SURFACE XPLORER

Version 4.2
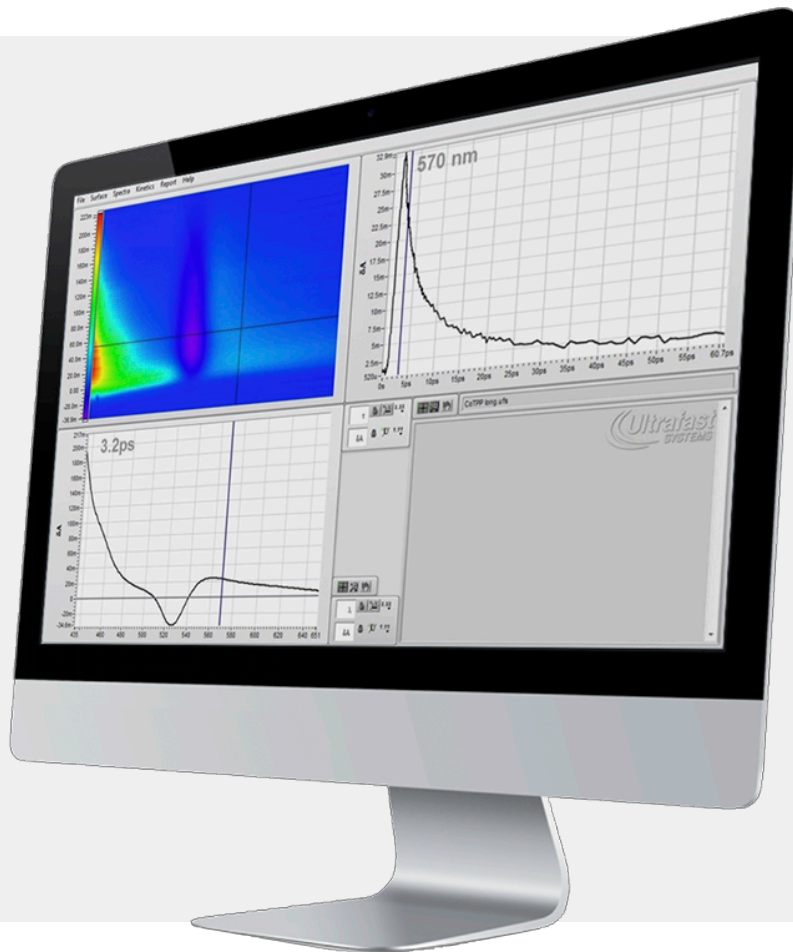
USER MANUAL

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System Requirements

- **CPU > 1 GHz** recommended

- **RAM > 1024MB** recommended

- Microsoft **Windows XP/Vista/7/10** operating system

- Display resolution of **at least 1260x768 pixels**

Introduction to SURFACE XPLORER

Surface Xplorer was designed to make working with 3-dimensional time-resolved spectroscopic data simple and time-efficient. It is extremely advantageous for the analysis of ultrafast transient absorption data. It contains features that allow for the visualization of the experimental data, correction for artifacts (e.g., chirp correction), as well as advanced analysis procedures such as singular value decomposition (SVD) and global fitting. Surface Xplorer (SX) is written in a LabVIEWTM environment; therefore most of its graph controls are standard LabVIEWTM controls. In order for you to have full benefit of the features of Surface Xplorer you will need to accustom yourself to working with graphs. Once you are used to it, working with your data will become a quick and easy process.

First, open the Surface Xplorer application by double clicking the shortcut icon you placed on your desktop. Now let's view the main screen (Figure 1). At the top of the screen there is a menu bar, File name bar shows currently-loaded data file.
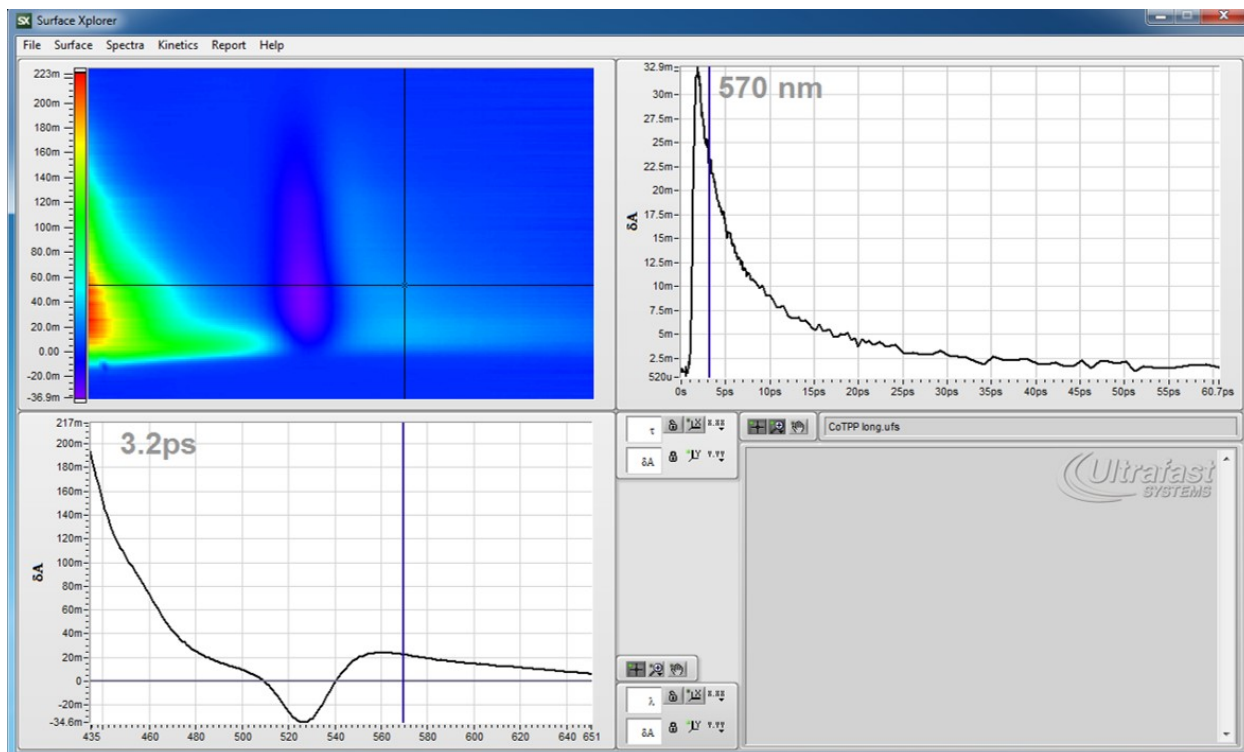
Figure 1 - Main SX panel.

The upper left panel is the 3D Surface Panel. This panel represents a bird's eye view of the Amplitude-wavelength-time (A(l,t), ΔA(l,t), I(l,t),) surface; it is composed of many transient spectra acquired at a succession of closely-spaced time delays. An individual transient spectrum is shown on the Spectrum Panel immediately below the surface; an individual time profile is displayed on the Kinetic Panel located on the upper right. The

lower right quarter of the main screen contains information about experimental details and user comments saved during the measurement.

3D Surface control

NOTE: You are recommended to put your original 3D data file into a dedicated folder. The reason is that some of the files you create will automatically be saved into the folder from which the original 3D data file was opened. This is convenient because while creating reports you will not have to load up numerous files manually because it will be done automatically from the folder you are working with. Furthermore it is much better to have all your data and analysis files for a particular sample/experiment stored in a single folder.

Load a 3D data file to work with by clicking on **File** on the menu bar and selecting **Open File...**from the drop down menu. In the opened File dialog window navigate to the .ufs data file that you wish to work with. Opening a file results in a display similar to that shown in Figure 2.

The transient surface is displayed on the upper left panel and comprises the data collected during the instrument run. This is a "raw" representation of the data collected by the detector.

NOTE: The 3D surface time scale (Y) is in delay steps instead of time units. This may seem unusual at first but such a time scale allows viewing all transient changes at once without having to change the scale, provided the time steps during experiment were chosen appropriately. Additionally, the wavelength scale (X) is in the steps of the original data file (e.g., in CCD pixels).

When you initially load your data file into Surface Xplorer, some parts of the displayed surface, (particularly the blue and red edges of the TA spectrum)may show only noise or no data because of the lack of probe light. Cutting those data out (**Crop...** command from **Surface** menu – page 15) and saving the resulting surface will help cleaning up the data matrix for future analysis.
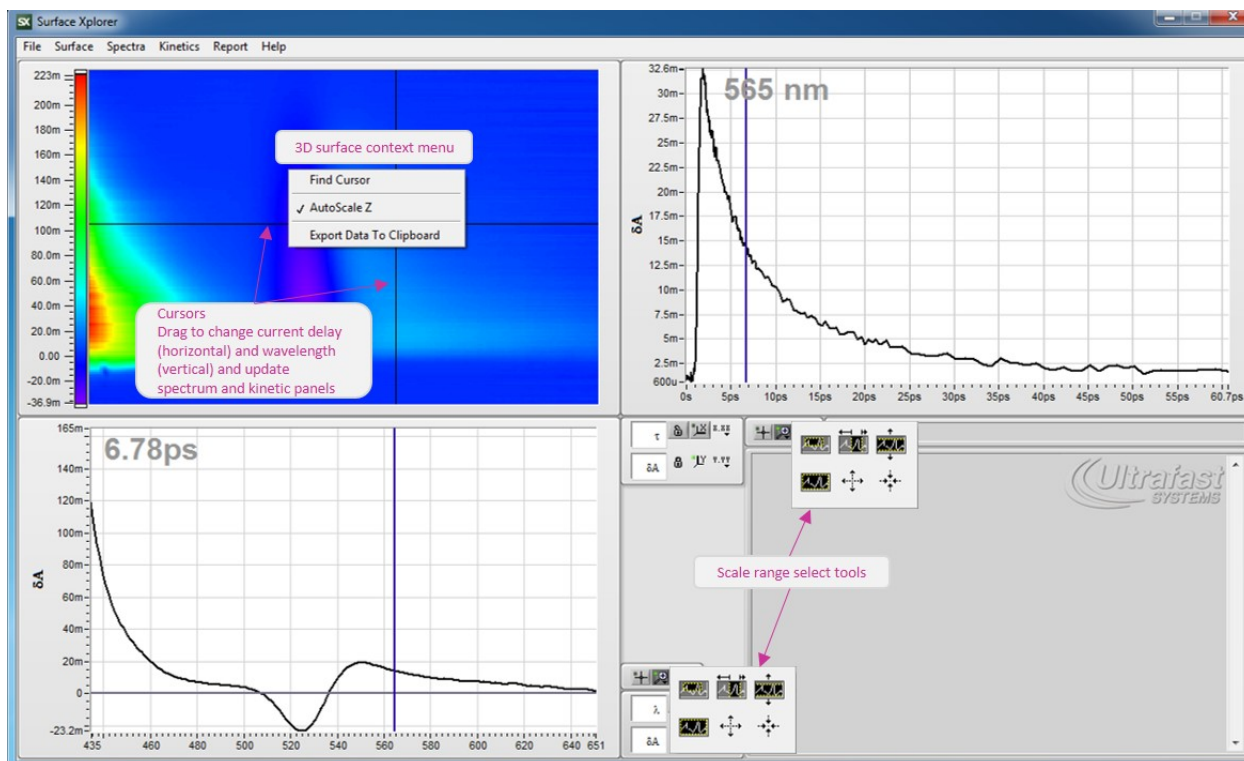
Figure 2 - Main SX panel. Surface View Controls.

By using different zooming tools you are able to select particular segments of the surface to be displayed.

NOTE: The Zoom affects only what is displayed in main window. All functions (kinetics fit, global analysis, chirp correction, etc) work with full set of data not limited with by the selected scale ranges on the main SX panel. The only exception is the **Report -> Surface** tool - it shows surface only within selected temporal and spectral ranges.

The color bar to the left of the surface panel shows the correlation between the amplitude and the color. By default the color bar is automatically scaled so that you can instantly see the maximum and minimum amplitude values for the data set. To manually control the scale, uncheck **Autoscale Z** item in context menu of the surface.

The black vertical and horizontal cursors on the surface are used to select individual spectral (horizontal) or kinetic profile (vertical) slices from the surface. For example, dragging the horizontal cursor up the surface causes spectra for different delay times to be displayed on the Spectrum Panel (lower left) Figure 3.

Spectrum Panel

This panel displays spectra from the 3D data matrix at times selected by the horizontal cursor on the surface panel (Figure 3).The Spectrum panel can also display any spectra the user chooses to save (Spectra menu, Save Selected Spectra command, page 27).

At the top left of the panel there is a time indicator showing the delay time to which the currently displayed spectrum corresponds. The vertical cursor position on the spectrum panel is linked to the surface panel's black vertical cursor to accurately show its wavelength position.
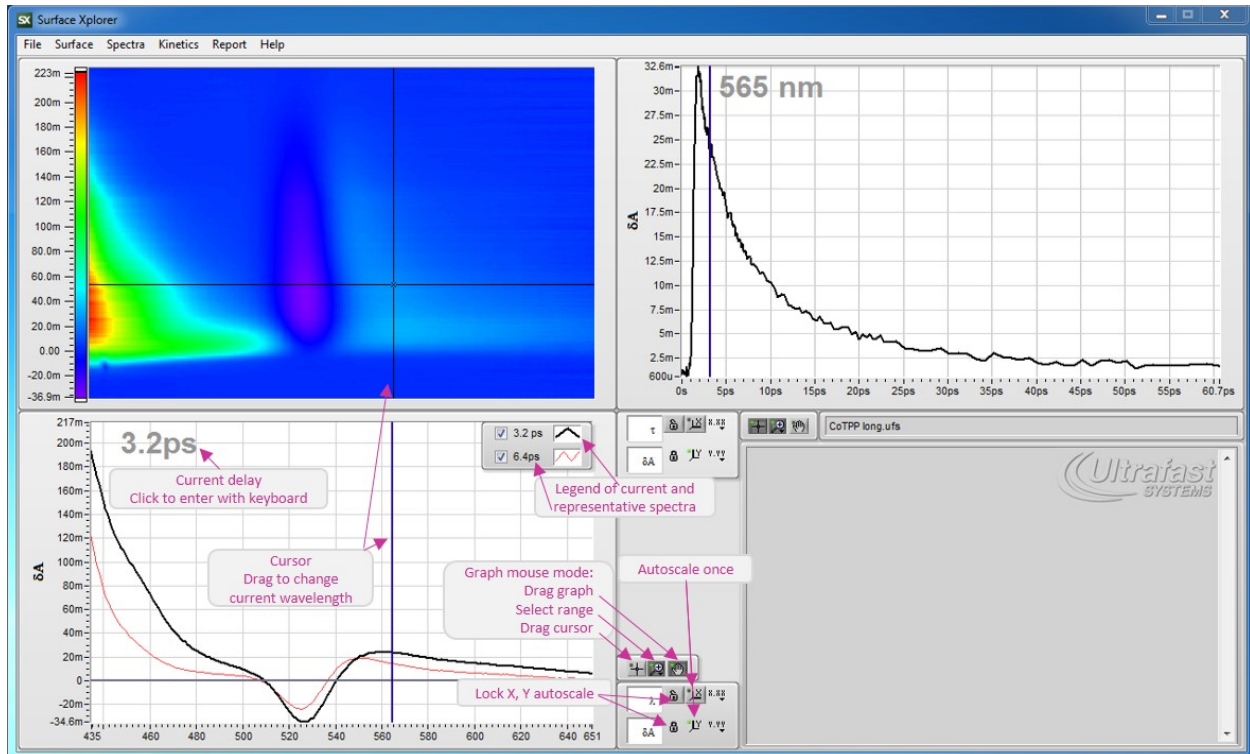
Figure 3 - Main SX panel. Description of the Spectrum Panel.

Kinetic Profile Panel

This panel displays the kinetic profiles from the 3D data matrix as selected by the black vertical cursor on the surface panel (Figure 4). The Kinetic Profile panel can also display any kinetics the user chooses to save (Kinetics Menu, Save Selected Kinetics... command, page 30).

