

## QUANTIFY - Multivariate Calibration

Training Material  
Quebec, June 2007

HORIZON MB<sup>TM</sup> FTIR SOFTWARE

Take advantage of your MB3000



### Quantify – Multivariate Calibration

#### Calibration Models

- Multiple Linear Regression (MLR)
- Partial Least Squares Regression (PLS1)
- PLS2 (Multi Component)
- SIMPLS

## Quantify – Multivariate Calibration Overview

### Calibration Model Development Steps

- Load calibration spectra
- Merge all spectra into one data view
- Create a project (and a folder)
- Add labels with concentrations (Label Editor)
- Start the calibration wizard
- Create and optimize a calibration model
- Save the model in the project

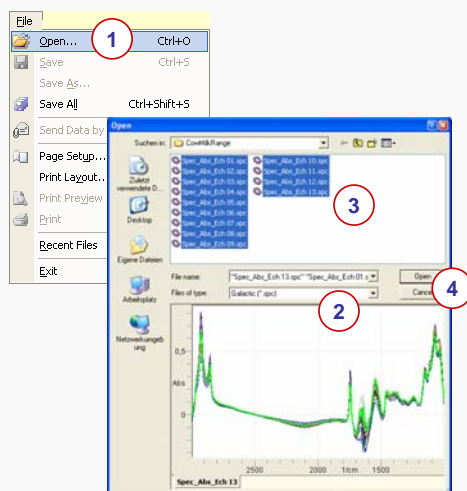
### Editing/Copying Calibration Models



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## Load Calibration Spectra



1. Click „File->Open“
2. Select the file type (\*.spc)
3. Select all calibration and validation spectra  
(Multiple selection with **CTRL** or **SHIFT** key held down)
4. Click „Open“



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### Merge and Split Spectra

The screenshot shows a menu with options: Auto Scale (Ctrl+Shift+A), Sticky View (Ctrl+Shift+K), Clear View, Merge Views (Ctrl+Shift+M), Split View (Ctrl+Shift+U), Show Data in Table, Show Favorite Area, Preferences..., and Create 3D Data. The 'Merge Views' and 'Split View' options are circled in red. Below the menu, two spectral plots are shown. The left plot is labeled 'Multiple tab windows' and the right is 'One tab window'. A red double-headed arrow labeled 'Merge Views' points from the multiple tabs to the single tab, and another red double-headed arrow labeled 'Split Views' points from the single tab back to the multiple tabs.



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### Add Labels to Calibration Spectra

The screenshot shows the 'Label Editor' window with a list of labels and a 'New Label' dialog box. Step 1 points to the 'Options' button in the 'Tools' menu. Step 2 points to the 'Display Options' button. Step 3 points to the 'New Label' button. Step 4 points to the text input field in the 'New Label' dialog where 'Fa' is entered. Step 5 points to the 'OK' button in the 'New Label' dialog.

1. Click „Tools->Label Editor“
2. Click „Display Options“ to customize visible labels
3. Click „New Label“ to add a new label
4. Enter a label name
5. Click „OK“



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## Add Concentration Values to Labels



1. Load concentration values, e.g. from MS-Excel worksheet
2. Select the **concentration range** in the worksheet and copy to clipboard with **CTRL-C**
3. Select the field in the upper left corner of the table in the label editor.
4. Insert contents with **CTRL-V**
5. Click „OK“ to leave the Label Editor



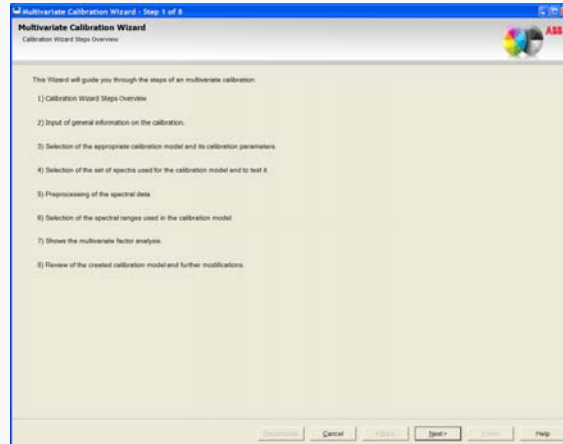
## Multiple Linear Regression (MLR)

