Three-Way PLS Regression Analysis of Fluorescence Excitation-Emission Spectra (Tutorial H)

Description of Tutorial H

General Context of Tutorial H

In this tutorial we will utilize Fluorescence Excitation-Emission spectra to study the process of refining wood into fibres for the production of fibreboards by steam treatment of various severities.

The original data are from the Institute of Applied Research (Prof. Kessler), Reutlingen University, Germany.

Detailed Context of Tutorial H

Steam treatment of wood at different temperatures results in a softening of the fibre composite structure as well as in a separation of the wood into the main products cellulose, hemicelluloses and lignin. The flexibility of this process allows to produce a broad range of products ranging from fibreboards up to pulp. Due to the complexity of this process it is important to know in detail the kinetics of degradation of the wood composite. There are numerous investigations to characterize the raw material and reaction products by means of FTIR or NIR spectroscopy, but little work has been done on Fluorescence spectroscopy, although Fluorescence is an extraordinary sensitive tool.

Fluorescence spectroscopy is able to distinguish similar molecules and can discriminate identical molecules in different chemical environments. This is due to the possibility to scan excitation spectra at specified emission wavelengths and to scan emission spectra at specified excitation wavelengths (EEM-scans). This procedure results in 3-D graphs of the fluorescence intensity with respect to different excitation and emission wavelengths. But the EEM data are strongly inter correlated and difficult to interpret. Standard unfolding methods often give

unsatisfactory results. We will use a three-way analysis approach to overcome this problem.

What You Will Learn in Tutorial H

This tutorial contains the following parts:

Toggle 3D layouts in the 3D Editor;

Plot 3D data;

Define a Primary Variable set and a Secondary Variable set;

Build a three-way PLS regression model;

Find an outlier and recalculate;

Interpret a three-way PLS regression model

Tutorial H - Data Tables

The data for this tutorial are stored in files **Tutor_h_X3D** and **Tutor_h_Y2D** in the **Examples** directory on your computer.

Wood Samples (X and Y Data)

The samples (objects) are common for the X and Y data tables. They consist of 32 fibre samples of steam treated and refined woodchips. Two types of wood are studied: beech (B) is a hard wood and spruce (S) is a soft wood. The wood samples were either fresh (F, 3 months) or old (O, 6 months). Two plate gaps of grinding were used: fine (Fi) and coarse (C).

The sample names indicate this information. For example:

"BFFi" means Beech, Fresh and Fine

"SOC" means Spruce, Old and Coarse

Fluorescence Excitation-Emission Spectra (X Data)

The X-variables are fluorescence excitation-emission spectra. They are saved in a 3D data table (**Tutor_h_X3D**) with 32 rows for the 32 woodchip samples, and 2046 columns corresponding to 66 Primary Variables (Excitation) x 31 Secondary Variables (Emission). This is a so-called OV^2 table as it contains one Object mode and two Variable modes.

The fluorescence spectra were measured in the following ranges: Excitation 250 - 575 nm with a step of 5 nm, Emission 300 - 600 nm with a step of 10 nm.

Severity (Y Data)

The Y data is found in table **Tutor_h_Y2D**, consisting of 32 rows for the 32 woodchip samples and one column, Severity.

Severity of steaming is a measure reflecting the duration and temperature of steam treatment. The spruce and beech samples were treated with steam at temperatures from 160°C to 220°C. The Severity values range from 1.7 to 3.5.

Toggle 3D Layouts in the 3D Editor (Tutorial H)

3D tables can be displayed in 12 different layouts. By easily changing the layout of a table, you will be able to organize your data set as best suits your analysis needs.

Task

Toggle 3D data layouts.

How to Do It

Open the data file **Tutor_h_X3D** by selecting **File - Open**. It is a file of type **3D Data.**

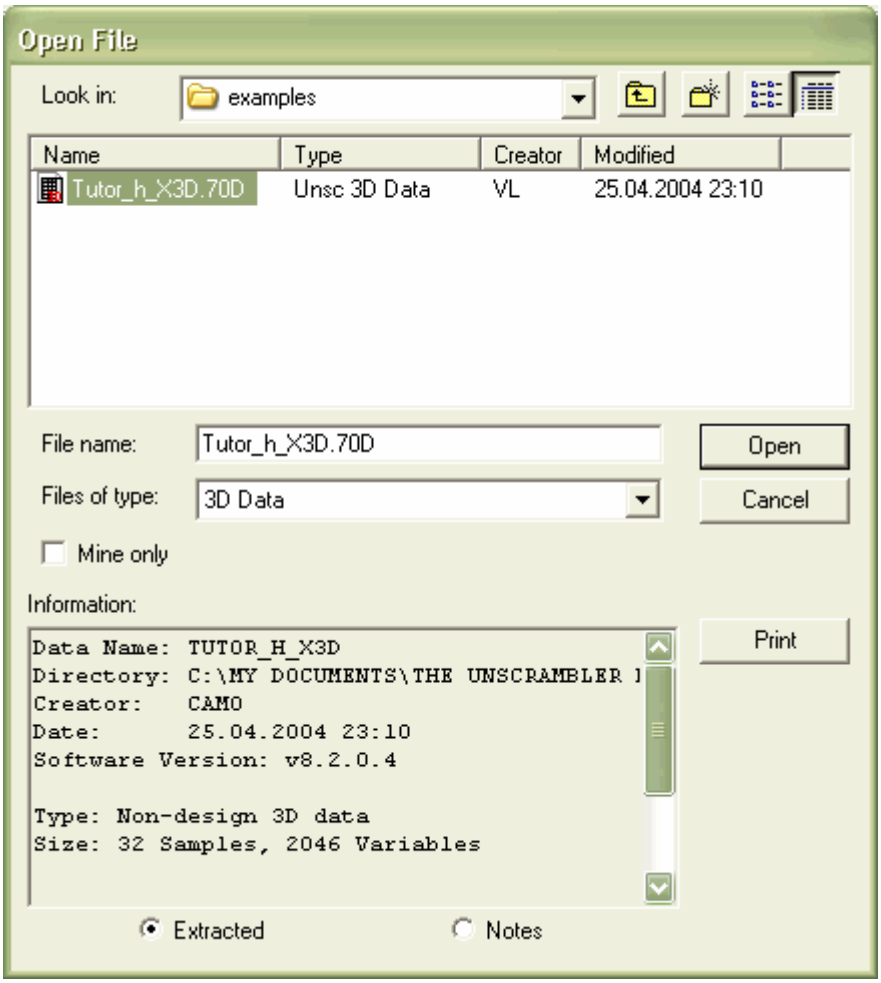
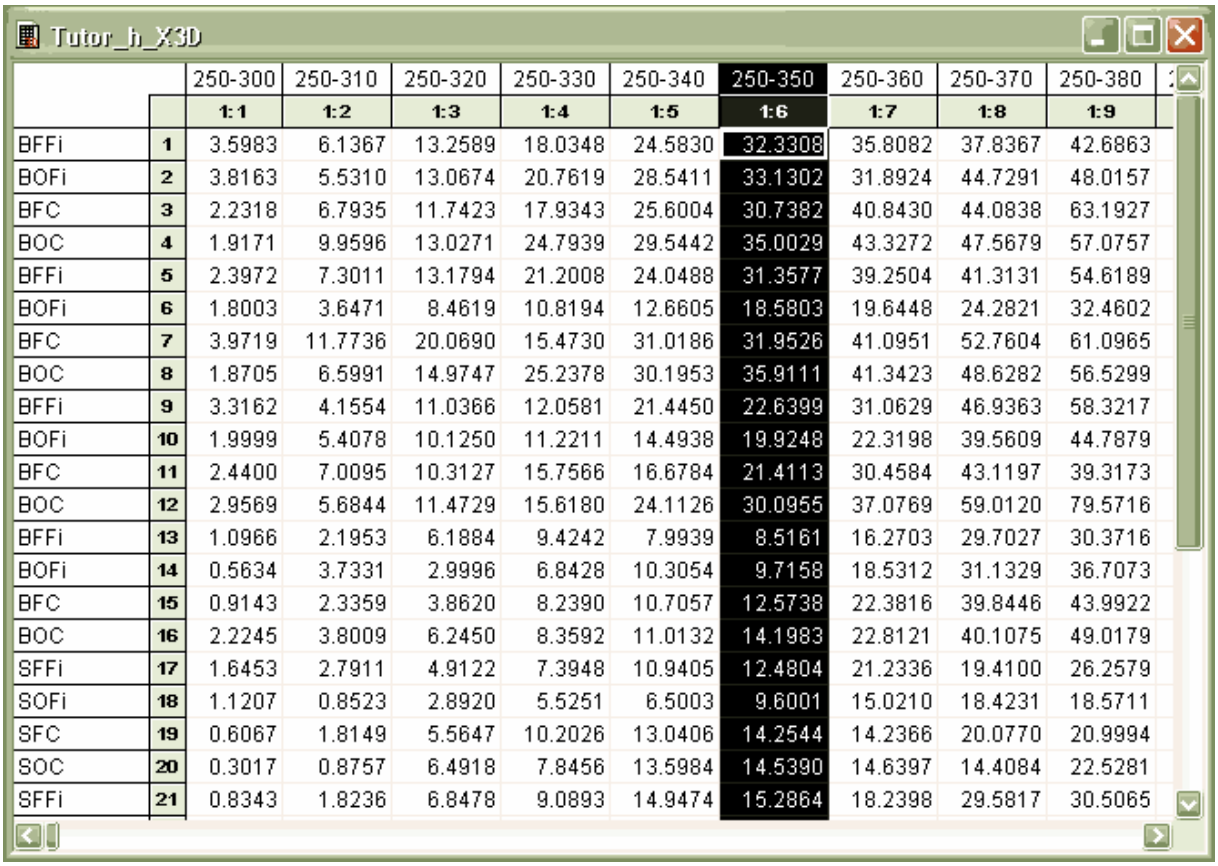


Figure 1: Open File dialog, opening the data file of type "3D Data" for tutorial H

The table opens in the 3D Editor. It is a table of OV^2 layout (1 object mode, 2 variable modes), therefore its column numbers are two-fold. For example, column **1:6** corresponds to primary variable number 1 (Excitation wavelength 250 nm) and secondary variables number 6 (Emission wavelength 350 nm).