At the top right of the panel there is an indicator showing the wavelength of the currently selected time slice. The vertical cursor position on the kinetic panel is linked to the surface panel's black horizontal cursor to show the point on the kinetic profile that corresponds to the currently displayed spectrum on the Spectrum Panel.
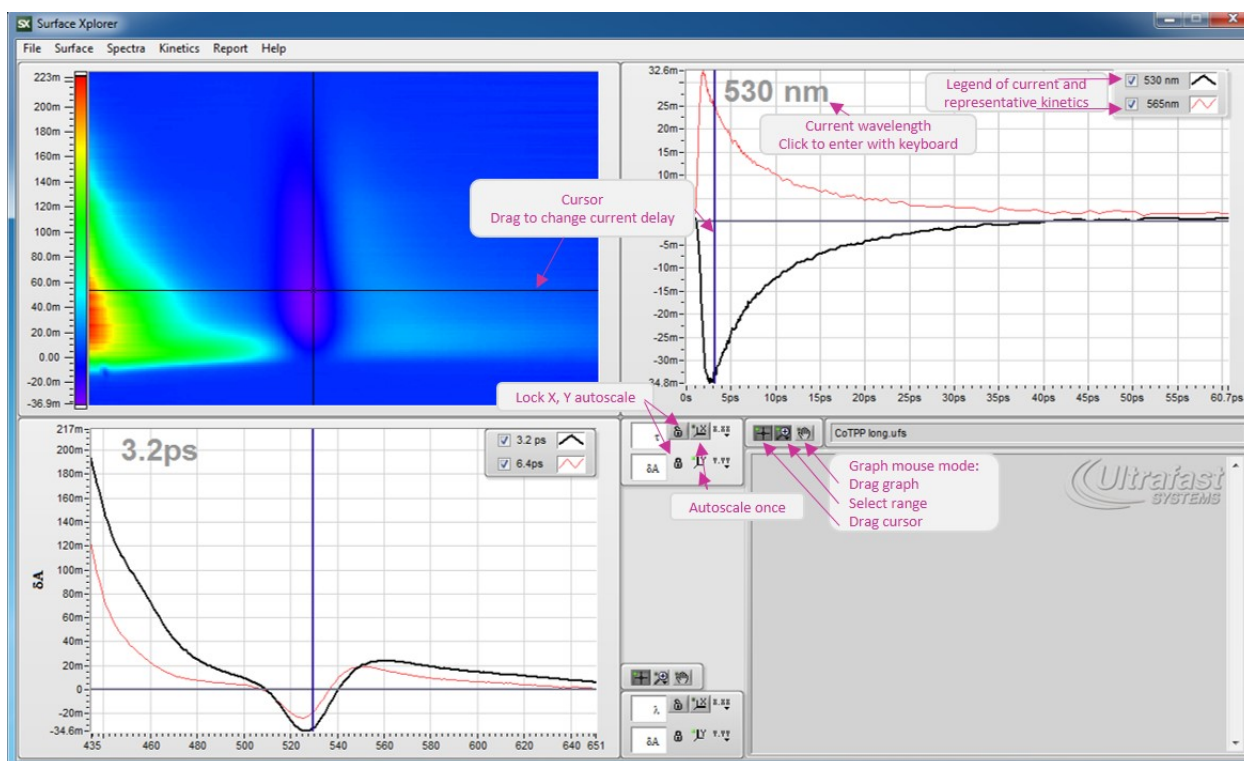
Figure 4 - Main SX panel. Description of the Kinetic Panel.

File Info
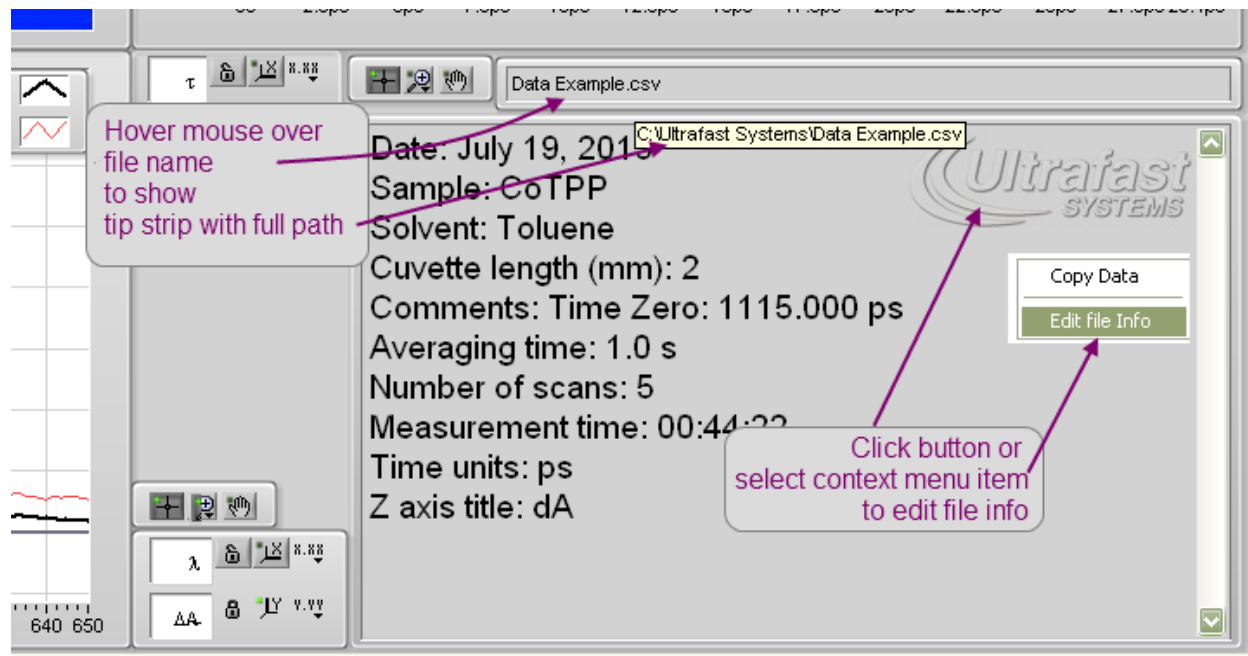
Figure 5 - File info panel.

The right bottom panel contains any additional information stored in the data file like sample and solvent details, experiment description, etc. Users can edit this information by switching to editing mode. To switch, press **Edit File Info** button, select context menu command or press any key in file info text field. The **Edit File Info** button is displayed when the mouse pointer is hovered over the logo in right top corner of the file info panel. This option is also available from the context menu.

File Menu

This category is for loading and saving 3D data files with extension .ufs
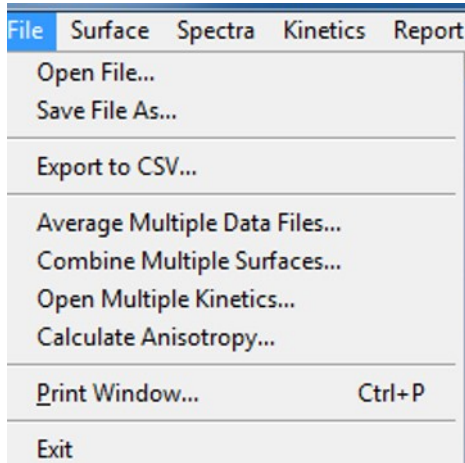
Figure 6 - SX Menu panel and File menu.

Open File…

Use this function to open new surface data files.

Save File As…

Use this function every time you want to save the currently opened 3D surface, for example after you have applied chirp correction.

Average multiple data files…

Use this function to average multiple data files, usually different Helios or Eos scans. Surface Xplorer will prompt you to save current data if there are any changes. After a standard Windows dialog window is opened where multiple data files can be selected (hold Ctrl and Shift to select several items).

NOTE: Files should have identical time and wavelength scales.

Combine multiple surfaces…

Use this function to open and combine multiple surfaces with different spectral or time range. Surface Xplorer will prompt you to save current data if there are any changes. For more details see Combine multiple surfaces section (page 54).

Open multiple kinetics...

This feature uploads multiple files in kinetic format for different wavelengths and builds a 3D surface. Surface Xplorer will prompt you to save current data if there are any changes.

NOTE: Files should have identical time scales.

Calculate Anisotropy

To calculate the anisotropy matrix, r(λ,τ), first open the parallel polarization data set, then choose **Calculate Anisotropy** menu item and choose the perpendicular polarization data file when asked by the program. The initially opened parallel polarization data will be substituted by the anisotropy matrix:

$$R = \frac{S_{II} - S_{\perp}}{S_{II} + 2 \cdot S_{\perp})},$$

where S_{II} is a parallel polarization data set and $S_{\perp}$ is a perpendicular one.

Surface Menu Functions

The Surface menu (Figure 7) is divided into two main categories:

- The first category contains tools for 3D surface manipulation.

- The second category includes global analysis tools.

Figure 7 - SX Menu panel and Surface menu.

Crop…

This function can be used to crop data to a region of interest. It crops data to those displayed on the 3D surface panel, which are determined by kinetic and spectrum graph X scale ranges.
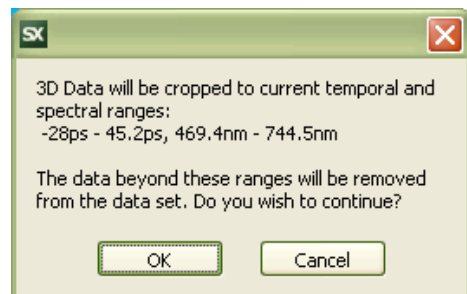
Figure 8 - Crop confirmation window.

Subtract 3D Surface

Use this function to subtract 3D surface data (i.e. solvent response) from the opened surface. Both surfaces have to be taken using the same scales.

Subtract Background

CAUTION: Do not choose this option to correct for scattered excitation light. For that choose Subtract Scattered Light option from the Surface menu.

Some data files may have a non-zero spectral baseline, which appears throughout the whole data set even before t0. Such background is usually a result of long-lived species present in the sample (species with lifetimes longer or on the order of the temporal interval between the excitation laser pulses). Additionally such background may be a result of thermal lensing caused by the excitation beam. When you choose the Subtract Background option a new window will pop up (Figure 9).

The spectrum displayed is the first spectrum that appears on your 3D surface. Use the increment button to add as many spectra as needed to produce an averaged background spectrum which will be automatically subtracted from the original data when you hit the Accept button.

Close Subtract Background window if you want to return to the main screen without subtracting the background.

Choose Save File... to save the new surface under a new name.
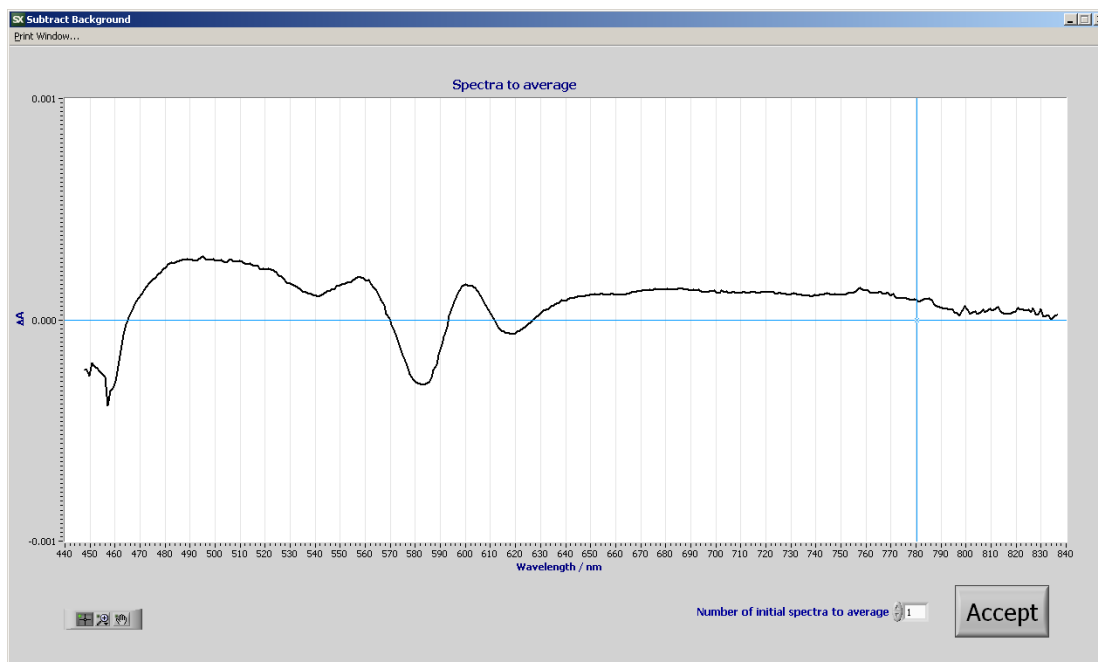
Figure 9 - Subtract Background window.

Subtract Scattered Light

CAUTION: Choose this option when performing correction for scattered pump light or scattered fluorescence (but not stimulated emission).

In some cases your detector picks up scattered light from the sample. This often happens when the excitation wavelength falls into the probing range of your experiment. To correct for such scattered pump or scattered emission (not stimulated emission) you cannot simply subtract the pre-zero background from the data, particularly if the amount of scattered light is significant.

The corrected transient absorption is expressed as follows:

$$\Delta A = -\log(10^{-S} - 10^{-S(t<0)} + 1),$$

Where S is the experimentally observed ΔA, and S (t<0) is the average of experimentally observed ΔA before time zero.
When you choose the Subtract Scattered Light option a new window will pop up,

similar to Figure 9. The spectrum displayed is the first spectrum that appears on your 3D surface. Add as many spectra as needed to produce an averaged background spectrum.

Long time-window data. Sometimes the time window can be larger than camera integration time that is synchronized with a probe light source. This causes the scattered light to disappear after some delay time on the 3D surface as it is shown on Figure 10.
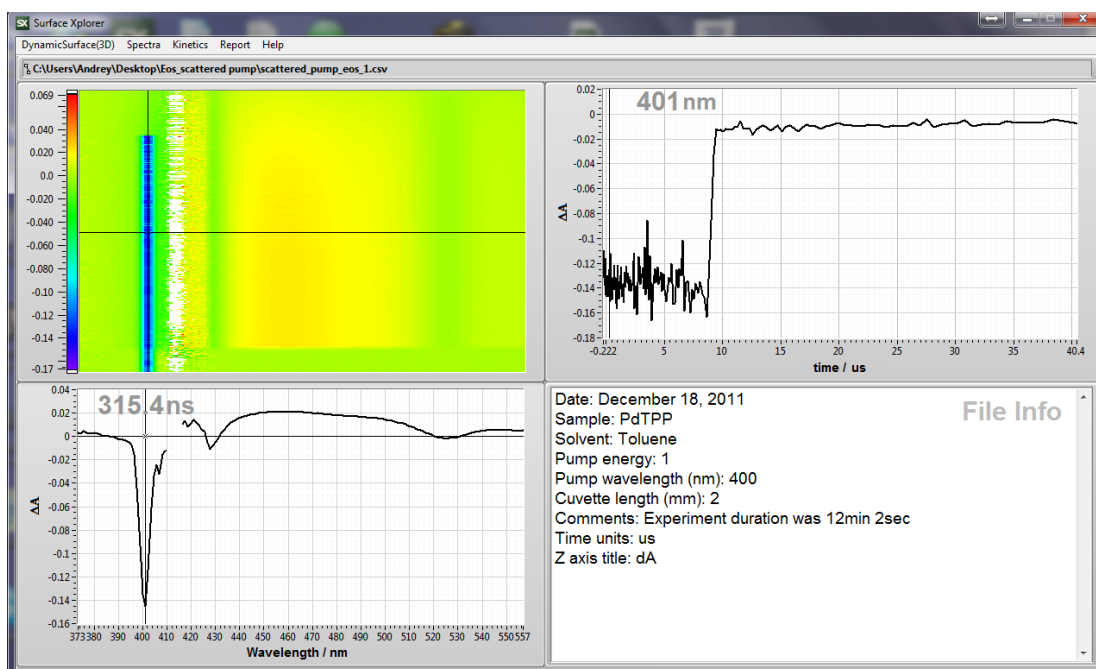
Figure 10 - Long-time-window data.

To take this effect into account click the "Set time Range" button on the bottom left of the "Subtract scattered light" window. This shows a plot that displays the kinetic profile corresponding to the wavelength selected on the "spectra to average" plot cursor (see Figure 11). Use two cursors on the kinetic plot to select the range where the scattered light disappears.

The software subtracts the averaged pre-zero spectrum from the part of the 3D surface that is before the first cursor position.