### Multiple Linear Regression (MLR)

- Steps of calibration modelling
- Spectrum selection
- Math preprocessing
- Selection of spectral ranges
- Model calculation and results



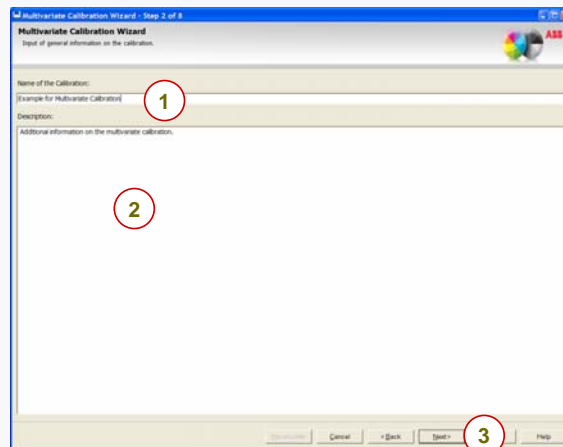
## MLR Step – 1 - Overview



This step provides an overview over the subsequent steps for calibration modelling

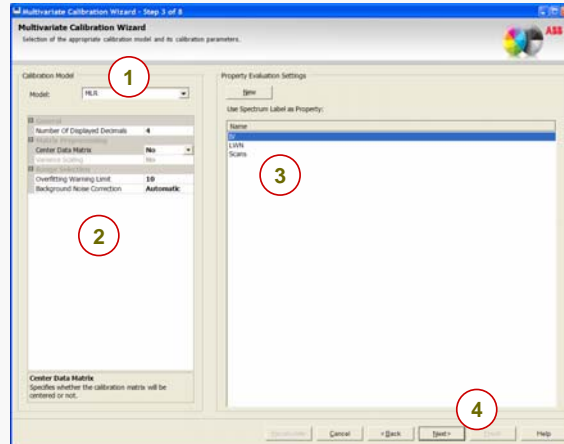
Click „Next“

## MLR Step – 2 – General Information



1. Enter a **calibration name** (e.g. Model Type plus calibrated label)
2. Enter **additional information** like experiment information, sample preparation, applicable range. Etc.
3. Click „Next“

## MLR Step – 3 – Calibration Model Selection



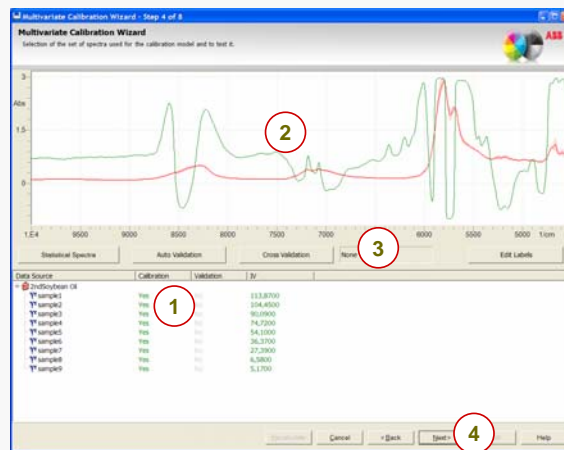
1. Select the **MLR** calibration model
2. Adjust additional parameters
3. Select a calibration label
4. Click „**Next**“



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## MLR Step – 4 – Selection of Spectra



1. Set calibration and validation spectra to „**Yes**“ in the respective column
2. Spectrum selection with statistical information (green line and red shape) indicate highly correlated areas in the data view
3. Adjust **cross validation** settings
4. Click „**Next**“

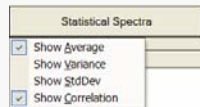


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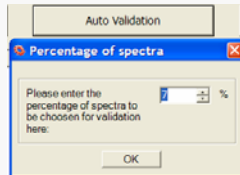
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## MLR Step – 4 – Function Buttons