Tutor_h_X3D

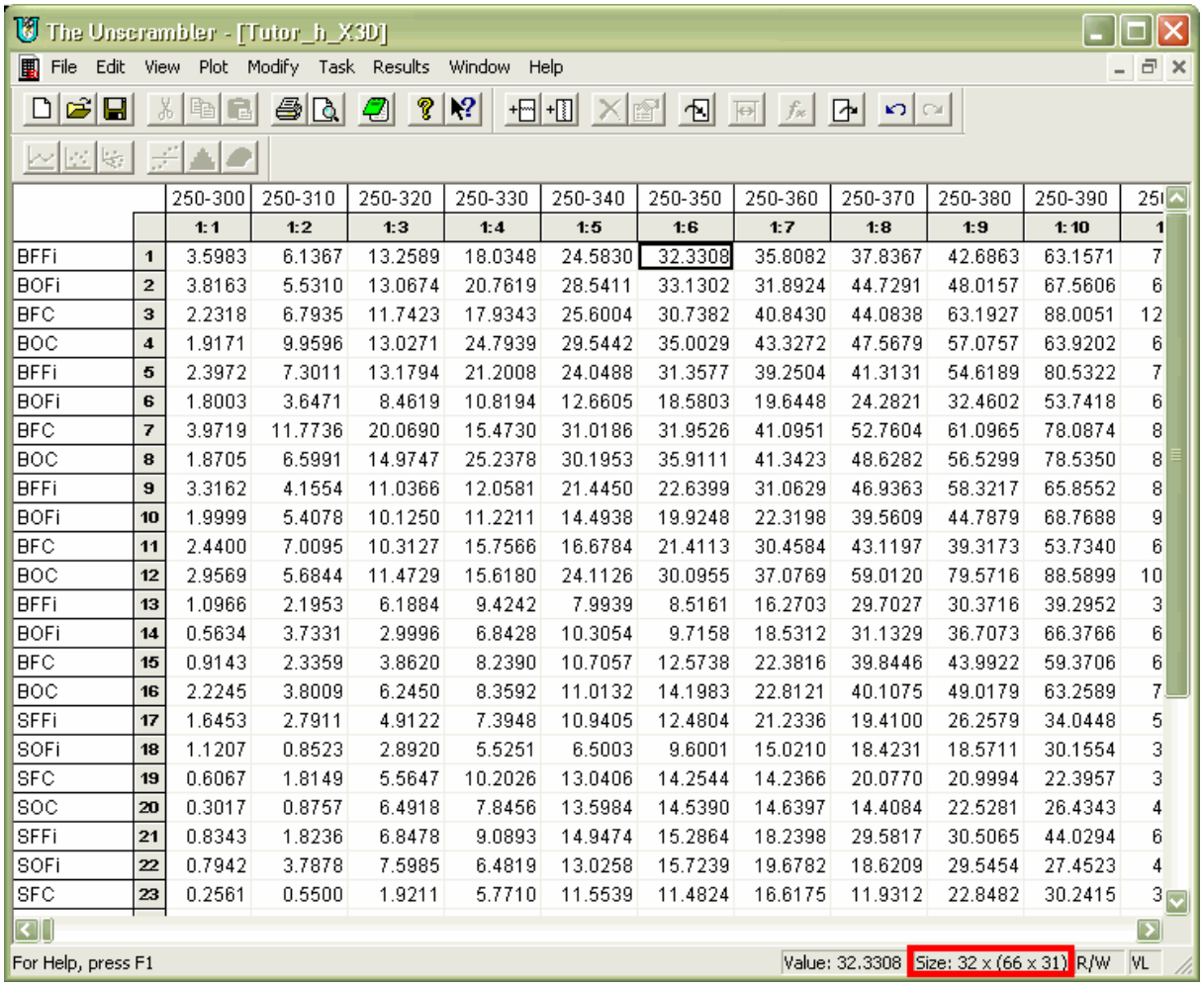
		250-300	250-310	250-320	250-330	250-340	250-350	250-360	250-370	250-380
		1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
BFFi	1	3.5983	6.1367	13.2589	18.0348	24.5830	32.3308	35.8082	37.8367	42.6863
BOFi	2	3.8163	5.5310	13.0674	20.7619	28.5411	33.1302	31.8924	44.7291	48.0157
BFC	3	2.2318	6.7935	11.7423	17.9343	25.6004	30.7382	40.8430	44.0838	63.1927
BOC	4	1.9171	9.9596	13.0271	24.7939	29.5442	35.0029	43.3272	47.5679	57.0757
BFFi	5	2.3972	7.3011	13.1794	21.2008	24.0488	31.3577	39.2504	41.3131	54.6189
BOFi	6	1.8003	3.6471	8.4619	10.8194	12.6605	18.5803	19.6448	24.2821	32.4602
BFC	7	3.9719	11.7736	20.0690	15.4730	31.0186	31.9526	41.0951	52.7604	61.0965
BOC	8	1.8705	6.5991	14.9747	25.2378	30.1953	35.9111	41.3423	48.6282	56.5299
BFFi	9	3.3162	4.1554	11.0366	12.0581	21.4450	22.6399	31.0629	46.9363	58.3217
BOFi	10	1.9999	5.4078	10.1250	11.2211	14.4938	19.9248	22.3198	39.5609	44.7879
BFC	11	2.4400	7.0095	10.3127	15.7566	16.6784	21.4113	30.4584	43.1197	39.3173
BOC	12	2.9569	5.6844	11.4729	15.6180	24.1126	30.0955	37.0769	59.0120	79.5716
BFFi	13	1.0966	2.1953	6.1884	9.4242	7.9939	8.5161	16.2703	29.7027	30.3716
BOFi	14	0.5634	3.7331	2.9996	6.8428	10.3054	9.7158	18.5312	31.1329	36.7073
BFC	15	0.9143	2.3359	3.8620	8.2390	10.7057	12.5738	22.3816	39.8446	43.9922
BOC	16	2.2245	3.8009	6.2450	8.3592	11.0132	14.1983	22.8121	40.1075	49.0179
SFFi	17	1.6453	2.7911	4.9122	7.3948	10.9405	12.4804	21.2336	19.4100	26.2579
SOFi	18	1.1207	0.8523	2.8920	5.5251	6.5003	9.6001	15.0210	18.4231	18.5711
SFC	19	0.6067	1.8149	5.5647	10.2026	13.0406	14.2544	14.2366	20.0770	20.9994
SOC	20	0.3017	0.8757	6.4918	7.8456	13.5984	14.5390	14.6397	14.4084	22.5281
SFFi	21	0.8343	1.8236	6.8478	9.0893	14.9474	15.2864	18.2398	29.5817	30.5065

Figure 2: Tutor_h_X3D data table displayed in the 3D Editor

Use menu **Modify – Toggle 3-D Layouts** or its corresponding shortcut **Ctrl+3**. Using this menu once will exchange Primary (now Emission spectra) and Secondary variables (now Excitation spectra). For example, column **1:6** will now correspond to Emission wavelength 300 nm and Excitation wavelength 275 nm.

Several sub-menus of the **Modify** menu allow you to change the layout of a 3-D table, for example by exchanging Primary and Secondary variables, or swapping layout from OV^2 to O^2V (2 object modes, 1 variable mode). You may freely try some of these menus and observe how the table is "transposed" in 3 dimensions.

Toggle the layout several times (**Ctrl+3**) until you are back to an OV^2 table of size 32 x (66 x 31), that is to say 32 samples, 66 Primary Variables and 31 Secondary variables. The size of the table is shown at the bottom right corner of the Editor.



		250-300	250-310	250-320	250-330	250-340	250-350	250-360	250-370	250-380	250-390	25
		1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9	1:10	1
BFFi	1	3.5983	6.1367	13.2589	18.0348	24.5830	32.3308	35.8082	37.8367	42.6863	63.1571	7
BOFi	2	3.8163	5.5310	13.0674	20.7619	28.5411	33.1302	31.8924	44.7291	48.0157	67.5606	6
BFC	3	2.2318	6.7935	11.7423	17.9343	25.6004	30.7382	40.8430	44.0838	63.1927	88.0051	12
BOC	4	1.9171	9.9596	13.0271	24.7939	29.5442	35.0029	43.3272	47.5679	57.0757	63.9202	6
BFFi	5	2.3972	7.3011	13.1794	21.2008	24.0488	31.3577	39.2504	41.3131	54.6189	80.5322	7
BOFi	6	1.8003	3.6471	8.4619	10.8194	12.6605	18.5803	19.6448	24.2821	32.4602	53.7418	6
BFC	7	3.9719	11.7736	20.0690	15.4730	31.0186	31.9526	41.0951	52.7604	61.0965	78.0874	8
BOC	8	1.8705	6.5991	14.9747	25.2378	30.1953	35.9111	41.3423	48.6282	56.5299	78.5350	8
BFFi	9	3.3162	4.1554	11.0366	12.0581	21.4450	22.6399	31.0629	46.9363	58.3217	65.8552	8
BOFi	10	1.9999	5.4078	10.1250	11.2211	14.4938	19.9248	22.3198	39.5609	44.7879	68.7688	9
BFC	11	2.4400	7.0095	10.3127	15.7566	16.6784	21.4113	30.4584	43.1197	39.3173	53.7340	6
BOC	12	2.9569	5.6844	11.4729	15.6180	24.1126	30.0955	37.0769	59.0120	79.5716	88.5899	10
BFFi	13	1.0966	2.1953	6.1884	9.4242	7.9939	8.5161	16.2703	29.7027	30.3716	39.2952	3
BOFi	14	0.5634	3.7331	2.9996	6.8428	10.3054	9.7158	18.5312	31.1329	36.7073	66.3766	6
BFC	15	0.9143	2.3359	3.8620	8.2390	10.7057	12.5738	22.3816	39.8446	43.9922	59.3706	6
BOC	16	2.2245	3.8009	6.2450	8.3592	11.0132	14.1983	22.8121	40.1075	49.0179	63.2589	7
SFFi	17	1.6453	2.7911	4.9122	7.3948	10.9405	12.4804	21.2336	19.4100	26.2579	34.0448	5
SOFi	18	1.1207	0.8523	2.8920	5.5251	6.5003	9.6001	15.0210	18.4231	18.5711	30.1554	3
SFC	19	0.6067	1.8149	5.5647	10.2026	13.0406	14.2544	14.2366	20.0770	20.9994	22.3957	3
SOC	20	0.3017	0.8757	6.4918	7.8456	13.5984	14.5390	14.6397	14.4084	22.5281	26.4343	4
SFFi	21	0.8343	1.8236	6.8478	9.0893	14.9474	15.2864	18.2398	29.5817	30.5065	44.0294	6
SOFi	22	0.7942	3.7878	7.5985	6.4819	13.0258	15.7239	19.6782	18.6209	29.5454	27.4523	4
SFC	23	0.2561	0.5500	1.9211	5.7710	11.5539	11.4824	16.6175	11.9312	22.8482	30.2415	3

Figure 3: Tutor_h_X3D data table in OV2 layout, size 32x (66x31)

Plot 3D Data (Tutorial H)

It is always recommended to study your raw data before engaging into modelling. Let us plot the raw spectra of a few wood samples of Beech and Spruce and compare these. We will use a **Matrix 3-D** plot to display the fluorescence spectra.

Task

Study the raw data by plotting the fluorescence spectra of a few wood samples.

How to Do It

Go to menu **Plot-Matrix 3-D** and select sample 13, BFFi (Beech, Fresh wood, Fine grinding). The excitation-emission spectrum for this sample is displayed in the Viewer.

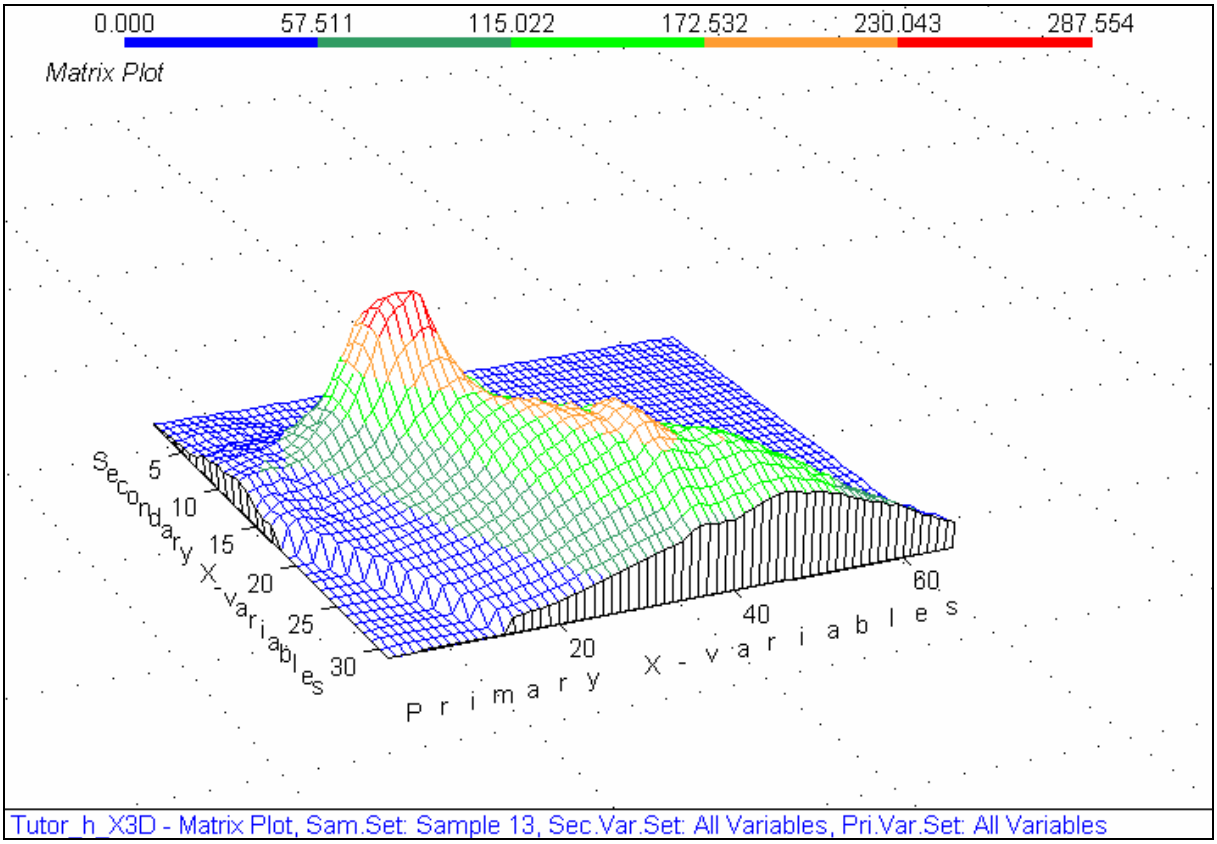


Figure 4: Fluorescence spectra of sample 13 (Beech, Fresh, Fine, high Severity treatment) in Landscape layout. Variables are in their series, not in real wavelength

You may use the Rotate option ( or **View- Rotate**) to view the spectral landscape from various angles. Use either the mouse or the arrow keys on your keyboard to rotate the plot. Holding your finger on an arrow key will allow a continuous rotation of the plot; pressing the **AltGr** key at the same time will slow down the rotation.

Menu **Edit-Options…** (or ) allows you to change the Plot Layout from a 3-dimensional Landscape view into Contour or Map.

