NIRS Vision 4.1

Vision

Tutorial – Instrument Operation

8.105.8052EN / 2015-12-04

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Although all the information given in this documentation has been checked with great care, errors cannot be entirely excluded. Should you notice any mistakes please send us your comments using the address given above.

Table of Contents

1 Introduction

NOTE

This tutorial describes the use of the Vision Software, it does not include specialties of the Vison Pharma version.

1.1 Prerequisites for this Tutorial

In order to be able to follow this tutorial, the following prerequisites must be met:

- The Vision Software must be installed on your computer.
  Information on how to install Vision can be found in the document "*Vision installation" (8.108.8006EN)* delivered with the software. This document can also be downloaded from the internet (*http://documents.metrohm.com*), search for *"8.108.8006EN"*.
- Your computer must be connected to a NIRS XDS instrument via direct cable connection, or via network connection *(see instrument manual)*.
- The instrument must be:
  - connected to the computer,
  - switched on,
  - running for at least 30 minutes.

1.2 Symbols and conventions

The following symbols and formatting may appear in this documentation:

Symbol or Example	Conventions
Login	Dialog texts and parameters are printed in semi bold type
File ▶ Project	Menu and menu items are printed in semi bold type and separated by ▶
[Next]	Buttons or keys are printed in semi bold type and surrounded by square brackets.

1.3 Field and parameters

In this tutorial all fields and other screen elements (parameters) are not explained. Only parameters which need to be filled or changed are listed.

Do not fill in fields or change parameters not mentioned in the following instructions. Explanations for all parameters can be found in the *Vision Reference manual*.

2 Getting Started

2.1 Starting Vision

Once the software is installed, a Vision Icon can be found on the desktop of your computer.

1. Double click on  .
2. In the **Login** dialog box, enter the following default login information:
   - **User ID** NIRS
   - **Password** NIRS
3. Press **[Enter]** or click on **[OK]**.

2.2 Setting-up a Project

In Vision, a Project must be set up, before any data (instrument diagnostics or spectral data) can be collected.

If this is your initial entry to Vision the **Create New Project** dialog box is automatically opened. If the **Create New Project** dialog box is not open:

Open the Create New Project dialog box

1. **Click on File ▶ Project ▶ New**.
2. When asked to close the open project, click on **[OK]**.

Define Project information

1. In the **Create New Project** dialog box enter the following information:
   - **Project ID** "tutorial"
   - Click on **[OK]**
   - When asked **"Create database C:\Vision\tutorial?"** Click on **[Yes]**.

Configure Data Sources

1. In the **Configure Data Sources** dialog box
   - In the **Instrument** section, select **Metrohm NIRS XDS-series Instrument Driver**
   - Click on **[Configure]**
2. In the **NIRSystem XDS-series Instrument Configuration** window
   - select the **IP Address** of your instrument
   - Click on **[OK]**

3. Back in the **Configure Data Sources** window, click on **[OK]**

Connect to the instrument

1. In the **Connect to Instrument** dialog box

   - Select **Acquire New Data**

The **Select Data Collection Method** dialog box appears.

Before the Data Collection Method can be defined, project options and system options must be established.

2. In the **Select Data Collection Method** dialog box

   - Click on **[Cancel]**.

3. If asked "**Turn Lamp Off?",** click on **[No]**.

2.3 Define Project Options and System Options

Project Options apply only to the current (open) Project. System Options apply to all future Projects.

Open the Edit Options dialog box

1. Click on **Configure ▶ Options**

2. In the **Project Options** tab enable the following options:

   - **Instrument must stabilize before data acquisition**.
   - **Run performance test after wavelength linearization**
     For DS instruments only.
   - **Use Auto-Linearization**
     for Process instruments only.
   - **Reference Standardization** (for reflection measurements only)
   - **Blank Correction** (for transmission measurements only)
   - **Use Instrument Calibration**
     for XDS instruments only.

3. Click on the **System Project** tab

4. In the **System Options** tab

   - enable the same options as in the **Project Options** tab
   - In the **System Type** section enable **Use XDS System**.
   - Click on **[OK]**

2.4 Setting up the Data Collection Method – DCM

A Project must have at least one Data Collection Method (DCM). Data Collection Method (DCM) defines all instrument specifications (instrument

model, module and amplifier type, number of scans) and selected performance test parameters (like pass/fail limits).

Connect the instrument

1. Click on **Acquire ▶ Connect**

Create Data Collection Method

1. In the **Select Data Collection Method** dialog box click on **[New]**
2. In the **Edit Data Collection Method** dialog box enter a name for the DCM.
   We recommend the following structure for DCM naming: "name of instrument" – "detector type". For example:
   - **Method** e.g. "RCA_solids_reflectance"
3. Apply module specific parameters, e. g. spot size.
4. Click on **[OK]**

From this point, creating a DCM depends on the type of instrument connected. Detailed descriptions of the procedures can be found in the *NIRS Vision Tutorial Instrument Calibration (8.105.8054EN)*.

Wait until instrument stabilization has finished.

2.5 Instrument Setup Diagnostics

For each instrument the following diagnostic tasks must be performed:

1. Wavelength Linearization
2. Reference Standardization
3. Instrument Calibration
4. Performance Test

The procedure of the instrument setup diagnostics depends on the type of instrument connected.

Please follow the detailed descriptions for each instrument type in *the NIRS Vision Tutorial Instrument Calibration (8.105.8054EN)*.

3 Acquiring Spectral Data

At this point you should have performed the necessary instrument diagnostic tests and be in your Project "tutorial".

If you are not:

Open Project „tutorial"

1. Click on **File** ▶ **Project** ▶ **Open**
2. In the **Open Project** dialog box,
   - select "tutorial"
   - click on **[Open]**
3. Click on **Acquire** ▶ **Connect**
4. In the **Select Data Collection Method** dialog box,
   - Select your method e.g. "RCA_solids_reflectance"
   - Wait until the message "**Waiting for the Instrument to become Stable**" disappears
   - Click on **[OK]**

Scan a reference

1. Click on **Acquire** ▶ **Reference** or click on 

Scan and save samples

When the reference scan is completed, place the first sample in the instrument sample holder (*see instrument manual*).