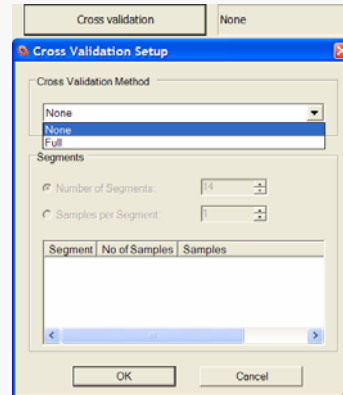
Statistical information on selected spectra



Automatic selection of validation spectra



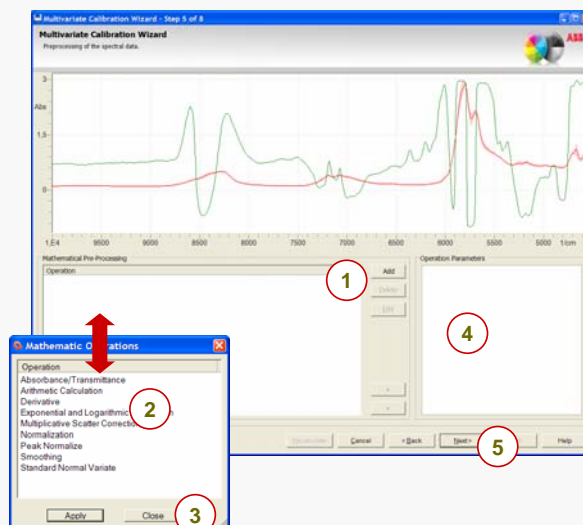
Cross validation settings



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## MLR Step – 5 - Math Preprocessing



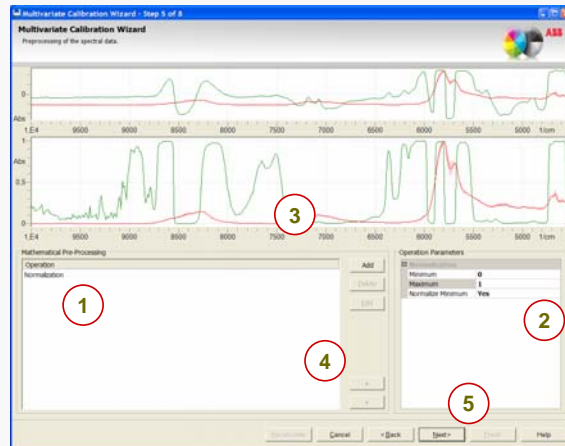
1. Click „Add“ to add new math function
2. Select a math function and click „Apply“
3. Click „Close“
4. Adjust math function parameters (see next page)
5. Click „Next“



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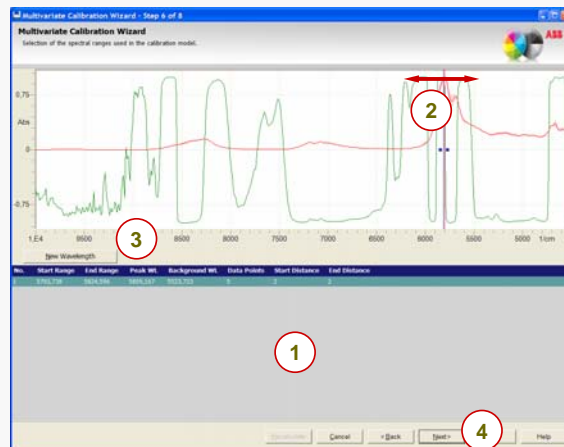
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## MLR Step – 5- Setup of Math Function Parameters



1. Select the math function in list
2. Adjust math function parameters
3. Instantly see the result in the data view
4. Change the order of math functions
5. Click „Next“

## MLR Step – 6 – Selection of Spectral Ranges

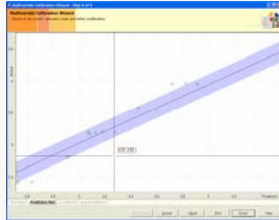


1. The first range is proposed automatically
2. Move the range to the desired location (Optional)
3. Click „New Wavelength“ to add a new range or the DEL key to delete a range
4. Click „Next“