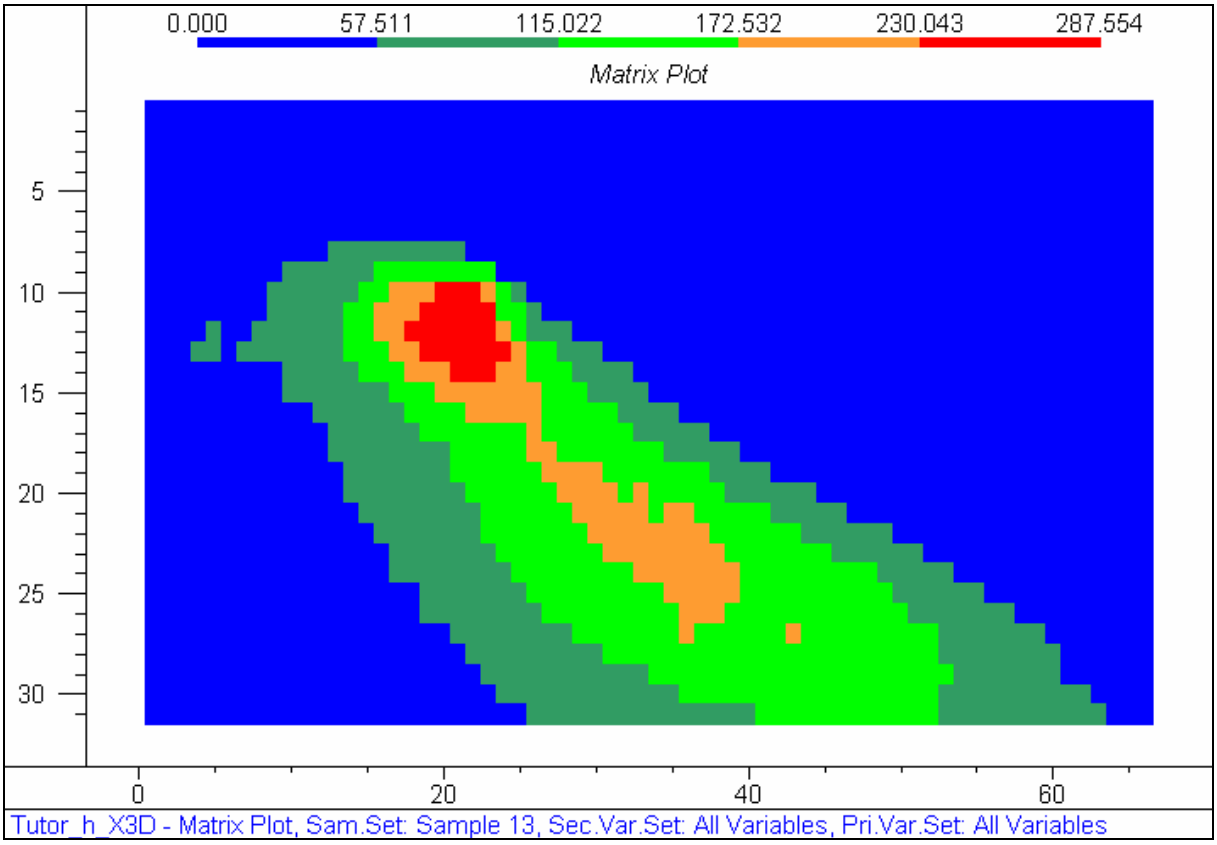


Figure 5: Fluorescence spectra of sample 13 (Beech, Fresh, Fine, high Severity treatment) in Map layout. Variables are in their series, not in real wavelength

Go back to the 3D Editor and use menu **Plot-Matrix 3-D** to plot sample 29, SFFi (Spruce, Fresh wood, Fine grinding).

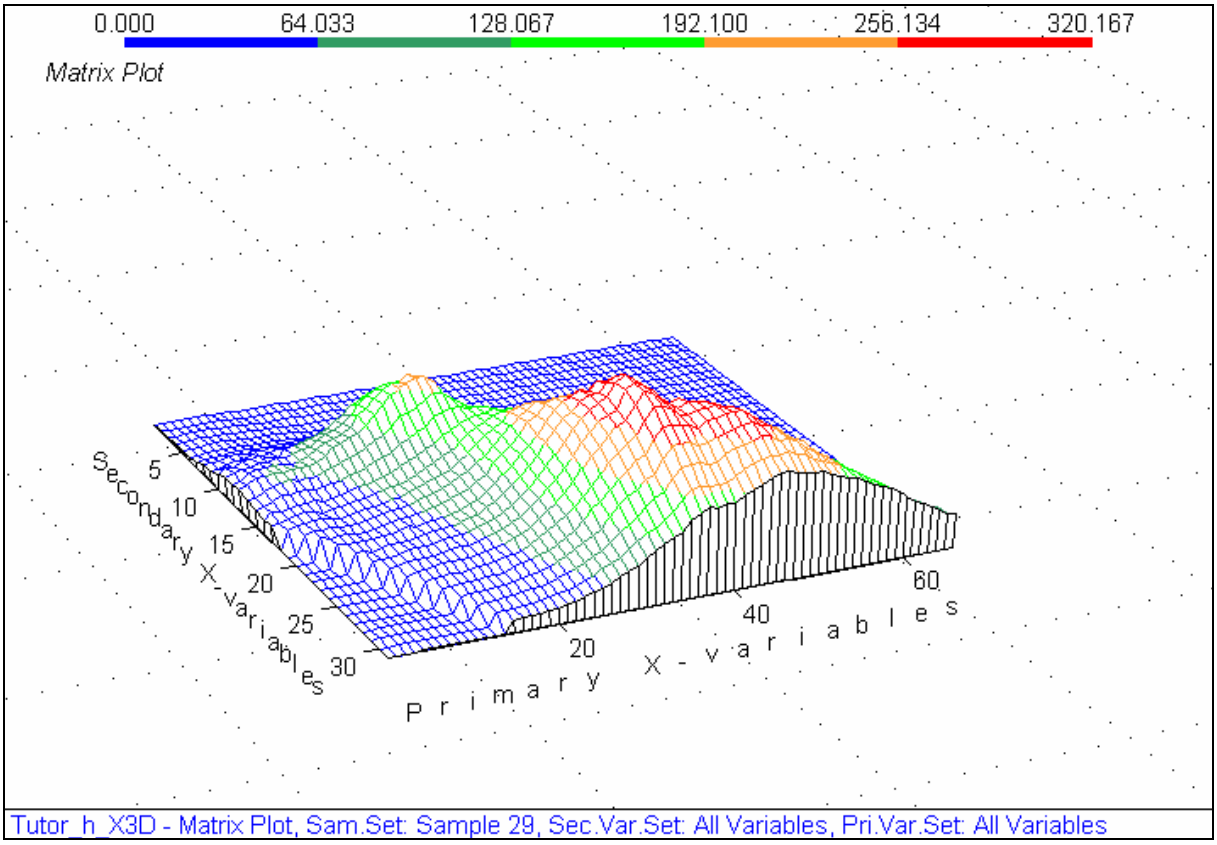


Figure 6: Fluorescence spectra of sample 29 (Spruce, Fresh, Fine, high Severity treatment) in Landscape layout. Variables are in their series, not in real wavelength

Interpretation of the Raw Fluorescence Spectra Plots (Tutorial H)

Both sample 13 and sample 29 were submitted to a high severity treatment (Severity values are indicated in the Y-data table, **Tutor_h_Y2D**). Yet, they have very different spectra, showing that the degradation process of the wood (softening of lignin with destruction of the hemicellulose lignin complex) is very different in soft- and hardwood.

We can also notice that only excitation wavelengths number 1-14 and 60-66, and emission wavelengths 1-7 do not contain information. We will define a Primary Variable set with variables 15-59 (Excitation 320-540 nm) and a Secondary Variable sets with variables 8-31 (Emission 370-600 nm) only.

Close your various matrix plots before proceeding with the tutorial.

Define a Primary Variable Set and a Secondary Variable Set (Tutorial H)

Defining variable sets allows you to keep the full spectra in the table, yet to work on just the relevant part of the data.

Task

Define a Primary Variables set and a Secondary Variables set.

How to Do It

Go to menu **Modify-Edit Set** or use the corresponding shortcut **Ctrl+E**. This opens up the **Set Editor** dialog.

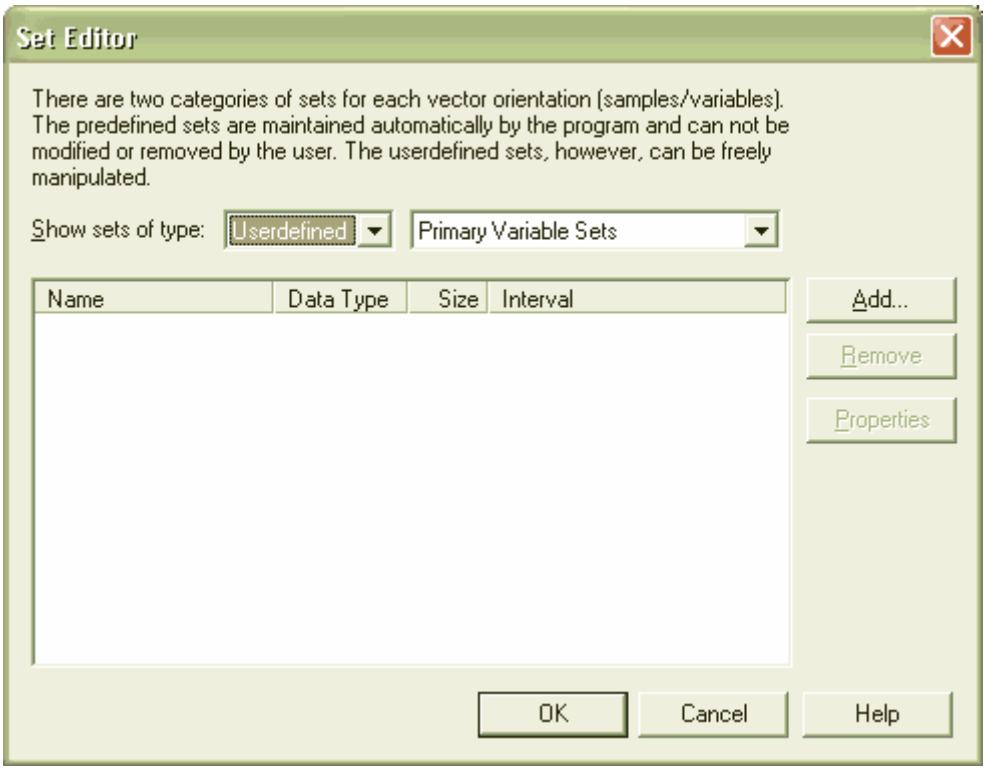


Figure 7: Set Editor dialog for an OV2 data table. Primary Variable Sets, Secondary Variable sets and Sample sets can be defined

Click on the **Add…** button to open the **New Primary Variable Set** dialog. Use the following settings:

Name: Excitation 320-540 nm

Data type: Spectra

Interval: 15-59

Alternatively, click the **Select…** button and select wavelengths 320 to 540 nm in the **Select Variables** dialog.

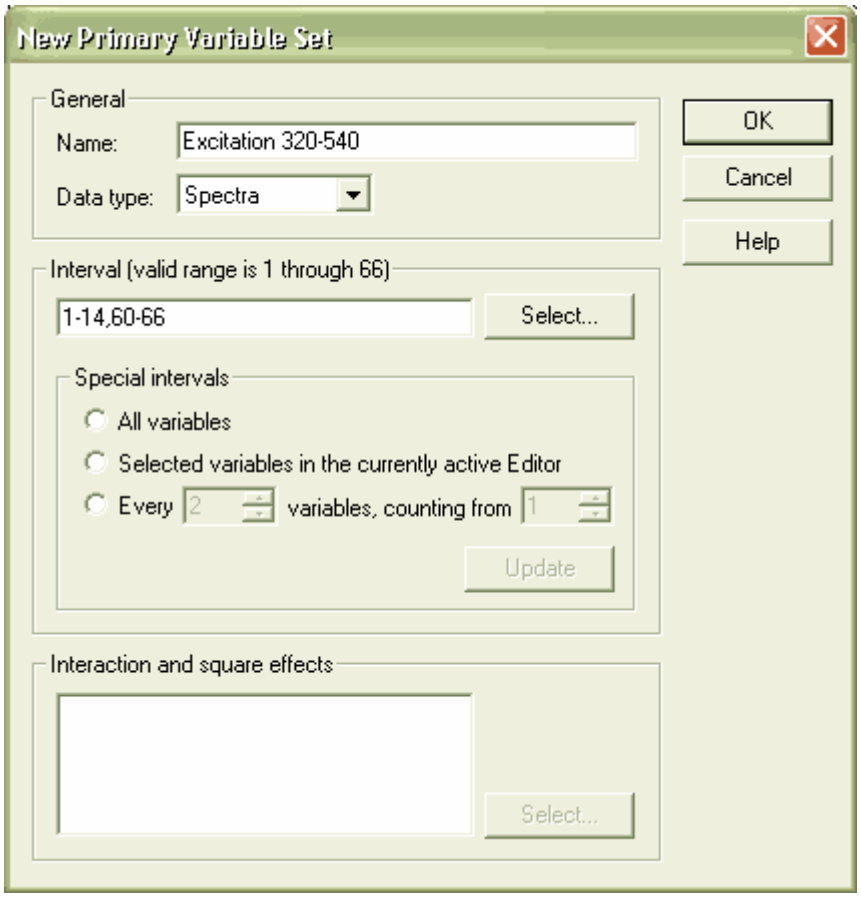


Figure 8: New Primary Variable Set dialog

Click OK; you are back in the **Set Editor** dialog where you can see your Primary Variable Set.

Use the drop-down list and select option **Secondary Variable Set**.

