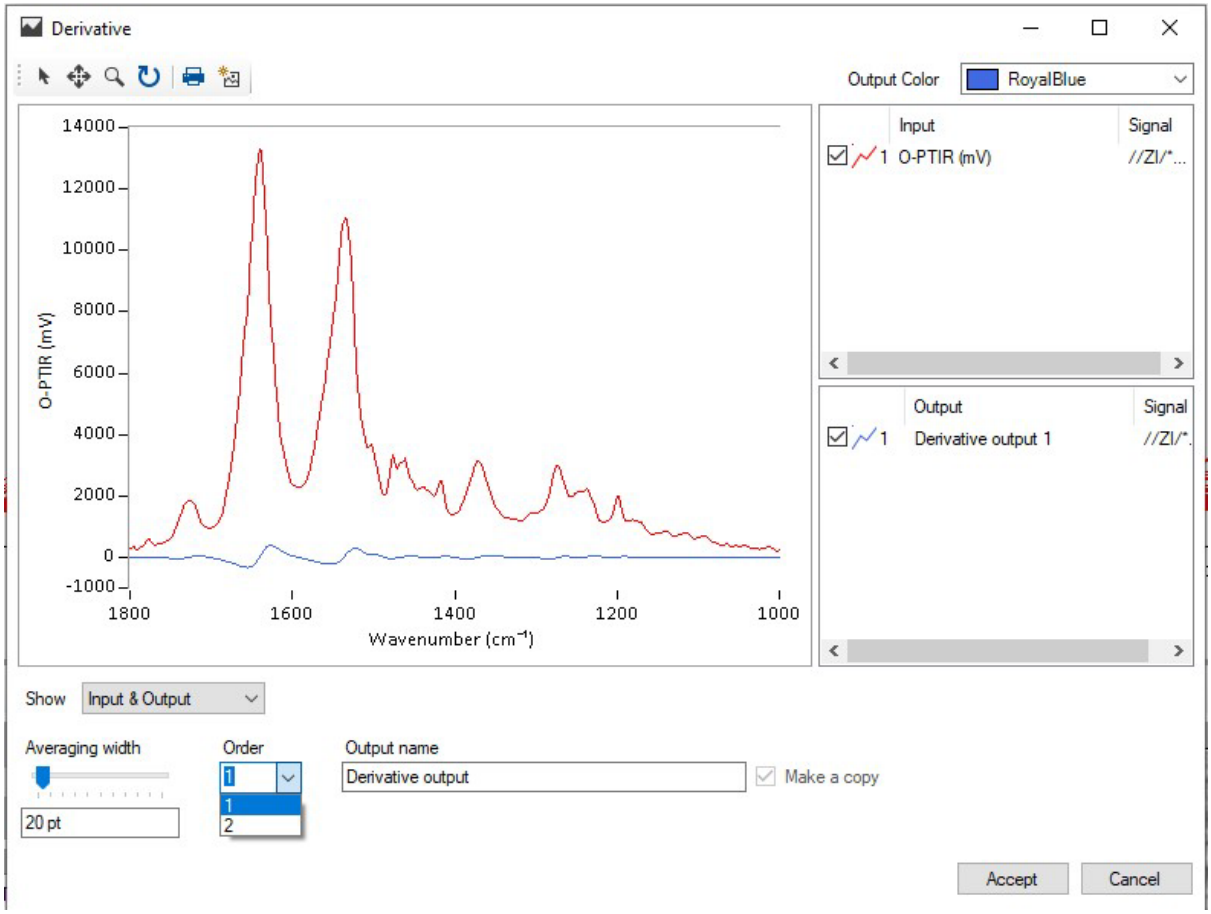


Figure 2-22. Derivative function, input and output shown

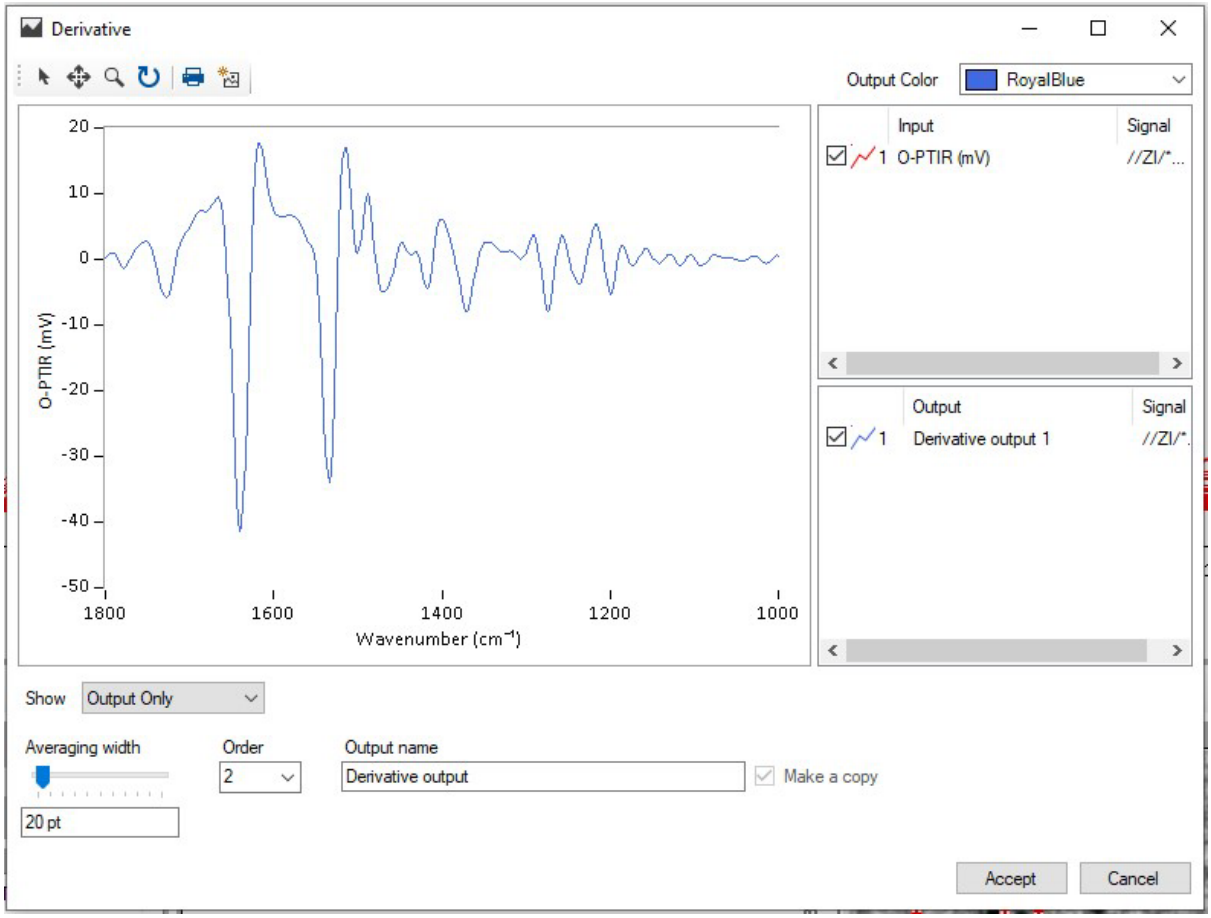


Figure 2-23. Derivative function, output only shown.

2.10. Label Peaks

The Label Peaks tool is a useful tool for identifying and labeling spectral peaks. To use this function, select Analysis/Label Peaks on the menu bar. Adjust the Threshold at the lower left to a desired value and then click Calculate Peaks. If too few peaks are shown, lower the Threshold. If too many peaks are shown, increase the Threshold and/or increase the Smoothing Intensity or Peak Consolidation settings. The Smoothing Intensity sets the level of smoothing before the peak identification algorithm is run. The Peak Consolidation parameter sets the minimum distance between labeled peaks.

Figure 2-24. Label Peaks tool

2.11. Peak Height

The Peak Height tool calculates the height of a selected peak in a spectrum. To use the Peak Height tool, click on a spectrum and then select Analysis/Peak Height from the menu bar.

In the Peak Height tool, use the two red cursors to position the left and right edges of the base of the peak for which you want to calculate the height. The software will display a linear baseline between the two cursors that indicates a baseline signal that will be subtracted before the peak height calculation. Move the black cursor to the peak you want to measure. The baseline corrected height and uncorrected height are displayed at the bottom of the window.

