VISION*lite* Software
Operator Manual

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This application program serves for the purpose of processing, presenting and storing spectral data, which have been acquired by a spectrophotometer system. The program offers comprehensive functions for formatting and evaluating data. Resulting from the various options, it cannot be excluded that a report generated by a user presents the data in a form that does not agree with the intended purpose, so that a false interpretation is possible. It is the responsibility of the user to verify, that a report presents the measured data in the intended manner without falsification. If manipulation by third persons is to be excluded, the user should utilize the security functions of the Microsoft Windows operating system to check the right of access to the program, and in particular writing permission for the Method directory should be severely limited.

Contents

Introduction

VISION*lite*™ is a PC-software package to control UV-Vis- and Vis-spectrophotometers of the Thermo Scientific Evolution, GENESYS, Helios and UV- (all ™) series and compatible instruments. The package includes various data recording and data evaluation functions. **VISION*lite*** is a 32-bit PC-software running under current Microsoft Windows™ versions.

The software consists of 4 separate application modules:

Scan	Spectrum recording
Rate	Recording time dependent readings at a fixed wavelength and is dedicated for measuring rate curves.
Fixed	Recording spectrometer readings at different dedicated wavelengths and performing simple calculations
Quant	Quantifying sample concentrations via calibration, based on Lambert-Beers law

The application modules present themselves very similarly and they differ only in their specific functions. Each module also performs the measurement in a consistent manner. This allows defining measurement parameters within a re-usable method and allows simple data evaluations like peak picking or enzyme activity calculations as well as data storage and printout. Measurement parameters are stored as an easily addressable method.

Parameters and details of the measurement procedure vary according to the spectrophotometer type, e.g. wavelength range, and the attached accessory, e.g. sipper settings. Notes to the applicable instrumentation are given in the appendix.

An autosampler system can be setup with the extended software version **VISIONlite auto**, a spectrophotometer equipped with a sipper and a CETAC™ autosampler.

VISIONlite is also available as **VISIONlite SE**, a version that offers extended data documentation and data security following requirements of 21 CFR Part 11.

The additional software packages **VL ColorCalc** and **VL MaterialsCalc** support spectrum recording and dedicated spectrum evaluation for routine measurements. This includes a large number of predefined characteristic figures like Apha/Pt-Co color value, transmission coefficients, solar protection factors, CIE color values and so on. In addition, user-defined calculations with spectrum data are possible.

You will receive further information about these packages with **Thermo Scientific** or its local dealer.

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1 Installation and Start-up

1.1 Hardware- and Operating System Requirements

The software requires a PC with a 32-bit Microsoft Windows® operating system Windows 2000 or XP. Windows XP Professional is recommended. Screen graphics resolution must be at least 800 x 600 pixels.
The software requires the Microsoft Internet Explorer® 5.0 (or higher versions), delivered with newer Windows versions. An Internet connection is not required.

The PC must have an RS-232C interface (serial interface) for connecting the instrument. If not available, a USB to RS-232 adapter cable can be checked.

VISIONlite requires a CD drive for software installation and about 20 MB space on the PC´s hard disk. Additionally sufficient space should be available for storing data and methods.

1.1.1 Applicable Instruments and Accessories

The various instruments that can be used with **VISION*lite*** exhibit different technical functions. For example, these include the wavelength range, the scanning speed, or the accessory installations and options. Additionally the behavior of the instruments, e.g. the instrument live display under PC control, is different. The appendix describes instrument aspects that are relevant to the use of the software.

The spectrophotometer models can be divided in 3 groups:

- GENESYS 6, GENESYS 10 Vis/ 10 Bio/ 10 UV/ UV*scanning*, BioMate 3, Evolution 60
- GENESYS 20, Helios Epsilon
- Evolution 100/300/600, UV 1/3xx/5xx,, Helios Alpha/Beta/Gamma/Delta, BioMate 5, AquaMate

The following accessories can be used and are supported by the software:

- 6-position (group 1) or 7/8-position (group 3) cell changer
- remote control sipper: Smart sipper (Evolution 300/600), Mini-sipper or Super sipper (other instruments of group 3)

An autosampler system may be put together based on the **VISION*lite* auto** software, a sipper and a CETAC autosampler (see more information in the manual supplement, if applicable).