Spectra corresponding to delay times within the interval between two cursors will be removed from the data set.
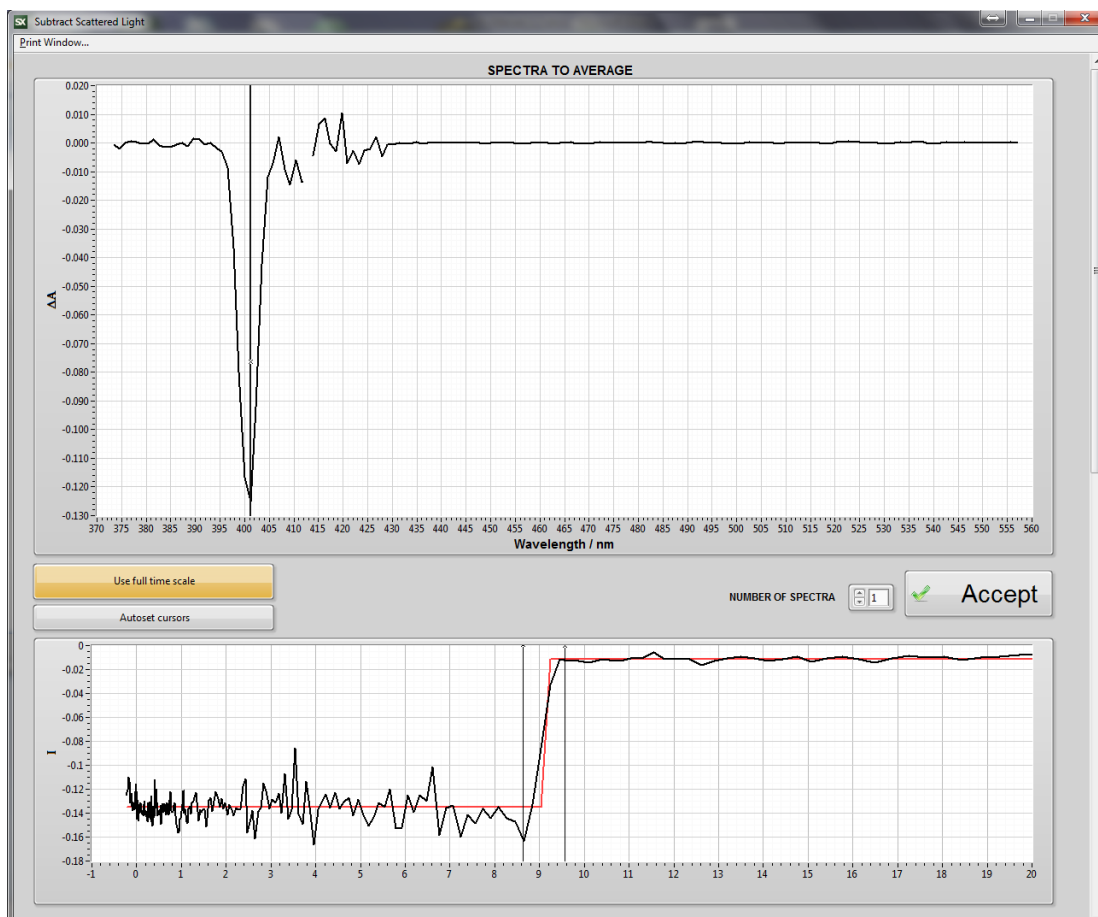
Figure 11 - Subtract Scattered Light window.

The 3D surface will be automatically corrected after you click the Accept button.

Close Subtract Scattered Light window without accepting if you want to return to the main screen without subtracting any background.

Choose Save File... to save the amended surface under a new name.

Data Conversion...

This function can be used to recalculate measured values to different representation. If currently opened data has ΔA or ΔT/T representation (Z-axis title) the user is asked if he wants to use automatic ΔA ↔ΔT/T conversion. Data values conversion can also be defined by a custom formula. The dialogue box (Figure 12) also allows changing the Z axis units (see **Z-Axis Title** section below − page 25).
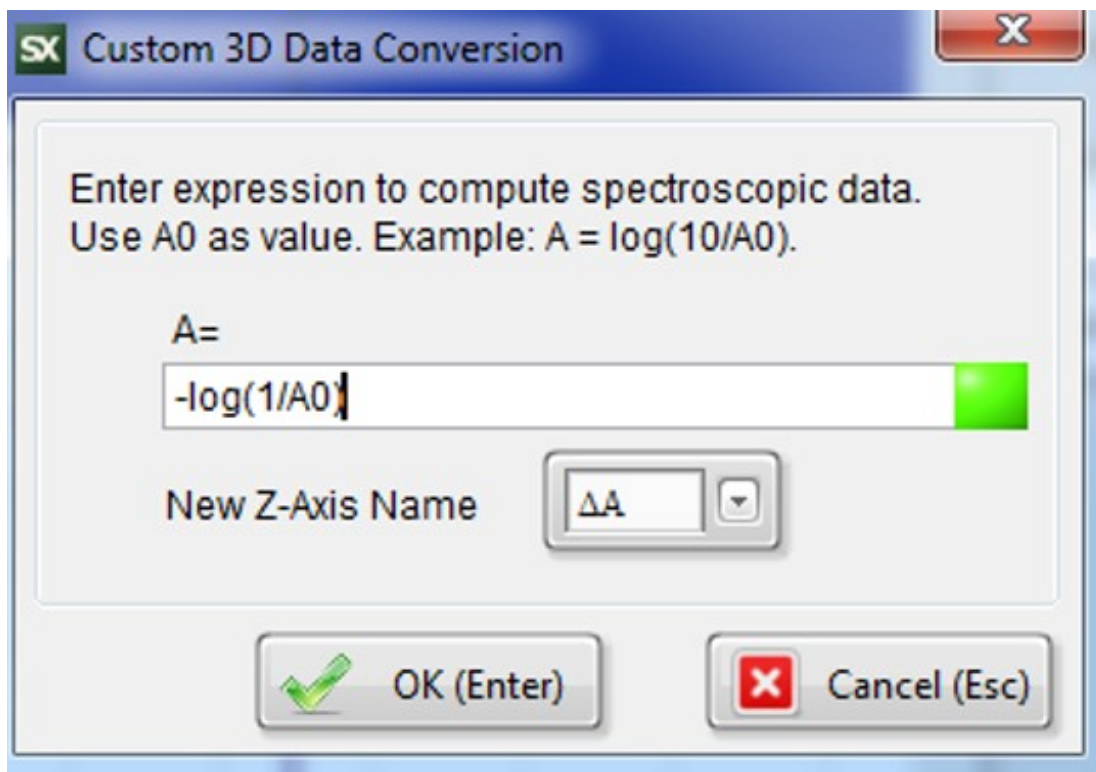
Figure 12 - D data Z axis unit conversion.

Chirp correction

This function allows you to correct the 3D data surface for the chirp of the probe pulse. The main window of the chirp correction utility is shown on Figure 13. It is similar to Surface Xplorer main window. 3D surface, kinetic and spectrum panels with cursors function similarly.

NOTE: To improve the precision of chirp correction, prior to applying the procedure, trim the data matrix on the wavelength axis to include only the useful wavelength range (e.g., to remove spectral regions without sensible data).
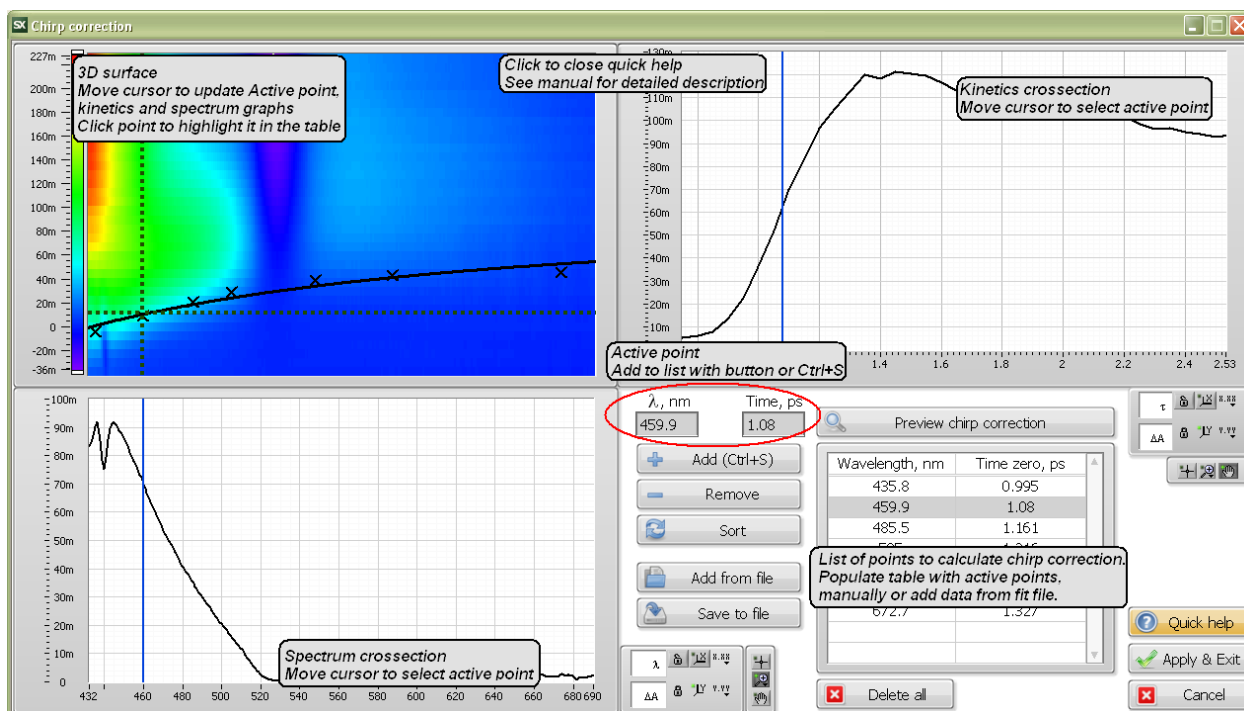
Figure 13 - Chirp correction window with quick help tips.

To apply chirp correction to the data it is necessary to specify the correct time zero values for every wavelength. The software requires specifying at least 4 time zero–wavelength pairs, others are determined by approximation with a theoretical function.

You can specify points to fit in any of the following ways:

- select an active point with cursors and press **Add** button (or Ctrl+S shortcut);

- manually populate the table with values;

- load fit coefficients file (**Add from file** button). Usually results of the **Fitting solvent response** or **Resonant fit** (see page 30) are used for that purpose. File format is described in Appendix (page 53).

These points are shown as crosses on the Surface graph. When 4 or more points are

$$t_0 = a \cdot \sqrt{\frac{b \cdot w^2 - 1}{c \cdot w^2 - 1}} + d,$$

specified, the software fits them with the following function:

where a, b, c, d – parameters to determine during the fit, w – wavelength, t_0 – time zero. The fit is shown as a black curve on the surface. Buttons **Preview chirp correction** and **Apply & Exit** also become available only after enough points have been created.

Use the following elements to control chirp correction tool:

- Click to select point in the table. This will highlight the clicked row, making the point active: move cursors to the corresponding position, update spectrum and kinetic graphs. It will also adjust the graph scales to show this point if it is beyond scale ranges.

- Click existing point on the 3D surface graph to highlight it in the table.

- Button **Sort** arranges the points in wavelength increasing order.

- Button **Remove** erased the selected point.

- Button **Save to file**. Save points to text file for future use. File format is described in Appendix, Fit files section (page 56).

- Button **Preview Chirp correction** calculates chirp correction, and switches interface to preview mode. Click **Back to Setup** button to undo changes and return to original surface.

- Button **Quick help** shows short help tips (Figure 13). Click anywhere within the screen to close help.

After chirp correction, each kinetic profile is shifted based on its time zero value, thus moving them out of the previous borders and causing undefined data – white space on the 3D surface (Figure 14). If this shift is larger than the step in the end of the kinetics, then undefined data will appear also on top of the image (long delay values).
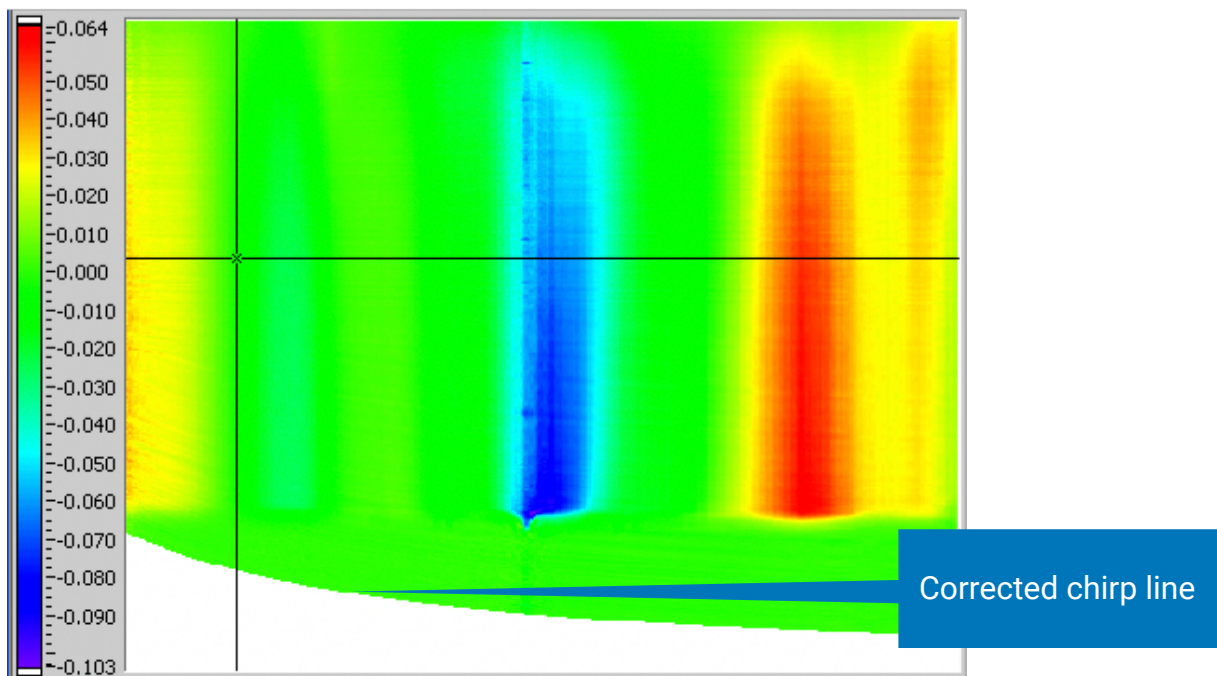
Figure 14 - 3D Surface Panel with chirp corrected data.

Click **Apply & Exit** button to perform chirp correction, return to main window and update the data.

Time zero correction

Use this function to shift the time profile on the time axis in order to adjust the zero time setting.

First decide what time corresponds to the instrument zero then choose it with a cursor on kinetics or 3D surface graph, then select **Time zero Correction** from the Surface menu. A new window will pop up prompting you to enter a new value with default at current delay.

After you click OK the time scale will be adjusted.

Adjacent Averaging...
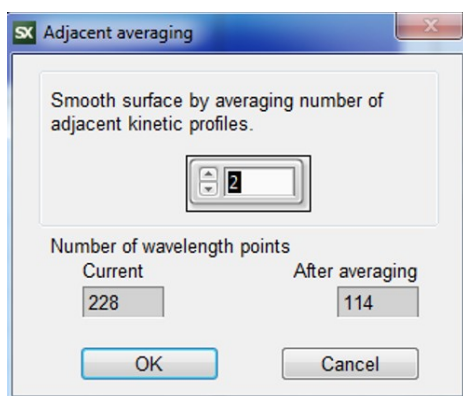
Figure 15 - Adjacent averaging window.

This command opens window (Figure 15) to specify the number of adjacent kinetic profiles to average. After you check wavelength scale reduction and confirm values, data spectral resolution will be reduced by averaging every N kinetic profiles (data matrix columns). This serves to improve signal-to-noise ratio.

CAUTION: Use this feature with data having broad spectral bands only.