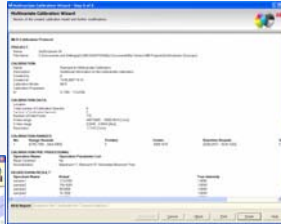


## MLR Results

Click „**Back**“ to go back to a previous step for optimization



Hold down **CTRL** key and click markers to select spectra



Click the tabs on the final wizard page to see the results



Click „**Recalculate**“ to recalculate the model with actual settings

## Editing a Calibration Model

Sample Name	Calibration	Validation	Use
CowMilkRange	Yes	Yes	11.034
Sample Outlier	Yes	Yes	0.000
Spectra	Yes	Yes	0.000
Example for Multivariate Co	Yes	Yes	0.000
Calibration Data	Yes	Yes	0.000
Validation Data	Yes	Yes	0.000
Reports	Yes	Yes	0.000
MLR Report	Yes	Yes	0.000
Prediction Plot	Yes	Yes	0.000
2D Delta Plot	Yes	Yes	0.000

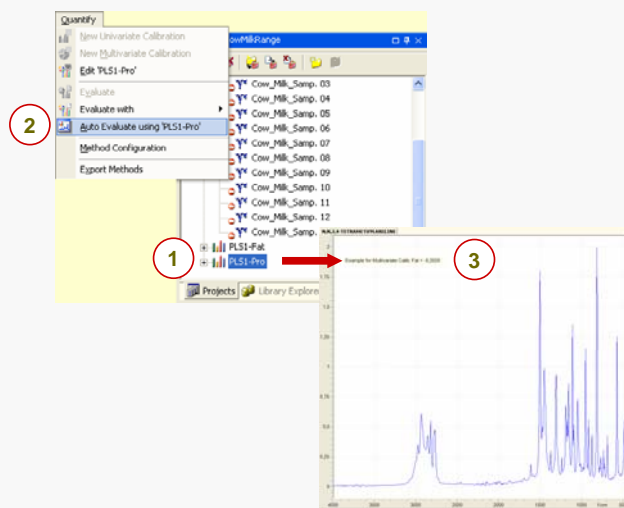
1. Select the calibration node in the project explorer
2. Click the right mouse button and choose „Edit“
3. Click „Next“ in the wizard and proceed to the page of interest.

## Predictions of unknown Samples

### Prediction methods

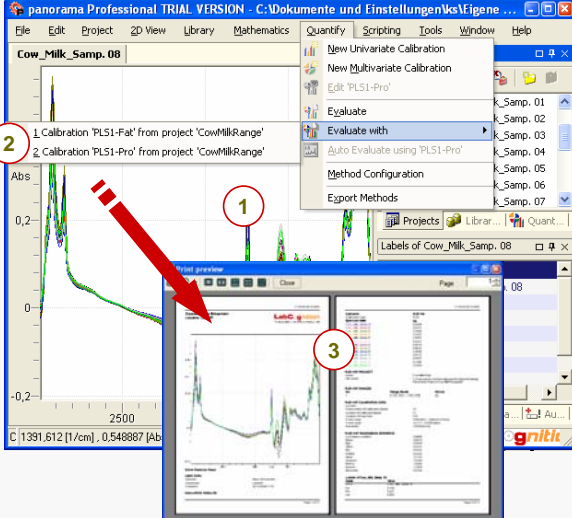
- Auto-evaluation and online display for the active spectrum
- Evaluation with comprehensive report

## Auto-Prediction with Online Display (Active Object)



1. Select the calibration node in the project explorer
2. Click „Quantify-> Auto Evaluate using...“ to enable or disable automatic evaluation
3. Evaluation result is shown instantly for the active spectrum