1.1.2 Connection PC – Spectrophotometer

As a default, the instruments that are connected via RS-232 use the COM interface selected during installation (COM1 as a default). A different COM port may be defined during installation or during software operation. There is no further configuration of the interface.

Please only use the interface cable as recommended by the spectrophotometer manufacturer (see *appendix*).

1.2 Installing the Software

In order to install the software you have to be registered in Windows NT/2000 and XP as the administrator. First close all other active applications. Normally the installation routine starts automatically when you insert the software CD into the PC. If it does not start, please start the *setup.exe* routine in the main CD directory. Thereafter the installation routine will guide you through the installation procedure.

The following steps are performed. Make your choice and accept the entries via the OK button:

- You are reminded to close all running applications. Exit the installation if this is not yet the case.
- According to Windows conventions, the software installs to the *c:\Program Files\VISIONlite* directory. Use the **Change directory** button to choose a different directory or define a new directory.
- In the next window, select **English** as the language for the user interface.
- Thereafter select your type of spectrophotometer from the given list.
- Then select the COM-port where the instrument is attached, typically **COM1**.
- The software usually is installed in its own program group. Optionally select a different program group.

After the queries, data transfer from CD to the hard disk is initiated.

If the software is updated or has already been installed before, the system will overwrite all files which are older than those of the new installation or which have the same date and time of generation. Files, which have been modified in previous usage, for example the initialization file or the default method files, might be newer than those on the installation disk. In this case, you are queried, if you want to keep these files.
Select **Yes**, if you want to keep the file, or **No**, if you want to have it updated. Select **No to all**, if you want to have a completely new installation.

At the end of the installation, you are asked if you want to see the *readme* file. This text file holds the latest information not yet included in this manual. After reading, close the editor window.

When the software is going to be used by a PC-user with limited access rights, make sure that the program directory and the application data directory with subdirectories are accessible with all user rights.

Before starting the first measurement, refer to the *appendix* of this manual and to the user's manual of your spectrophotometer, regarding special features of your instrument type. E.g. for some models it is required to switch the instrument to a REMOTE operation mode for PC operation.

After installing or dismantling accessories, it is necessary to close and re-start the software. Otherwise, the system can not detect the new configuration.

1.3 Installation in Demonstration Mode

For test purposes, reviewing or learning the software operation, the software can also be installed in the demo mode without entering a serial number. In this mode the instrument control functions are disabled. Instead of actual readings, the software uses simulated measurement values that correspond to typical instrument parameters.

1.4 Customising the Software Installation

It is possible to designate the program window with an instrument serial number, workbench designation or some other text. This text is attached to the program call in square brackets. To do so open the **Properties** window of the program link and extend the **Target** entry for example like this

C:\Program Files\ VISIONlite \ VISIONlite.exe [Instrument 20 A]

The attached text in square brackets will then be listed in the title bar of the software's application window.

Instructions for installing a symbol on your desktop and adding additional applications to the start routine are given in the section *Software Configuration*.

The program call can include the opening of a method within the respective application. Other parameters may be used as well. Example: the automatic printout of the results or an automatic termination of the software after the completion of the analyses; see section *Software Configuration*.

The user may define further settings by editing the software configuration file; see the sections *Software Configuration* and the *appendix*.

1.5 Update Information

This section provides information for users, who already have been working with former software versions.

With the updates of VISIONlite, the proven user interface and the data format are unchanged. For existing methods, new parameters are set to defaults, so that also existing methods can be used with the new software version.

During installation of a new version in the place of the old version, the user is queried to overwrite existing files, which have been modified, e. g. the configuration file or example data. Make sure to keep important data.

Details of the software modifications have been incorporated into this manual.

1.5.1 Version 2.2 (March 2007)

Version 2.2 additionally supports the spectrophotometer model Evolution 60.