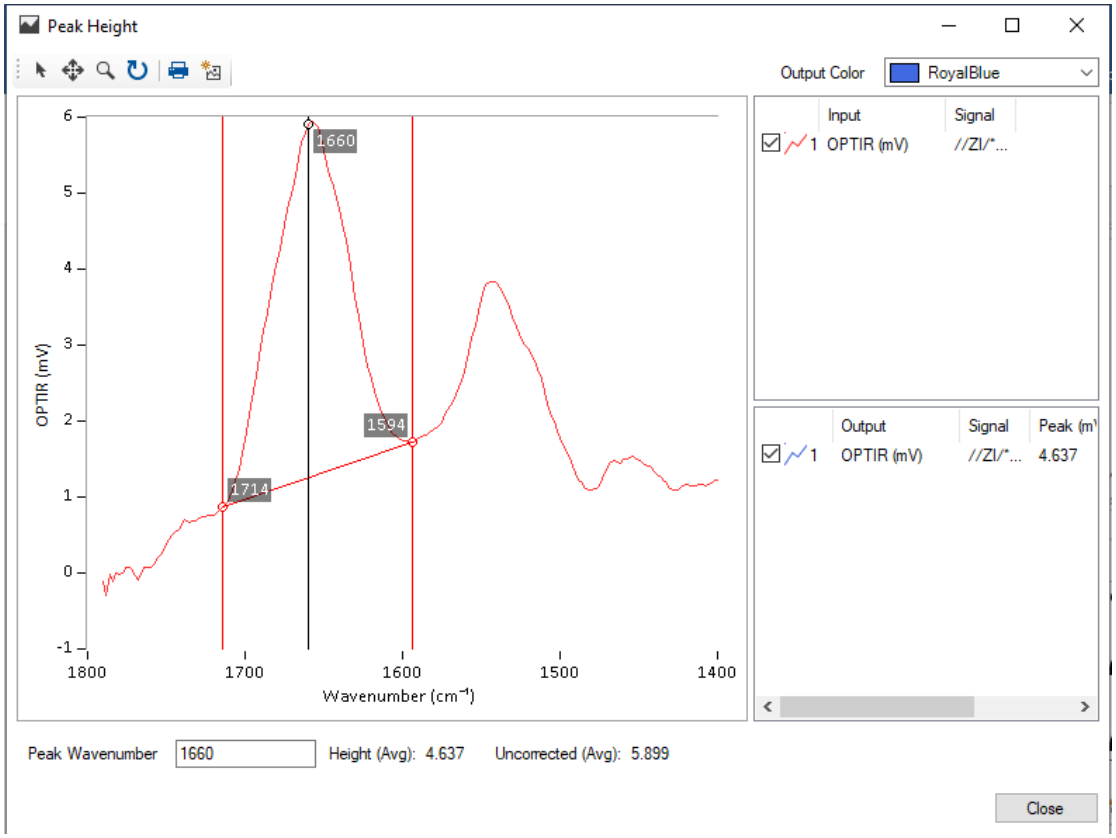


Figure 2-25 Peak Height tool

2.12. Peak Area

The Peak Area tool is used to calculate the integrated area under a peak. To use this tool, click to highlight a spectrum and select Analysis/Peak Area from the menu bar. Adjust the two red cursors to set the left and right edges to use for baseline subtraction and use the two blue cursors to select the width over which to calculate the peak area. The baseline corrected and raw peak areas are displayed at the bottom of the window.

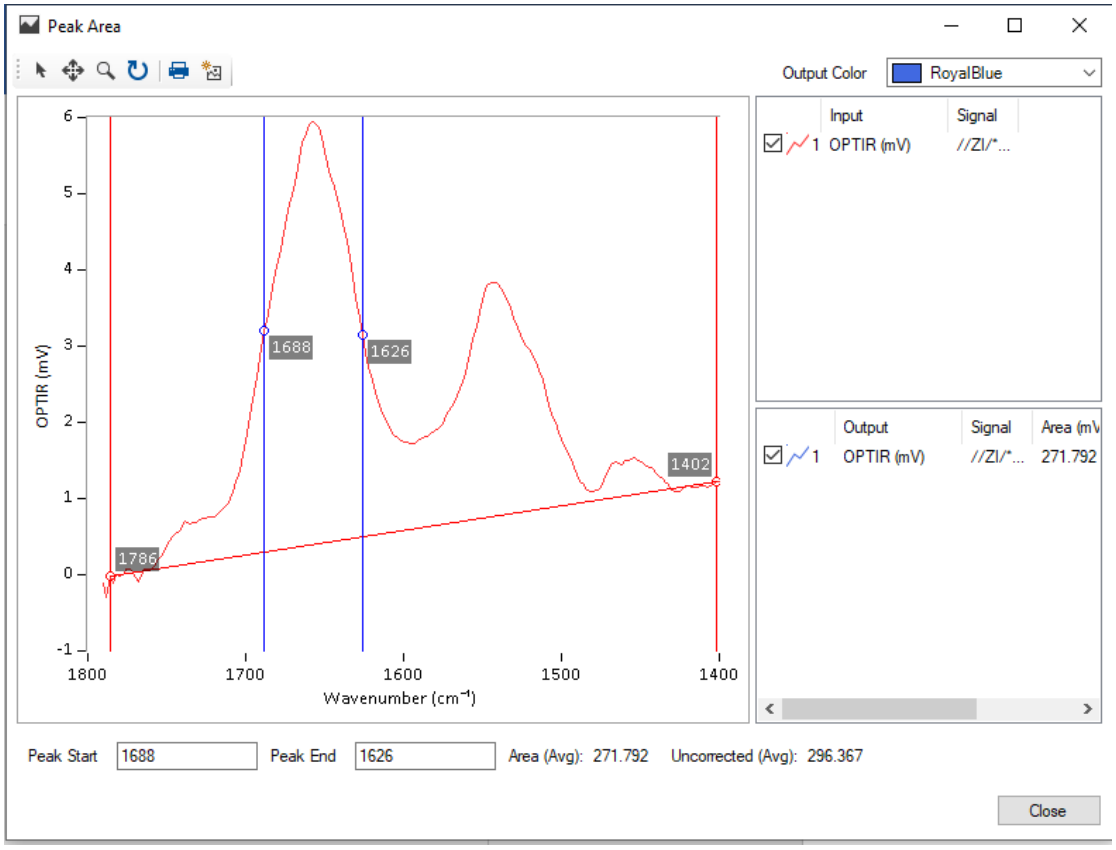


Figure 2-26. Peak Area tool

2.13. Recolor

The Recolor tool is an extremely useful tool for changing the colors of a group of spectra. To use the Recolor tool, select a group of spectra and then right click on the spectra and select Recolor or click on the Recolor icon on the toolbar.

The Recolor function has several options for the recoloring spectra.

2.13.1. Recolor-Color

The Recolor-Color option will let you recolor **all selected spectra the same color** as chosen from the drop-down menu as shown in the figure below.

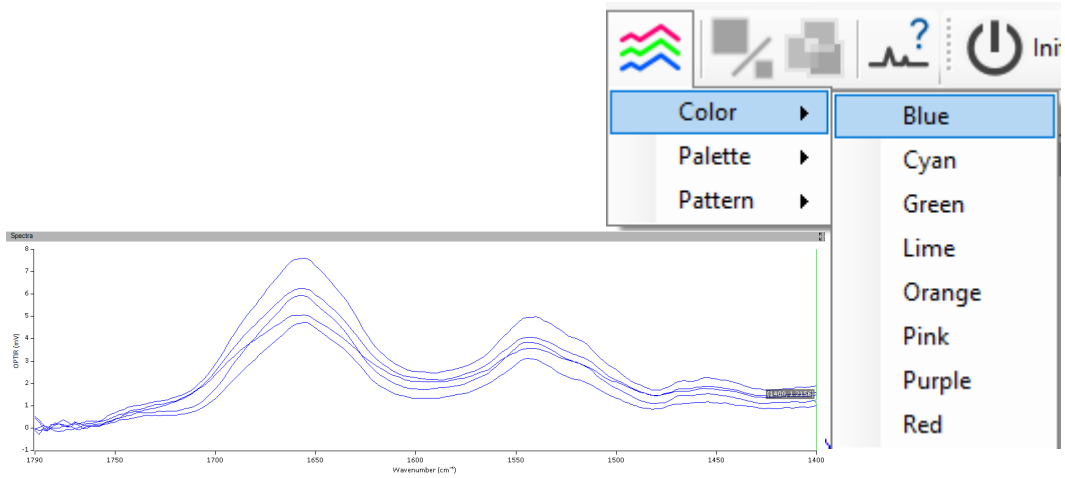


Figure 2-27. Recolor/Color function

2.13.2. Recolor-Palette

The Recolor-Palette function will recolor spectra using colors from the color palettes normally used to color O-PTIR images. The software will choose colors that are evenly distributed over the range of colors in the selected palette based on the number of selected spectra.

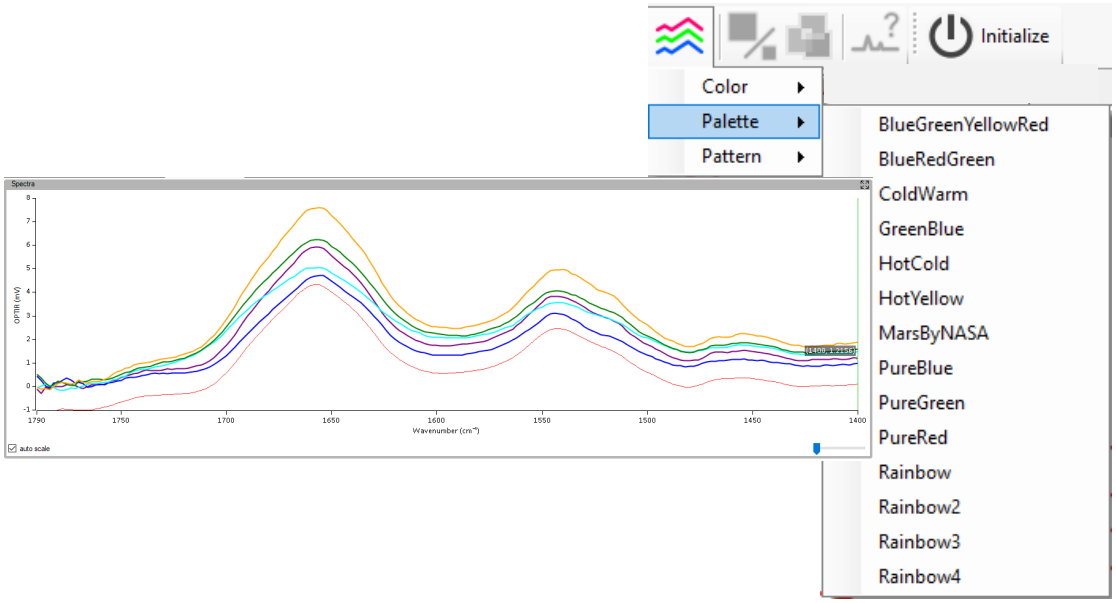


Figure 2-28. Recolor-Palette function

2.13.3. Recolor-Pattern

The Recolor-Pattern applies a pattern of one of two predetermined color patterns, RGB and Rainbow. The RGB option successively colors spectra with a pattern of red, green, and blue and the pattern is

repeated as needed. The Rainbow option distributes the selected spectra over a range of rainbow colors: red, orange, green, cyan, blue, and violet. (Yellow is omitted due to low visibility.)