1. Click on **Acquire** ▶**Sample** or on 
2. When the scan is completed, click on **Acquire** ▶ **Save** or on  .
3. In the **Enter Sample Information** dialog box enter the following information. Give proper description of the sample series or the single measurement like vial type, module etc.
   - **Product ID** e.g. "Test Samples"
   - **Sample ID** e.g. "C-05"
   - **Comment** optional
   - Click on **[OK]**
4. If asked "**The product ID Test Samples does not exist. Do you wish to create a new product?**" click on **[YES]**.
5. In the **Add New Product** dialog box enter the following information:
   - **Product Description** e.g. Enter today's date.
   - Click on **[OK]**.

Scan other samples

1. Repeat the steps in the sections "Scan and save samples" and "Entering Constituent Values" until your entire test samples have been scanned and saved.
2. Scan a reference about every 10 samples (*see section "Scan a reference", page 6*)

Constituent Values are laboratory reference data which are only required for quantitative models. For each product, you can optionally add constituent values, while saving the first sample.

Entering Constituent Values

1. In the main window, right-click on the product. Select **Products…** from the context menu.
2. In the **Select** dialog box, under **Mode** select **Constituents**.
3. Click on **[Edit]**.
4. In the **Edit Constituents** dialog box click on [**Add Constituents**].
5. In the **New Constituent** dialog box enter the name of your constituent.
6. Click on **[OK]**

   You will now see the constituent name listed.
7. Back in the **Edit Constituents** dialog box enter
   - **Units** e.g. %.
   - **Decimal Pts** e.g. 2
   - Click on **[OK]**.

3.1 Accessing Tutorial Data

For the continuation of this tutorial we will use a project prepared especially for this purpose. It contains data collected and prepared to demonstrate the tasks that follow.

This project is stored in the file folder "Tutorials Data" which is copied automatically as a separate folder when you install Vision software.

Restore Tutorial Data project

1. Click on **Mode ▶ Data Acquisition**
2. Click on **File ▶ Restore ▶ Restore Project**
3. In the **Select project zip file to restore** dialog box,
   - go to the folder C:\vision\Tutorial Data\TutProj
   - Double click on open the file **xdsstart.zip**

- In the **Preview project to be restored** dialog box click on **[OK]**
  The Project **xdsstart** will be unzipped and restored automatically to your Vision software.

4. In the dialog box stating that the file has been restored successfully, click on **[OK]**.

Open Tutorial Data project

1. Click on **File** ▶ **Project** ▶ **Open**
2. When asked to close the open project, click on **[OK]**.
3. When asked to switch of the lamp, click on **[no]**.
4. In the **Open Project** dialog box select **xdsstart** and click on **[Open]**

You can now open any of the products in the project and display the individual spectra. Constituent data has been entered for later exercises.

4 Qualitative Model Development

A qualitative model consists of a library of spectra of known products. A spectrum of an unknown product can then be compared with the spectra in this library. If a matching spectrum is found in the library, the unknown product can be identified. The following steps are required for developing a qualitative model:

1. Creating a library
2. Sample Selection
3. Developing an Identification Method
4. Developing a Qualification Method
5. Library Validation

1.1 Creating a Library

In Vision, a library is required for qualitative model development. For more information on vision libraries, please read the *Vision Reference manual (8.102.8010EN).*

Create a library

1. In the Data Acquisition Mode, click on **Mode** ▶ **Qualitative Analysis** ▶ **Sample Selection**
2. Click on **File** ▶ **Library** ▶ **New**
3. In the **Create New Library** dialog box, enter the following information:
   - **Library ID** "xdslib"
   - **Reference Standardized** enabled
   - Click [**OK**]
4. If asked "**Create database C:\vision\xdslib**", click on **[YES]**.
   The name of the library will appear next to a grey cuvette in the Samples View.

1.2 Sample Selection

The purpose of sample selection is to identify outliers as well as redundant samples.

Open Project "xdsstart"

1. Click on the + sign next to the green cuvette icon.

Add Product to the Library

1. Drag the product "AcDiSol" from the products folder into the library folder.
2. In the **Library Product** dialog box confirm the product ID and click on **[OK]**.

The product "AcDiSol" now appears under the library name next to a gray (empty) cuvette. This indicates that "AcDiSol" has been chosen for sample selection but has not yet completed the selection process.

Start Sample Selection

1. Click on "AcDiSol"
2. Click on **Select ▶ Apply Math**
3. In the **Edit Sample Selection Parameters** dialog box, enter the following information:
   - **Selected Method** "Mahalanobis Distance in Principal Component Space"
   - **Math Treatment** "2nd derivative"
   - Click on **[OK]**
4. In the **Edit Math Properties** dialog box in the **Principal Components ID Parameters** tab set the following wavelength regions:
   - **Region 1**
     Minimum: 416
     Maximum: 1080
   - **Region 2**
     Minimum: 1120
     Maximum: 2484
   - **Cum Var.** 95 %
   - Click on **[OK]**
5. In the **Sample Selection Options** dialog box, set the following:
   - **Find Outliers** enabled
   - **Outlier Threshold** 0.95
   - **Threshold Type** Probability Level
   - **Find Redundant** enabled
   - **By number of samples** enabled
   - **Training Set** 75 %
   - **Acceptance Set** 25 %
   - Click on **[OK]**

Vision now performs calculations according to the parameters defined in the previous steps.