The version features a number of improvement and extensions compared to VISIONlite 2.1:

- All applications: Sample list operation
- Scan/Rate/Fixed: Reflectance measurement modes %R, %Ra, f(R)
- Scan/Rate: File information window allows saving and transforming of all curves shown in the graph with a single function
- Scan/Rate: File information window allows copying to clipboard of all curves shown in the graph as a single table
- Quant: Modified reference wavelength entries with additional option wavelength with factor
- Advanced Parameters options:
  - Reflectance correction
  - Lamp change, if applicable
  - Slit, if applicable
- Evolution 300/600: Align command for alignment of accessories
- Load/Save spectrum: csv and dx format selectable
- External spectrum converter can be integrated.
- Further software configuration options
- The Sipper button/window renamed to Accessory
- Manual Control: Sipper volume calibration
- Operation toolbar shows lamp status and slit.
- Default Operator entry with Windows user name and PC name

The parallel software variant **VISIONlite auto** integrates the control of an autosampler for fully automated measurements.

Optional software **VL MaterialsCalc** and **VL ColorCalc** for specific spectrum evaluations available.

1.5.2 Version 2.0/2.1 (June/December 2004)

Version 2.1 additionally supports the spectrophotometer models Evolution 100/300/600.

The versions feature a number of improvement and extensions compared to VISIONlite 1.0:

- VISIONlite start module and „Change Application“ command in each application
- Graphics: „Read Cursor“ mode
- Operator entry field in the sample information window
- Advanced method options:
  - Start Delay (all applications)
  - Repetitive measurement (Scan and Fixed)
  - Result Limits (Rate, Fixed and Quant)
  - Reference Wavelength (Rate)
  - Output Options (Rate)
- Scan/Rate: 200 data sets can be evaluated together
- Rate: with cell changer autozero a each measurement cycle
- Rate: preliminary data storage each 10 min
- Rate: Graph/Table toggle button with graphics
- Fixed: Mode „wavelength(s) with factor“
- Fixed/Quant: Results are stored after each cycle
- Quant/Rate: 1000 results in a result file
- Quant: 1/2 reference wavelength(s)
- Quant: Curve type „Calibration parameter entry”
- Quant: Check box „Remeasure”
- Quant/Fixed/Rate: New results can be appended to existing result file

1.5.3 Version 1.0 (March 2002)

Initial version.

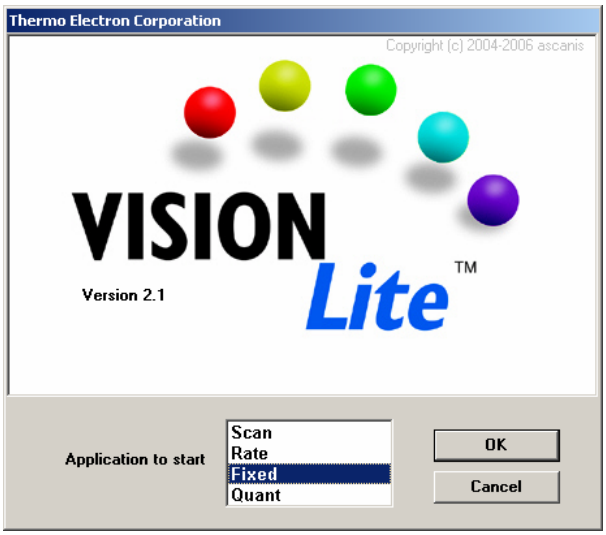
2 Basic Operation

This chapter describes general functions, which are necessary for a software overview and for the first simple measurements.

2.1 Starting VISIONlite

You start **VISIONlite** by a double click on the **VISIONlite** desktop icon or by clicking on the VISIONlite entry of the VISIONlite program group. This will open the VISIONlite Start Routine, which lists the available application modules. The recently used application is highlighted. This application is started after 10 seconds of inactivity. Select another application by clicking with the mouse or by moving the cursor to the desired application and pressing the Enter key. A new window is shown with the selected application.

When the connection has been established successfully, the software takes up the current instrument settings and activates the live display, showing the wavelength and ordinate readings. If unsuccessful, the software displays a respective error message and the live display remains inactive. After accepting this error message however, offline data and method handling are possible.



VISIONlite Start Window

When starting an application for the first time, the pre-installed method opens automatically. Every later application start will open the recently used method.

Only one application module can access the spectrophotometer at a time. Therefore, for changing to another measurement mode, you must close the currently used application at first. For that, use the **Change application** menu command in the **File** menu.