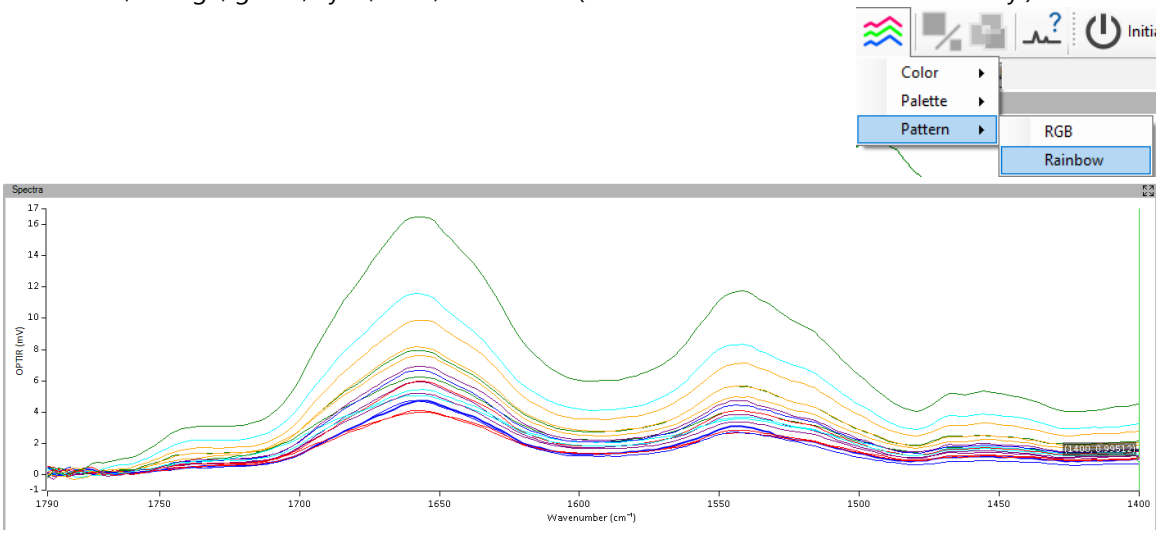


Figure 2-29. Recolor/Pattern function

Chapter 3

3.Image Analysis Functions

This chapter outlines image analysis functions that are used to modify, analyze, and change the display of images in the Document Window.

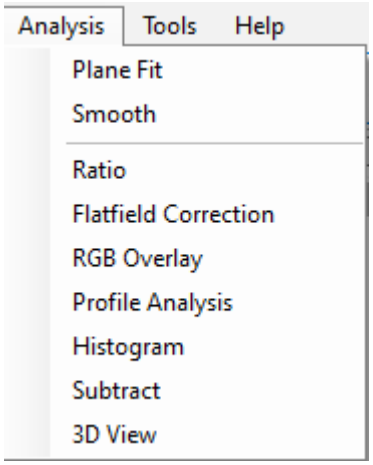


Figure 3-1. Image analysis functions

3.1. Plane Fit

The Plane Fit function performs a single fit to the entire image. It is used to remove offset or tilt from images. This function was originally written for atomic force microscopy images and is **generally not used for O-PTIR images**. The following section is maintained for any special uses of the Plane Fit function. To use this function, click on an image and then select Analysis/Plane Fit.

The type of plane fit, Linear (1st order) or Offset (0th order), is chosen from the Fit Order drop-down list. Whether to fit both axes or only X or Y is chosen via the radio buttons. The "Show original" and "Show modified" buttons allow the user to switch between viewing the original image and viewing the fitted image. To overwrite the original image with the fitted image, select the "Accept" button. The change will be reflected in the image displayed in the Image Map. To create a new image with the fitted data (and preserve the original image unchanged), select the "Make a copy" option and then select the "Accept" button. The new fitted image will appear in the channel image list directly above the original image. To leave the Plane Fit function without making any changes, select the "Cancel" button.

It can be useful to exclude areas of the image from the plane fit calculation. Select the exclusion box icon on the toolbar and use the cursor to draw one or more boxes around the area to be excluded. The plane fit will be calculated without using the data in those areas. An exclusion box can be removed by selecting it (clicking on one of its sides) and then hitting the Delete key on the keyboard.

3.2. Smooth

This function applies a user-defined level of smoothing to a selected image. To use this function, click on an image, and then select Analyze/Smooth from the menu bar. Adjust the Intensity parameter at the bottom left of the image to set the degree of smoothing. Click Accept or Cancel when done.

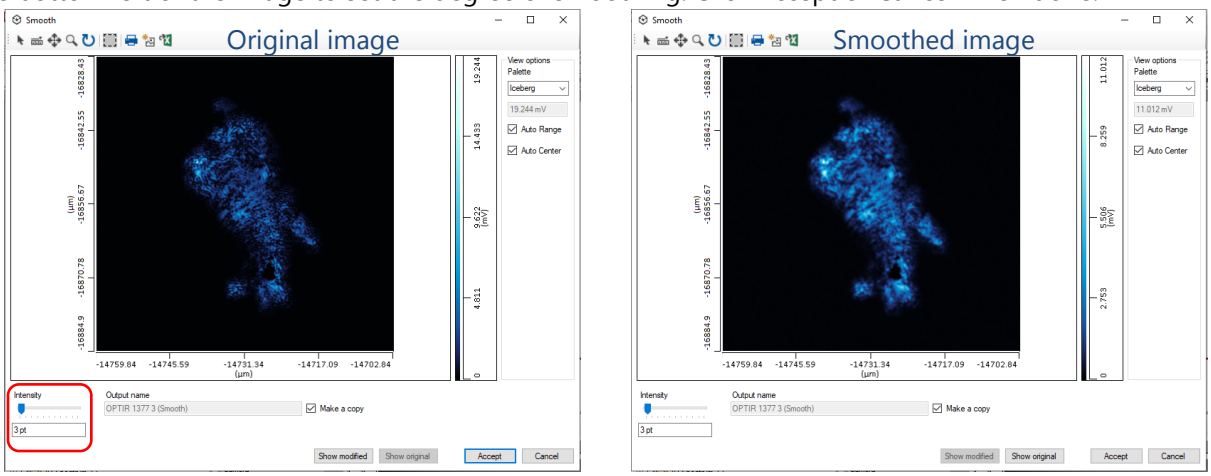


Figure 3-2. Smooth function

3.3. Ratio

3.3.1. Why calculate a ratio image?

The contrast in O-PTIR images can depend on several sample properties (e.g. reflectivity, thickness, and thermal/mechanical properties), thus a single wavelength O-PTIR image does *not* necessarily accurately portray variations in IR absorption alone. For this reason, it is often useful to calculate a image ratios that compare the O-PTIR signals at two different wavenumbers.

Consider an O-PTIR image with a signal intensity $S(x, y, \nu)$, where (x,y) are the locations on the sample, and ν is the IR wavenumber used to collect the O-PTIR image. This signal can be broken down into two separate functions:

Eq. 1: $S(x, y, \nu) = A(x, y, \nu)B(x, y)$

(1) $A(x, y, \nu)$ which represents the IR absorption as a function of (*x,y*) position and wavenumber ν
(2) $B(x, y)$ which represents the other sources of variation in the O-PTIR signal, for example reflectivity, sample thickness, and thermal/mechanical properties.

Calculating the ratio of the signal S at two different wavenumbers $\nu 1$ *and* $\nu 2$ *gives:*

Eq. 2: $$\frac{S(x,y,\nu 1)}{S(x,y,\nu 2)} = \frac{A(x,y,\nu 1)B(x,y)}{A(x,y,\nu 2)B(x,y)} = \frac{A(x,y,\nu 1)}{A(x,y,\nu 2)}$$

In this case the reflectivity, sample thickness, and thermal/mechanical properties are constant at each point in the sample, thus the $B(x, y)$ term in the numerator and denominator are the same and cancel out in the ratio. The ratio image thus reveals the variation in IR absorption while eliminating effects from other sample properties.

3.3.2. Calculating ratio images

To calculate a ratio image, do the following:

1) Capture two images at different wavenumbers, preferably using Interleaved Mode.

2) In the Document window image list, select the image you want to use as the numerator.

3) Select Analysis/Ratio or click on the ratio icon  .

4) Click and drag an image at a different wavenumber to the denominator selection "Ratio Image B" as shown in the figure below and then click Continue.