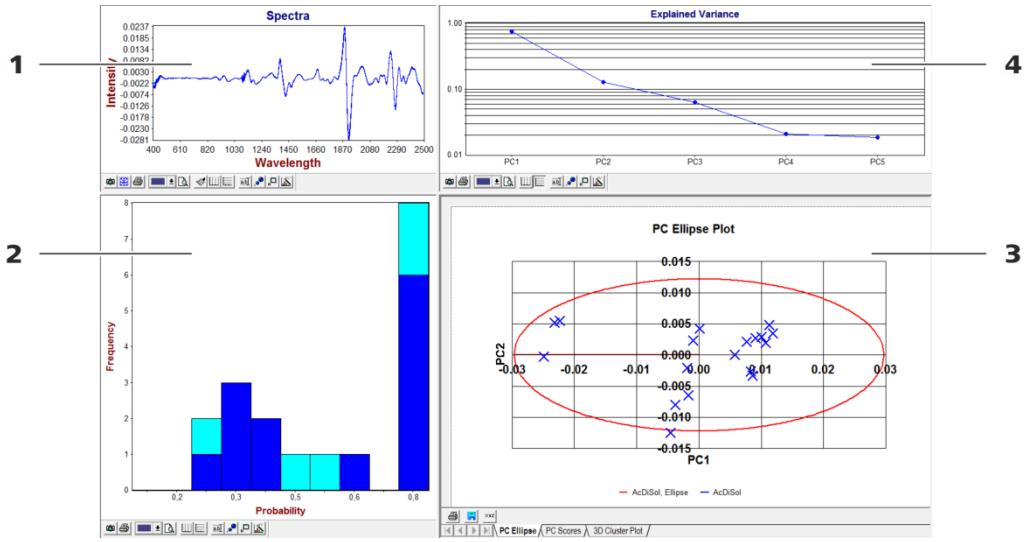

Figure 1 Result Sample Selection for qualitative Analysis

1	**Math treated spectra**	2	**Frequency versus Probability**
3	**Sample location in 2D Principal Component Space**	4	**Explained Variance**

Color Code:

- *Dark blue = Training Set*;
  samples determined to be suitable for library development.
- *Light blue = Acceptance Set*;
  samples suitable for library development but which are redundant compared with those in the Training set. The Acceptance set is used for Validation of the Library.
- *Red = Rejected*;
  samples determined to be unsuitable for library development because they differ significantly from those in the Training and Acceptance sets.

Since we know, that the samples contain only the desired variance, the Frequency versus Probability box should not show rejected samples (red boxes).

Save Results

1. Click on **Select ▶ Save Results**
2. If asked "**Do you need to Save previously selected samples?"** click on **[YES]**
3. If asked "**Do you want to save redundant samples as Acceptance Set?**" click on **[YES]**

The color of the cuvette next to the product name turns red.

Include other Products

1. Repeat the Sample Selection Process for all the products in the xdsstart project.

1.3 Developing an Identification method

An Identification Method is required to differentiate products which differ in chemical and/or physical properties.

Develop an Identification Method

1. Click on **Mode** ▶ **Qualitative Analysis** ▶ **Identify Method Development**
2. **File** ▶ **Library** ▶ **Open**, select the library **xdslib**
3. Click on **Identify** ▶ **Apply Math.**
4. In the **Edit Identify Method** dialog box, set the following:
   - **Method** Correlation in Wavelength Space
   - **Pre Treatment Method** 2nd derivative
   - **Region 1**
     Minimum: 416
     Maximum: 1080
   - **Region 2**
     Minimum: 1120
     Maximum: 2484
   - **Threshold** **type** = Match value; **value** = 0.95
   - **Identify Acceptance Set** enabled
   - **Identify Rejection Set** disabled
   - Click on **[OK]**
5. In the **Edit Math Properties** dialog box in the **Wavelength Regions** tab set the following wavelength regions:
   - **Region 1**
     Minimum: 416
     Maximum: 1080
   - **Region 2**
     Minimum: 1100
     Maximum: 2484
   - Click on **[OK]**
6. In the **Edit Math Properties** dialog box in the **2nd Derivative** tab set the following parameters:
   - **Segment**: 10
   - **Gap**: 0

7. In the **Library Method** dialog box optionally enter the following information:

   - **Comments** "Training and today's date".
   - Click **[OK]**.

Vision now computes the required information on all spectra for all products in the library. It automatically analyzes each spectrum in the training sets as if it were an unknown to determine if the method is capable of identifying it.

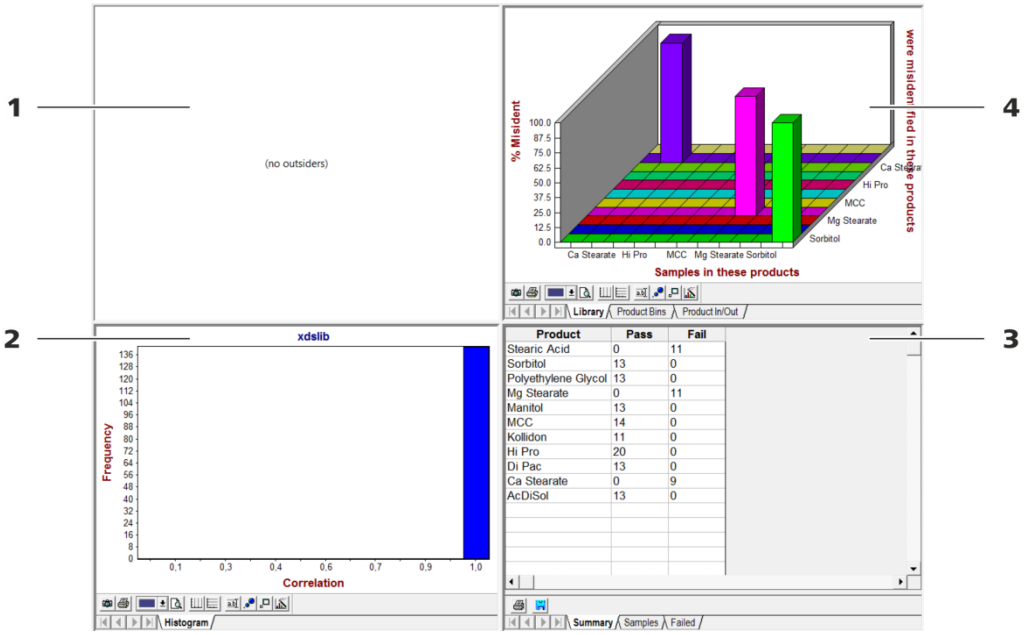

Figure 2: Results Identification Method

1	**Special Outliers**	2	**Histogram** „Correlation versus frequency"
3	**Identification results**	4	**Percent misidentified versus product**

Review of the results in box 2 (Histogram) shows that all products were identified with very high correlations. However, in box 3 (samples tab), the results for stearic acid, calcium stearate and magnesium stearate are listed as failed.

They are actually ambiguous. Although the samples were identified, one product had an acceptable match value with another product. This ambiguity can be handled by using a qualification method to further differentiate samples. This approach is covered in the next section.

Save Results

1. Click on **Identify** ▶ **Save Results**
2. If asked "**Save identify method #1 as library global identify method?"** click on **[YES]**

1.4 Qualifying a Product

The 3 stearate products are ambiguous.