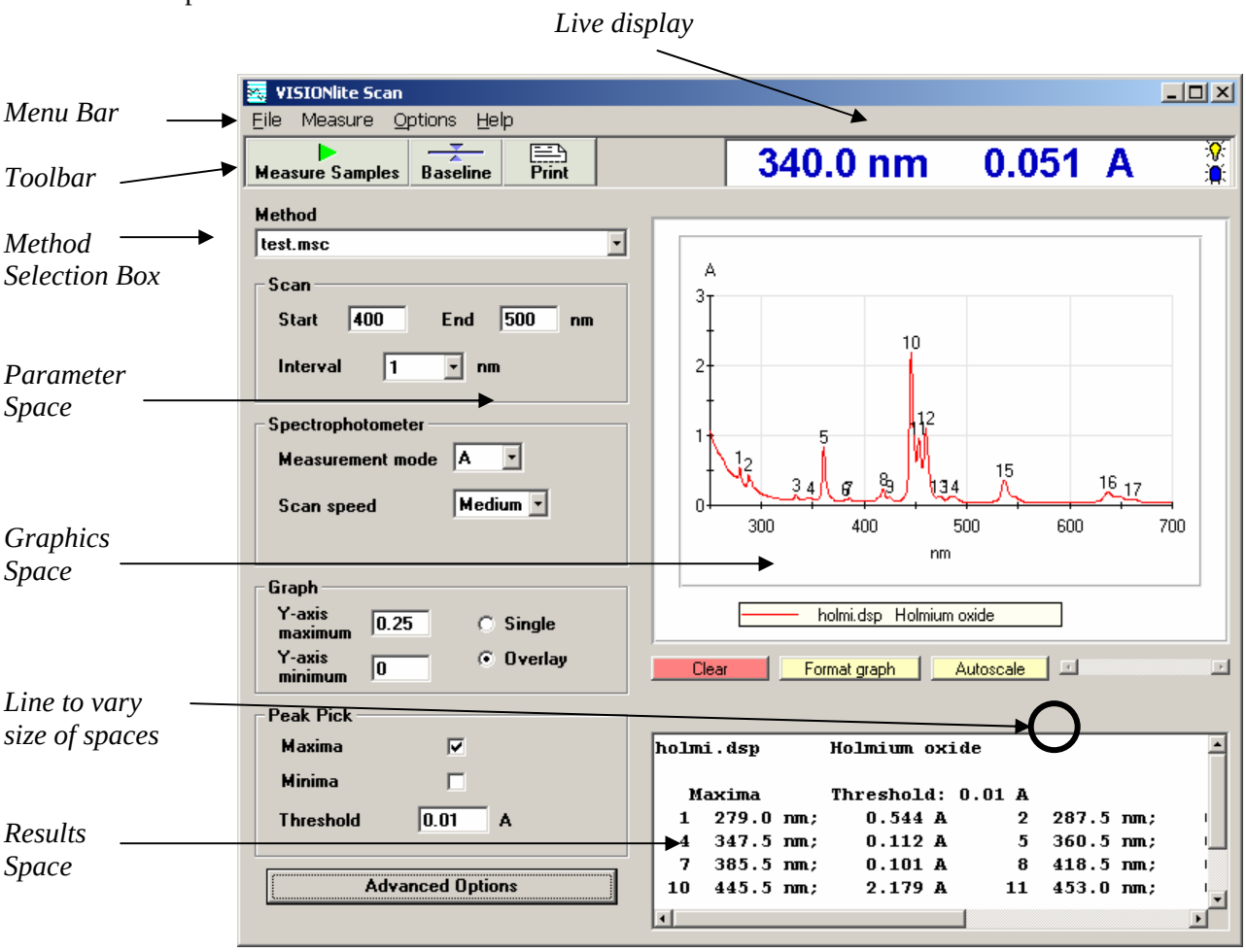
After installing or removing an accessory, it is necessary to restart the software to detect the new configuration.

See the appendix for the option to place links on the desktop for an immediate application start with a standard method. See also the appendix to add additional items to the list of applications, e.g. applications with a selected method.