Delete/Replace Bad Spectra

Sometimes in your data set there are spectra with extra noise, missed points (NaN), etc. Such data points may be caused by laser instabilities, damage in the continuum generator crystal, etc. In this case your data may look like as shown in Figure 16.
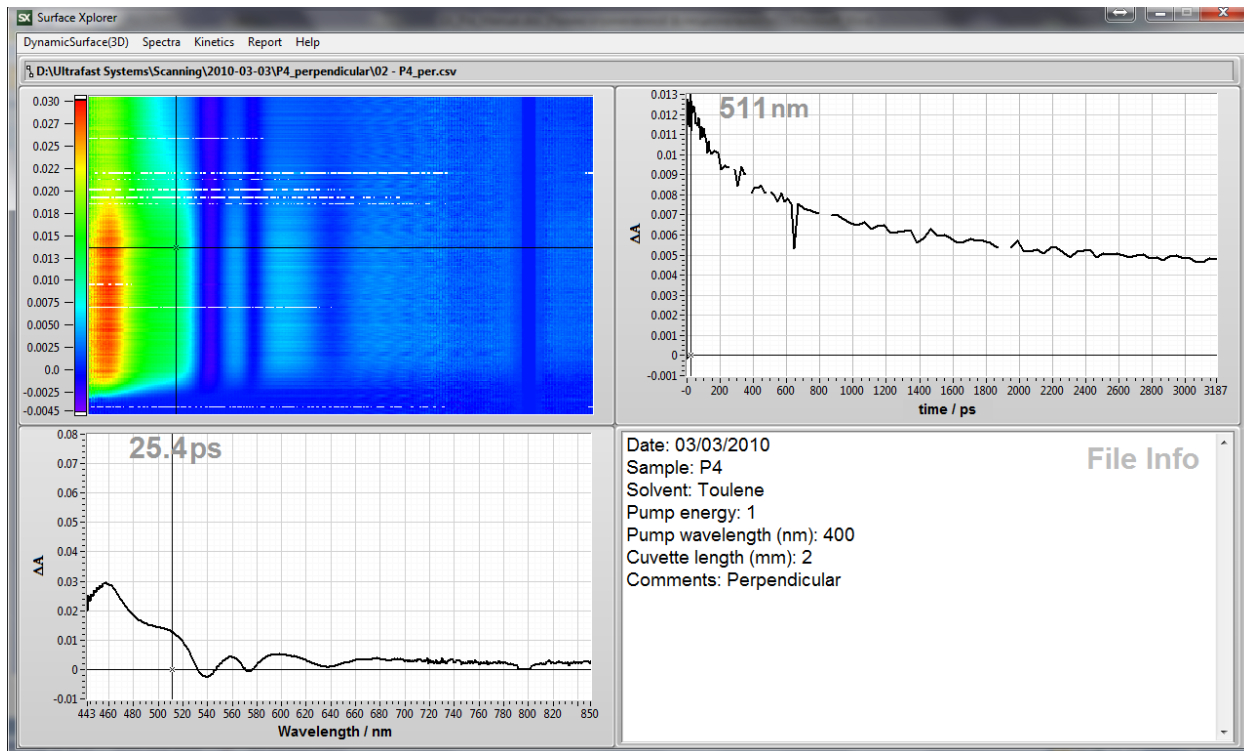
Figure 16 - A 3D Surface with missed data points.

In many cases it is sufficient to delete such spectra. However if you are trying to average several surfaces corresponding to different scans of the delay line, a single bad spectrum in one scan will result in a bad spectrum in the averaged data set. The Surface Xplorer "Replace Bad Spectra" function allows you to replace a bad spectrum with one interpolated from two adjacent spectra.

Once this option is chosen from the "Surface" drop-down menu, the dialog window as shown in Figure 17 will appear.
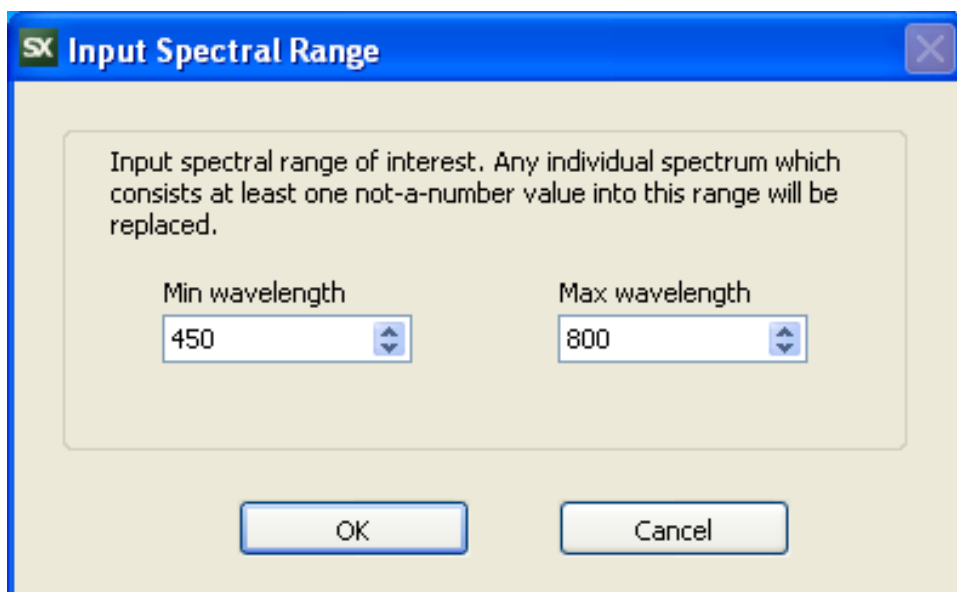
Figure 17 - Specify Spectral Range dialog window.

The spectral range to be analyzed for bad data points should be defined in this window. One the OK button is pressed the software will replace every spectrum in this range that contains at least one NaN value by an interpolated one (Figure 17).
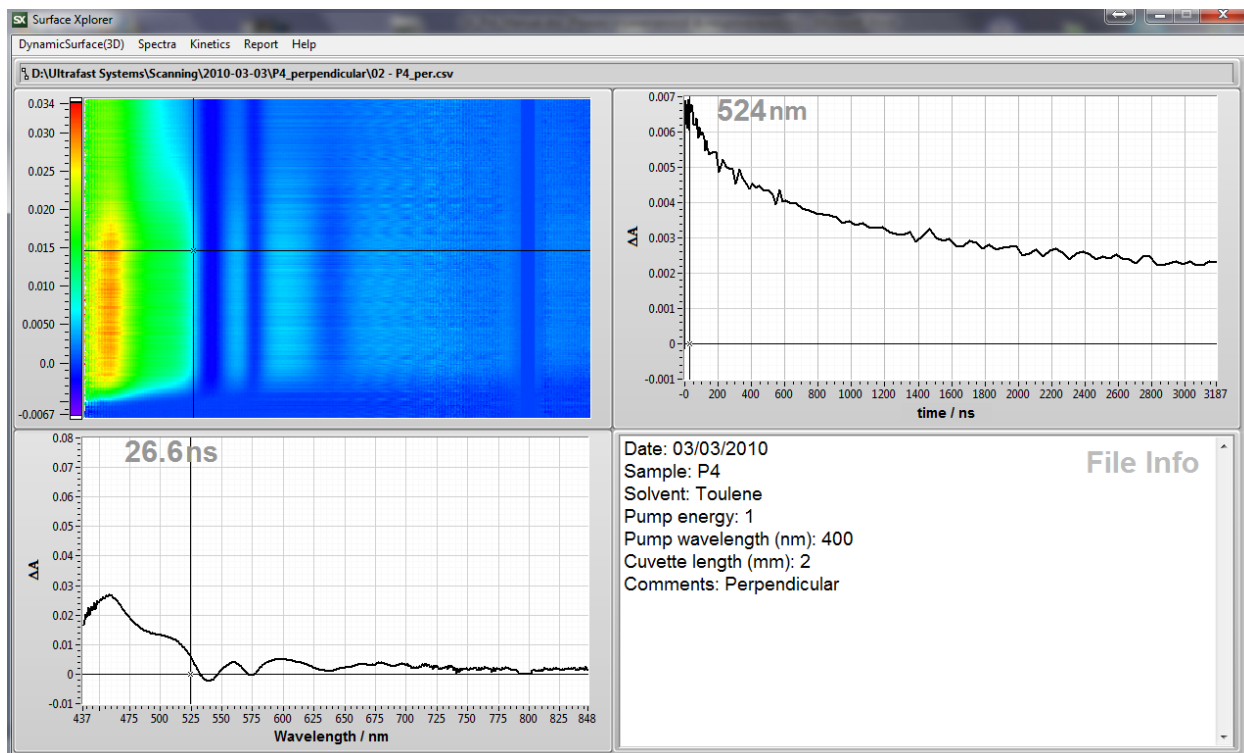
Figure 18 - Result of Replace Bad Spectra function.

Remove Spikes

Some data (say, from a CCD camera) may contain spikes as it is shown on Figure 19.
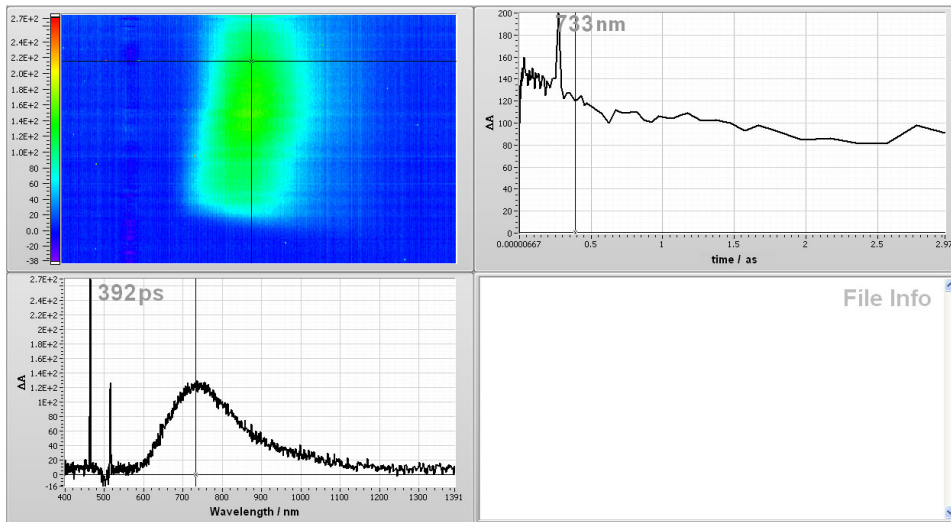
Figure 19 - Data distorted with spikes.

Remove spikes menu item opens the window shown on Figure 20. Adjust the slide to set the sensitivity of the spikes detection algorithm. Detected spikes are shown with blinking red points. Click the Accept button to apply the filter. Detected spikes will be replaced with interpolated values.
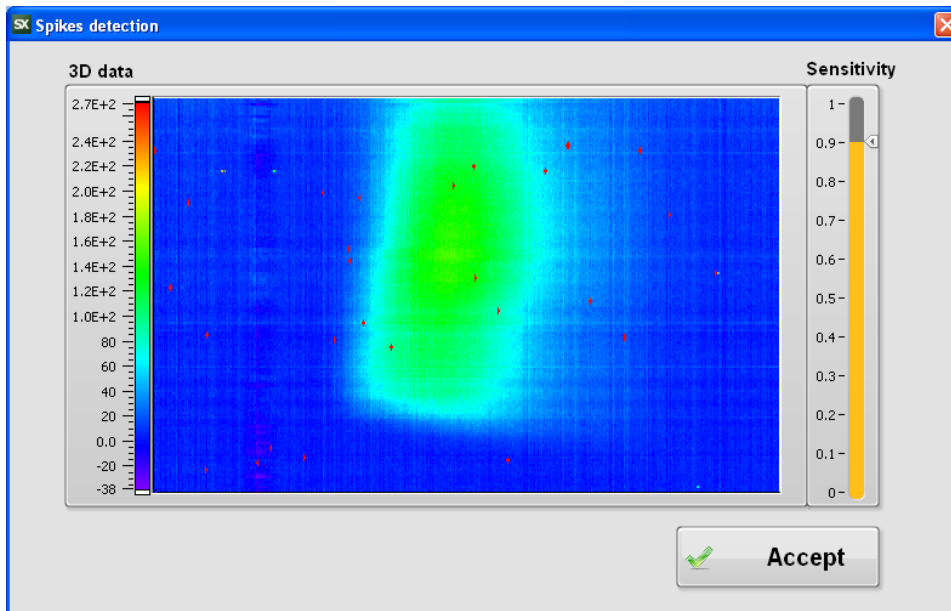
Figure 20 - Spikes detection window.

Global analysis functions

The following functions perform analysis of the

Principal Components via SVD

This function applies the singular value decomposition procedure to the 3D data matrix.

For more details see the Principal Components via SVD section (page 45).

Reconstruct From Principal Components

This function reconstructs the 3D Surface using the principal components from the SVD procedure. For more details see Principal Components via SVD (page 45) section.

Global Fit

This feature allows you to perform Global Fitting of your transient data. For details see the Global Fit (page 51) section.

Z-Axis Title

This feature allows choosing the name of the measured value. The options are: ΔA (absorption difference, e.g., transient absorption data), A (absorption, e.g., stopped flow data), I (intensity, e.g., fluorescence data), and ΔT/T (another representation for transient absorption data).

NOTE: this does not change data values. Use 3D Data conversion (page18) instead.

Spectra Menu Functions
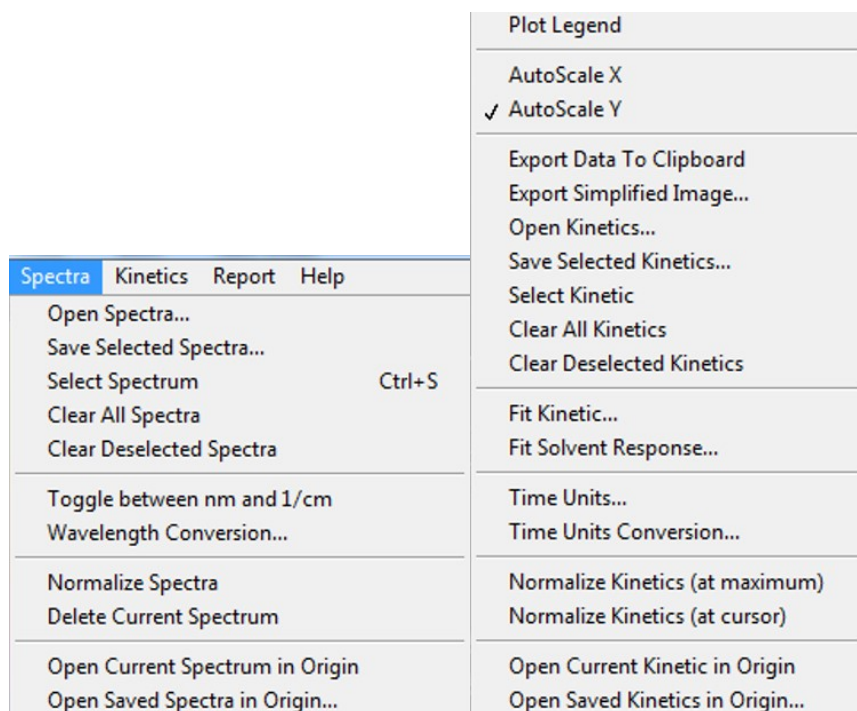
Figure 21 - Spectra menu functions.

Spectra functions are available through drop down menu and pop-up context menu context menu on the spectrum panel.

Open spectra

This command opens previously-saved spectral displays, adds them to currently shown representative spectra (from file **{original3Dsufacefilename} RepresentativeSpectra.csv**) and copies data into RepresentativeSpectra file. Both files have the same format described in Appendix (page 56).

Save Selected Spectra...

This function saves the currently selected spectra to **{original3Dsufacefilename} RepresentativeSpectra.csv**. File format is described in Appendix (page 56).

NOTE: To use this function a spectrum has to have been selected using the Select Spectrum function.

Select Spectrum

This function adds the currently displayed spectrum to **{original3Dsufacefilename} RepresentativeSpectra** which can be saved using Save Selected Spectra... This function allows the user to display multiple spectra for a more facile change comparison. By moving the horizontal cursor on the 3D graph to a different time point a new spectrum is displayed and selected.

Clear spectra

This function purges the currently displayed spectra from the Spectrum Panel.

Clear Deselected Spectra

This function purges the deselected spectra only from the Spectrum Panel.

Toggle between nm and 1/cm

This function allows the user to change the wavelength axis from nm to wave numbers and vice versa.

Wavelengths conversion

This feature allows recalculating the X scale, for example to take into account CCD pixel calibration.
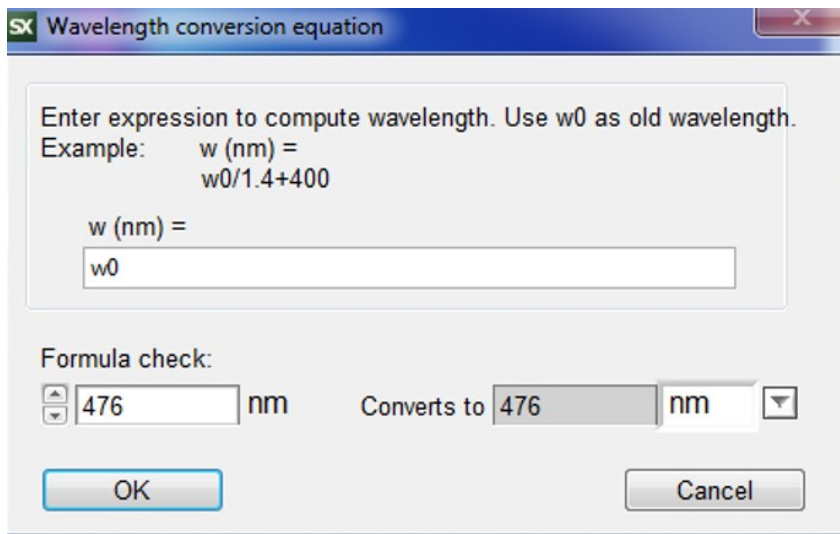
Figure 22 - Wavelength conversion.