## Prediction using specific Calibration



1. Open one or more spectra for evaluation in a data view

2. Click „Quantify-> Evaluate with-> Calibration model“

3. The comprehensive report shows the prediction result

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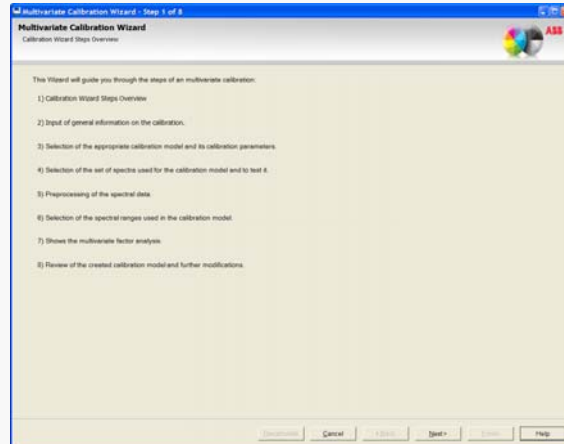
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## Partial Least Squares Regressions (PLS1, PLS2, SIMPLS)

### Partial Least Squares Regressions

- Steps of calibration modelling
- Spectrum selection
- Math preprocessing
- Selection of spectral ranges
- Model calculation and results

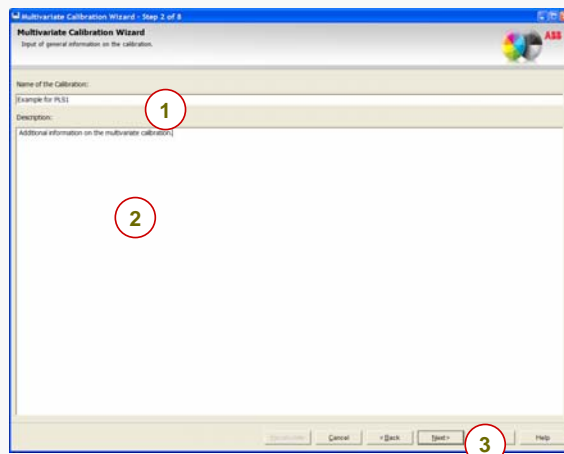
## PLS Step – 1 - Overview



This step provides an overview over the subsequent steps for calibration modelling

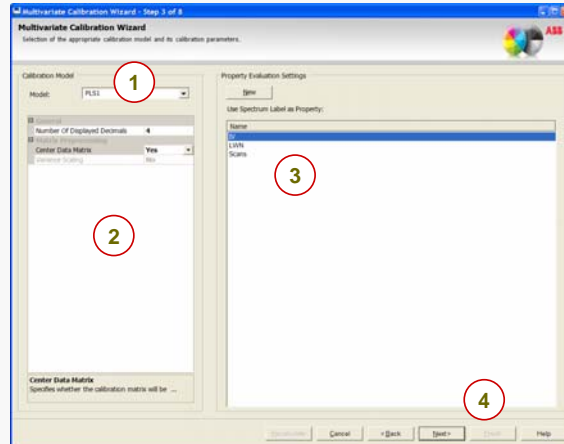
Click „Next“

## PLS Step – 2 – General Information



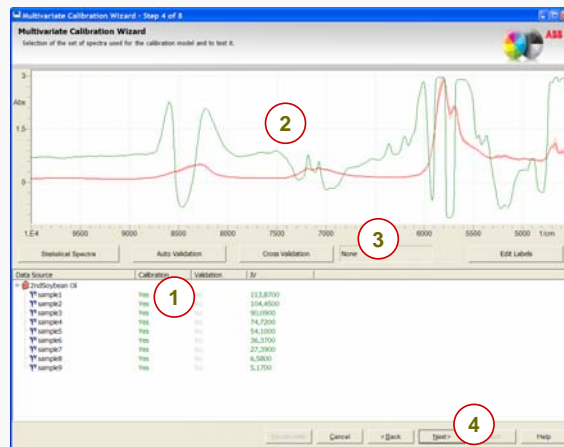
1. Enter a **calibration name** (e.g. Model Type plus calibrated label)
2. Enter **additional information** like experiment information, sample preparation, applicable range. Etc.
3. Click „Next“