2.2 Layout of the Program Window

As well as the standard Windows menu toolbar, the program window for each application has an operating toolbar with buttons. Below this toolbar the program window is divided vertically into two areas:

- Parameter area
- Data output area



VISIONlite Scan application window

The grey parameter area on the left hand side of the window has entry boxes and selections to define all settings and options for measurement and evaluation.

The right hand window section is reserved for the output of data. In the Scan and Rate applications and in the calibration section of the Quant application it is used for the graphical presentation of the data. Buttons are available to modify the graph. If optional numerical results are generated they are displayed in combination with sample information in the results space below the diagram.

Drag and drop the upper results space border with the left mouse button pressed to resize the spaces.

In the Fixed and Quant applications the full output area is used for the numerical report.

2.2.1 Parameter Entry

In the parameter area, you choose the method or the settings for the measurement and evaluation. The exact presentation of parameters depends on the respective application and spectrophotometer. Further information is provided in the chapter on the applications and spectrophotometer types.

Depending on the specific parameters, selection boxes or selection buttons are available. An error message is displayed if the allowed range of a parameter is exceeded.

You can also use the Tab keys to change from one entry field to another.

2.3 Operating Toolbar

The operating toolbar below the menu bar contains buttons to operate basic functions: measurement, printing and data evaluation.

The arrangement of buttons depends on the application selected. The buttons can change their designations, e.g. the change from **Measure Samples** to **Stop** button. Grayed-out buttons are inactive at the time. The toolbar functions are identical to the corresponding **Measure** menu commands.

Note In contrast to the **Print** command, the **Print** button will not show the printer window. Instead, it will immediately actuate printing.

The button **Recalculate** appears with the Rate and Quant application (calibration mode) to repeat the calculation of enzyme activity values or calibration data after modification of settings.

With the Quant application the additional button **Go to Samples Mode**/**Go to Calibr. Mode** is available to switch between the two operation modes.

Toolbar with live display

A live display appears at the right hand side of the toolbar. The live display continuously (not possible for some models; only with restrictions during autozero/baseline measurements) displays the current wavelength and ordinate and it depicts the current position of the cell changer or the autosampler, if applicable. For instruments incorporating continuous light sources (not pulsed Xenon sources) the current status of the lamp(s) shows as a pictogram. Additionally, for spectrophotometers with adjustable slit width the current slit setting is shown.

***Important*:** The ordinate readings could be erroneous if a new measurement uses wavelengths outside the previous range (Rate, Fixed and Quant). Perform a new autozero/baseline run for accurate measurements.

Note *The* **Autozero/Baseline** *button is inactive, when operating the instrument with a sipper or an autosampler. You can perform an autozero/baseline only as a part of the measurement sequence or after pumping the blank sample. In this case, use the* ***Manual Control*** *function to actuate the autozero function.*

2.4 Methods Concept

Each VISIONlite measurement is performed with defined parameters. These parameter settings are treated as method files. The name of the active method is shown in the method selection box of the parameter space in each application.

You can save and restore methods containing your measurement parameter settings. Each application uses a specific method type, which is characterized by its filename extension:

- Scan .msc
- Rate .mra
- Fixed .mfx
- Quant .mqa

When an application is used for the first time, the basic method *test.mxx* is opened. You can modify the settings of the basic method and you can save these settings as the modified basic method.

You can generate further methods under new method names by saving a method using the **Save Method** command. The method directory as defined in the **Preferences** command is offered as the default directory. If you select an existing method filename, an overwrite message will warn you about overwriting the existing method file.

The method comprises all instrument and measurement settings as defined on the applications window. This includes the settings, summarized in the **Advanced Parameters,** the **Accessory** and the **Advanced Options** window.

Note *Variable settings like the activated lamps, cell changer positions, user name, the graphics text label or the data directory are not included as method parameters.*

Method files maintain audit trail information that allows reconstructing the history of a method. The **Show Method** command lists the audit trail information together with all method parameters in a print preview window.

2.4.1 Opening Methods

You may recall existing methods in 2 ways:

- Select a method in the method selection box in the parameter space,
- Use the **Open Method** command in the **File** menu.