Normalize spectra

This function normalizes the displayed spectra at the wavelength selected by the vertical cursor on the 3D Surface Panel.

Delete Current Spectrum

This feature deletes the currently selected spectrum from the data set.

Open current spectrum in Origin

Opens the currently displayed spectrum in Origin software. This feature requires Origin Lab Corporation Origin or Origin Pro version 8.6 or later to be installed on the computer.

Open saved spectra in Origin

Opens the spectra saved with *Save spectra* function in Origin software. This feature requires Origin Lab Corporation Origin or Origin Pro version 8.6 or later to be installed on the computer.

Export Data to Clipboard

NOTE this command is available only in shortcut menu.

This command copies all displayed graphs data into the text table of tab separated values to paste into third party programs (Microsoft Excel, Origin, notepad, etc). Use this function to copy data to Origin if your Origin version is not supported (8.5 or earlier). See data format in Appendix (page 53).

Export simplified image

NOTE this command is available only in shortcut menu.

This command allows you to save spectra panel view into image file.

Kinetics Menu Functions

Figure 23 - Kinetics functions in pull-down and context menus.

Kinetics functions can be accessed via drop-down menu and pop-up context menu of the kinetic panel.

Open Kinetics

This command opens previously saved kinetics displays, adds them to currently shown representative kinetics traces (from file **{original3Dsufacefilename} RepresentativeKinetics.csv**) and copies data into Representative Kinetics file. Both files have the same format described in Appendix (page 53).

Save Selected Kinetics…

This function adds the currently selected time profile and its calculated parameters into the working folder under the name **{original3Dsufacefilename} RepresentativeKinetics**. File format is described in Appendix (page 53).

NOTE: To use this function a spectrum has to have been selected using the Select Spectrum function.

Select Kinetics

This function adds the currently displayed spectrum to **{original3Dsufacefilename} RepresentativeKinetics** which can be saved using Save Selected Kinetics... This function allows the user to display multiple kinetics for a more facile change comparison. By moving the vertical cursor on the 3D graph to a different wavelength a new kinetic trace is displayed and selected.

Clear Kinetics Traces

This function purges the currently displayed time profile(s) from the Kinetics Panel.

NOTE: The corresponding Representative Kinetics file from the working folder will be deleted.

Clear Deselected Kinetics

This function purges the deselected kinetic traces only from the Kinetics Panel.

Fit Kinetics

This feature allows fitting currently selected time profile with a sum of convoluted exponentials. This feature can be used to fit regular transient decay profiles. For details see **Fitting Kinetics** section (page 40).

Fit Solvent Response

This feature allows fitting currently-selected time profile with a sum of Gaussian functions or the first two derivatives of a Gaussian. Use this feature to fit the solvent response profiles prior to chirp correction. For details see **Fit Solvent Response** section (page 37).

Time Units

This function allows choosing data time units from the following options: fs, ps, ns, us, ms, and s.

Time Units Conversion

This function allows recalculating the time scale using a custom formula using the following dialog:
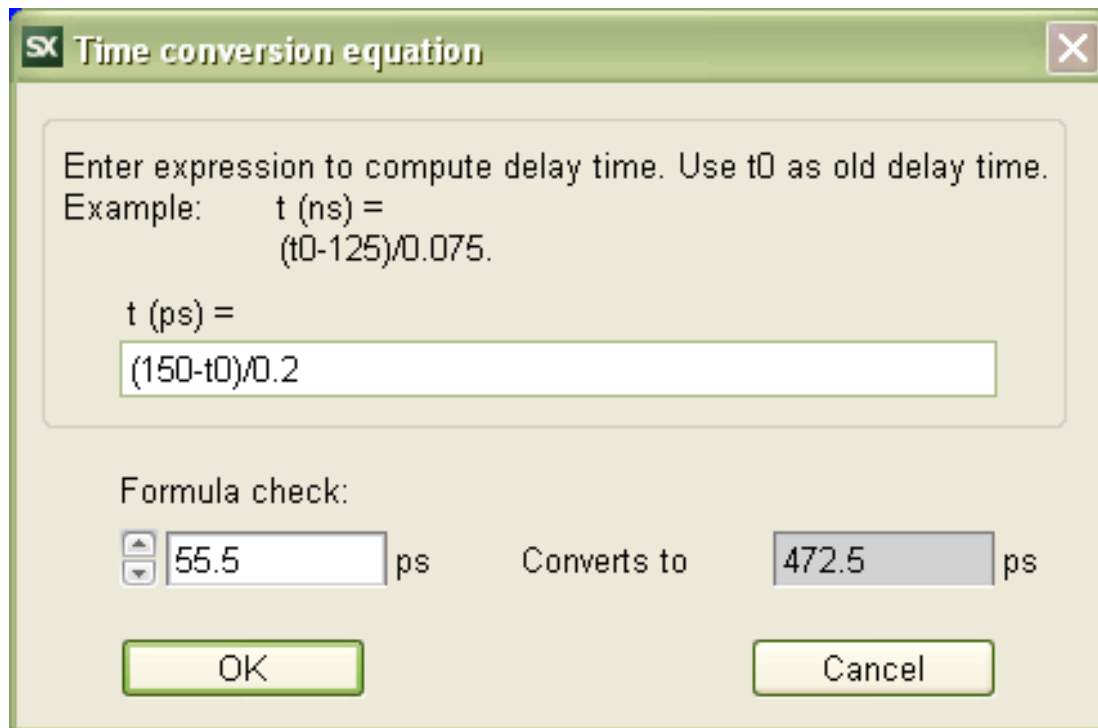
Figure 24 - Time conversion window.

Normalize Kinetics (at maximum)

This tool normalizes the time profiles saved by the "Save Kinetics" function to equal maxima.

Normalize Kinetics (at cursor)

This function normalizes saved time profiles at the time selected by horizontal cursor in the 3D Surface panel.

Open Current Kinetics in Origin

Opens the current time profile in Origin software. This feature requires OriginLab Corporation Origin or Origin Pro version 8.6 or later to be installed on the computer.

Open Saved Kinetics in Origin

Opens time profiles saved with the **Save Kinetic** feature in Origin software. This feature requires OriginLab Corporation Origin or Origin Pro version 8.6 or later to be installed on the computer.

Export Data to Clipboard

NOTE: This command is available only from the shortcut menu.

This command copies all displayed graphs data into the text table of tab separated values to paste into third party programs (Microsoft Excel, Origin, notepad, etc). Use this function to copy data to Origin if your Origin version is not supported (8.5 or earlier). See data format in Appendix (page 53).

Export simplified image

NOTE: This command is available only from the shortcut menu.

This command allows you to save spectra panel view into image file.

Report Menu Functions

Surface

This function opens a 3D view of the data surface (Figure 25), allows you to set up a view, add comments and print it on any installed printer.

NOTE: By default this report shows only the data displayed in main window. You do not need to crop data prior to using this function.

Use the following tips to manipulate the surface:
- Click+drag freely rotates the surface.
- Shift+click+drag works as a panning tool – drags surface.
- Alt+click+drag or mouse wheel: changes zoom.

The top menu on the 3D Surface report window contains the following options:
- Print Window – open standard printer select dialog to print the current view.
- View – contains a list of options to control the 3D surface appearance
    - 3D – rotates surface to the 3D view
    - Bird's eye – rotates the surface to the bird's eye view
    - LightingSwitch – toggles external lighting
    - Fast Draw – toggles fast draw in the rotation mode (works much faster for large surfaces)
    - Color bar – show/hide color bar
    - AutoscaleZ – set Zand color bar to autoscale or lock to current values.
- Time Scale – opens the dialog to enter time scale limits.

The comment field below the surface contains file information from the main SX window.
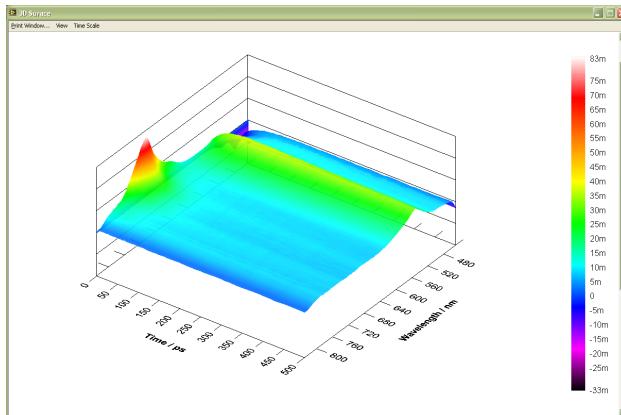
Figure 25 - The 3D Surface report window

Spectra

This function displays the previously saved spectra (Figure 26). In the top right corner of the screen there are delay times corresponding to each spectrum. You can re-scale the spectrum panel the same way as described for the main window on page 10.
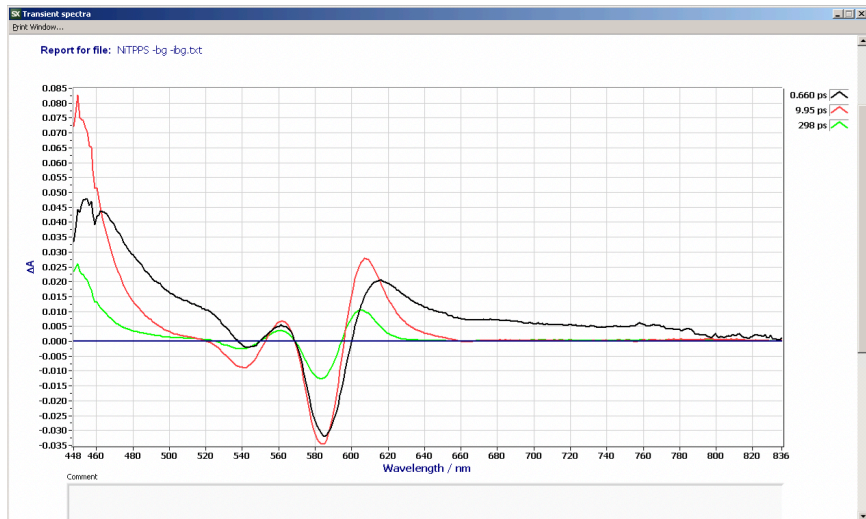
Figure 26 - Representative Spectra report window.

Kinetics

This function displays the previously saved time profiles (Figure 27). The legend in top right corner of the screen shows wavelength corresponding to each kinetic trace. You can rescale the kinetic panel the same way it is described for the main window on page 11. At the bottom of the page you can add comments to the file.
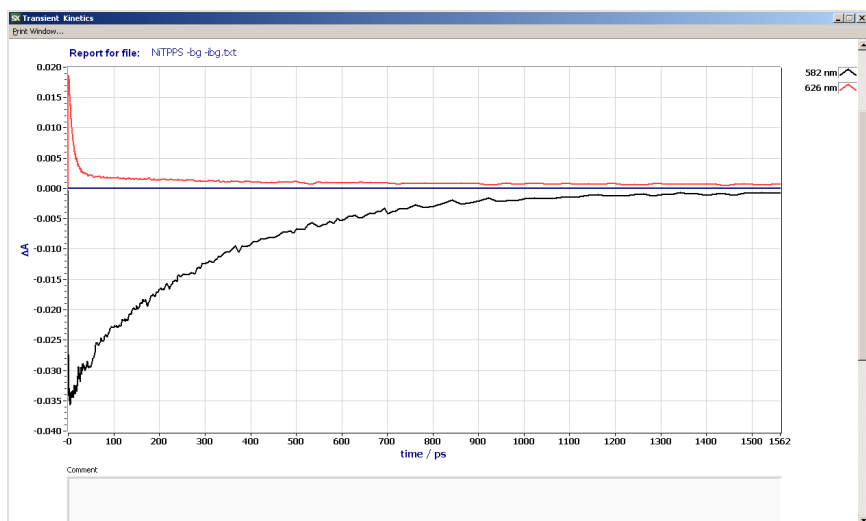
Figure 27 - Kinetics report window.

Spectra + Kinetics

This function displays the previously saved spectra and kinetic profiles. In the top right corner of each panel screen there are the corresponding times and wavelengths. You can rescale the spectrum or kinetic panel the same way as described for the main window on page 10.
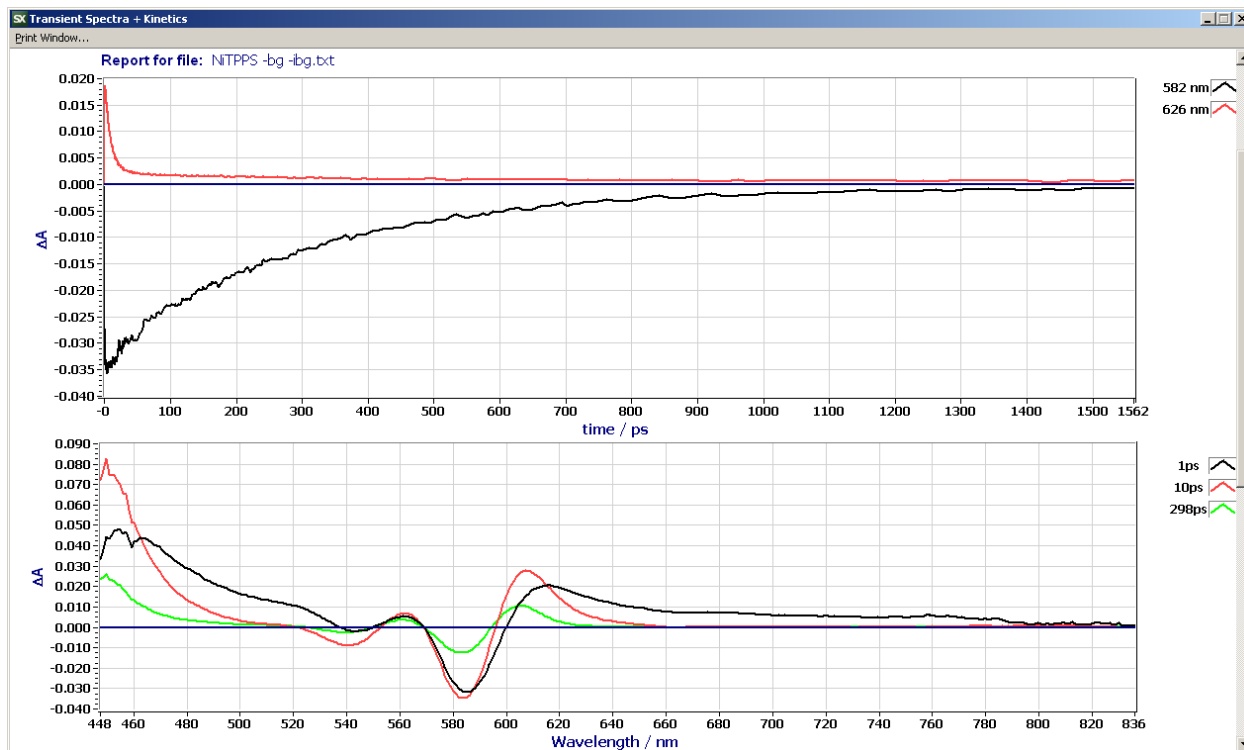
Figure 28 - Spectra + Kinetics report window.

Spectra + Kinetics + Global Fit

This function displays the previously saved spectra, kinetic profiles and the results of global analysis (Figure 29).