A Qualification Method is used to determine how similar a sample is it to what has been accepted in the past. It can also be used to distinguish very similar products, e. g. Stearates.

Create a Qualification Method

1. Click on **Mode** ▶ **Qualitative Analysis** ▶ **Qualify Method Development**.
   The currently selected library appears next to a red cuvette in the Samples View.
2. Double click on the **xdslib** library
   all products are listed under the selected library.
3. Double-click on the "Calcium Stearate"
   The cuvette turns gray, indicating that the product has been selected.
   NOTE: the cuvette must turn gray before the math can be applied.
4. Click on **Select** ▶ **Apply Math**.
5. In the **Edit Qualify Method** dialog box apply or confirm the following settings:
   - **Method** Maximum Distance in Wavelength Space
   - **Pre Treatment Method** 2nd derivative
   - **Threshold** **type** = Match value **value** = 3
   - **Library Stabilization** 1
   - Click on **[OK]**
6. In the **Edit Math Properties** dialog box in the **wavelength distance** tab set the following wavelength regions:
   - **Region 1**
     Minimum: 1870
     Maximum: 2000
   - Click on **[OK]**

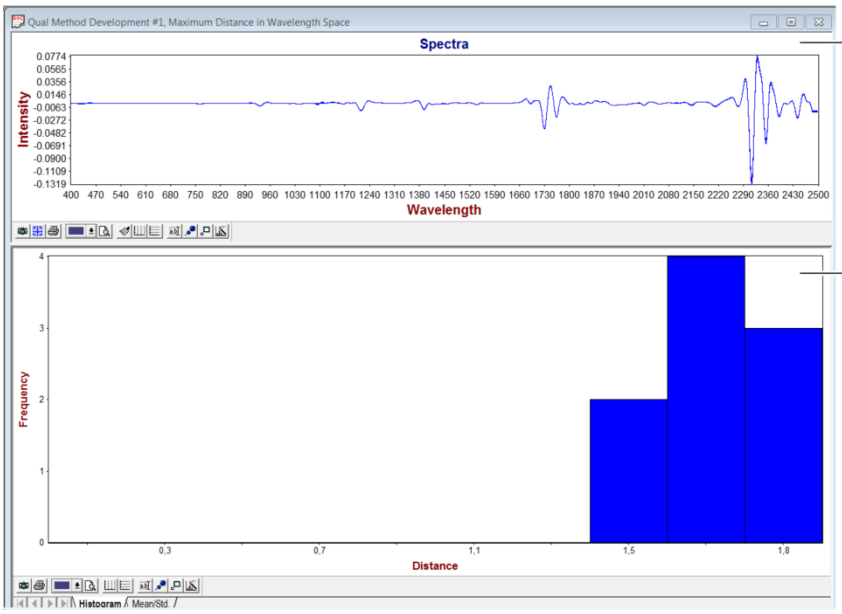

Figure 3: Results Qualification Method

1 **Sample Spectra**

2 **Histogram**
Distance versus Frequency

Color Code:

- *Dark blue = Training Set*;
  samples determined to be suitable for library development.
- *Light blue = Acceptance Set*;
  samples suitable for library development but which are redundant compared with those in the Training set. The Acceptance set is used for Validation of the Library.
- *Red = Rejected*;
  samples determined to be unsuitable for library development because they differ significantly from those in the Training and Acceptance sets.

Save Results

1. Click on **Select ▶ Save Results**
2. If asked "**Do you need to save Developed Qualify Method?"** click on **[YES]**
3. In the **Library Method** dialog box, click on **[OK]**

Repeat Qualification

1. Repeat the Qualification steps for "Magnesium Stearate" and "Stearic Acid".
2. Save the Results.

1.5 Library Validation – Testing the Library Model

Library validation is used to test the library model.

Open the Library

1. Click on **Mode ▶ Qualitative Analysis ▶Library Validation**
2. In the **Open Library** dialog box,
   - click on **xdslib**
   - click on **[Open]**

Vision opens the library and enters the Library Validation program.

Validate the Library

1. Click **Library ▶Run Validation**.
2. In the **Library Validation** dialog box, click on **[Start]**.

Vision will now start the Library Validation routine. In it, each sample in the library is tested as an unknown for internal validation of the library. Additionally, if samples have been saved in the Acceptance and/or Rejection Set, they are also tested to verify that the library analyzes them correctly.

NOTE: If an Acceptance Set sample "Fails", this is an indication that more samples of that Product are needed. If a sample Passes ID and Fails qualitative analysis, this suggests that the Product is indeed the correct Product but is not of the same "Quality" as accepted in the past. This sample could be from a new source or supplier.

Save Results

1. In the **Library Validation** dialog box, click on [**Save**].
2. The software will tell you "Validation results saved in library". Click on **[OK]** then click on **[Close]**.

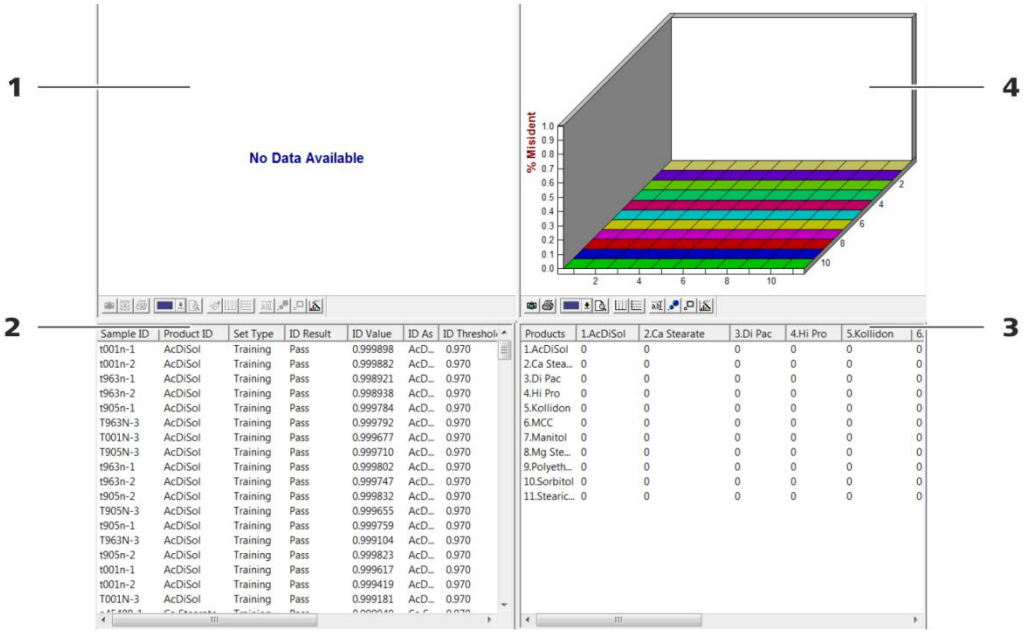

Figure 4: Results Library Validation

1	**Individual Sample Spectra**	2	**Validation Results for each sample**
3	**Summary of failures**	4	**Percent misidentified**

Examining summary data shows how the Qualify Method eliminated the ambiguity that occurred in the Identify Method among stearic acid, calcium stearate, and magnesium stearate.