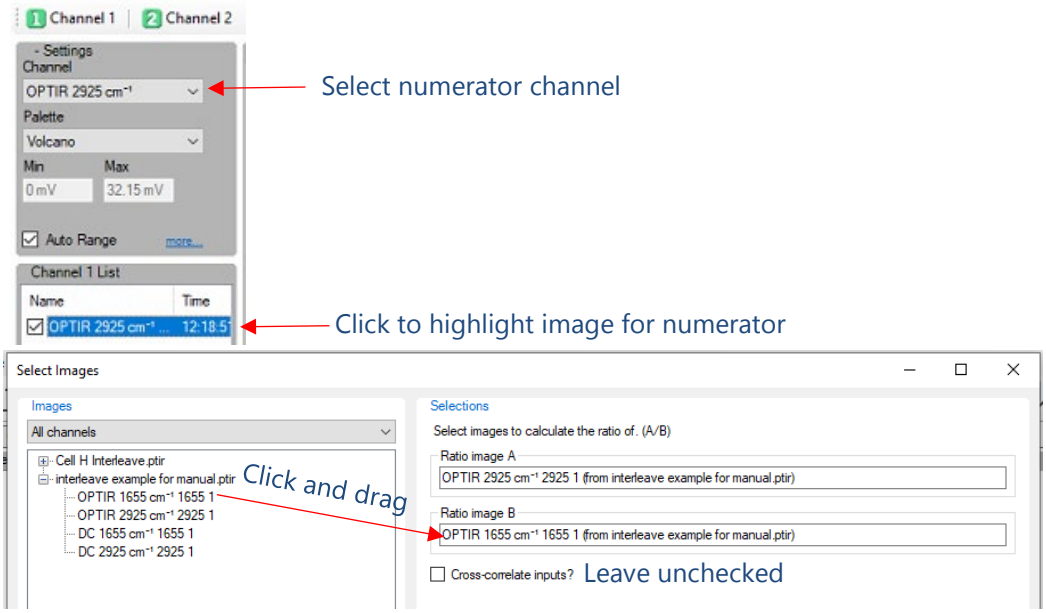


Figure 3-3. Setting up an image ratio calculation.

5) An image ratio display will open, an example shown below.

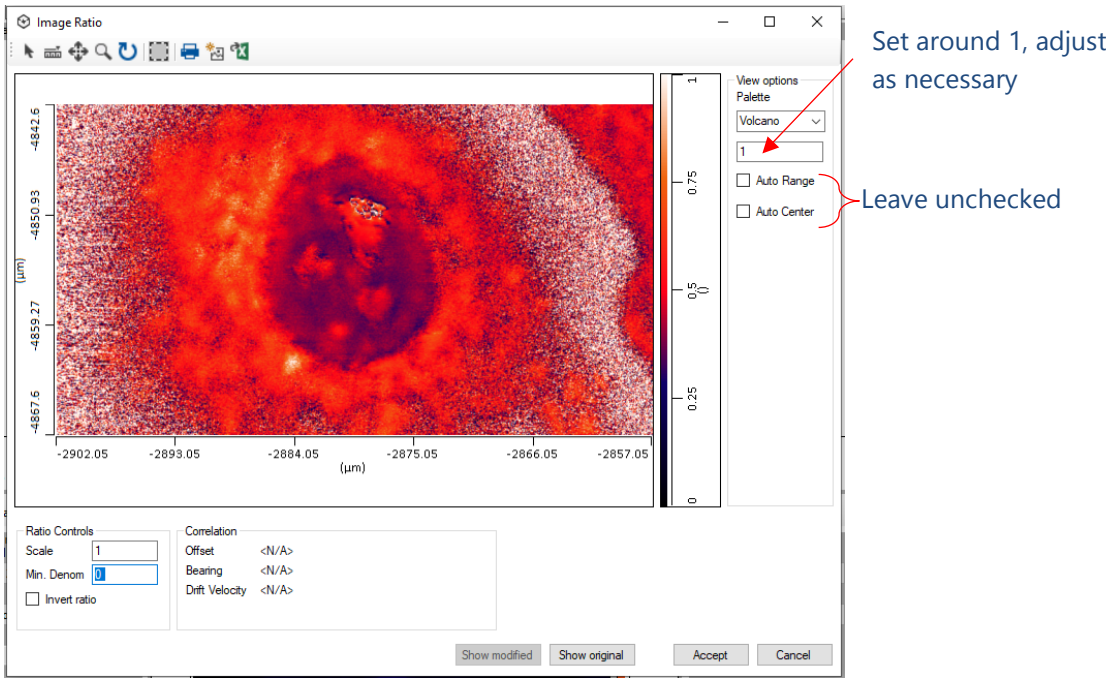


Figure 3-4. Image ratio calculation

6) If desired, adjust the Min. Denom to exclude areas from the ratio calculation where the denominator has minimal O-PTIR signal. This will avoid excessive noise in a ratio image due to dividing by a number close to zero. The figure below illustrates ratio images calculated with different minimum denominator settings.

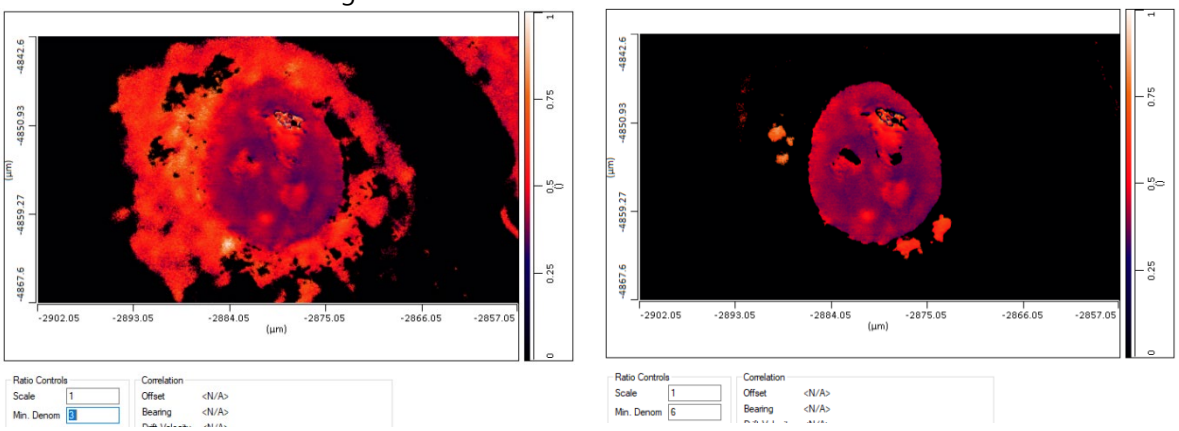


Figure 3-5. Adjusting the minimum denominator setting in an image ratio calculation

7) Click Accept to accept your selection and save the ratio image to the document. The image will be saved in the document under the Ratio channel.

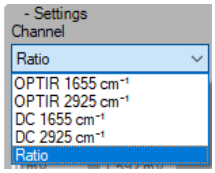


Figure 3-6. Image ratio in the channel list

3.4. Flatfield Correction

Flatfield correction can be used to reduce effects from image illumination non-uniformity and vignetting. To apply flatfield correction, select an optical microscope image and click on Analysis/Flatfield Correction. Click on Calculate to create a corrected image. Click Accept to write the corrected image to the document. If desired, adjust the Kernel Size and/or Sigma parameters.

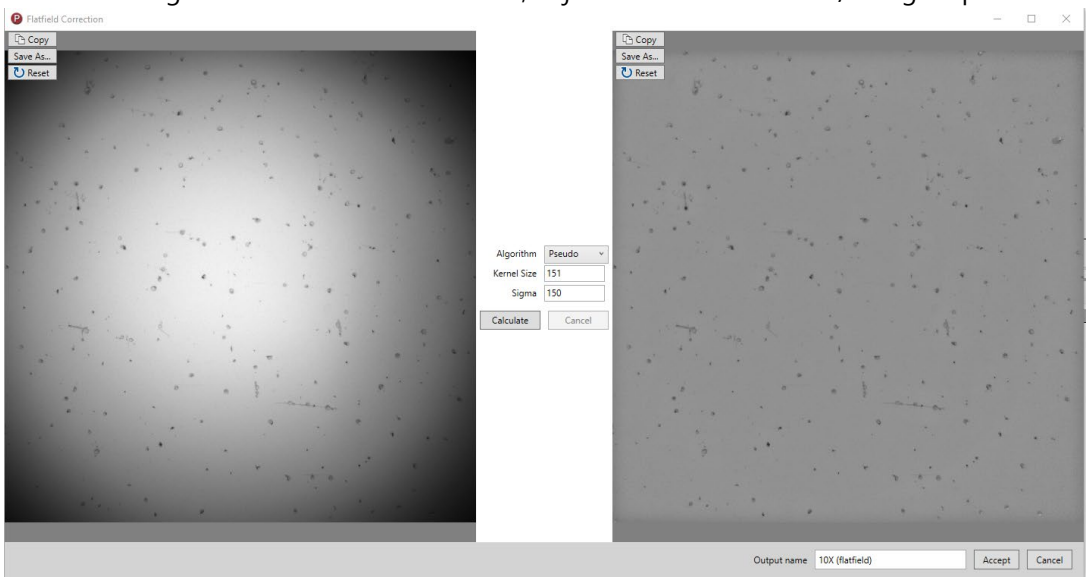
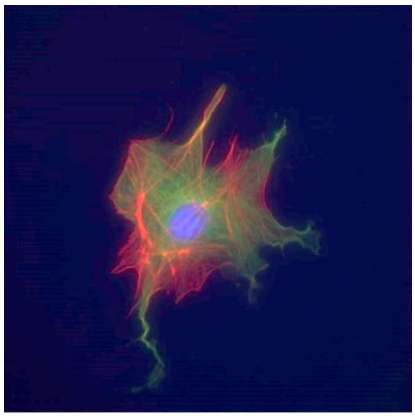


Figure 3-7. Image flatfield correction

3.5. RGB Overlay



The RGB Overlay tool can be used with O-PTIR or fluorescence microscopy images to create multi-color overlays, for example showing IR absorption at different absorption bands or emission of different fluorophores, or overlays of brightfield and fluorescent images.