## PLS Step – 3 – Calibration Model Selection



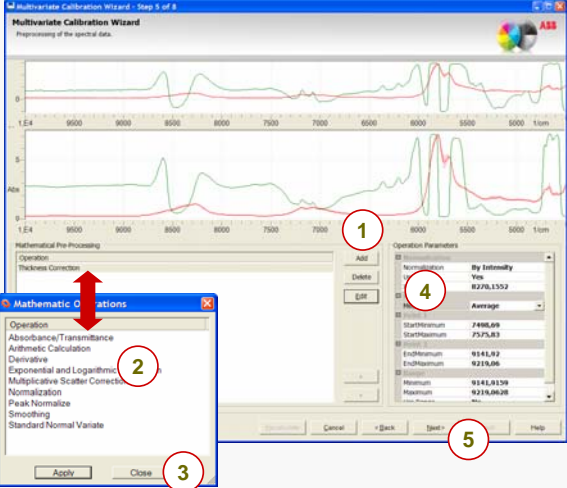
1. Select the **PLS1** calibration model
2. Adjust additional parameters
3. Select **ONE** calibration label
4. Click „Next“

## PLS Step – 4 – Selection of Spectra



1. Set calibration and validation spectra to „Yes“ in the respective column
2. Spectrum selection with statistical information (green line and red shape) indicate highly correlated areas in the data view
3. Adjust **cross validation** settings
4. Click „Next“

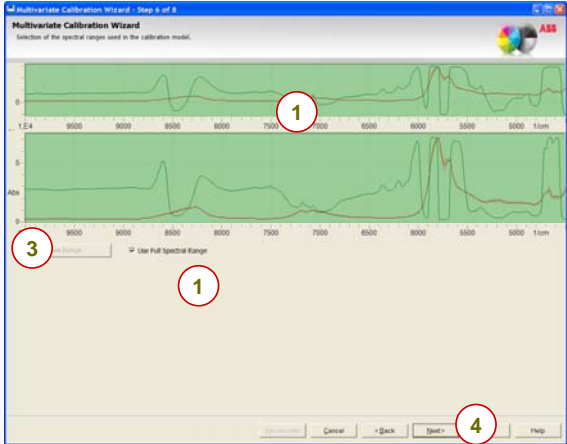
## PLS Step – 5 - Math Preprocessing



1. Click „Add“ to add new math function
2. Select a math function and click „Apply“
3. Click „Close“
4. Adjust math function parameters (see MLR)
5. Click „Next“

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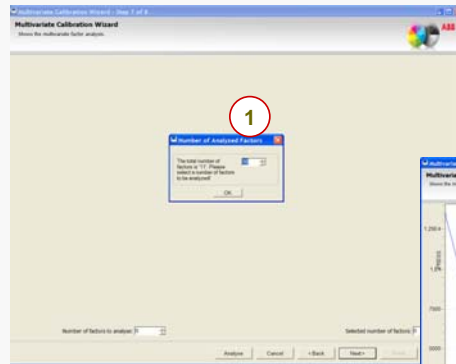
## PLS Step – 6 – Selection of Spectral Ranges



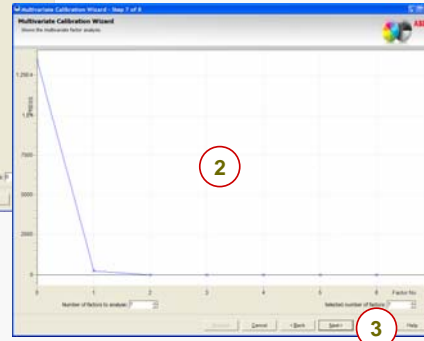
1. Initially, the **full range** is selected automatically
2. Uncheck „Use Full Range“ to select individual ranges
3. Click „New Range“ to add a new range or the **DEL** key to delete a range
4. Click „Next“

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## PLS Step – 7 – Factor Analysis