Print the Report

1. Click on **Library ▶ View Report**
2. Click on the **printer icon** on the bottom of the report.
3. To close the report click on **Library ▶ Close Report.**

5 Quantitative Model Development

Quantitative Analysis mode is the part of Vision where calibration equations are developed and tested. The created calibration equations can be used in Routine Analysis to predict unknown sample composition in real time.

Vision offers two types of calibration development: Multilinear Regression (MLR) and Partial Least Squares (PLS).

1.6 Multilinear Regression

Multilinear Regression (MLR) is a least squares method that uses spectral information at one or several wavelengths. In the simplest case (one wavelength), the method reduces to simple Linear Regression.

1.6.1 Sample Selection

When developing a calibration equation to predict a constituent's concentration in a sample, Sample Selection must be performed on the spectral dataset for each equation to be developed. Equations/models must be developed for 1 constituent at a time and for each constituent you must go through Sample Selection

Select a Product

1. Click on **Mode** ▶ **Quantitative Analysis** ▶ **Sample Selection**.
2. Double click on the "xdsstart" project
3. Click and drag the product "MCC" onto the red EQ and release the mouse button.
4. In the **Select Appropriate Constituent** dialog constituent *Caffeine* is listed. Enter:
   - **Unit** %
5. Click on **[OK]**
6. In the **Calibration Equation** dialog box, add a proper equation name (e. g. caffeine_mlr), click on **[OK]**

Notice Caffeine now appears under the red **Eq** and the cuvette is gray.

Perform Sample Selection

1. Click on **Select ▶ Apply Math**.

2. In the **Edit Sample Selection Parameters** dialog box enter the following settings:

   - **Selection Method** Mahalanobis Distance in Principal Component Space
   - **Math Treatment** 2nd derivative
   - Leave all other settings unchanged

3. Click on **[OK]**.

4. In the **Edit Math Properties** dialog box in the **Principal Components ID Parameters** tab set the following wavelength regions:

   - **Region 1**
     Minimum: 416
     Maximum: 1080
   - **Region 2**
     Minimum: 1120
     Maximum: 2484
   - **Cum Var.** 95 %
   - Click on **[OK]**

5. In the **Edit Math Properties** dialog box in the **2nd derivative** tab set the following values:

   - **Segment**: 10
   - **Gap**: 0

6. In the **Sample Selection Options** dialog box apply or confirm the following settings:

   - **Find Outliers** enabled
   - **Outlier Threshold** 0.95
   - **Threshold Type** Probability Level
   - **Find Redundant** enabled
   - **By number of samples** enabled
   - **Training Set** 75 %
   - **Acceptance Set** 25 %
   - Click on **[OK]**

Vision now performs calculations according to the parameters defined in the previous steps.

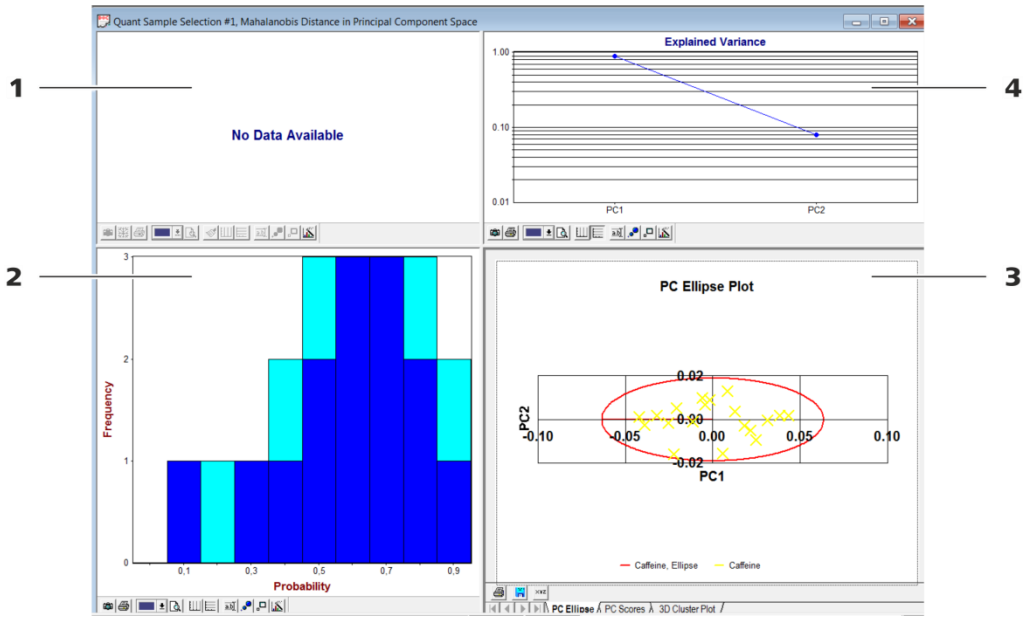

Figure 5 Result Sample Selection for quantitative Analysis

1	**Math pre-treated spectra**	2	**Explained Variance**
3	**Sample location in 3-D Principal Component Space**	4	**Frequency versus Probability**

Color Code:

- *Dark blue = Training Set*;
  Samples suitable for calibration development.
- *Light blue = Acceptance Set*;
  Samples suitable for calibration but redundant.
- *Red = Rejected*;
  Samples unsuitable for calibration development.