To create a multi-color overlay, select a fluorescence (or brightfield) image in the Document window and then click on the RGB overlay icon or Analysis/RGB Overlay on the menu bar as shown below.

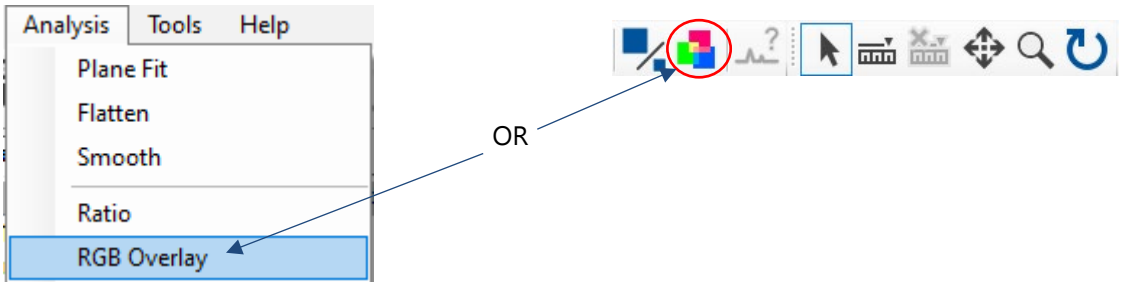


Figure 3-8. Selecting a RGB Overlay tool

Next, drag the images to use for the RGB overlay into the selection box and click Continue.

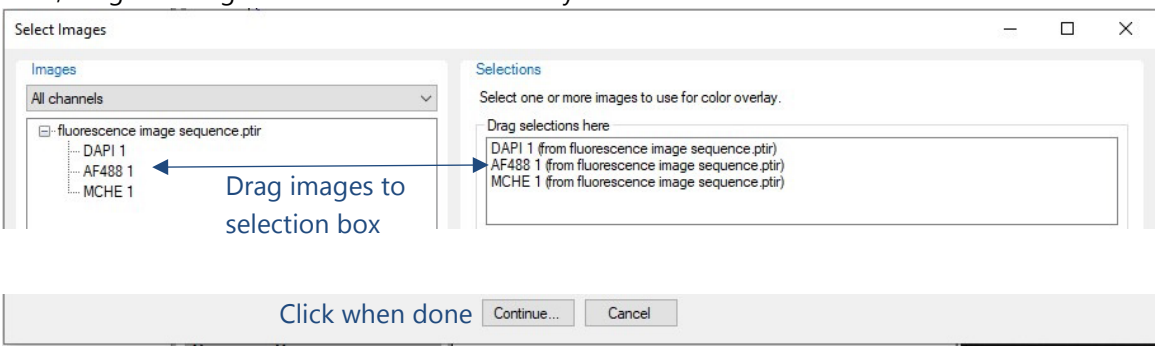


Figure 3-9. Selecting images for RGB overlay.

The RGB Overlay panel will then open. Adjust the color selection and Max/Min of the display range for each fluorescence image as desired. If you wish to copy to the clipboard use, the Copy button, or Save As... to save a copy of the image outside the document. To save the RGB overlay into the current document, click Accept.

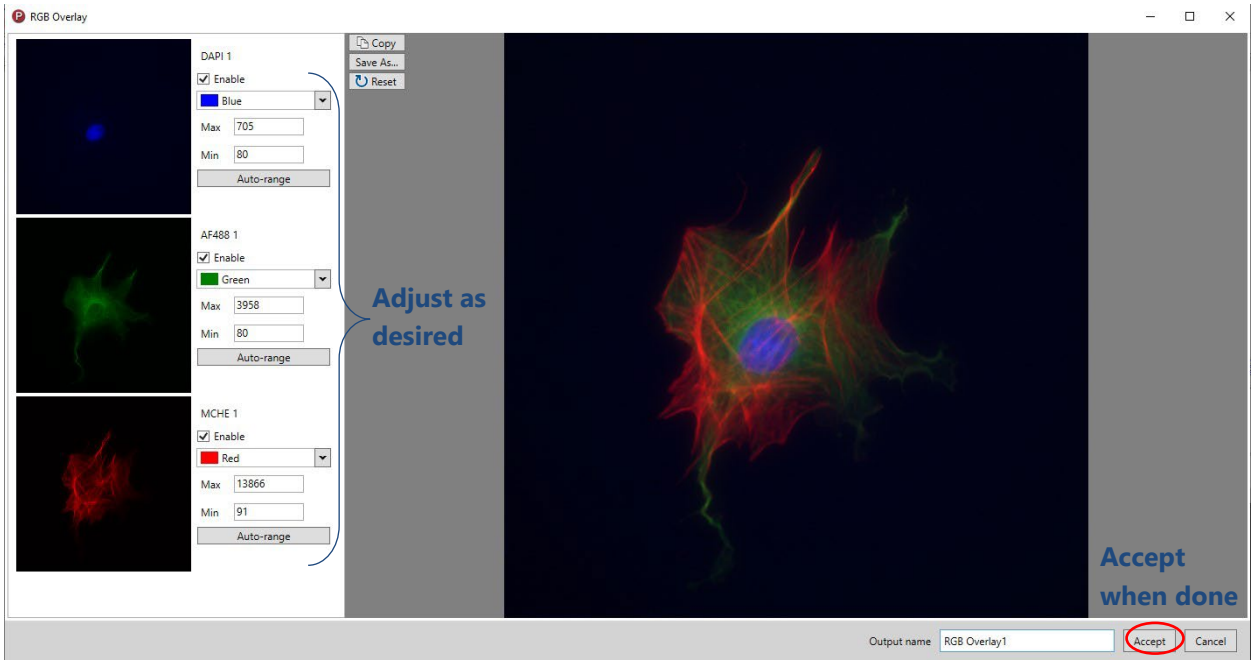


Figure 3-10. Image overlay controls.

The RGB overlay will now appear in the current document under the Camera channel.

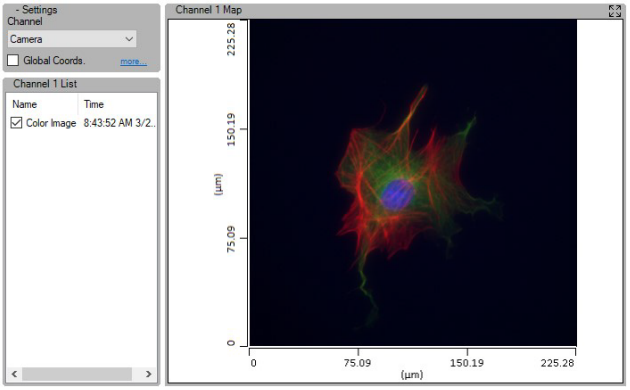


Figure 3-11. RBG overlays of optical images in the Camera channel

The resulting overlay image can be copied to the clipboard using the Copy button or saved to disk as a graphics file using the Save As... button. RGB overlays can also be performed using O-PTIR images as shown in Figure 3-12.

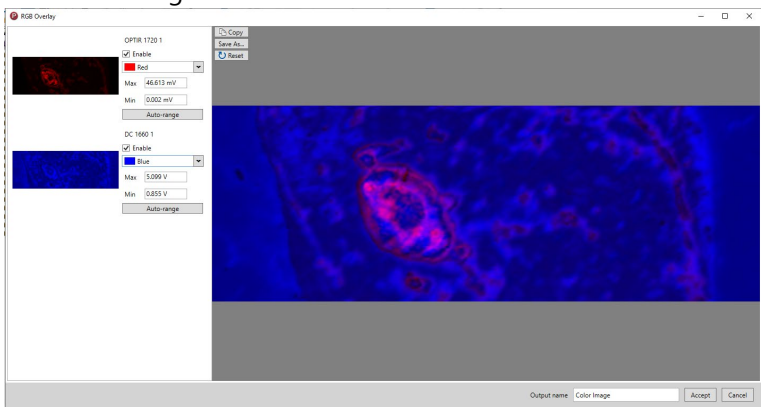


Figure 3-12 RGB overlay example using two O-PTIR images.

3.6. Profile Analysis

The Profile Analysis tool creates one or more cross-sections through an image. To use this function, click on an image and then select Analysis/Profile Analysis from the menu bar. Click and drag to select a cutting line for the cross-section analysis. To draw a horizontal line, position the cursor at the desired vertical position in the image and then hold shift and click the left mouse button. If desired, enter a number larger than 1 for the Perpendicular Averaging setting. This will average the cross-section in the direction perpendicular to the cutting line for the selected number of pixels. Move the crosshairs to different positions on the cross-section to measure relative in plane (horizontal) and vertical distances. More than one cutting line can be created. The cross-section can be exported as an image using the Save Image icon or exported as a CSV file using the Export to CSV icon .