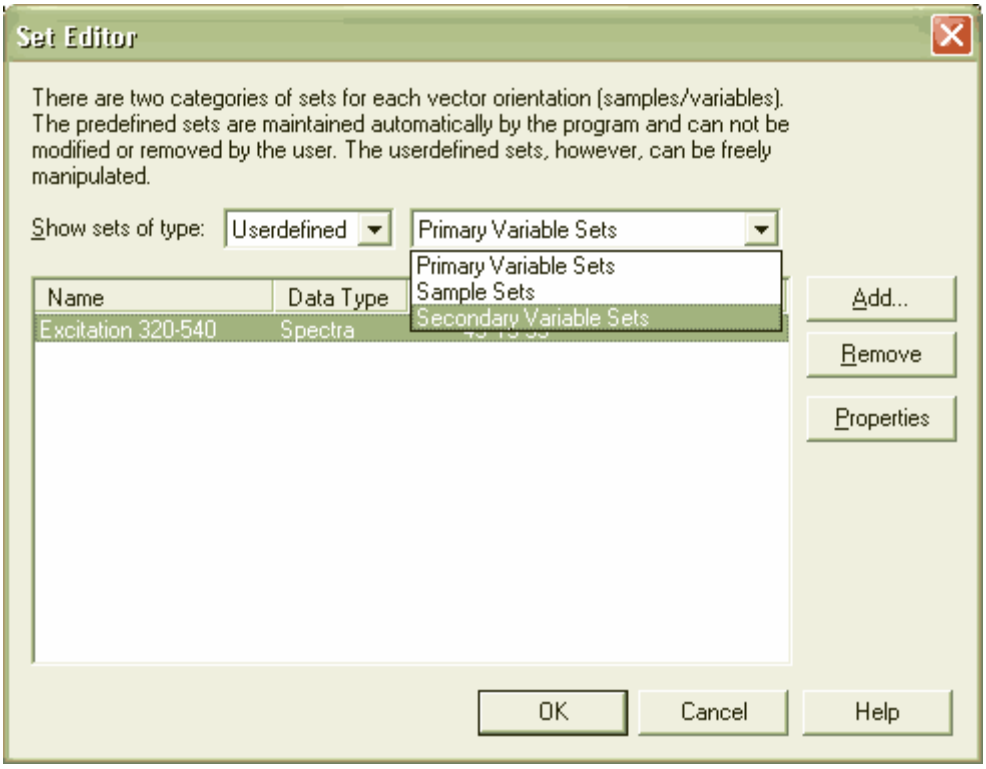


Figure 9: Set Editor dialog for an OV2 data table. A Primary Variable Set was defined, now the Secondary Variable Sets option is selected to define a new set

Click on the **Add…** button to open the **New Secondary Variable Set** dialog, and define a set as follows:

Name: Emission 370-600 nm

Data type: Spectra

Interval: 8-31

Alternatively, click the **Select…** button and select wavelengths 370 to 600 nm in the **Select Variables** dialog.

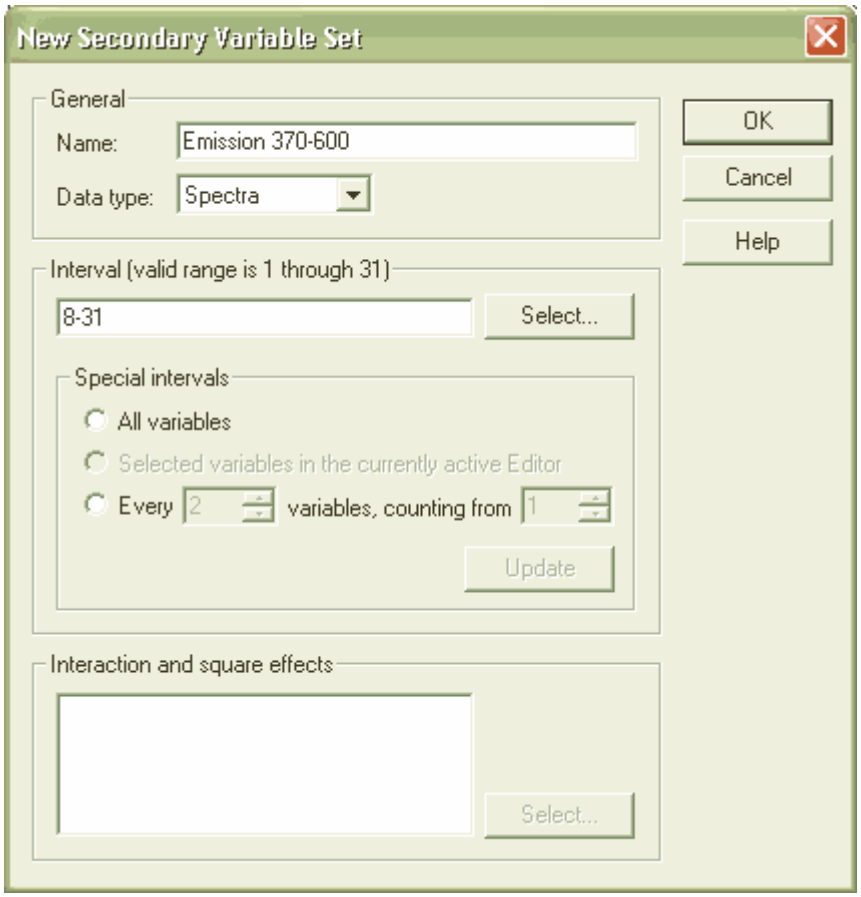


Figure 10: New Secondary Variable Set dialog

Click OK; you are back in the **Set Editor** dialog where you can see your Secondary Variable Set.

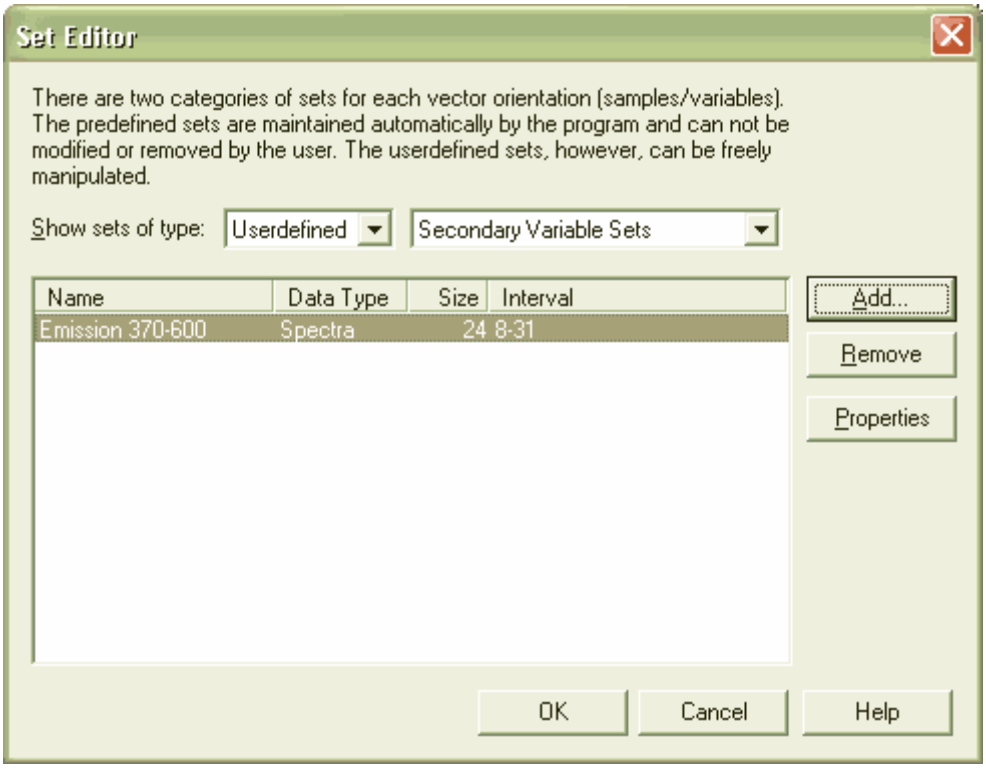


Figure 11: Set Editor dialog for an OV2 data table. A Secondary Variable Set was defined

Note!

If you made any mistake in defining the variable sets, use the **Properties…** button to return to the **New Primary/Secondary Variable Set** dialog and make corrections accordingly.

Click OK; you are back in the 3D Editor. Use menu **File-Save As…** to save the data sets information. You may call your new table **Tutor_h_X3D with sets**.

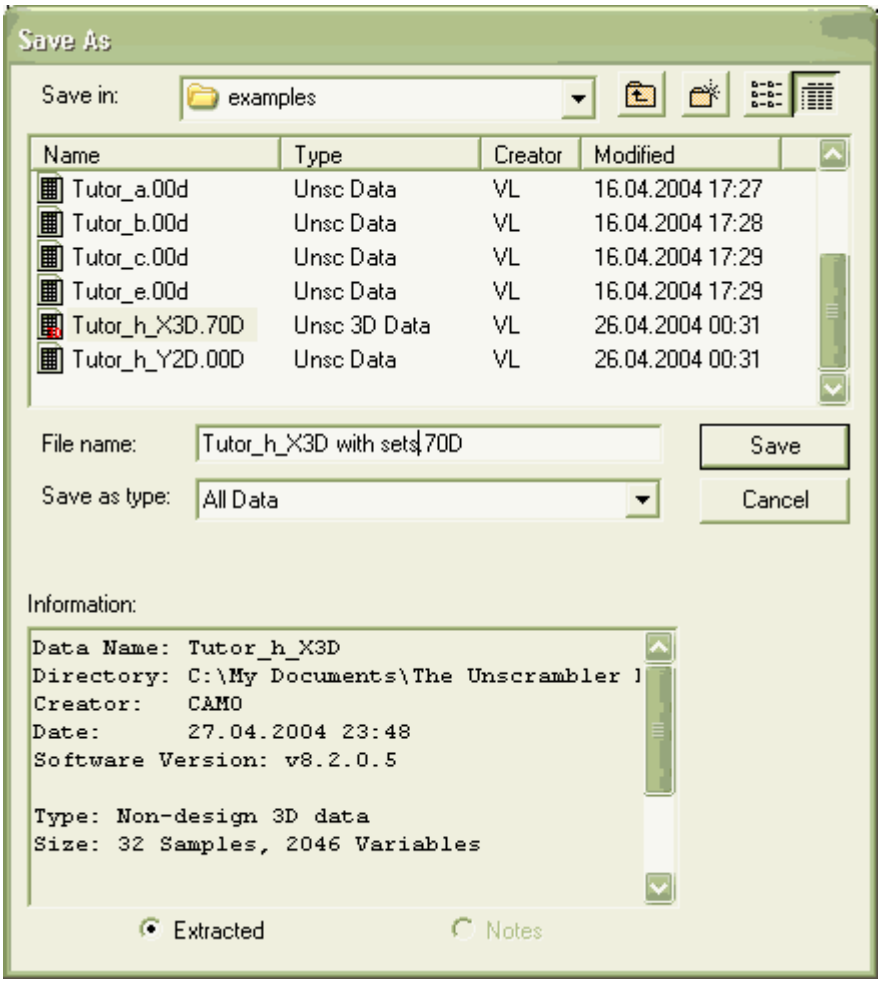


Figure 12: Save As dialog

Build a Three-Way PLS Regression model (Tutorial H)

A Three-Way PLS regression relates a three-way X-data array (here: fluorescence intensity) to a one-way or two-way Y-data array (here: Severity of steam treatment).

Task

Setup the options for a Three-Way PLS Regression and launch the model calculations.

How to Do It

Make sure that your 3D data table **Tutor_h_X3D with sets** is on screen. Select **Task-Regression…** to open the **Regression (Three-Way PLS)** dialog. Choose the following options:

Sample Set: All Samples [32]

Pri. X-Vars: Excitation 320-540 [45]
Weights: All 1.0

Sec. X-Vars: Emission 370-600 [24]
Weights: All 1.0

Y-Variable File: Tutor_h_Y2D
Variable Set: Severity [1]
Weights: All 1.0

Validation Method: Cross Validation. Use the **Setup…** button to choose Full Cross Validation

Num PCs: 10

Center Data: selected

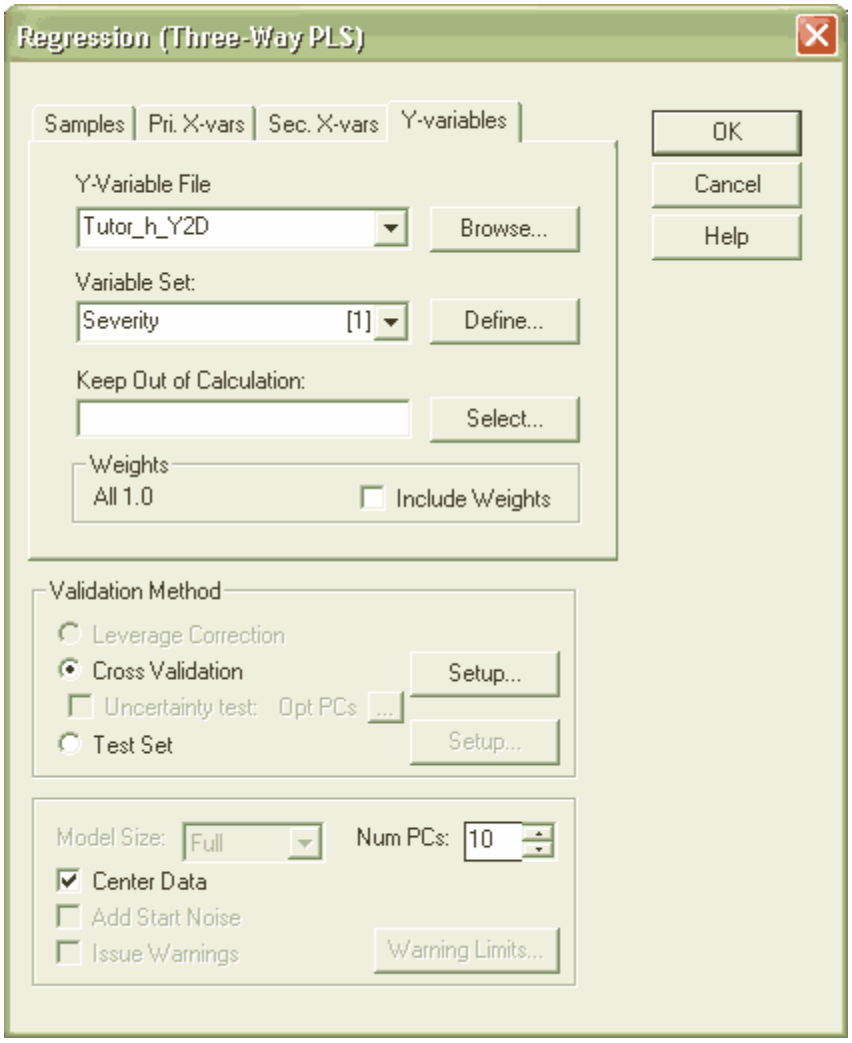


Figure 13: Regression (Three-Way PLS) dialog

Note!