The method selection box lists all methods of the current method directory as defined using the **Preferences** command in the **Options** menu. Additionally, those methods are listed which were loaded from other directories during the current working session. Select a method by clicking on the entry.

Using the **Open Method** command will also allow you to access method files from other directories. After you have done so, the method is also listed in the method selection box during the current session. It will not appear in the list if the software has been closed and restarted.

2.4.2 Protected Methods

If a method file is “write protected”, you cannot change any parameters or overwrite the method.

To set a method “write protected”, in the Windows **Explorer** select the active method directory, then right-click on the required method filename to display the popup menu. Select the **Properties** menu command to open the **File Properties** window and select the **Read-only** option.

If you open a write protected method, all method parameters in the **Parameters** group are displayed as usual, but there will be no reaction, when clicking on it. Above the method selection

window the message **write-protected** is given in red. Additionally, the group titles in the method selection window are grayed out.

When trying to save a method under the filename of an existing protected method, an error message is given.

Further information on special properties of protected methods within the Quant application: see section.

Important: Protected methods will prevent you from unintentionally changing method parameters. To avoid intended misuse and falsification, the access permissions on the method directory and method files must be restricted to authorized users by an administrator.

2.5 Results and Methods Storage

Recorded spectra, Rate data sets and results are automatically stored to hard disk as separate files. The files are differentiated by a user-entered name and a system-added file extension.

The software stores data/results and methods in 2 different directories, which can be located at any position in the directory tree. Initially, the directories are selected within the installation procedure.

As a default, the directories named *Data* and *Method* are established under the program directory.

2.6 Measurement Sequence

The basic measurement sequence is identical to all applications and instrument configurations.

1. After selection of a method or modification of parameters, you start a measurement with the **Measure Samples** button or the menu command **Measure/Samples** or by pressing F5 on your keyboard.
2. A sample information window is presented. After performing an autozero/baseline correction, you enter the characteristics of the sample or sample group to be measured. In sipper or autosampler operation, the autozero/baseline run typically is integrated into the measurement series.
3. Then in manual or cell changer operation, place the sample(s) into the instrument and close the sample compartment cover. In sipper or autosampler operation, prepare the samples to be aspirated.
4. Press **Measure**, **Sip & Measure** or the **Enter** key to actually start the instrument data recording.
5. The results are displayed during and after the measurement.
6. After the run, a new sample information window is presented to define the next sample/sample group (not autosampler and sample list operation). Enter the data for the next sample or check the sample list entries. Proceed as above or click on the **Close** button to terminate your measurements.

See the sections "*Performing Measurements*" and about the applications for further details and specifics.

3 General Functions

This chapter describes those functions, which are identical or similar for the applications. Details of operation are described for each application separately.

3.1 Menus and Menu Commands

Each application has a menu bar with the following menus:

- **File** Load and save data; methods; printing; changing application and exit.
- **Measure** Start of measurement and autozero/baseline.
- **Options** Selection of various basic preferences and direct control of the instrument.
- **Help** Access to the help system and software information.

The commands in each menu vary slightly according to the application selected.

3.1.1 File Menu

The **File** menu contains commands for data handling and output:

- **Open Method**
- **Save Method**

- **Load Spectrum/Data Set** (Scan and Rate applications only)
- **Save Spectrum/Data Set as** (Scan and Rate applications only)

- **Export Results** (not Scan application)
- **Load Results** (not Scan application)

- **Print**
- **Print Preview**
- **Printer Setup**

- **Change Application**
- **Exit**

***Note*:** A command to save the results is not available with the Fixed and Quant application, since these data are stored to disk automatically. Peaks lists of the Scan application cannot be saved. An additional function to save spectra and Rate data sets is available in the **File Information** window of the graph.

Open Method Command

The **Open Method** command presents a window listing all methods of the respective type in the pre-selected method directory. A different directory can be selected. Select the requested method and confirm by clicking on the **Open** button. The method settings are transferred to the various parameters.
Alternatively, you may select a method from the method selection box.

Save Method Command

This command is used to save the current measurement and evaluation parameters to disk as a method. The method can be applied for later use of the conditions. The command opens a **File Save** window, where you enter the method filename. The respective method file extension is attached automatically. The current method directory is offered as the directory to save the method; however, you can select different directories.