Save Results

1. Click on **Select** ▶ **Save Results**.
2. If you are asked "**Warning – By saving this calibration equation you will invalidate the regression method which you previously developed for this equation. You must develop the regression again to use this equation. Do you want to continue?**", click on **[YES]**.
3. If you are asked "**Do you want to save redundant samples as Validation Set?**" Click on **[YES]**.

1.6.2 Regression

Configure Regression Method

1. Click on **Mode** ▶ **Quantitative Analysis** ▶ **Regression**.
2. Double-click on the **xdsstart** project name.
3. Double-click on the **MCC** product
   the tree expands to show all the products and their associated samples, constituents, and equations when available.
4. Double-click on **(f(x)) Equations** under MCC.
5. Double-click on **f(x) Caffeine**
   the name given for the equation turns light blue indicating that it has been selected for Calibration Development.
6. Click on **Regression** ▶ **Edit Regression Method**.
7. In the **Edit Regression Method** dialog box, enter the following:
   - **Regression** Multilinear Regression
   - **Pre-Treatment Method** 2nd derivative
   - Leave all other settings unchanged
   - Click on **[OK]**.
8. In the **Regression Treatment Properties** dialog box, confirm the following settings:
   - **Segment Size** 10
   - **Gap** 0
   - Click on **[OK]**.

Vision performs calculations according to parameters defined in preceding steps.

Save Equation

1. Click on Regression ▶ Save Equation
2. Click on **[OK]**

Results

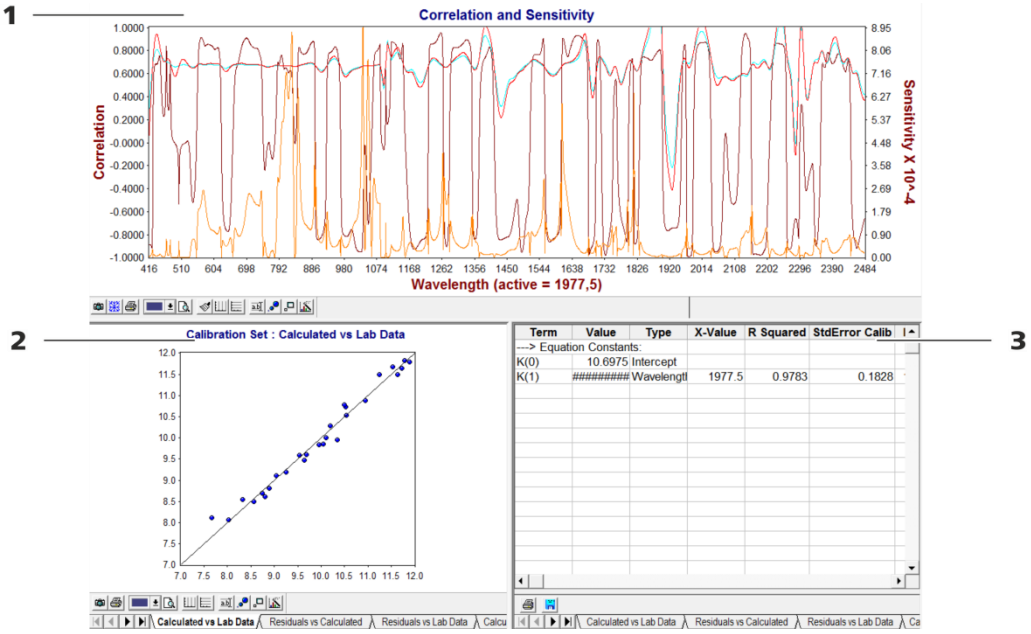

Figure 6 Result Multi Linear Regression

1	**Correlation and Sensitivity**	2	**Statistical results**
3	**Statistical results** with statistical summary		

Color code In the Correlation and Sensitivity view

- *Black or brown:*
  Correlation: The correlation coefficient for spectral value versus constituent value
- *Orange*
  Sensitivity: The slope for constituent value versus spectral value
- *Red*
  Spectra of the sample with the highest entered constituent value
- *Light blue*
  Spectra of the sample with the lowest entered constituent value.

Wavelength Selection Criteria

To select the optimum wavelength, select a wavelength that is high in correlation and low in sensitivity, and where the spectra of high and low constituent values are far apart.

- Correlation:

  Generally, the selected wavelength should be where the absolute value of correlation is high.
  When using 2nd derivative spectra, locate an area of *high negative correlation*. (When using raw spectra, use a wavelength with high positive correlation.) The wavelength should be selected in a region where correlation is relatively broad and flat, not narrow and spike-like.

- Sensitivity:

  Selecting a wavelength with *low sensitivity* makes the calibration more robust, because small changes in absorbance will not result in large relative changes in the reported constituent value.

- Statistical values:
  High F-value, low K(1), R^2 close to 1, small SEC around 1-1.5 times Laberror of reference method.

Edit Equation

1. Click on **Regression ▶ Edit Wavelength**
2. In the **Edit Wavelength** dialog box enter:
   - Wavelength 1675
   - Click on **[OK]**

1.7 Partial Least Squares Regression

Partial Least Squares (PLS) regression allows a user to use the spectral information of a whole range of wavelengths, even the full spectrum. This method eliminates colinearity (high correlation between wavelength terms) that is difficult to overcome with classical methods.

1.7.1 Sample Selection

A second Product, HiPro, has been chosen for developing a Partial Least Squares Regression. Sample Selection must be performed on this dataset prior to developing the equation.

Select a Product

1. Click on **Mode** ▶ **Quantitative Analysis** ▶ **Sample Selection**.
2. Double click on the "xdsstart" project
3. Click and drag the product "HiPro" onto the red EQ and release the mouse button.
4. In the **Select Appropriate Constituent** dialog constituent *Protein* is listed. Enter:
   - **Unit** %
5. Click on **[OK]**
6. In the **Calibration Equation** dialog box, click on **[OK]**

Notice Protein now appears under the red **Eq** and the cuvette is gray.