In the **Y-variables** sheet, you may have to **Browse...** to find the Y-Variable File **Tutor_h_Y2D**.

Click OK to launch the calculations. The **Three-Way PLS Regression Progress** dialog appears. As the calculations run, the Y-Validation Residual Variance curve per cross validation segment is shown. When the calculations are over the Residual Y-Validation Variance curve for the global model is displayed.

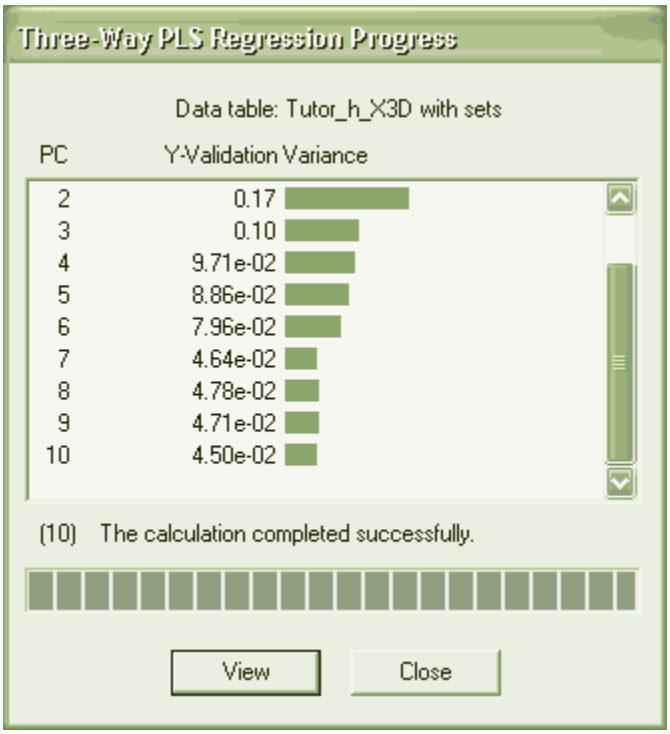


Figure 14: Three-Way PLS Regression Progress dialog, Model 1 calculations

Hit the View button. The **Regression Overview** opens, showing four default plots. These are (clockwise): Scores, X1-Loading Weights and Y-Loadings, Predicted vs. Measured, Residual Y-Validation Variance.

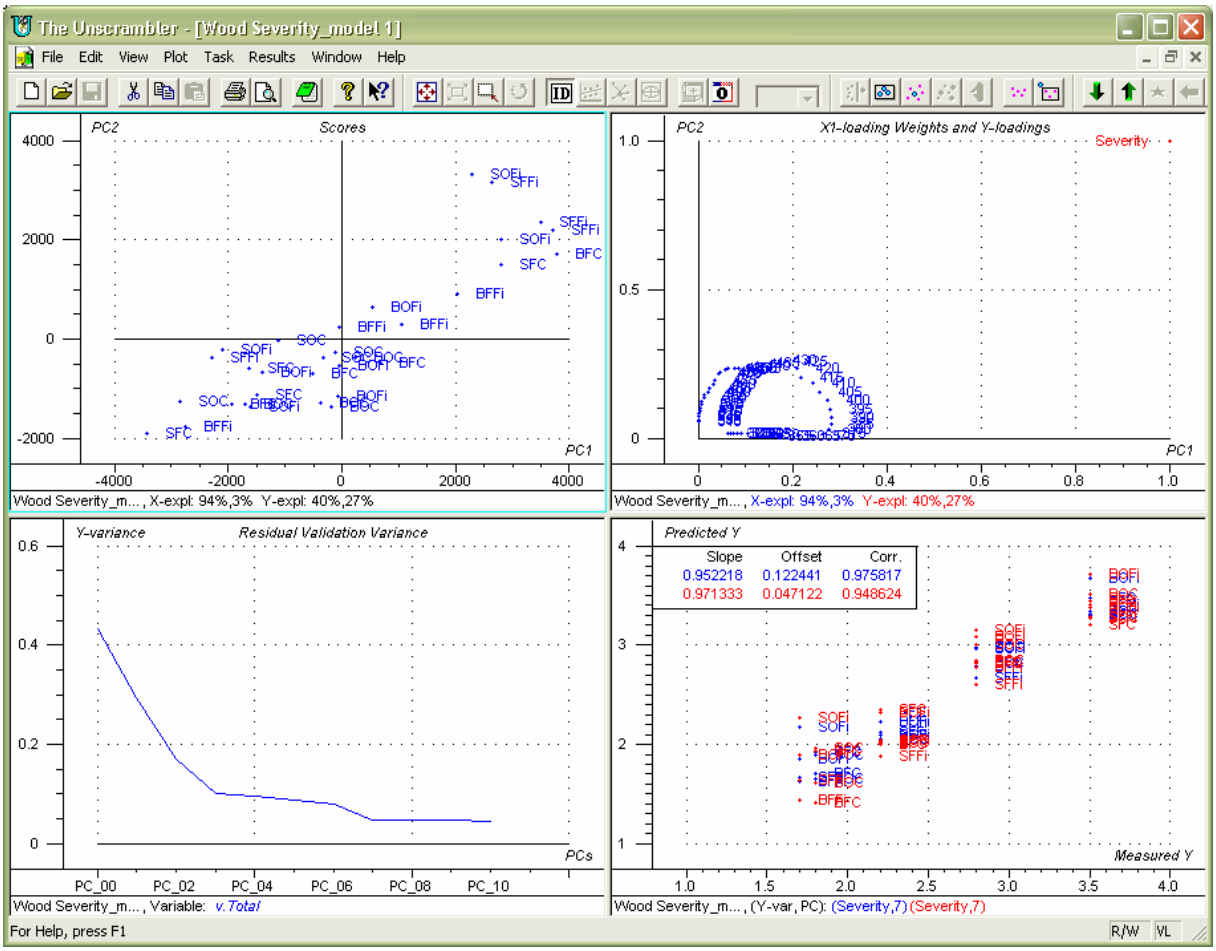


Figure 15: Three-Way PLS Regression Overview, Wood Severity_model 1

Go to menu **File-Save** and save your model as "**Wood Severity_model 1**"

Find an Outlier and Recalculate (Tutorial H)

Before interpreting a model, one should always check the model for potential outliers.

Tasks

Detect an outlier and recalculate the model without it.

How to Do It

Go to menu **Plot – Sample Outliers**. Keep the default settings and click OK. Four plots appear in the **Viewer**: Scores, Influence, Y-Residual Sample Variance and X-Residual Sample Variance.

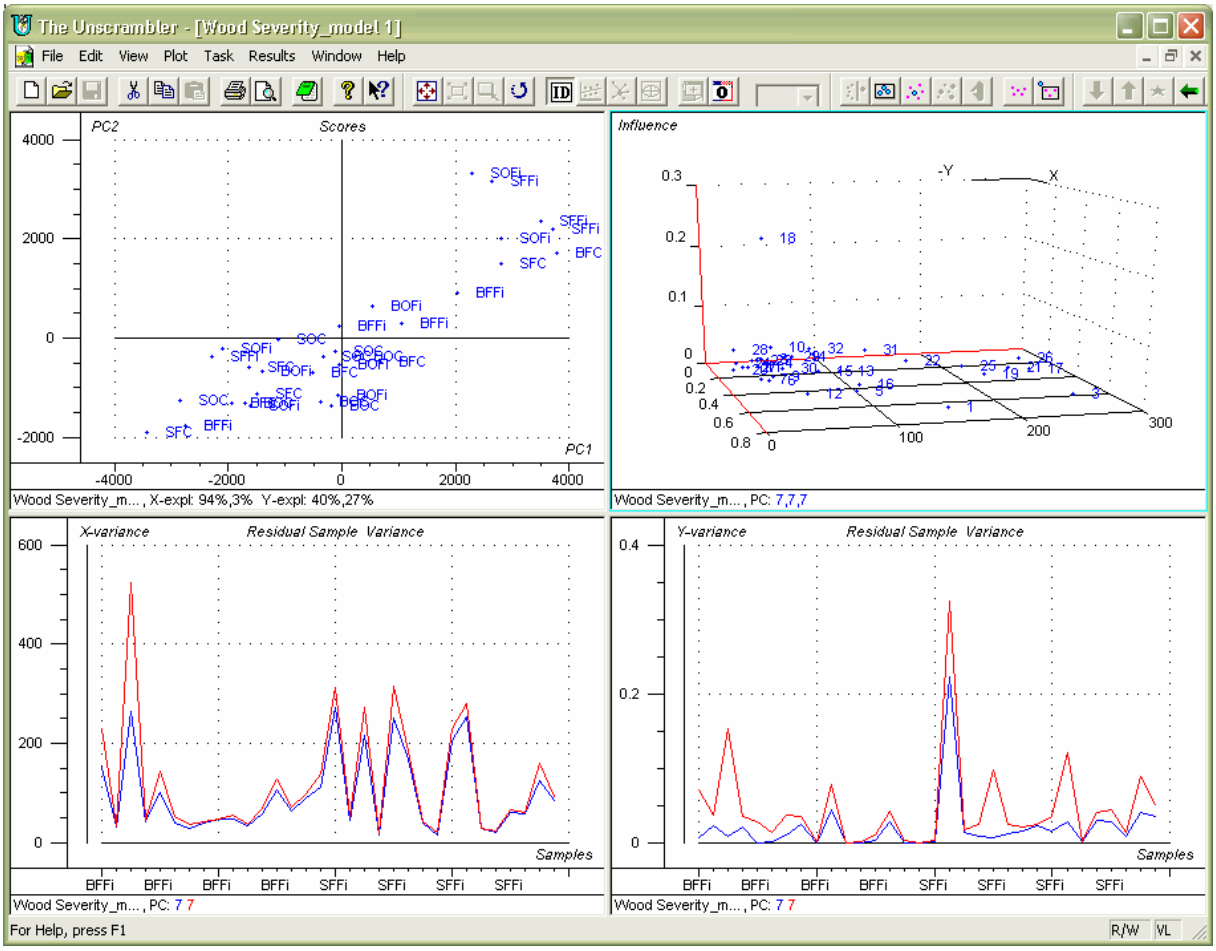


Figure 16: Sample Outliers plots, Wood Severity_model 1

Click on the **Influence** plot so that it is active, then use the X and Y buttons () to display only X information, or only Y information, or both. Sample 18 (SOFi) is an outlier with a high Residual Y-Variance.

Go to menu **Edit-Mark-One By One** or use the corresponding shortcut  , then click on sample 18 in the **Influence** plot. This sample is now marked by a circle on all plots.

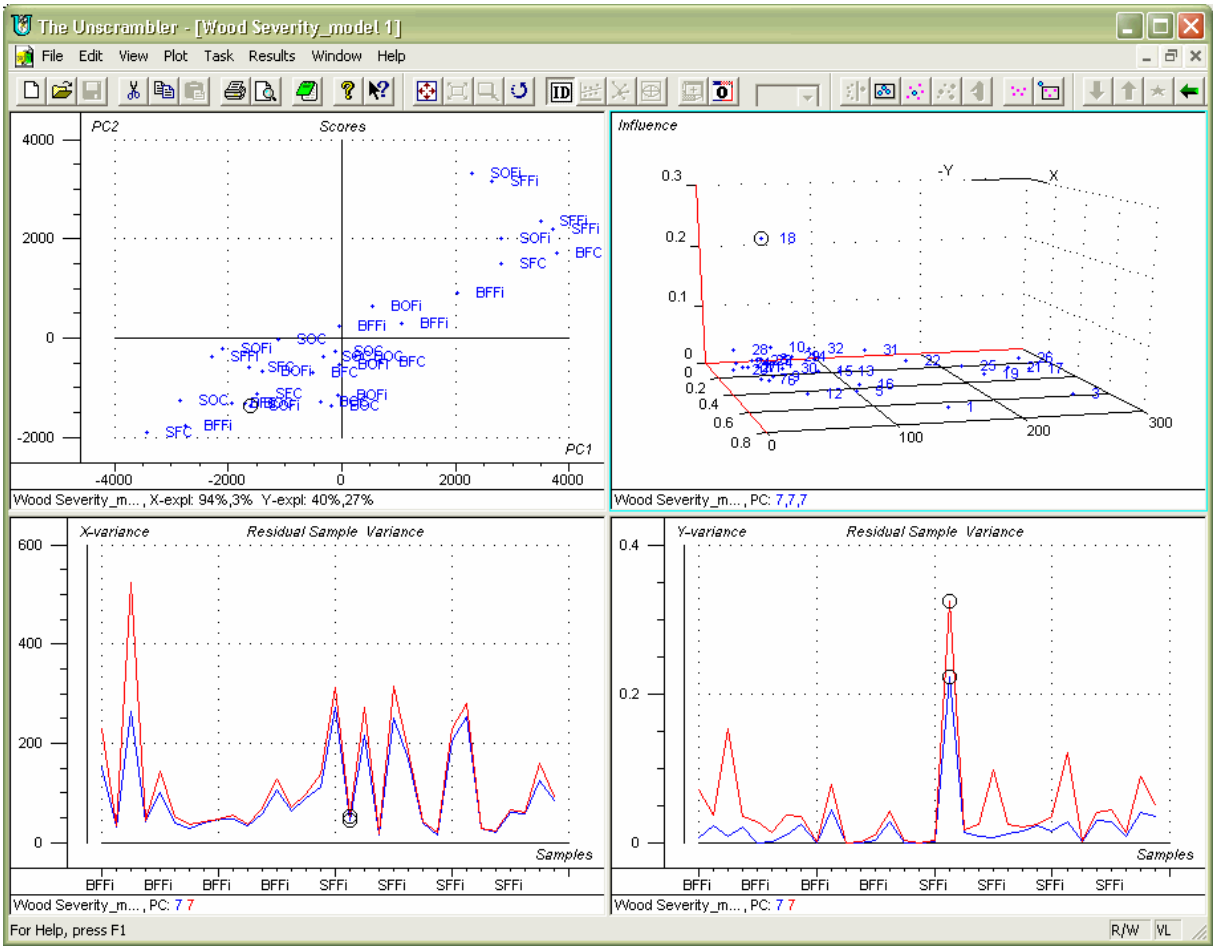


Figure 17: Sample Outliers plots, Wood Severity_model 1, with sample 18 marked with a circle

Go to menu **Task-Recalculate Without Marked**. This brings up the **Regression (Three-Way PLS)** dialog, and you can observe that sample 18 is shown in the Keep Out of Calculation field.