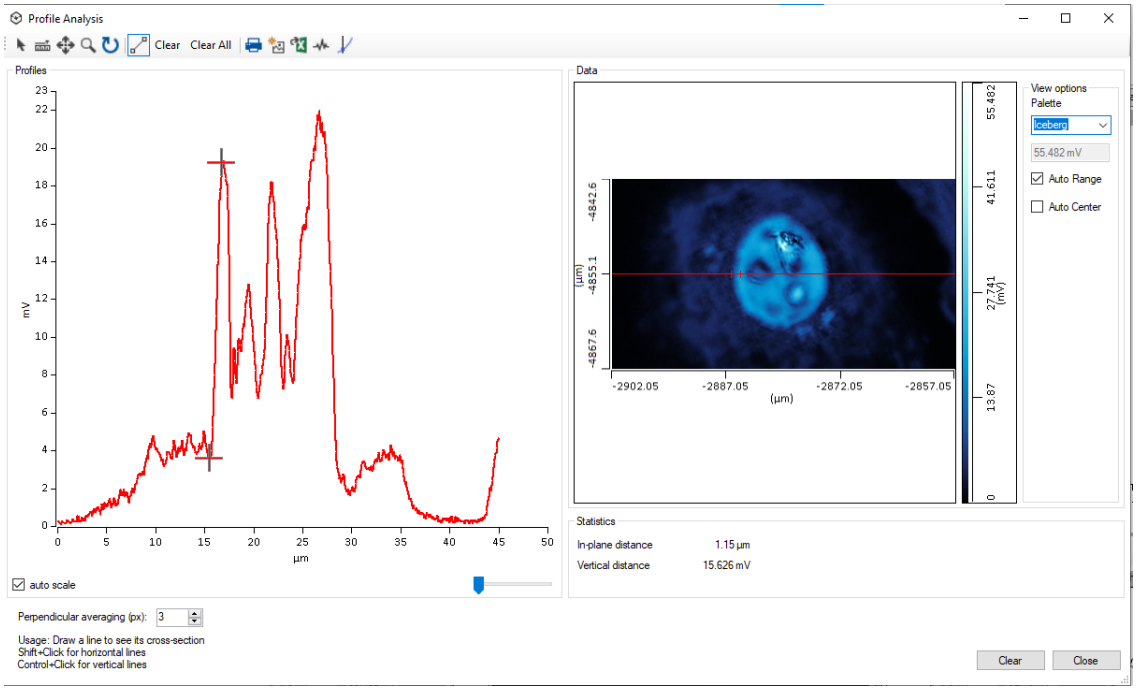


Figure 3-13. Profile Analysis tool

3.7. Image Histogram

The Histogram analysis tool creates a histogram of the intensities in the image. An image histogram is a plot of the number of pixels within given ranges of intensities. The image intensity range is broken down into bins which comprise different intensity ranges and then the Histogram analysis calculates the numbers of pixels within each bin. To use this tool, click on an image and then select Analysis/Histogram from the menu bar. The Add Cursor icon on the top toolbar can be used to add a cursor for example to determine the peak or min/max intensity values.

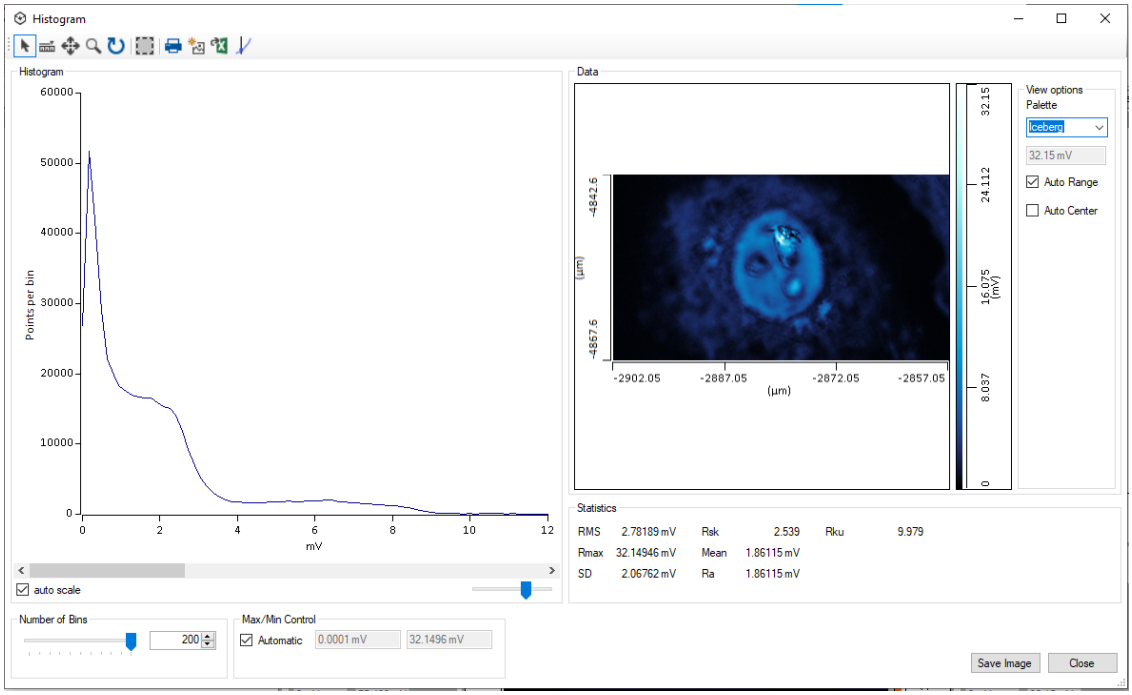


Figure 3-14. Image Histogram analysis tool

The user specifies the Number of bins to be used in the plot. By default, the width of each bin is the range of the image data (Rmax) divided by the number of bins. The histogram's x axis represents image intensity and each bin spans a subset of the overall range of intensities. The histogram's y axis is the number of points in the image that have an intensity value within the range spanned by each bin. The Max/Min Control defaults to Automatic which sets the upper and lower limits of the x axis equal to the limits of the image's data range. The limits of the x axis can be adjusted manually by disabling the Automatic option. Note that this will directly affect the width of the bins (bin width = plot width/# of bins). The histogram plot can be saved as a graphics file via the "Save Image" button. It can be useful to exclude areas of the image from the histogram and statistics. Click the Select Regions button on the Histogram toolbar. Then click and drag on the image to create one or more exclusion boxes. The data inside these boxes will not be included in the histogram plot or the related statistics. To remove an exclusion box select it (click on one of its sides) and then click the Clear button. To remove all exclusion boxes, click Clear All. Various statistics from the image data are reported as described below.

RMS – Root Mean Square of the image data. This will be the same as the Standard Deviation unless the Mean is non-zero.
Rmax – Range of the image data (Zmax – Zmin).

SD – Standard Deviation.
Rsk – Skew.
Mean – Average value of the image data.
Ra – Mean Absolute Deviation.
Rku – Kurtosis.

The histogram data can be exported to a CSV file or the plot can be saved as a graphics file via buttons on the toolbar.

3.8. Image Subtract

The image subtract function subtracts one image from another. To use this function, click on one or two images and then select Analysis/Subtract from the menu bar. If you only selected one image to start, click and drag from the image list at left of the Select Image window into the Subtract image B box. If the two images were obtained in interleave mode, leave Cross-correlate inputs unchecked. If the two images were taken at different times, you can optionally check Cross correlate inputs to adjust for position drift between the two images.
Click continue when done. The subtracted image will then appear. Click Accept to add the subtracted image to the current document.

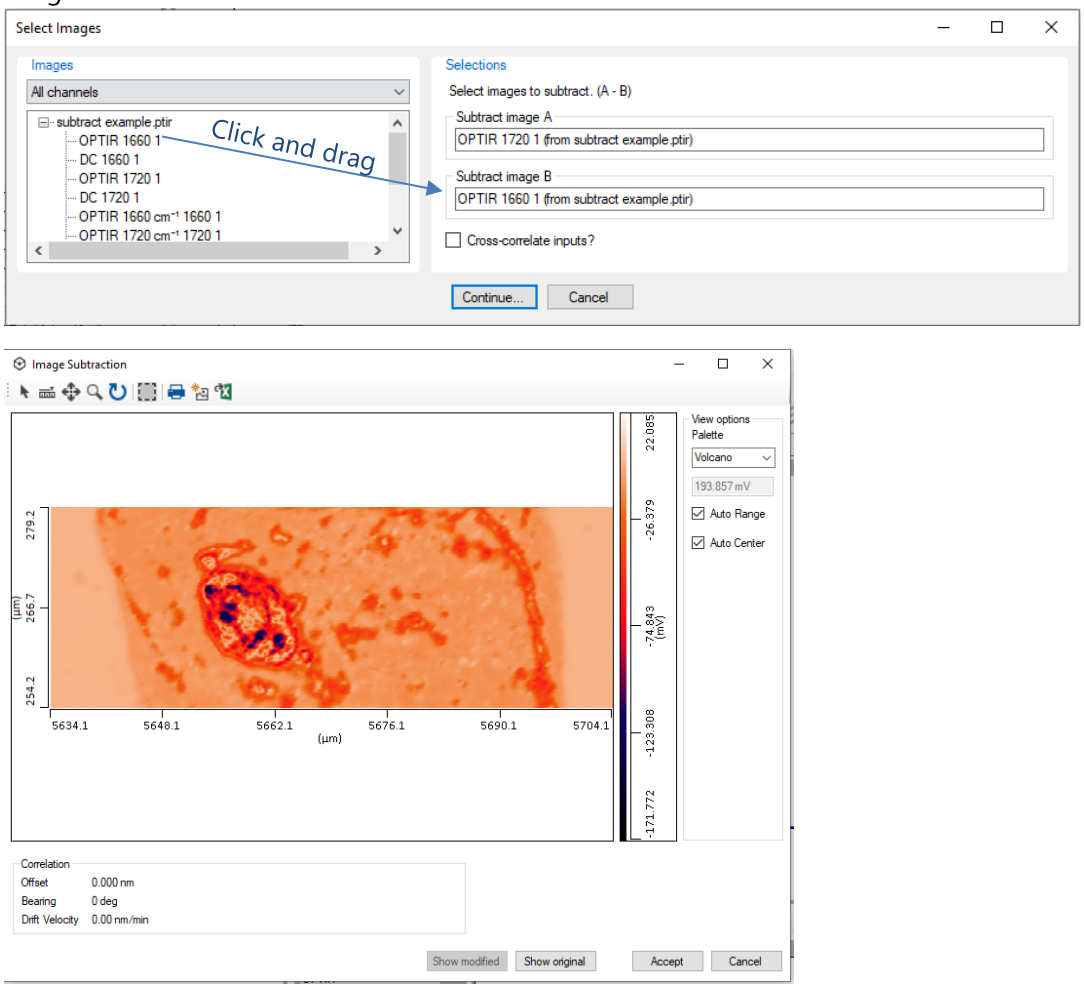


Figure 3-15. Image subtract function

3.9. 3D View

This function displays an image in a 3D view. The intensity information of the image is used to create contours as well as the color scale.