The top and bottom left panels are similar to those described in the **Spectra** (page 10) and **Kinetics** (page 11) sections. The bottom right panel contains the results of the global fitting and displays the spectral distributions of the pre-exponential coefficients and the corresponding lifetimes. Each spectrum on that panel corresponds to the lifetime located next to the corresponding color line.
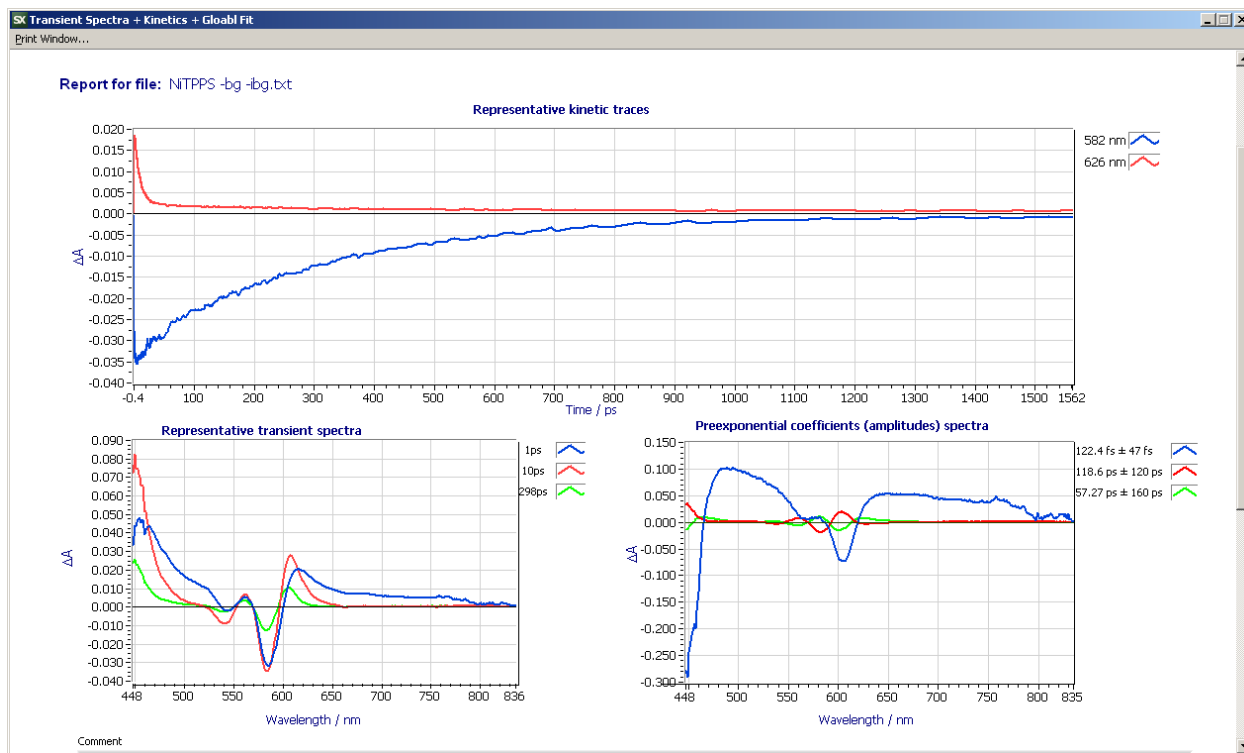
Figure 29 - Spectra + Kinetics + Global Fit report window.

Fitting Solvent Response

To perform the chirp correction or to extract the solvent response kinetic parameters one needs to use **Fit Solvent Response**. This function is also useful when you want to determine the instrument response time. The function fits the kinetic trace at the current wavelength to the sum of Gaussian (optional) and first two derivatives of the Gaussian. To perform the non-resonant kinetics fit one needs to have collected the $A(\lambda,t)$ data of neat solvent under the same experimental conditions as the sample being studied. The solvent response kinetics profile should look like the one shown in Figure 30. For Raman solvent response the signal looks like a Gaussian directed upward or downward.
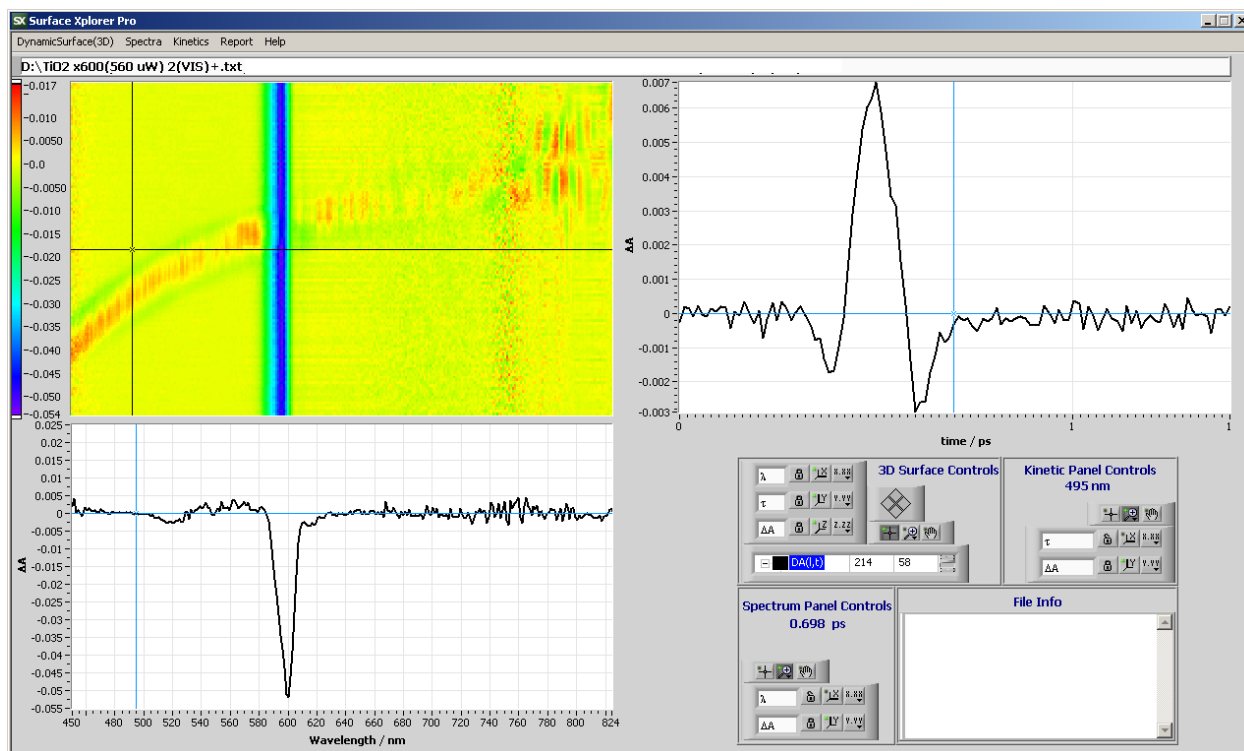
Figure 30 - Example of solvent response surface.

After the wavelength for fitting is selected, go to the drop down menu on the top control panel (Figure 31) titled **Kinetics** and select **Fit Solvent Response**.
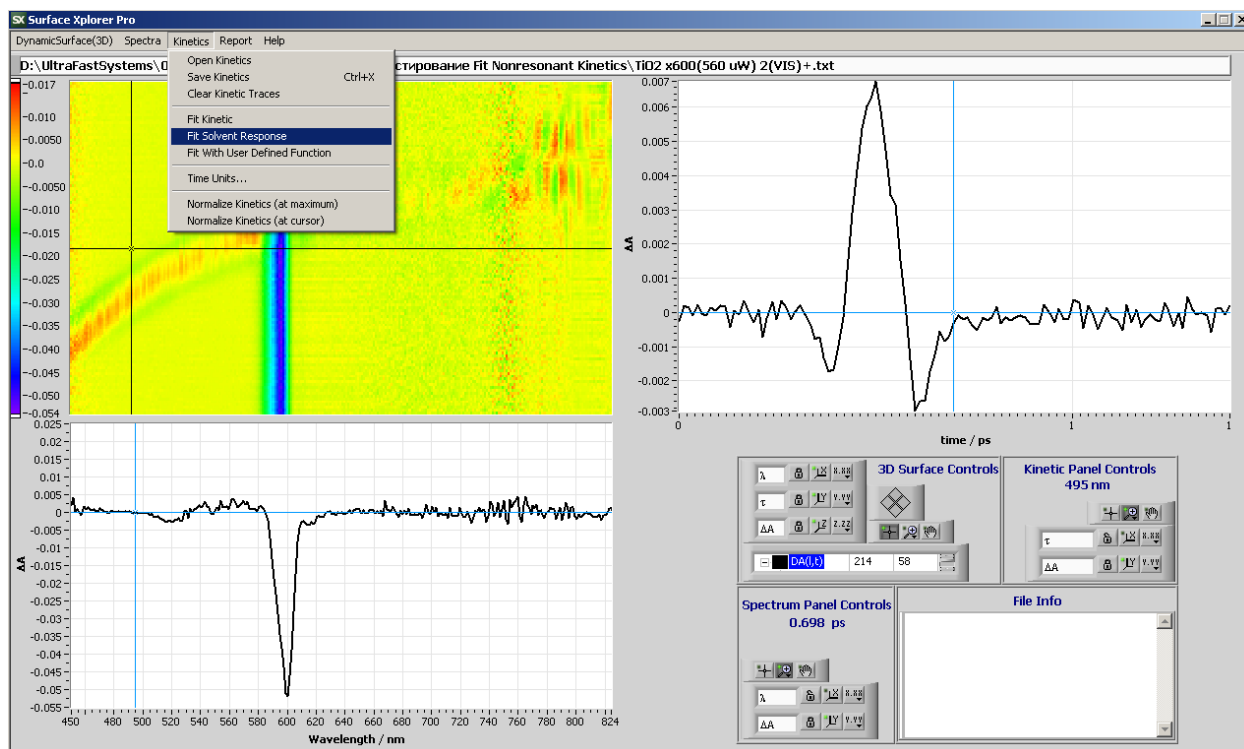
Figure 31 - Selecting the Kinetics Menu.

The fitting window panel will open (Figure 32).
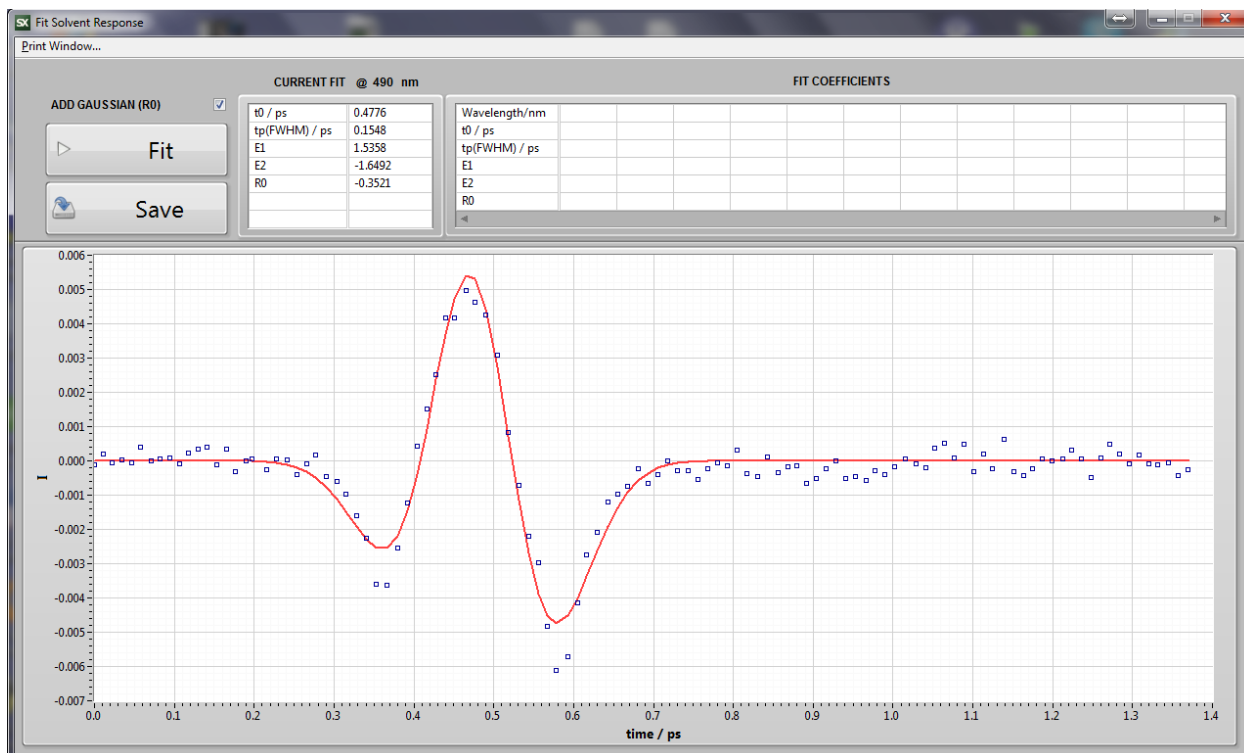
Figure 32 - Fit Solvent Response window.

Time zero – point of signal sign inversion (point of max amplitude in the case of Raman band, e.g., 0.88ps in this case)

E1 and E2 – amplitudes of the firstand the second derivatives.

R0 is the amplitude of the Gaussian (this component is optional, necessary only for Gaussian profiles; check the square "Use R0" to activate)

Pulse width.

Figure 33 - Coefficients table.

Press the **Fit** button to activate the fitting process. The fitting result will be displayed as a red curve on the kinetics panel (Figure 32). To exit the fitting window without saving the results just close the Fit Solvent Response window. Press the Save button to save the results of the fitting procedure. Close the Fit Solvent Response window to return to the main window.

In the main window select other wavelengths for fitting and repeat the same procedure for at least 5 wavelengths if the chirp correction operation is to be done.

The fitting results are saved in the same directory as the source 3D surface data file. The resulting file name is **{original3Dsufacefilename} FitCoefficients.csv**. This file can be used for the chirp correction procedure (for more details see the **Chirp Correction** section at page 18).

Fitting Kinetics

This function allows you to fit a kinetic trace for the selected wavelength with a sum of convoluted exponentials:

$$S(t) = e^{-\left(\frac{t-t_0}{t_p}\right)^2} * \sum_i A_i e^{-\frac{t-t_0}{t_i}}, \; t_p = \frac{IRF}{2 \cdot ln2}$$

whereIRF isthe width of instrument response function (full width half maximum), t_0 is time zero, A_i and t_i are amplitudes and decay times respectively, * is convolution.

Much like in the previous section, before proceeding with the fitting operation one needs to select the desired time profile by using the vertical cursor on the 3D Surface Panel.

NOTE: Surface Xplorer operates with all the data independent of main window kinetics panel range. All the unwanted segments of the kinetics can be trimmed off by using the **Crop** function from the Surface menu (see page 14).

To activate the fitting procedure, select **Fit Kinetics** option from **Kinetics** drop down menu. The new **Fit Kinetics** window will open (Figure 34 Fitting kinetics. Figure 34). Central panel displays the kinetic trace to be fitted S_{exp} (blue dots) and the S(t) fit itself (red solid line), below is a residuals graph (S_{exp}-S). You can adjust scale range using the graph control pallet (by default it appears automatically when hovering the mouse over graph). Standard deviation value below Residuals graph is the numerical characterization of the fit quality:

$$\sigma = \sqrt{\frac{\sum (S_{exp} - S)^2}{n-1}},$$

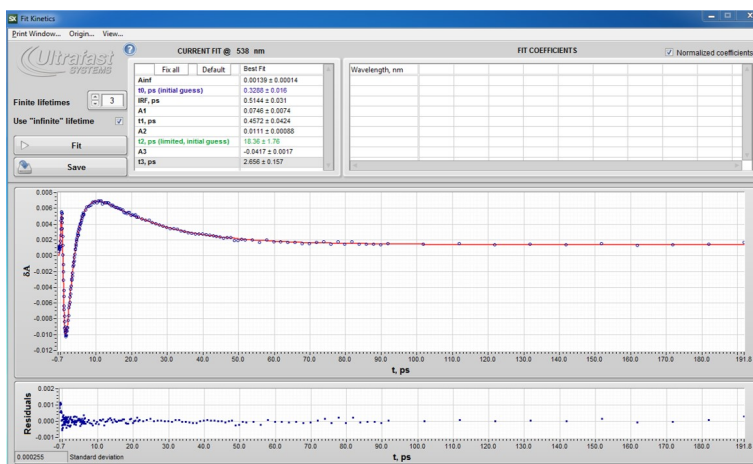where n is the number of data points.

Figure 34 - Fitting kinetics.

Top left portion of the screen is used to control the fit procedure. To run fit you should provide basic information about kinetics: number of components to use (Finite lifetimes control), whether to use component with infinite lifetime or not. This information will be displayed in **Current fit** table. At first launch all amplitudes (Ainf, Ai) are 0 and fit graph shows straight fit line.

NOTE: In order to obtain good fit results, number of components set should be reasonable. Trying to fit data with excessive number of components can yield inconsistent results.

NOTE: As there is no constant offset in the fit formula, background should be corrected in main window before running the fitting function.

You can press **Fit** button to run the procedure with default parameters. When the fitting finishes, graphs and **Current fit** table will be updated with fit results. Table shows the optimal coefficient values ± corresponding confidence intervals found by the algorithm. Confidence interval is a factor that shows both fit quality and model sensitivity to a given parameter. Confidence intervals are always calculated with some probability - in SX it is set to 90%.It roughly means that modifying parameters within confidence intervals will not change fit criteria (standard deviation) more than 10%.