Check that the **Cross Validation setup** is still Full Cross Validation, and that the number of components (**Num PCs**) is 10.

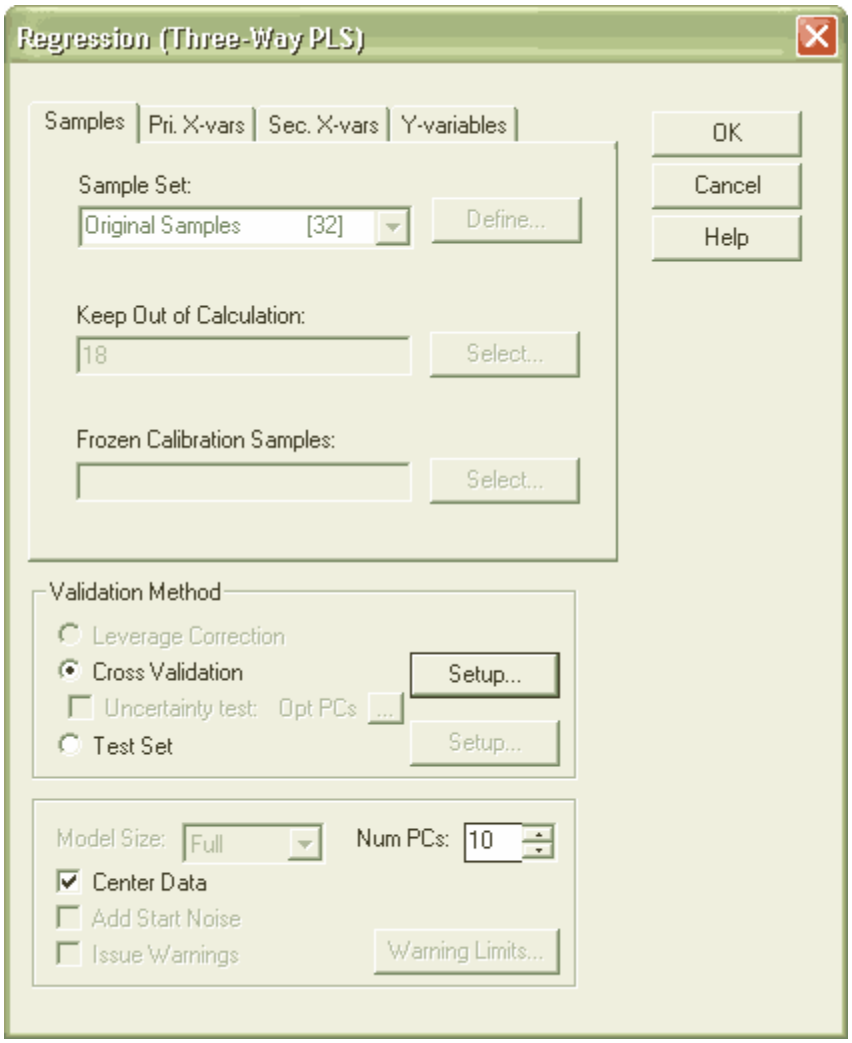


Figure 18: Regression (Three-Way PLS) dialog with sample 18 kept out of calculations

Click OK to compute a new model without sample 18.

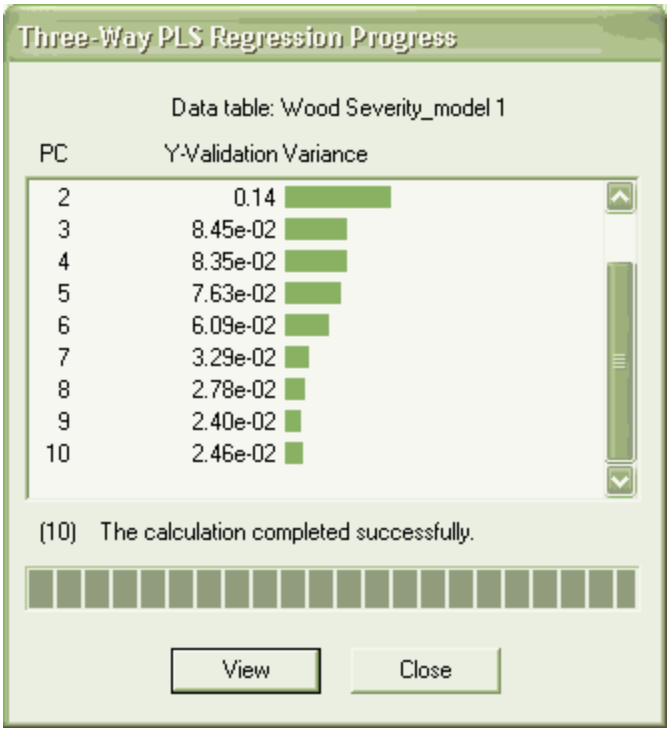


Figure 19: Three-Way PLS Regression Progress dialog, Model 2 calculations

. Click View to display the Regression Overview. Go to menu **Plot – Sample Outliers** and check that no sample is outlying in this new model.

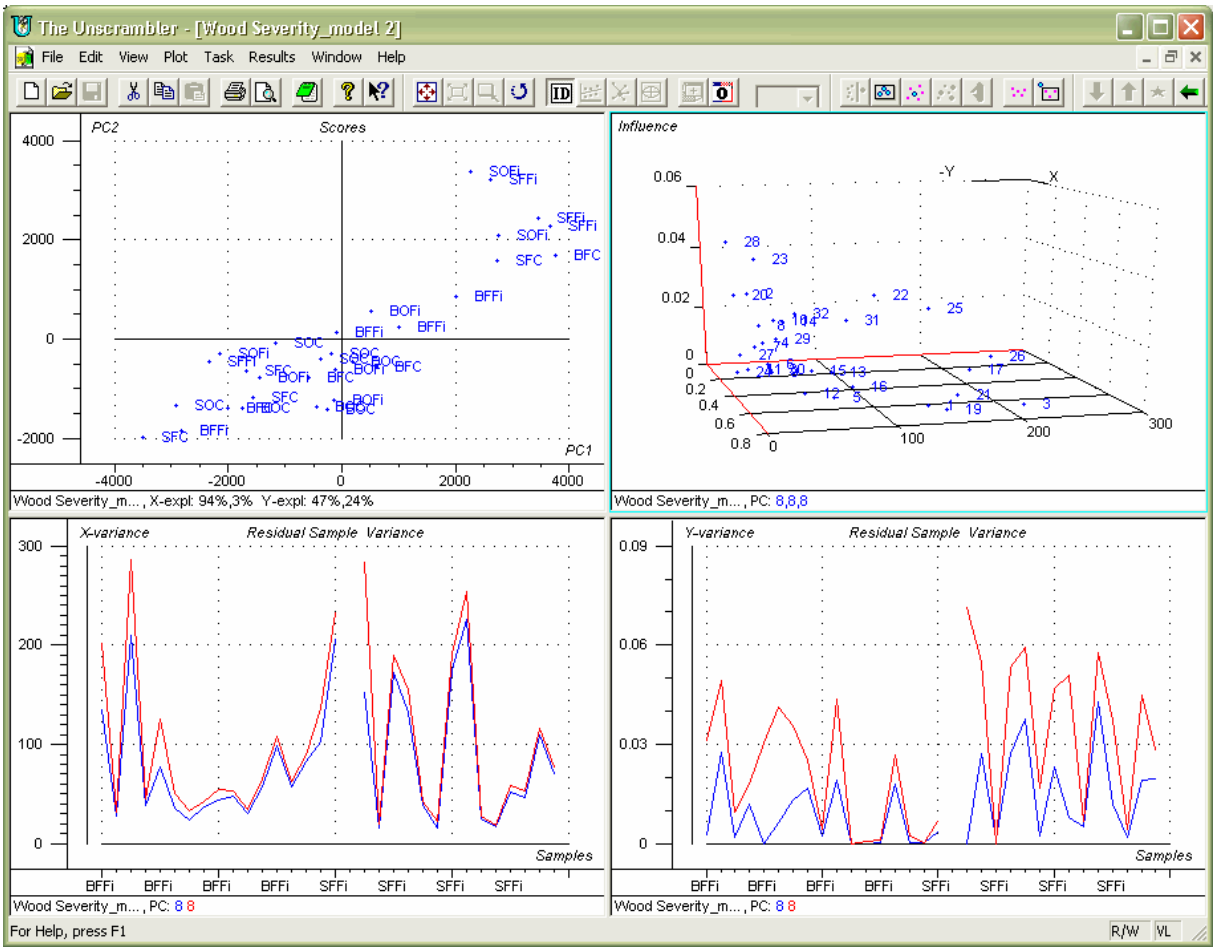


Figure 20: Sample Outliers plots, Wood Severity_model 2

Go to menu **File-Save** and save the new model as "**Wood Severity_model 2**"

Interpret a Three-Way PLS Regression Model (Tutorial H)

Three-Way PLS regression models are interpreted in very much the same way as (2-way) PLS-R models. We will focus our interpretation on some of the available pre-defined plots.

Tasks

Determine optimal number of components (PCs), Interpret the Scores, the X-Loading Weights, the regression coefficients and the Predicted vs. Measured plot.

Determine the Optimal Number of PCs for the Model (Tutorial H)

How to Do It

Go to **Plot-Regression Overview**. This opens the **Regression Overview** dialog. In the last section of this dialog, you can observe that the Suggested number of Components is 8. Keep the default settings and click OK.

In the **Regression Overview**, study the bottom left plot: Y-Residual Validation Variance.

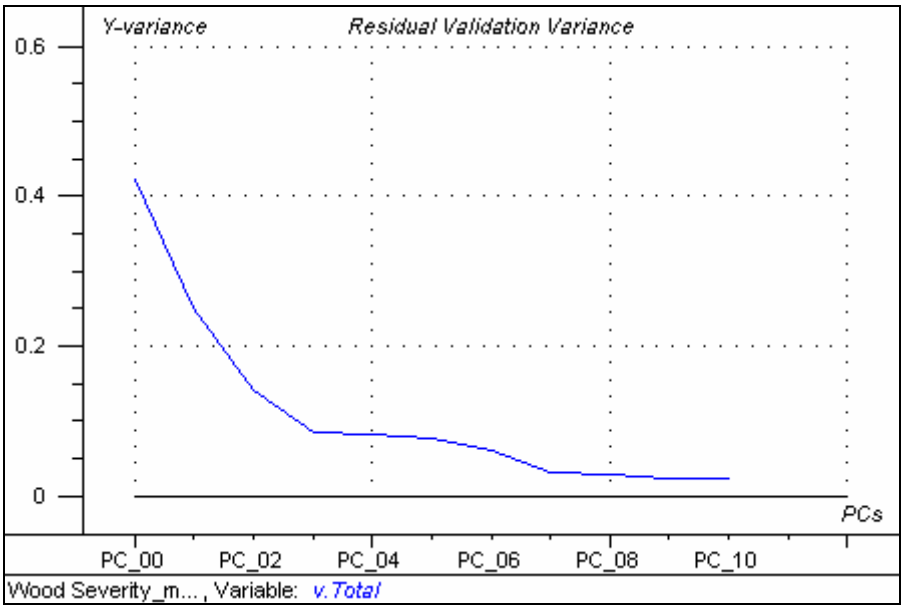
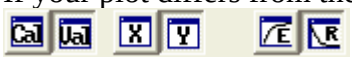


Figure 21: Y Residual Validation Variance plot, Wood Severity_model 2

.

Note!

If your plot differs from the picture, you may adjust it using this set of buttons:  which control Calibration and/or Validation results, X or Y variables display, and Explained or Residual variance. To determine number of PCs for the model, you should look at the **Y Validation Variance** (Residual or Explained).

The Y-residual validation variance shows a plateau from PCs 7-8, in agreement with the suggested number of components given by the software. We decide to be conservative and use 7 PCs for this model.