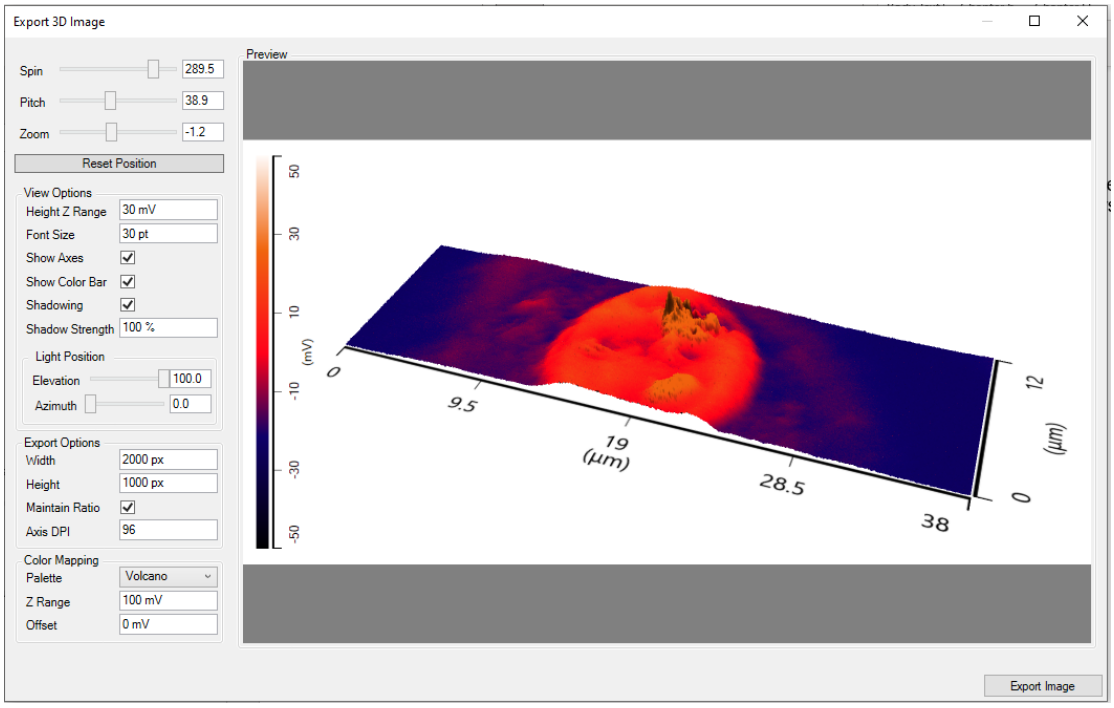


Figure 3-14: 3D View of an O-PTIR image.

The 3 primary controls of the 3D View can be changed via the slider bars, entering a value, or moving the mouse.

Spin – rotates the image in xy around the center point (click and hold, move mouse left/right).

Pitch – tilts the image from side view to top view (click and hold, move mouse up/down)

Zoom – resizes the displayed image (mouse scroll wheel).

There are separate controls for the Z Ranges of the contours and the color scale.

Height Z Range – sets the scale of the Z contours (a smaller Range makes taller contours).

Z Range – sets the range of the color palette.

Chapter 4

4. Hyperspectral Data Analysis

Hyperspectral arrays are acquired into a special document that has somewhat different behavior than the standard document. Tools for viewing/analyzing hyperspectral data sets are described below.

4.1. Hyperspectral document overview

The basic layout of the hyperspectral document window is shown in the figure below.

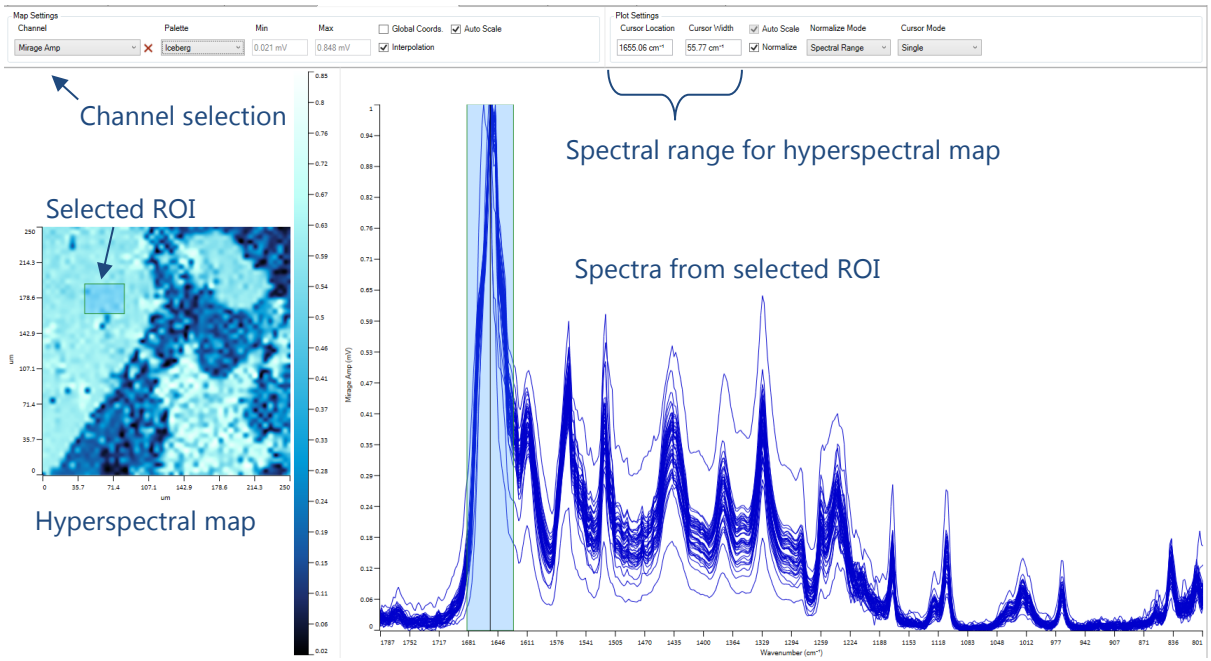


Figure 4-1. Hyperspectral document overview

4.1.1. Hyperspectral map

The hyperspectral map is a plot of the intensity of the spectral amplitude (IR or Raman) at a given wavenumber or over a range of wavenumbers. Slide the cursor on the spectra plot to change the center wavelength for the hyperspectral map. Drag the cursor width to increase or decrease the range over which the hyperspectral map integrates the spectral amplitude.

4.1.2. Spectral range text boxes

The current spectral range for the hyperspectral map is displayed above the spectra plot. New center wavelengths and spectral integration widths can be also entered into these text boxes.

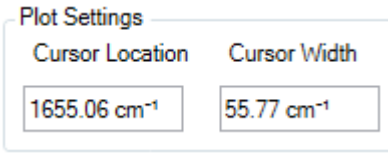


Figure 4-2. Spectral range for integration to generate hyperspectral map

4.1.3. Region of interest (ROI) selection

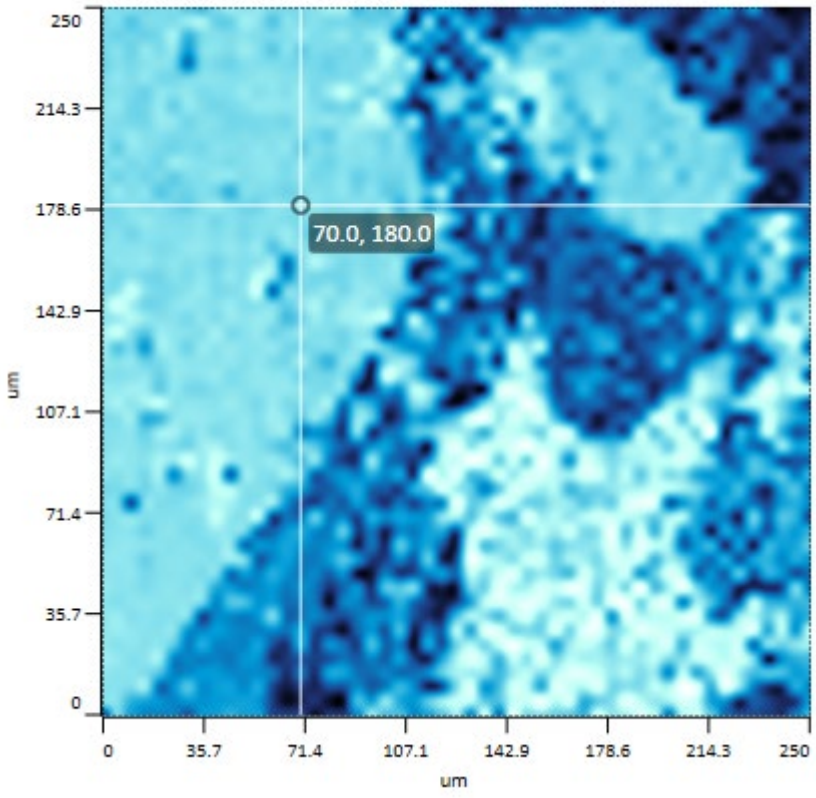
A region of interest on the hyperspectral map can be created by clicking and dragging. After ROI creation, all spectra for the selected data channel within the ROI are shown on the spectra plot. In general it is useful to first select a representative region of the hyperspectral map for the ROI. This will then display representative spectra which can be used to identify central wavenumbers that will be useful for displaying on the hyperspectral map. The ROI can be moved by clicking in the center of the ROI and dragging it to a new region as desired.