NOTE: Mouse cursor is disabled until fitting procedure is completed.

After fit procedure ends, please review parameters for consistency:
- Fit and experimental data should be close giving low residual values
- t0 can be visually estimated from the graph and found value should be close to it.
- IRF width should be reasonable: it is determined by probe and pump pulse widths.
- Components' amplitudes should be reasonable. For example, 2 components with very close lifetimes and huge opposite sign amplitudes indicate bad fit setup.

You can save resulting fit parameters into the table (and automatically into FitCoefficients.csv file). Please see file description in Appendix **Fit Files** (page 53). Table shows normalized coefficients: all amplitude modules sum up to 1. To show absolute values, remove checkmark **Normalized coefficients** in the right corner of the window.

Advanced fitting

If fit results are not consistent, you may need to use advanced fitting tools to restrict parameters.
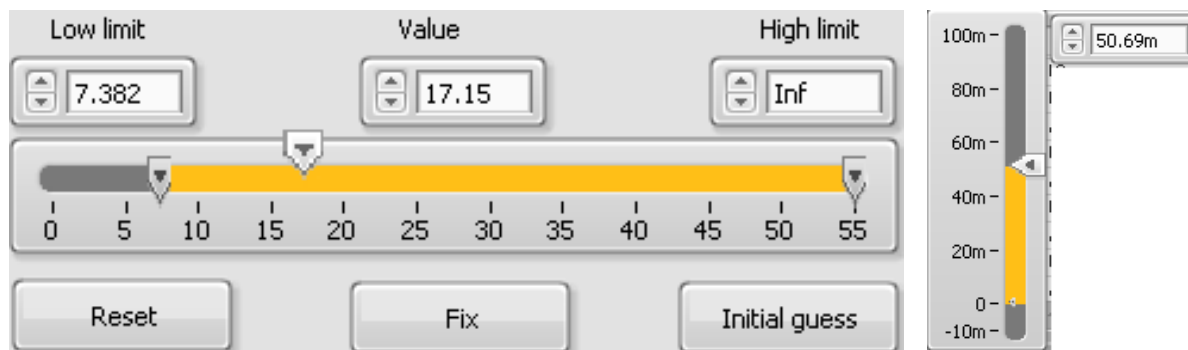
Figure 35 - Component time and amplitude parameter control tools.

Click **Current Fit** table row to show component control tools (Figure 35).

If you click any of the time parameter (t_0, **IRF**, lifetimes t_i) rows in the **Current Fit** table, a control will pop up allowing you to individually limit these parameters. It contains the following elements:

- **Scale** with sliders. Contains (left to right) **Low limit**, **Value** and **High limit** sliders. Scale range is determined by graph X scale range. Zoom in to the beginning of the rise on the kinetics and then set **IRF**, t_0, short lifetimes t_i more precisely. Click **Autoscale once** button in the X axis control panel to show all graph and then set long lifetimes. You can also type in scale limits in the control, they do not affect slider values.

- **Value** numeric field and large slider – current parameter value. Value displayed in the table, fit and residuals graphs will be updated automatically as you change it.

NOTE: You cannot set **Value** outside limits, change limits first.

- **Low limit** and **High limit** numerical fields and left and right sliders' values. These controls allow you to set the parameter limits during the fit, result will never go out of the defined range. By default **High limit** is **Inf** and **Low limit** is 0, except for t_0 - it is determined by the time scale low limit. Type a value into numerical fields to set parameter limit outside scale range. Table row will be colored yellow and header will say limited. Obviously this parameter does not play any role if parameter is fixed. But you can set initial guess and limit parameter at the same time. This is also a very powerful tool – it can be used to order parameter lifetimes, ignore noisy signal in the beginning, etc.

- **Fix** – fix current value not to be modified during the fit. Table row will be colored red and say fixed in row header.

- **Initial guess** – use current value as initial guess for the algorithm. Fit results can be improved dramatically if you enter correct initial guess values. If parameter was fixed, it becomes variable automatically and **Fix** button returns to the original state. Table row is colored blue and row header says I**nitial guess**.

- **Reset**. Clears all restrictions of the parameter: makes it variable in unlimited ranges (0, inf) with default guess value.
  Click anywhere outside the control to hide it. Changes are applied automatically. The following buttons to the left of the table control all time parameters:

- **Fixed**. All time parameters are fixed at their current values.

- **Initial guess**. Initial guesses for all time parameters that are not fixed are set at their current values.

- **Variable**. Clears all restrictions for all parameters: all become variable in unlimited range with default guess values.
  If you click any of the **Ainf**, or **Ai** rows in the table a control (Figure 36) will pop up, allowing you to change component amplitude using slider or by entering a value. Fit and residuals graphs will be updated automatically to reflect new amplitude. You can also type slider limits for better slider range.

NOTE: You cannot limit or fix amplitude values.

Figure 36 shows parameters with multiple restrictions.

All parameter restrictions and values are saved if you close the **Fit Kinetics** window and then load it with new data.
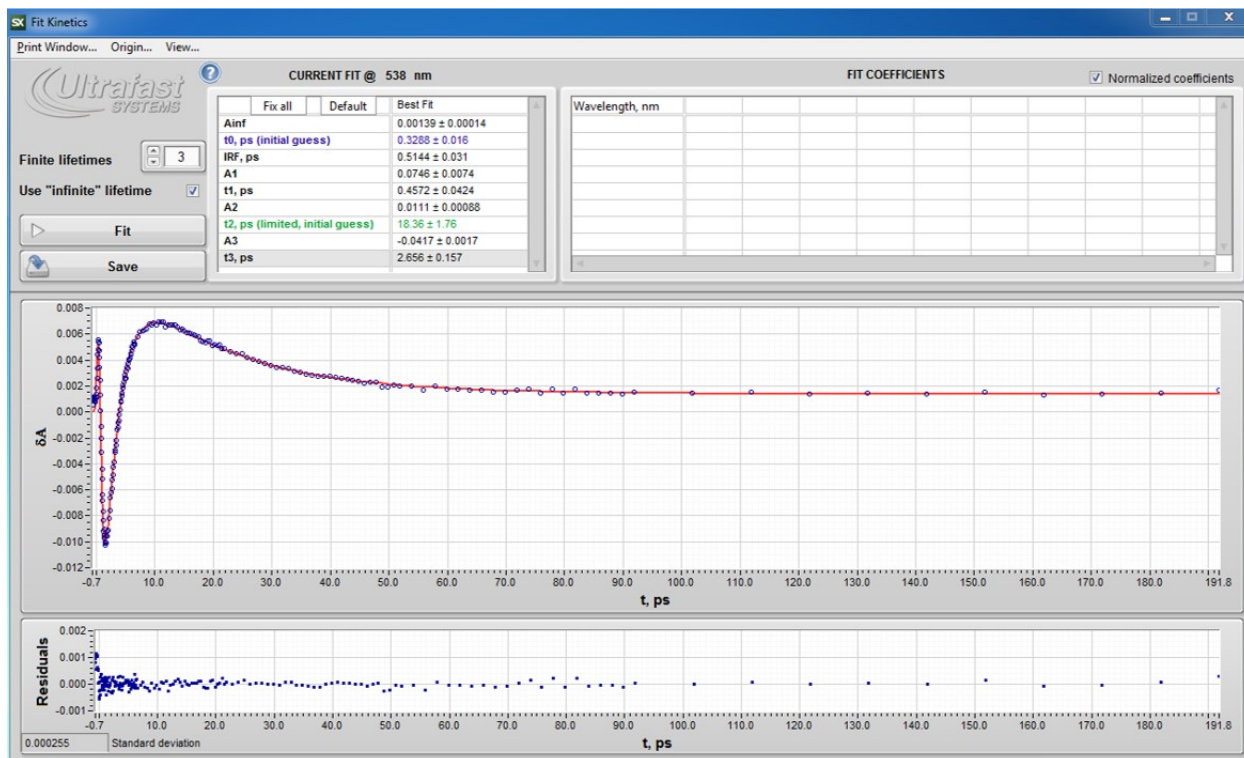

Figure 36 - Results of a nonlinear fit.

Principal components via SVD

This function implements the singular value decomposition (SVD) procedure on the 3D data matrix. Before use, make sure to:

1. Correct the data for chirp;

2. Cut out all the spectra or kinetic traces containing NaN ("NotaNumber") elements from the 3D surface in the main window (to review of the 3D Surface controls refer to page 7). These elements are represented by white field (no data) on the 3D surface.

The Singular Value Decomposition (SVD) procedure results in a basis set of eigenvectors whose matrix product Δ**A'** is given by:

$$\Delta A' = U \times S \times V,$$

is the least squares best estimator of the original 3D data set (ΔA). The SVD method produces a linearly independent set of eigenvectors with all of the spectral information stored in the few first eigenvectors with highest significance coefficients (S, the diagonal elements of the S matrix), plus some additional noise eigenvectors that can be excluded from further consideration. See the literature reference in the end of the chapter for more information.

To facilitate determination of the number of principal components, SX employs several plotting modes to display the results of SVD graphically. First, it displays the individual eigenvectors: the resulting principal spectra (bottom left graph) and the principal kinetics (upper right). This is especially useful for discerning how many vectors represent variations that arise from chemical processes, and how many vectors can be best described as experimental noise. Surface Xplorer also plots the differences between the original data and the factored scans (ΔA-$\Delta A'$)2 (the ($\Delta\Delta A$)2 or o matrix – the upper left panel) to allow for better assessment of the nature of the residuals that were factored from the principal eigenvectors by the SVD procedure.

To start the SVD analysis, select Principal Components via SVD from the upper drop down menu (Figure 7). The SVD procedure window will open (Figure 37).
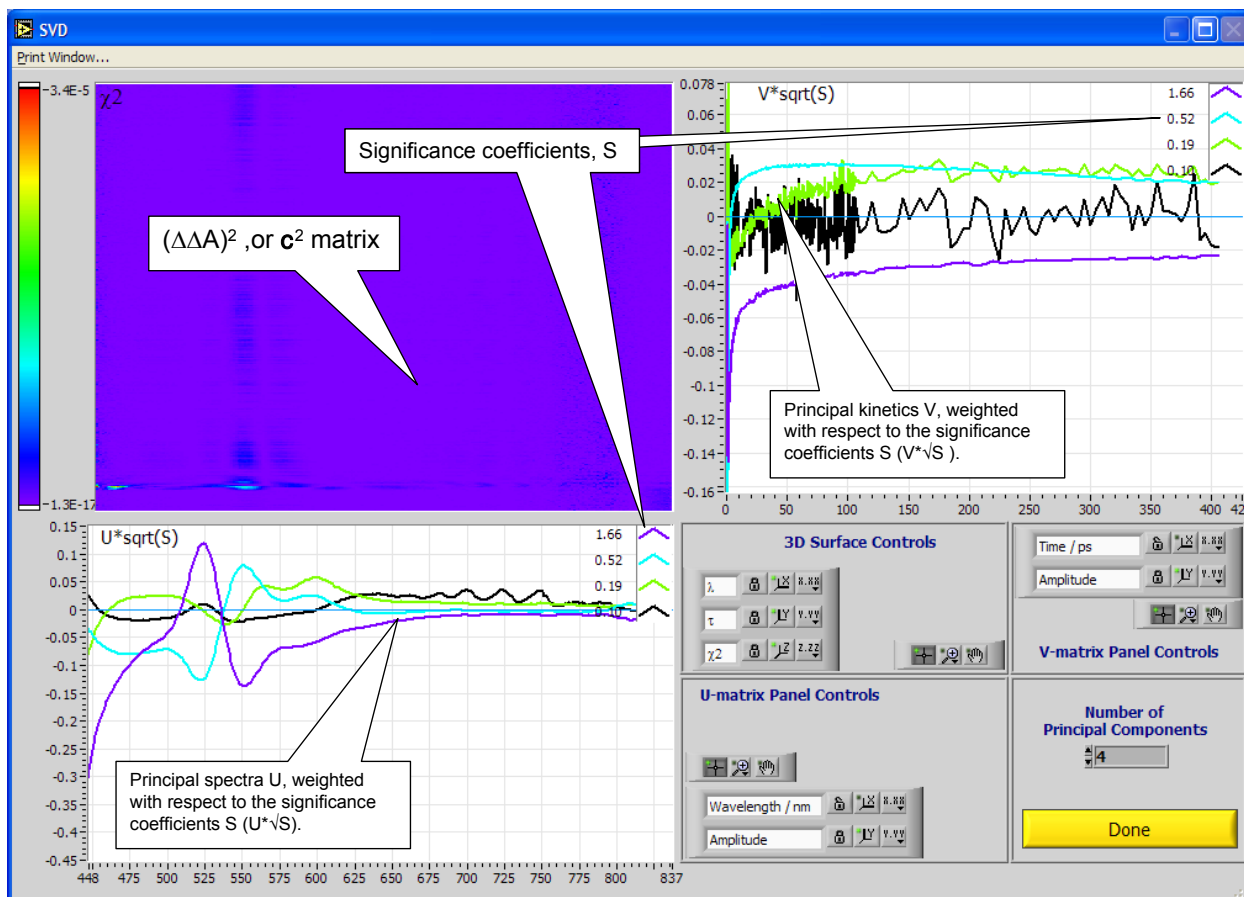
Figure 37 - View of the SVD window.

You can change the Number of Principal Components to display only the most significant components (the significance coefficients S are displayed on the U-matrix and V-matrix panels).To do this increase the number of principal components until the additional components look more like noise. In the example in Figure 37the black component (S=0.10) contains more noise than signal and therefore is not significant. Moreover, its significance coefficient is more than an order of magnitude less than that

for the first component. Thus, this data set can be represented by three sets of significant eigenvectors, while the fourth and subsequent ones mostly represent noise that could be factored out from the data.

Once you have selected the number of significant components based on the data from the graph panels and the values of the significance coefficients press the **Done** button to save the PrincipalSpectra.csv and PrincipalKinetics.csv data files. The saved files containing the principal spectra and kinetics data will be used by the global analysis procedure and the Reconstruct from Principal Components feature. In case you want to use the generated principal components files with any other software, note the file formats:

- In the Principal Spectra file the 1st column is the wavelengths, the 1st row contains the significance coefficients, and the rest of the matrix contains the corresponding amplitudes. The (1,1) element does not mean anything.

- In the PrincipalKinetics file the 1st row is the times, the 1st column contains the significance coefficients, and the rest of the matrix contains the corresponding amplitudes. The (1,1) element does not mean anything.

To display the 3D surface based on the results of the SVD choose the Reconstruct from principal components from the drop down menu (Figure 37) while having the original data used for SVD open. The new 3D surface will be displayed instead of the original data. The data matrix is saved once **Done** is pressed. The default name for the new data file will be: {original file name} ReconstrSVD.csv, indicating that the surface is reconstructed from principal components obtained via the SVD procedure.

Reference

"Factor Analysis In Chemistry", Second Edition, Edmund R. Malinowski, 1991, Wiley-Interscience, John Wiley & Sons, Inc., New York. U.S.A.

Global Fit

Make sure the following are performed:

1. The A(λ,t) data matrix is corrected for the chirp of the super-continuum probe pulse. See the chirp correction section for more details.

2. The time zero is adjusted for the real time zero using the coherent signal from the solvent. See the Time Zero Correction section for more details.

3. Theresultingmatrixisdecomposedintoitsprincipalspectralandkineticcomponents via SVD (singular value decomposition). See the **Principal components via SVD** section for more details.

4. The selected principal kinetic traces are globally fitted to a multi-exponential decay law convoluted with the Gaussian instrument response function using the nonlinear Marquardt algorithm.

5. The pre-exponential coefficients as functions of the wave length are calculated by the linear fit of the data obtained after step 1 to the exponentials obtained in step 3. Refer to the literature reference at the end of this chapter for more specific information on the employed methodology.