1. Choose an initial **number of factors**
2. From the PRESS plot refine selection



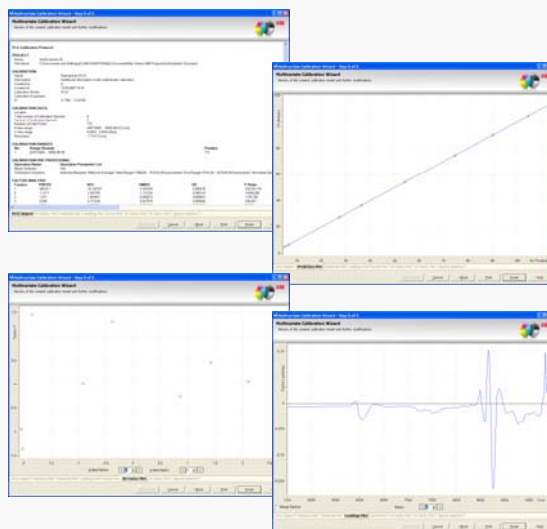
3. Click „Next“



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## PLS Results



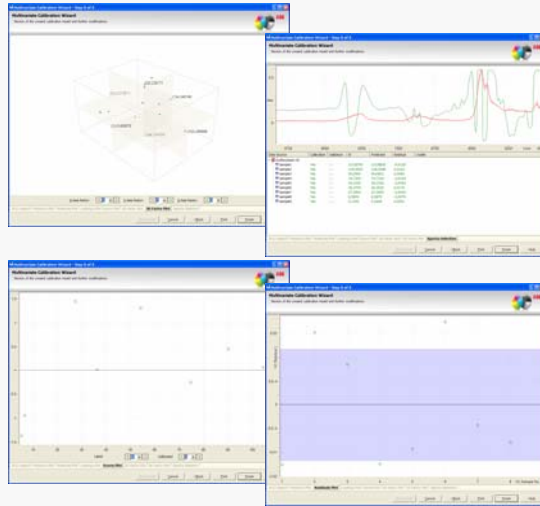
1. Calibration model result report
2. Predicted vs. Actual plot
3. 2D Factor plot
4. Loadings plot
5. Click other tabs to see more results



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## More PLS Results



1. 3D Scores Plot
2. Spectra Selection with numerical results
3. 2D Scores Plot
4. Delta Plot with residuals
5. Click „Back“ to previous steps to optimize the model

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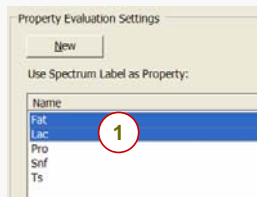
  
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## PLS1 vs. PLS2 and SIMPLS

The overall calibration wizard handling is the same for PLS1, PLS2 and SIMPLS.

### Differences:

1. Step 3 of 8: it is possible to **select multiple labels** for evaluation
2. Step 8 of 8: **Calculation results** show predicted and residual values for all selected labels.



Data Source	Calibration	Validation	Fat	Predicted	Residual	Outer	Lac	Inner	Predicted	Residual
Copy of Calibration										
SP1079										
Y*Spec_Abs_Ech10	Yes		2,2780	2,2078	0,0712		4,9244	4,8178	0,0066	
Y*Spec_Abs_Ech12	Yes		2,9060	2,9027	-0,0033		4,9403	4,9421	-0,0018	
Y*Spec_Abs_Ech13	Yes		3,6000	3,6035	-0,0035		4,9600	4,9600	0,0000	
Y*Spec_Abs_Ech14	Yes		3,3980	3,3965	0,0015		5,0000	5,0071	-0,0071	
Y*Spec_Abs_Ech15	Yes		3,3930	3,3945	-0,0015		6,0000	6,0035	-0,0035	
Y*Spec_Abs_Ech16	Yes		4,3120	4,3130	-0,0010		4,9800	4,9852	-0,0052	
Y*Spec_Abs_Ech17	Yes		5,0340	5,0350	-0,0010		4,9850	4,9856	-0,0006	
Y*Spec_Abs_Ech18	Yes		5,0040	5,0072	-0,0032		4,9400	4,9440	-0,0040	
Y*Spec_Abs_Ech19	Yes		5,0260	5,0294	-0,0034		4,8800	4,8763	0,0037	
Y*Spec_Abs_Ech20	Yes		3,3360	3,3354	-0,0006		4,8800	4,8806	-0,0006	
Y*Spec_Abs_Ech21	Yes		2,2240	2,2209	-0,0029		4,9640	4,9645	-0,0005	
Y*Spec_Abs_Ech22	Yes		2,2310	2,2289	-0,0041		5,0000	5,0000	0,0000	
Y*Spec_Abs_Ech23	Yes		3,4280	3,4242	0,0038		5,0000	5,0074	-0,0074	

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