4.1.4. Clearing the ROI selection

To clear an ROI selection, right click on the hyperspectral map and select Clear Selection

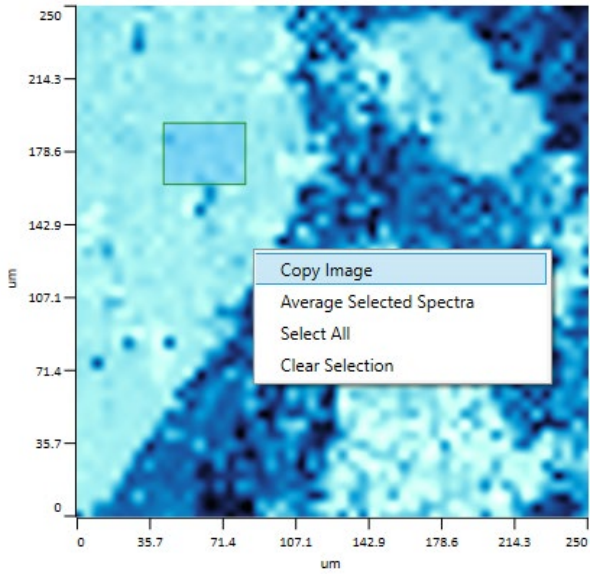
4.1.5. Single point spectra examination

To view the spectrum from a single point in the hyperspectral array, click on any point in the array outside of a currently selected ROI (or clear the ROI selection and click anywhere.)



4.1.6. Copy Image

This function will copy the currently displayed hyperspectral map such that it can be pasted into a traditional document in PTIR Studio. To use this, first set the spectral cursors as desired, then right click on the hyperspectral map and select Copy Image. Next create or select a document then right click in the document window and select Paste. It is recommended to rename the pasted hyperspectral map with information about the spectral peak and peak width used to create the image. (This information is not currently available in the metadata for the copy/pasted image.)



4.1.7. Average Selected Spectra

Selecting Average Selected Spectra will open a new document, copy the selected spectra into the new document and average them. It is then possible to use any of the other spectral filtering, processing, and analysis tools described elsewhere in this manual on the spectra in the new document.

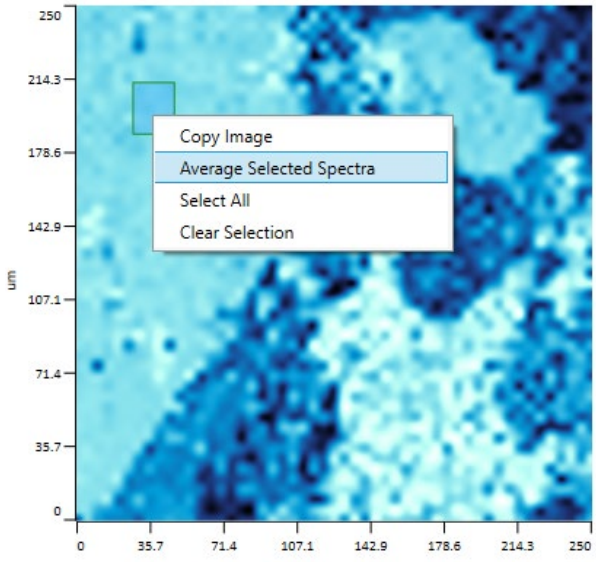


Figure 4-3. Average selected spectra

4.1.8. Select All

Right clicking on the hyperspectral map and clicking Select All will select all the spectra in the array. Note it may take up to 30 seconds to load all the spectra from a large array.

4.1.9. Normalize settings

If the Normalize check box is checked, spectra within the ROI will be normalized in one of two ways. If the Normalize Mode is set to Spectral Range, it will normalize all spectra such that their maximum value is 1. If set to Wavenumber, a cursor appears which can be move to a desired peak to normalize all spectra to 1 at that wavenumber.

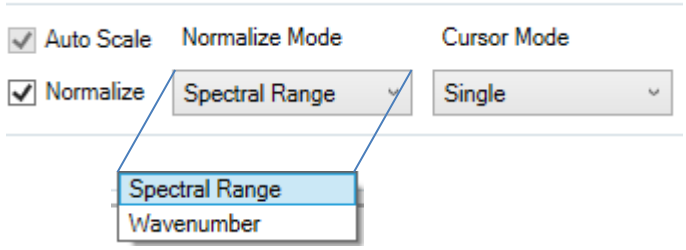
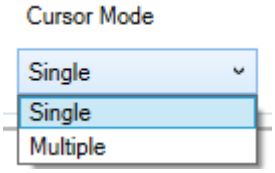


Figure 4-4 Hyperspectral map normalize options

4.1.10. Cursor Mode for hyperspectral map

The Cursor Mode drop down has two choices, Single and Multiple. When Single is chosen, the hypespectral map is colored based on the integrated spectral amplitude within the current cursor selection. When Multiple is selected, the hyperspectral map becomes a multi-color overlay plot show the integrated spectral amplitude for different spectral bands in different colors.



4.1.11. Multi-color hyperspectral maps

To create a multi-color hyperspectral map, choose Multiple for the Cursor Mode. Each cursor is used to integrate a separate spectral band and the integrated intensity is shown on the map by the brightness of the color associated with the cursor. Each cursor center position and width can separately be adjusted. It is generally a good idea to explore the hyperspectral array to determine which peaks most discriminate different components in your sample (or use any prior knowledge you have about your sample's components and spectral peaks).

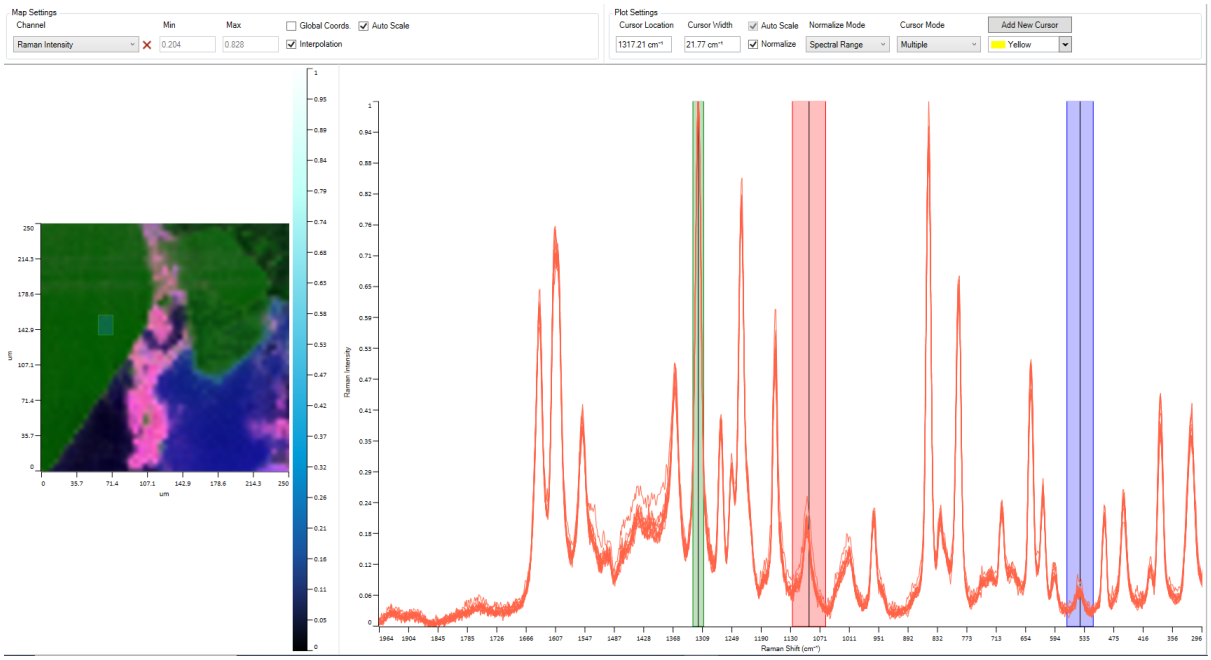
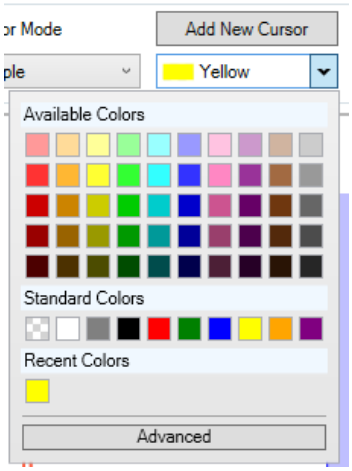


Figure 4-5. Multi-color hyperspectral map with multiple cursors

Adding a new cursor

Click on the Add New Cursor button to add an additional color cursor.



Deleting a cursor

To delete a cursor, select the cursor and then hit the delete key on your keyboard.

List of Figures

Index

O

P

R

S

T

V

Z