Interpret the Scores (Tutorial H)

How to Do It

Activate the **Scores** plot (map of samples) by clicking on it; it is the plot situated in the first quadrant. The sample names contain a lot of information. Let us focus on Wood type.

Go to **Edit-Options** or click on this shortcut:  . This opens the **Options** dialog. In the Markers Layout field, choose option Name, then click on the first box. This will disable the following boxes, so that only the first character in the sample name will be kept.

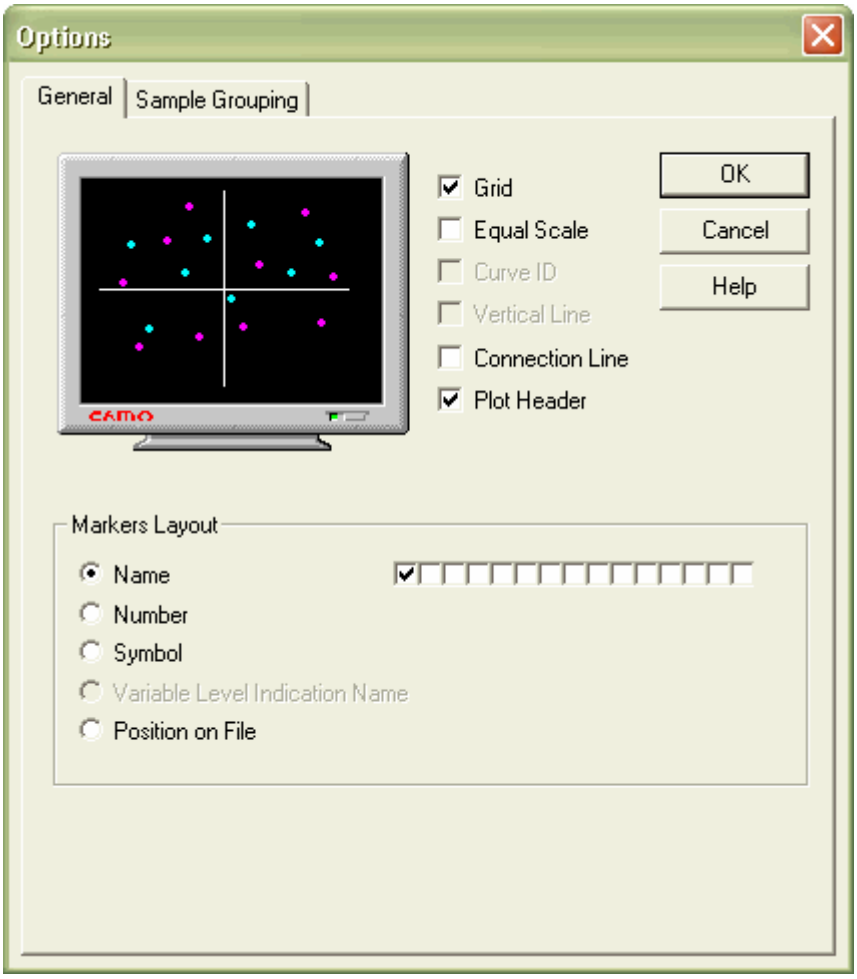


Figure 22: Options dialog

Click OK. The Sample names only indicate S for Spruce wood (soft) or B for Beech wood (hard).

Click on the **Next Vertical PC** button  , or use the **Up arrow key** on your keyboard to display the Scores for PC1 vs. PC3. We can observe that PC3 separates the Spruce samples (to the bottom) from the Beech samples (to the top).

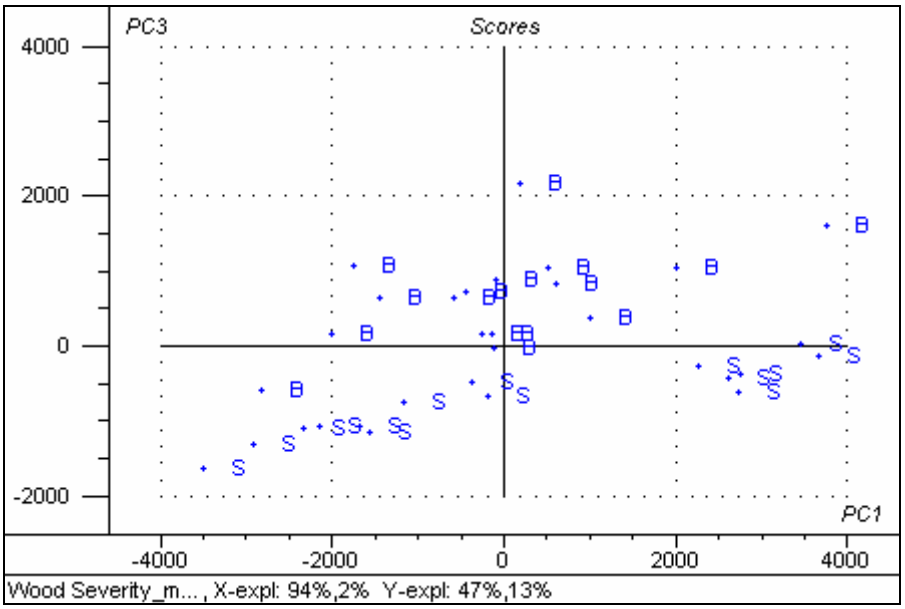


Figure 23: Scores Plot, PC1 vs. PC3, Spruce (S) and Beech (B) samples

.

Interpret the Loading Weights (Tutorial H)

How to Do It

Let us find out which fluorescence information is carried by PC3, which separates Spruce from Beech. Go to **Plot-Loading Weights**. In the **Loading Weights** dialog, select the following settings:

Plot type: Line

Vector 1: 1-3

Variables: X

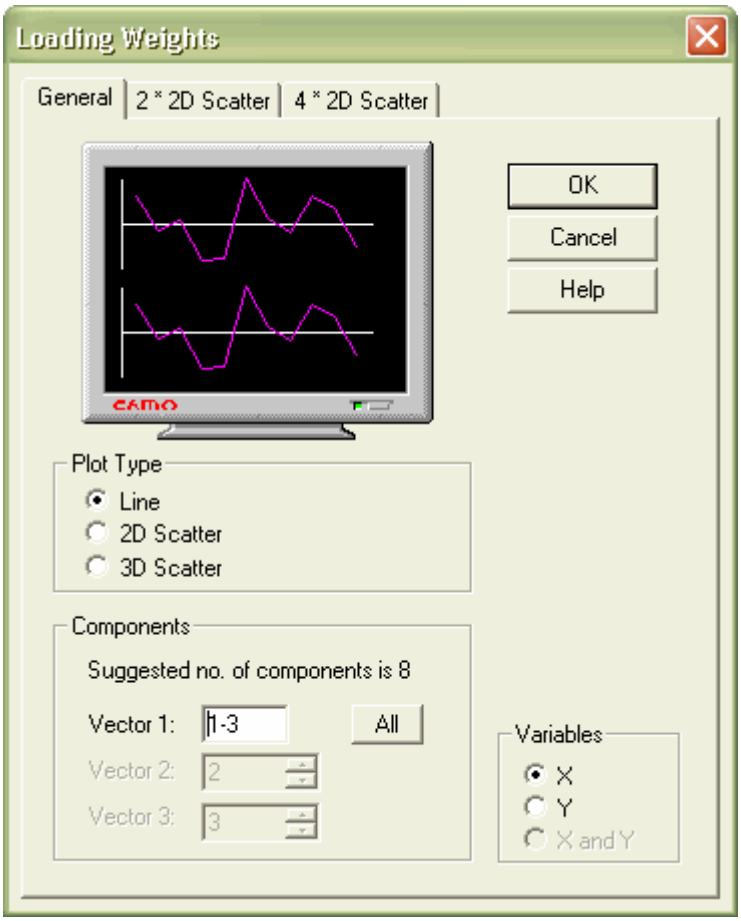


Figure 24: Loading Weights dialog

Click OK. The Loading Weights for excitation spectra (Primary variables, X1) appear in the top window and the Loading Weights for emission spectra (Secondary variables, X2) appear in the bottom window.

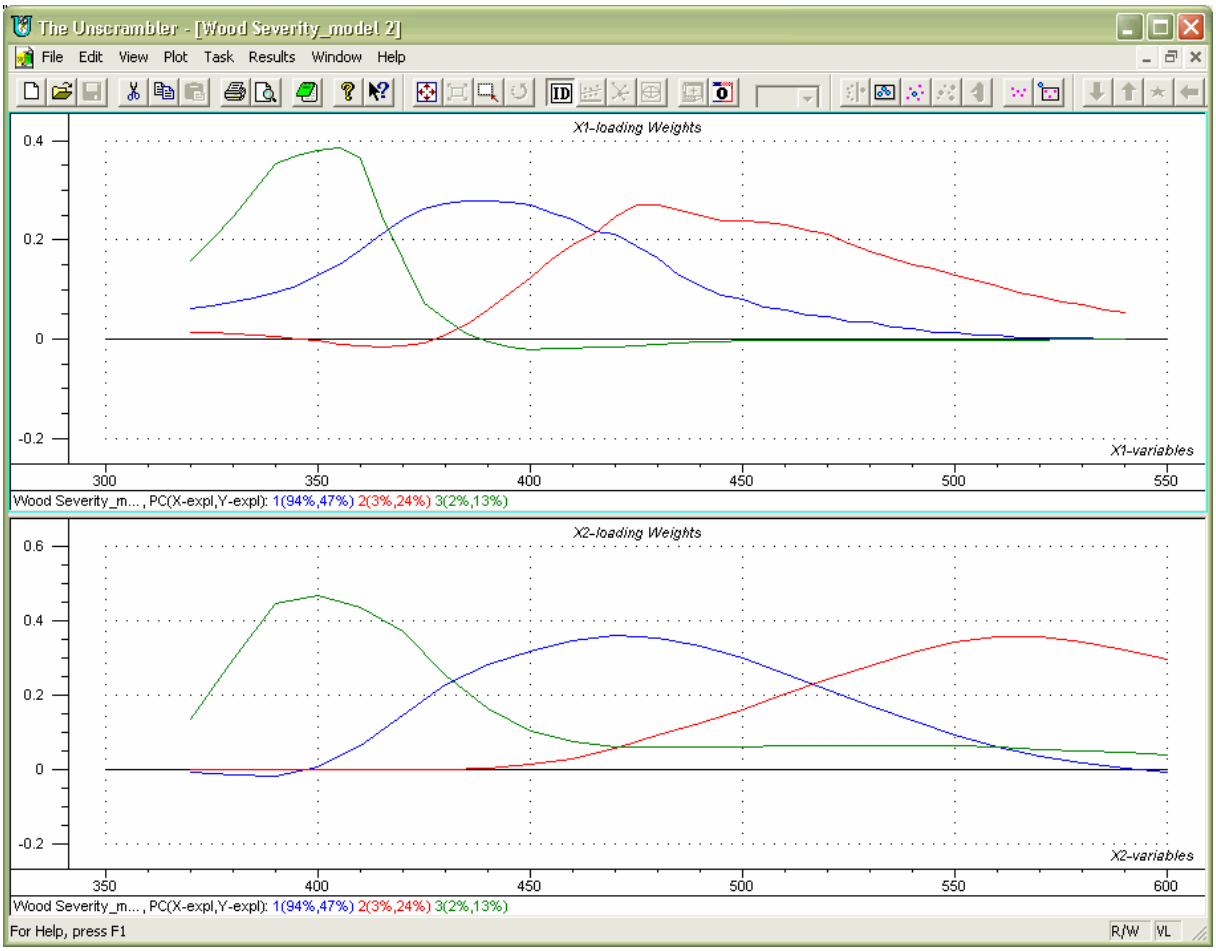


Figure 25: X1-Loading Weights (Excitation spectra) and X2-Loading Weights (Emission spectra) for PC1, PC2 and PC3

PC3 is represented in green on the plots. On the top plot, it shows a peak for excitation 355 nm. On the bottom plot, it shows a peak for emission 400 nm.

These peaks describe the CH3O functional groups of hardwood and softwood. The CH3O functional groups are higher in hardwood lignin than in softwood. This information is shown with PC3. The beech samples have higher scores than the spruce samples for this PC.