Prior to starting the Global Fit procedure make sure that you have the PrincipalSpectra.csv and PrincipalKinetics.csv (files acquired as a result of the SVD procedure) saved in the same directory as the main 3D surface data file. Select Global Fit from the upper drop down menu (Figure 38) to start the fitting procedure. The new window will open and its upper right panel will display the principal kinetic traces produced during the SVD operation (Figure 37).
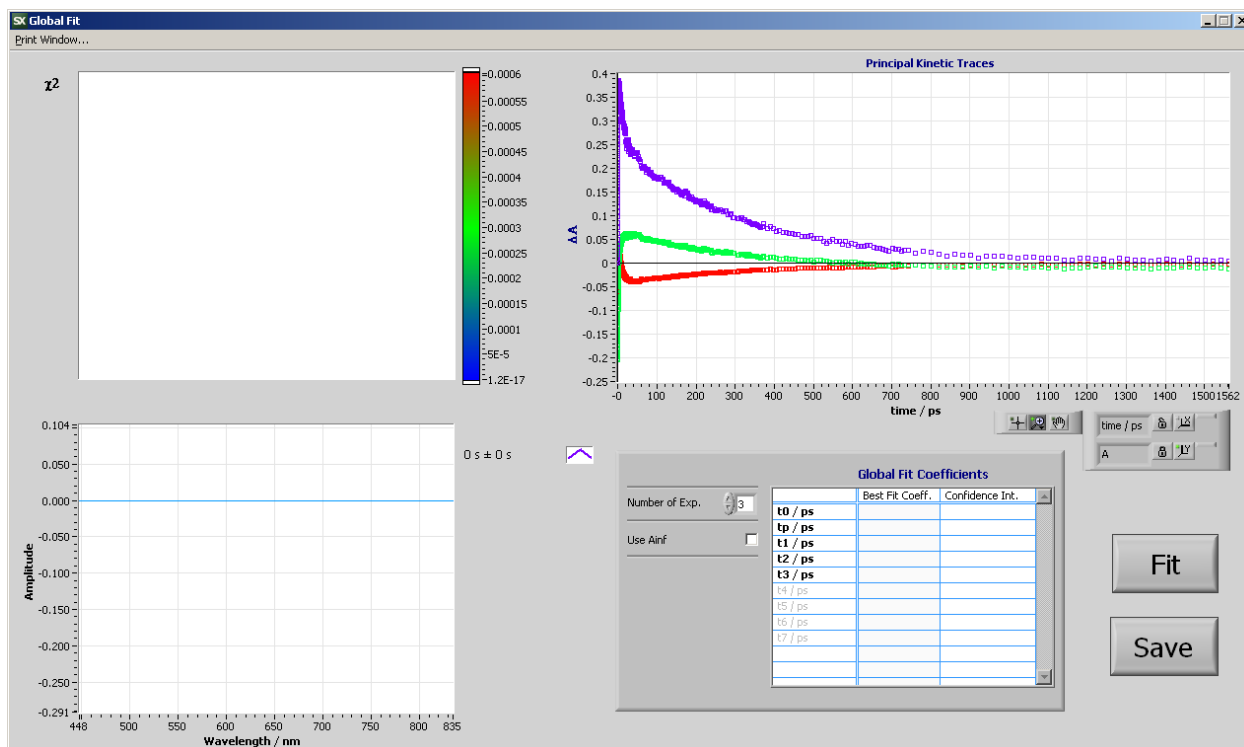
Figure 38 - View of the Global Fit window.

You need to specify the following fit parameters (if you are unsure about the feasible values, several resonant kinetics fits at different wavelengths may help (see the Fit Resonant Kinetics section, page 30):

> ***Number of exponentials*** – total number of expected lifetimes except infinite lifetime (Ainf);

> ***Use Ainf*** –check this option if the signal does not decay all the way to zero amplitude (adds an infinite exponential).

You can fix one or more parameters. This feature is similar to the one described in Fit Resonant Kinetics section.

Click the Fit button to start the global fitting procedure. A window with a progress bar appears for the duration of the fitting. Upon completion:

1. The computed parameters are displayed in the middle column titled **Best Fit Coeff**. In the right column confidence intervals for the Coefficients are displayed;

2. The solid curves representing the best fits for the kinetic traces (squares) of same color will be added to the upper right panel (Figure 39);

3. The bottom left panel will display the spectral distributions of the pre-exponential coefficients or amplitudes of each of the calculated lifetimes (specified in the legend with corresponding intervals). These lifetimes are the best result of the global fitting for the number of exponentials specified previously.

The quality of the 3D surface fit is represented by χ2 on the top left panel. If the results of the fitting procedure are found satisfactory, save the data by clicking the Save button.
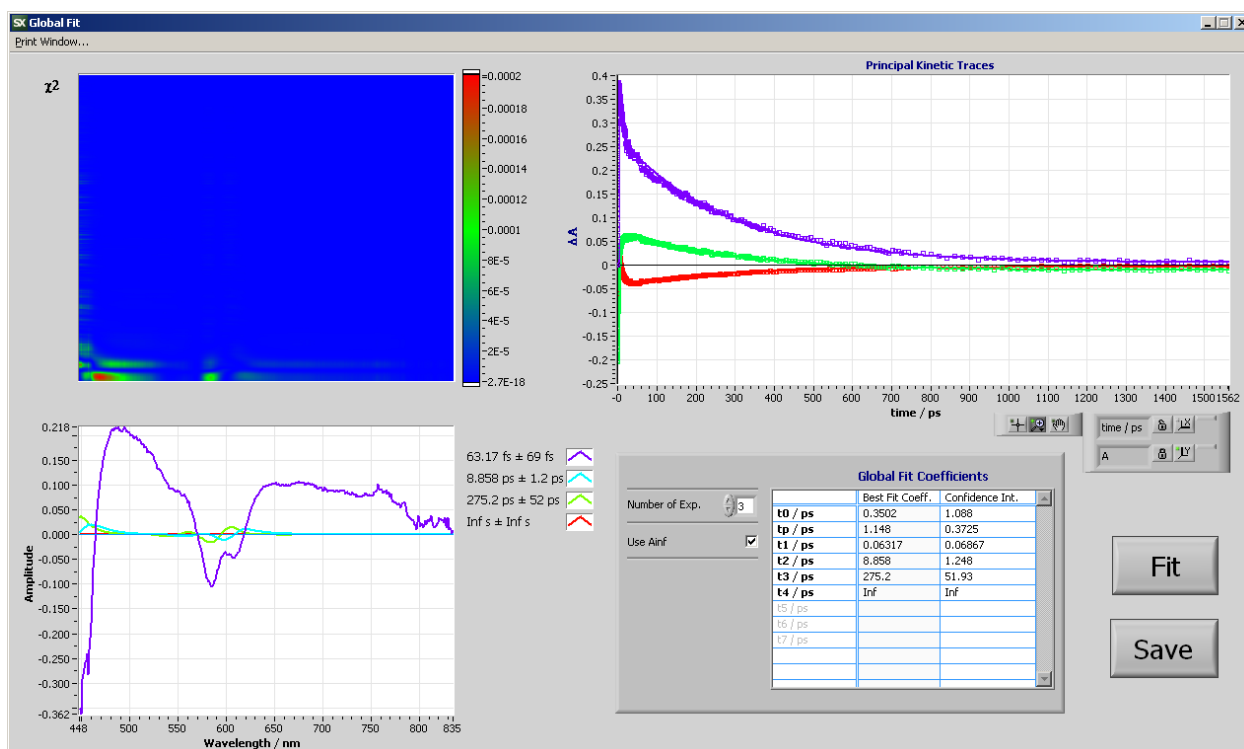

Figure 39 - View of the Global Fit window after fitting.

Reference

"Calculation Of Equilibrium Constants From Multiwavelength Spectroscopic Data - I, Mathematical Considerations", Harald Gampp, Marcel Maeder, Charles J. Meyer, and Andreas D. Zuberbühler, Talanta, 1985, 32, 95-101.

Combine multiple surfaces

This feature allows you to combine several 3D Surfaces having different spectral or time ranges. Surfaces are normalized by overlap range. Complete following steps before combining:

1. Adjust time zero for each surface. See Time Zero Correction, page 21.The time zero for all surfaces has to be the same.

2. Make sure that time units setting for each surface is correct. See Time units section, page 30.

3. If different time - resolution data are used (for example data obtained with Helios and with Eos) choose the appropriate time range for every surface. The time ranges of the data determine the overlap range.(For example, if the first surface has the time range of 0 – 3 ns and the second one has the time range 2 ns – 10 ns then the overlap range is 2 ns – 3 ns).The overlap range should be chosen so that there are no individual features (spikes, etc.). For example, a spike in the beginning of the Helios data can be perturbed by the Eos data (if the overlap range is 0 – 3 ns).

4. Save all data before combining.

5. Select menu item Surface ->Combine multiple surfaces and choose the files saved on step 4.
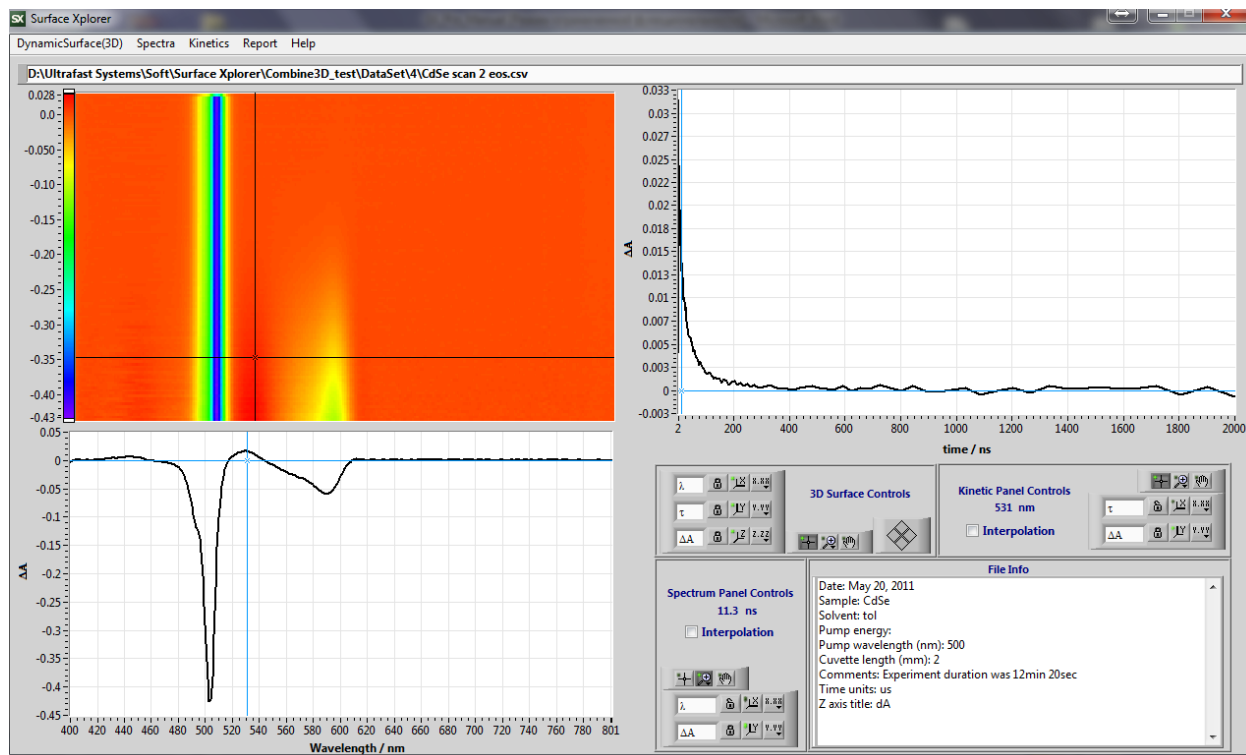

Figure 40 - Eos Data.

This example below is the result of combining date obtained by Helios and Eos instruments. The prepared Eos data are shown on Figure 40. The time zero was corrected and time range is set to 2-2000 ns. The range 0-2 ns was cut out as it can disturb the Helios data. The early part of the Helios data contains some spikes (see Figure 41) so the Eos range was chosen to not cover the beginning of the Helios data.

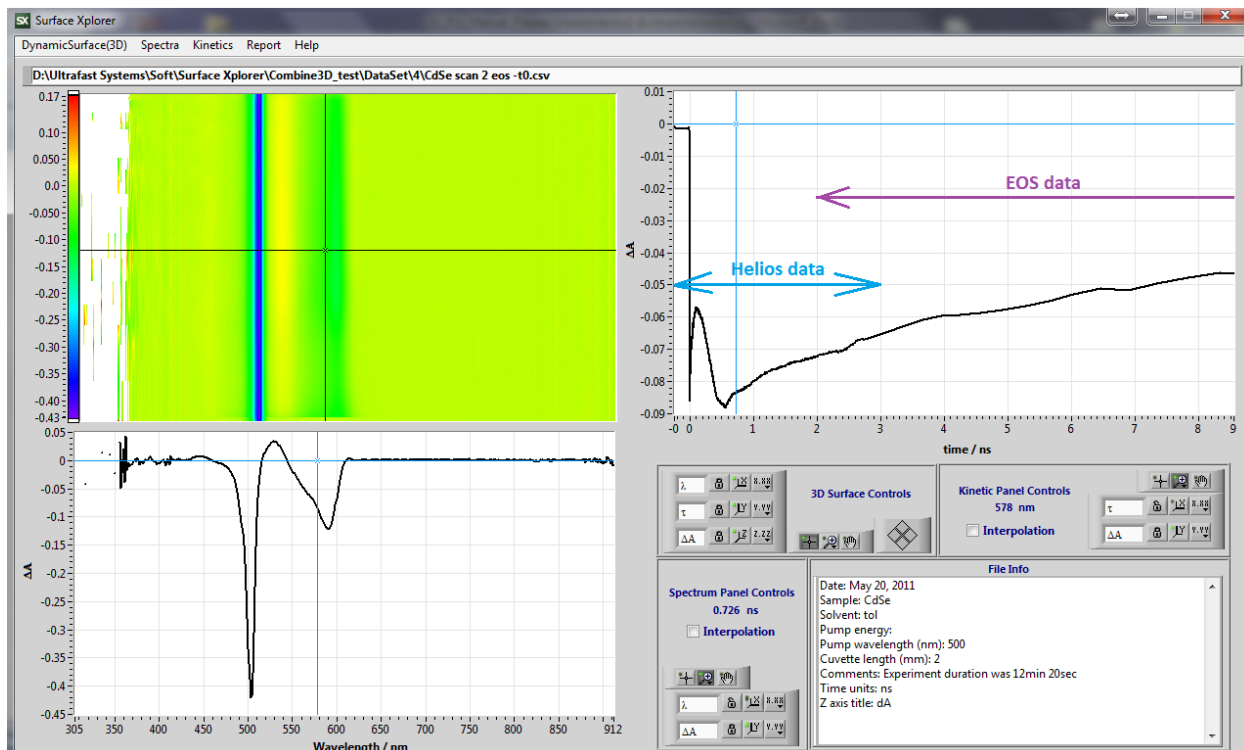The result of this procedure is shown on Figure 41.

Figure 41 - Result of Combine Multiple Surfaces function.

Appendix: File formats and filename convention

NOTE: All data files are in CSV format (comma separated values).

Raw data files

The 1st column is wavelength, the 1st row is time, and the rest of the matrix comprises the columns of the corresponding ΔA values. The (1,1) element does not mean anything.

Principle components files (* PrincipalSpectra.csv and *PrincipalKinetics.csv)

In the PrincipalSpectra.csv file the 1st column is wavelength, the 1st row is the significance coefficients, and the rest of the matrix comprises the columns with the corresponding amplitudes. The (1,1) element does not mean anything.

In the PrincipalKinetics.csv file the 1st row is time, the 1st column is significance coefficients, and the rest of the matrix contains the columns with the corresponding amplitudes. The (1,1) element does not mean anything.

Global fit files (* GlobalFit.csv)

The 1st column is wavelength, the 1st row is designated as follows: the 1st value (the (1,1) element) does not mean anything, the 2nd value is time zero (t0), the 3rd value is the instrument response function (fwhm), tp in ps, and the following values are lifetimes. The rest of the matrix contains the corresponding pre-exponential coefficients, except for those in the 2nd and the 3rd columns, which mean nothing.

Fitting files (* FitCoefficients.csv)

This file consists of rows of the fitting results, one row for each fitted wavelength. The order of elements in the row is as follows:

1. Wavelength
2. Timezero (t$_0$)
3. Instrument response function (FWHM), tp
4. First normalized pre-exponential coefficient
5. First exponential lifetime

6. The rest are pairs of pre-exponential coefficients and the corresponding lifetimes, same as #4 and #5.

Fitting solvent response files (* FitCoefficients.csv)

This file consists of rows of the fitting results, one row for each fitted wavelength. The order of elements in the row is as follows:

1. Wavelength
2. Timezero (t_0)
3. Instrument response function (FWHM), tp
4. Amplitude of the first derivative of Gaussian
5. Amplitude of the second derivative of Gaussian
6. Amplitude of Gaussian (optional).

Representative spectra files (* RepresentativeSpectra.csv)

This file consists of pairs of columns for each time point (the first element in both columns) with the 1st column of pair containing wavelength and the 2nd column of pair containing the corresponding ΔA values.

Representative kinetics files (* RepresentativeKinetics.csv)

This file consists of pairs of rows for each wavelength point (the first element in both rows) with the 1st row of pair containing the time and the 2nd row of pair containing the corresponding ΔA values.