Perform Sample Selection

1. Click on **Select** ▶ **Apply Math**.
2. In the **Edit Sample Selection Parameters** dialog box enter the following settings:
   - **Selection Method** Mahalanobis Distance in Principal Component Space
   - **Math Treatment** 2nd derivative
   - Leave all other settings unchanged
3. Click on **[OK]**.
4. In the **Edit Math Properties** dialog box in the **Principal Components ID Parameters** tab set the following wavelength regions:
   - **Region 1**
     Minimum: 416
     Maximum: 1080
   - **Region 2**
     Minimum: 1120
     Maximum: 2484
   - **Cum Var.** 95 %
   - Click on **[OK]**
5. In the **Sample Selection Options** dialog box apply or confirm the following settings:
   - **Find Outliers** enabled
   - **Outlier Threshold** 0.95
   - **Threshold Type** Probability Level
   - **Find Redundant** enabled
   - **By number of samples** enabled

- **Training Set** 75 %
- **Acceptance Set** 25 %
- Click on **[OK]**

Vision now performs calculations according to the parameters defined in the previous steps.

The Results of the Sample Selection are displayed *(cf. Figure 5, page 20).*Figure 5 Result Sample Selection for quantitative Analysis

Save Results

1. Click on **Select ▶ Save Results**.
2. If you are asked "**Warning – By saving this calibration equation you will invalidate the regression method which you previously developed for this equation. You must develop the regression again to use this equation. Do you want to continue?**", click on **[YES]**.
3. If you are asked "**Do you want to save redundant samples as Validation Set?**" Click on **[YES]**.

1.7.2 Regression

Configure Regression Method

1. Click on **Mode ▶ Quantitative Analysis ▶ Regression**.
2. Double-click on the **xdsstart** project name.
3. Double-click on the product "**HiPro"**
   the tree expands to show all the products and their associated samples, constituents, and equations when available.
4. Double-click on **(f(x)) Equations** under "HiPro".
5. Double-click on **f(x) Protein**
   the name given for the equation turns light blue indicating that it has been selected for Calibration Development.
6. Click on **Regression ▶ Edit Regression Method**.
7. In the **Edit Regression Method** dialog box, enter the following:
   - **Regression** Partial Least Square
   - **Pre-Treatment Method** 2nd derivative
   - Leave all other settings unchanged

8. Click on **[OK]**.

9. In the **Regression Treatment Properties** dialog box, in the **Wavelength Regions** tab, enter the following settings:

   - **Region 1**
     Minimum: 750
     Maximum: 1050
   - **Region 2**
     Minimum:1150
     Maximum: 1350
   - **Region 3**
     Minimum: 1500
     Maximum: 1830
   - **Region 4**
     Minimum: 1990
     Maximum: 2450

10. In the **Edit Validation** tab

    - **Method** Cross Validation
    - **Remove only one sample at a time** enabled
    - Click on **[OK]**.

Vision performs calculations according to parameters defined in preceding steps.

Evaluation of Method Performance

When the regression is complete, the screen displays 4 boxes in the Data View. With statistical information relating to the Cross-validation results:

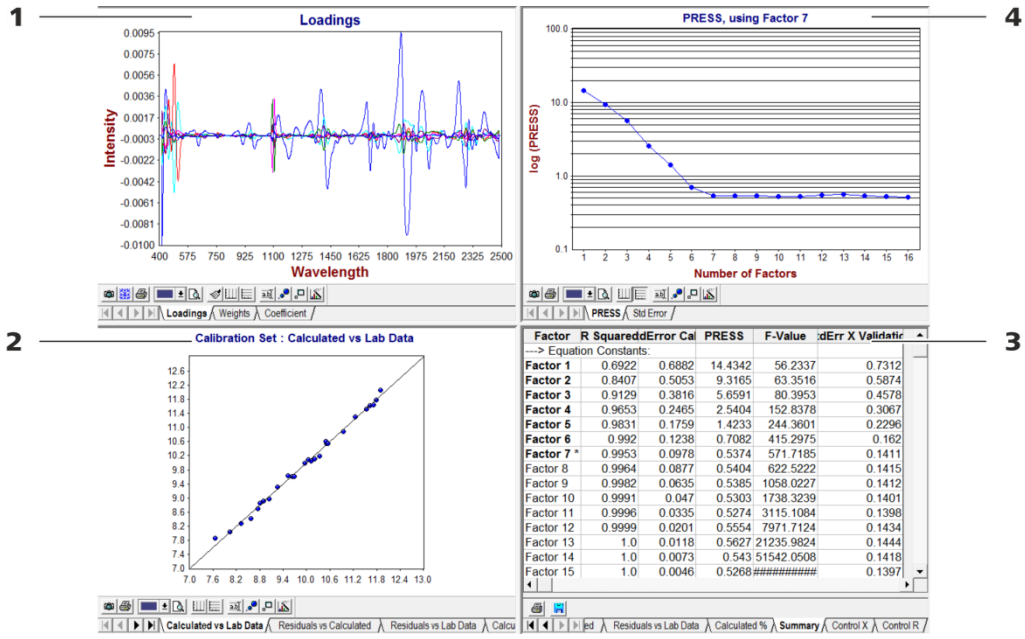

Figure 7 Result Partial Least Square Regression

1	**Loading** weights and coefficients	2	**Statistical results**
3	**Statistical results** with statistical summary	4	**PRESS and SEC**

The program automatically selects the number of factors for the method based on the approach of the PRESS (Predicted Residual Error Sum of Squares) value to a minimum. The number of factors actually appropriate for a method should be similar to the number of degrees of Freedom (chemical and physical variables) in the sample set. Thus, varying temperature and constituent concentrations represent variables that must be considered when selecting the number of factors.

Remember, robustness of a model is often reduced as more factors are added. The software selects the number of factors for the model to be the number of factors which the PRESS value is the lowest for the first time. At this point, the Standard Error of Cross Validation (SECV) should be visually compared with the laboratory error for the Reference data used in developing the model. The SECV should approach but not be lower than the lab error.

Save Equation

1. Click on Regression ▶ Save Equation
2. Click on **[OK]**

6 Routine Analysis

In Routine Analysis you use the models and equations you have built to analyze unknown samples. All Routine Analysis parameters are brought together in the Operations Method.

1.8 Setting up an Operations Method

Vision offers a wizard for setting up an operations Method.