Interpret the Regression Coefficients (Tutorial H)

How to Do It

Go to **Plot-Regression Coefficients**, an in the **Regression Coefficients** dialog choose the following settings:

Plot type: Matrix

X-variables: Primary X Vs Secondary X

Y-variable: 1, Severity

Components: 7

Double click on the preview screen at the top of the dialog to enlarge the plot: the plot will be displayed in Full Window

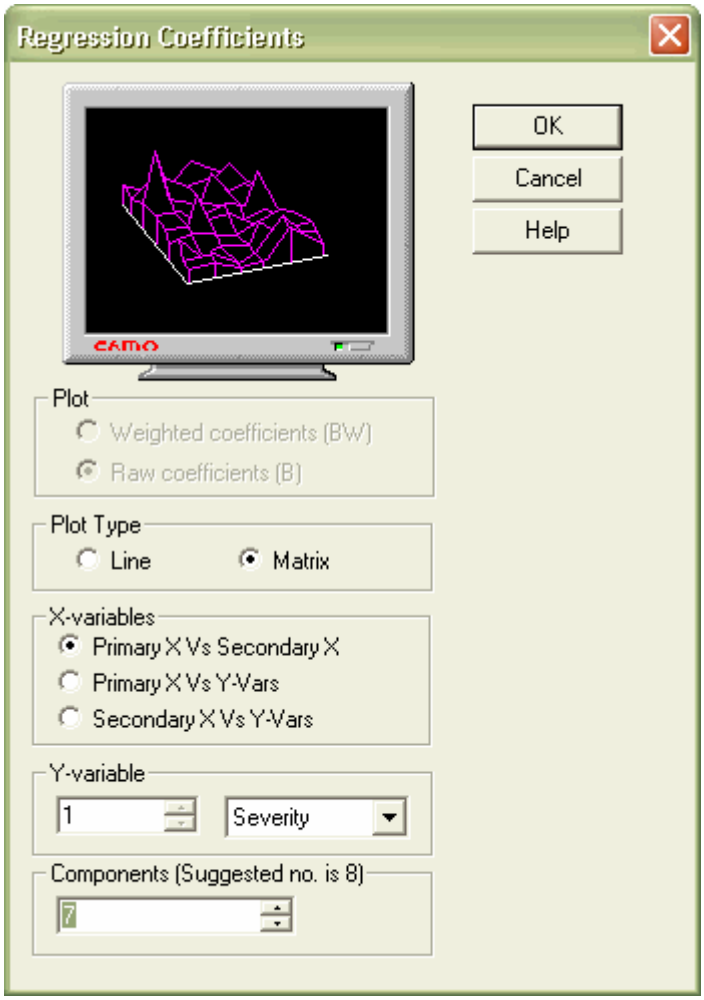


Figure 26: Regression Coefficients dialog

Click OK to display the regression coefficients plot. The plot is shown in landscape layout.

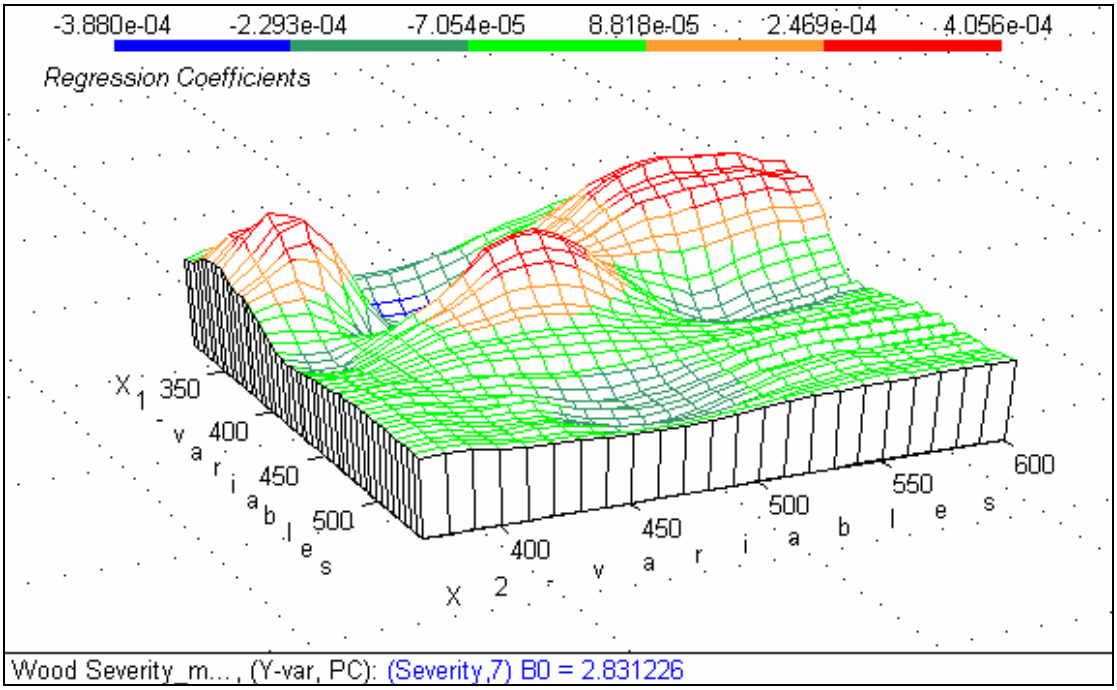


Figure 27: Regression Coefficients of Excitation Emission spectra for modelling Severity; 7 PCs, Landscape layout

We can observe four major areas presenting high regression coefficients (three positive, one negative). To better study the plot, use the rotate function ( or **View- Rotate**). Use either the mouse or the arrow keys on your keyboard to rotate the plot. Holding your finger on an arrow key will allow a continuous rotation of the plot; pressing the **AltGr** key at the same time will slow down the rotation.

Menu **Edit-Options…** (or ) allows you to change the Plot Layout from a 3-dimensional Landscape view into Map. Move your mouse over the Map plot to get the coordinates for excitation and emission wavelengths.

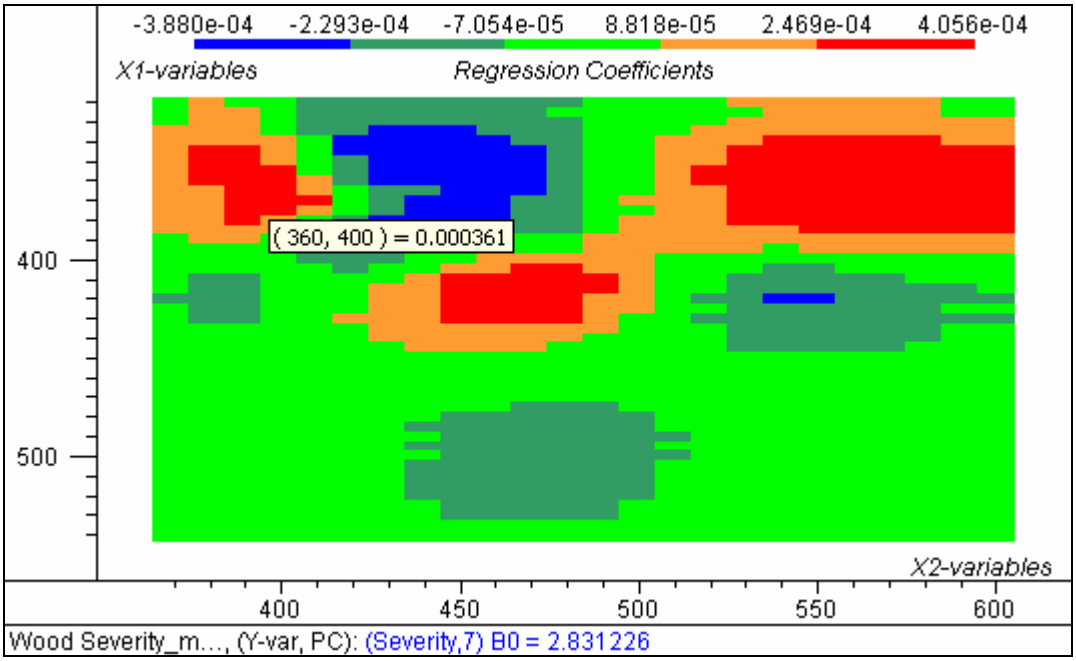


Figure 28: Regression Coefficients of Excitation Emission spectra for modelling Severity; 7 PCs, Map layout

.

Use the  buttons or corresponding keyboard arrows to navigate the regression coefficients plot for models of various numbers of components. This is available both in landscape and in map layouts.

As we navigate from PC1 over PC2, to PC3 we can see that the regression coefficients change corresponding to the absorption/emission band of the CH3O-functional groups (Excitation 360 nm, emission 400 nm) when we include the third component.

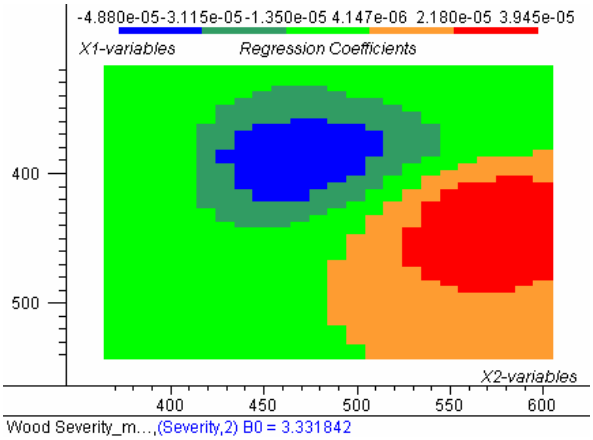


Figure 29: Regression Coefficients of Excitation Emission spectra for modelling Severity; 2 PCs (left) and 3 PCs (right), Map layout

Interpret the Predicted and Measured Plot (Tutorial H)

How to Do It

Go to **Plot-Predicted vs Measured**. In the dialog, choose the following settings:

Plot type: Predicted and Measured

Y-variable: 1, Severity

Components: 7

Samples: Calibration

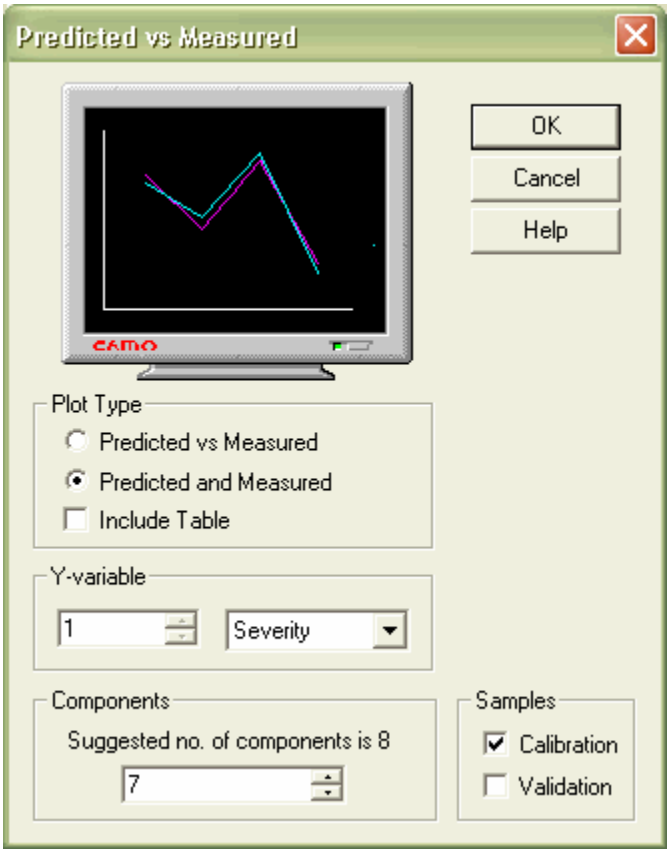


Figure 30: Predicted vs Measured dialog

Click OK to display the plot. The blue curve corresponds to our model, while the red curve corresponds to the measured values. There is a good fit of the model. Yet we can observe that several samples are not as well predicted as the others. By moving the mouse over these samples to identify them, it is seen that especially fresh wood samples (F) are generally better predicted than old wood samples (O).

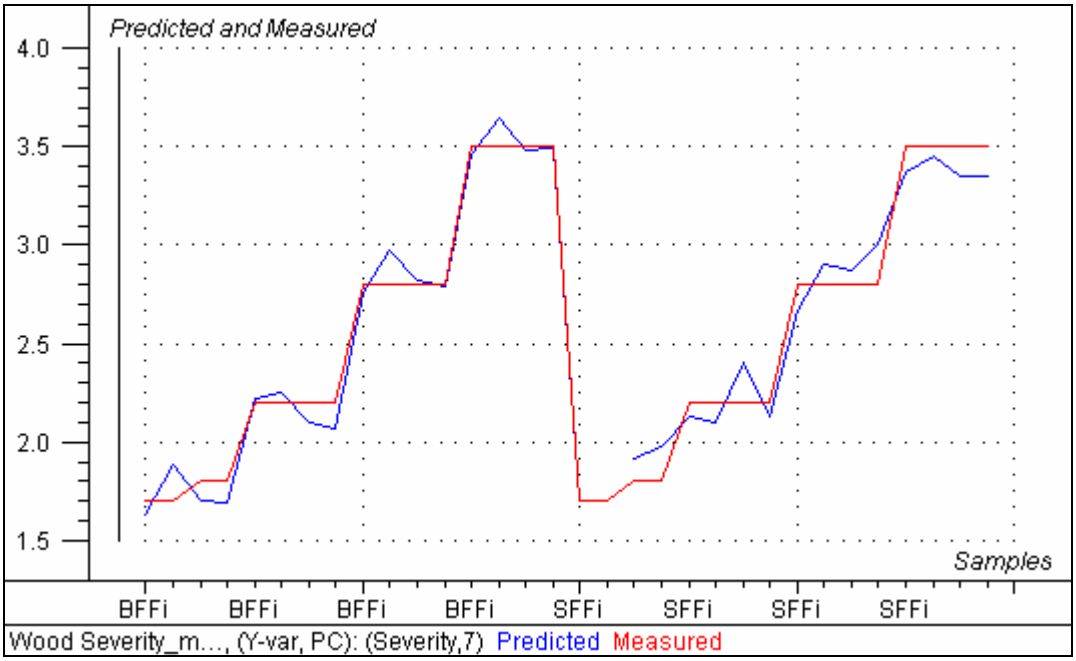


Figure 31: Predicted and Measured plot, Calibration results, 7 PCs

The RMSEC for the model is accessible from **Plot-Predicted vs Measured**. Choose settings:

Plot type: Predicted vs Measured

Y-variable: 1, Severity

Components: 7

Samples: Calibration

RMSEC is of 0.11, for steam treatments severity values that ranged from 1.7 to 3.5. This about the size for the reproducability of the severity measurement.