Start Operations Method Wizard

1. Click on **Mode ▶ Data Acquisition**
2. Click on **Mode ▶ Routine Analysis ▶ OM Wizard**.
3. In the **Select Operations Method** dialog box, click on **[New]**
4. In the **Select Operation Method Standardization** dialog box, set the following:
   - **Reference Standardization** enabled
   - Click on **[OK]**
5. In the **OM Wizard – OM Name** dialog box, enter
   - OM Name "Tutorial"
   - Click on **[Next]**

Select a DCM

1. In the **OM Wizard – Select DCM** dialog box, select
   - **DCM** Diags
   - Click on [**Timer]**
2. In the **Configure Timer/Reference Frequency** dialog box, set the following:
   - In the **Sample Frequency** box:
     Sample Collection Interval (sec.) 0
     No delay between enabled
   - In the **Time Mode** box:
     Timed Acquisition disabled
   - In the **Reference Frequency** box:
     Every x Sample enabled
     Frequency x 10
   - Click on **[OK]**

3. In the **OM Wizard – Select DCM** dialog box, click on **[Next]**.

Define an Output Project

1. In the **OM Wizard – Output Project** dialog box, click on **[Output Project]**
2. In the **Choose Output Project** dialog box, click on **[New Output Project]**
3. In the **Create New Output Project** dialog box, enter
   - Project ID "Testout"
   - Click on **[OK]**
   - If asked „Create database C:\vision\testout?", click on **[Yes]**
4. In the **OM Wizard – Output Project** dialog box, click on **[Next]**.

Select Reference

1. In the **OM Wizard – Select Reference** dialog box, click on **[Next]**.

Set Sample Average Options

1. In the **OM Wizard – Spl Average Options** dialog box, set the following:
   - All samples in average enabled
   - Click on **[Next]**.

Set Diagnostic Timer

1. In the **OM Wizard – Diagnostic Timer** dialog box, click on **[Next]**.

Set Save and Print Options

1. In the **OM Wizard – Save & Print Options** dialog box, set the following:
   - In the **Save** box:
     Results enabled
     Spectra enabled
   - In the **Maximum Number of…** box:
     leave everything unchanged
   - Click on **[Next]**

Select the Library

1. In the **OM Wizard – Select Library** dialog box,
   - **Library** xdslib
   - **Identify** enabled
   - **Qualify** enabled
   - Click on **[Next]**

Quantification Setup

1. In the **OM Wizard – Quant Setup** dialog box, click on **[Quant Setup]**
2. In the **Operations Method Product Selection** dialog box, do the following:
   - Select the **Product** MCC
   - Double-click on *xdsstart*
   - Double-click on *MCC*
   - Double-click on *Caffeine*
   - Double-click on *f(x) Caffeine*
   - Click on **[Save]**
3. Repeat for Product HiPro:
   - Select the **Product** HiPro
   - Double-click on *xdsstart*
   - Double-click on *HiPro*
   - Double-click on *Protein*
   - Double-click on *f(x) Protein*
   - Click on **[Save]**
   - Click on **[OK]**
4. In the **OM Wizard – Quant Setup** dialog box, click on **[Next]**

Skip Aux I/O Settings

1. In the **OM Wizard – Aux I/O** dialog box, click on **[Next]**

Configure Output

1. In the **OM Wizard – Configure Output** dialog box, click on **[Configure Views]**
2. In the **Configure Output Views** dialog box, set the following:
   - In the **Available Views** box:
     Sample Spectra enabled
     Results Report enabled
   - Click on **[Next]**
   - Click on **[Finish]**
3. Back in the **OM Wizard – Configure Output** dialog box, select
   - **Reports** RESULTS.XLS
   - Click on **[Next]**

Complete Operations Method Definition

1. In the **OM Wizard** dialog box, click on **[Finish]**

1.9 Analyzing Stored Data

In the Analyze Stored Data mode, it is not necessary to have an instrument connected. Vision will apply your previously configured Operations Method to the samples selected and predict the constituents. The results are displayed in the lower window. Results for the library will report in the ID Result and Qual Result.

Routine Analysis can be used to analyze samples in real time or to analyze sample spectra that were previously acquired and stored. This exercise will direct you in analyzing previously collected spectra.

1. Click on **Mode ▶ Routine Analysis ▶ Analyze Stored Data**

2. In the **Select Samples to Analyze** dialog box,

   - Click on the project "xdsstart"
   - Click on each product and select 3 samples from each of the product. Each selected sample will have a check mark to its left.
   - Click on **[OK]**

3. In the **Select Operations Method** dialog box,

   - Click on "tutorial"
   - Click on **[OK]**

4. In the **Enter Sample Information** dialog box, click on [OK]

5. In the Next Result dialog box, click on **[Next]** to analyze samples one-by-one or click on **[Non-stop]** to analyze all samples at once.

The results of the analyses are listed in the bottom part of the window in the **Results** tab.

View additional information

1. Click on the **Running Std** tab

   This tab displays the Running Standard Deviation and Running Average for the data. Because we entered 1 as Group Size, the Standard Deviation will be 0 and the average will be the same as the result.

2. Click on the **Details** tab

   This tab displays the Grand Average, the Grand Standard Dev. And the Grand Residual Standard Deviation

Exit Routine Analysis/Analyze Stored Data Mode

1. Click on **Mode ▶ Data Acquisition**

1.10 Recalling Stored Results

The Recall Results mode permits retrieval of Routine analysis results, provided the operations method specifies that results are to be saved. Samples stored in this fashion are identifiable by time and date.

1. Click on **Mode ▶ Routine Analysis ▶ Recall Results**.
2. In the **Select Results Type** dialog box,
   - select "Routine Analysis Results"
   - click on **[OK]**.
3. In the **Recall Stored Results** dialog box, set
   - From Date "current date" Time "00:00:00"
   - Until Date "current date" Time "23:59:00"
   - click on **[OK]**
4. In the Select Operations Method dialog box,
   - select "tutorial"
   - click on **[OK]**

Vision now searches the output project for results that meet the specified time and date limits.

5. In the Next Result dialog box, click on **[Next]** to analyze samples one-by-one or click on **[Non-stop]** to analyze all samples at once.

Exit Routine Analysis/Recall Results Mode

1. Click on **Mode ▶ Data Acquisition**

7 Appendix

7.1 21 CFR Compliant Mode

If you have chosen to operate in the 21CFR Compliant Mode, your Account Policy information should be entered at this time. Go to the Vision toolbar and select Configure followed by Account Policy and access the Account Policy setup screen. Your company establishes these policies. The Compliance feature of Vision can be activated or de-activated after installation by activating or de-activating the 21CFR Compliance box at the top of this screen. The exercises have been written without the use of this feature.