Load Spectrum/Data Set Command (Scan and Rate applications only)

The command is only available in the Scan and Rate applications. With this command, you load stored spectra and data set files from disk to the graph. All graph functions are available. It is

possible to inspect older data, to compare and to re-evaluate them. The data files must be in suitable data formats.

The command displays a selection window, which lists all stored data of the respective type in the data directory. A different directory can be selected. Select the required file or files (with Shift or Ctrl key) and confirm with the **Open** button.

Save Spectrum/Data Set as Command (Scan and Rate applications only)

The command is only available in the Scan and Rate applications. With this command you save spectra and Rate data sets to disk. This command displays the **File Save** window for saving the spectra/data sets with the extensions .dsp and .dti. Additionally the file formats csv and JCAMP-DX (spectra only) are available. After defining an extra data format via a spectrum converter (see *appendix*, *Data Formats*), this additional format will be displayed in the list and can be selected.

All spectra/data sets present in the graph will be presented for storage. A different name and directory can be selected. As a default, the pre-selected data directory is shown. With the Scan application the file extension dsp (Standard-Format), csv (Excel comma-separated values) or dx (ASCII JCAMP format) can be selected.
Note: Spectra and data sets are stored to disk automatically after recording. The command is mainly used to store data with a different name and directory. The **File Information** window of the graph offers an additional option to save single spectra/datasets.

Export Results Command (not Scan application)

This command allows results to be saved to disk as shown in the results space. The data are stored as *.csv* files (comma separated values). The files contain all general information with data and results in tabular form. The files are readable by spreadsheet programs (see: *appendix*).
A result list generated by the QUANT application comprises the additional column **Error**, which shows the error messages automatically generated by the system (e.g. data over-range). The result list generated by the Rate application lists the regression data of the fit in three additional columns.
Note: Spectra and Rate data sets can be exported in tabular form as *.csv* files.

Load Results Command (not Scan application)

This command allows stored results to be displayed. A selection window enables you to select the required filename and directory. The **Print Preview** window is used to display the results. A printout is possible. You can copy these data to the windows clipboard.
Note *Typical result files* demo.r** *are supplied with the install set where applicable.*

Print Command

The **Print** command prints the currently displayed results. You can select the printer, the number of copies, etc. in the print window. Additionally you can set the printer attributes.

Print Preview Command

The command opens the **Print Preview** window, which shows a preview of the printed report. The window allows you to copy the report to the clipboard and to print it, see section *Printing/PrintPreview*.

Printer Setup Command

With the **Printer Setup** command, you can preset various printer settings (as with the **Print** command). The possible options are determined by the printer driver.

Note *The main purpose of this command is to switch printers. Some printer drivers may not observe the selected print parameters. This should preferably be done in the print window or in the system settings.*

Change Application Command

The command closes the currently active application and opens the VISIONlite start routine to switch to another application. If you have modified any parameters of the current method, you are asked whether you wish to save these changes.

Exit Command

This command closes the currently active application. If you have modified any parameters of the current method, you are asked whether you wish to save these changes.

3.1.2 Measure Menu

The commands of the **Measure** menu are equivalent to the buttons on the toolbar. All application modules use the commands:

- **Samples**
- **Autozero** or **Baseline** (inactive for sipper and autosampler operation)

***Note*:** **Autozero**: Background correction for a single wavelength point (Rate, Fixed, Quant applications).
Baseline: Background correction for a wavelength range (Scan application).

The **Samples** command starts a measurement series and is equivalent to the **Measure Samples** button. The **Autozero**/**Baseline** command starts a background correction and is equivalent to the **Autozero**/**Baseline** button.
Note: With a cell changer, the autozero sample (blank) is always assumed in cell position 1, (or **B** with some spectrophotometer models).

You can also activate each command using a function key; the required function key is listed behind the command name. Using a function key is often more convenient while performing an analysis in the laboratory.

- **Samples/Stop** F5
- **Autozero**/**Baseline** F6
- **Manual Control** F4

The respective reactions, settings and procedures are described with the individual applications and in section *Performing Measurements*.

3.1.3 Options Menu

The **Options** menu includes the following commands in all applications:

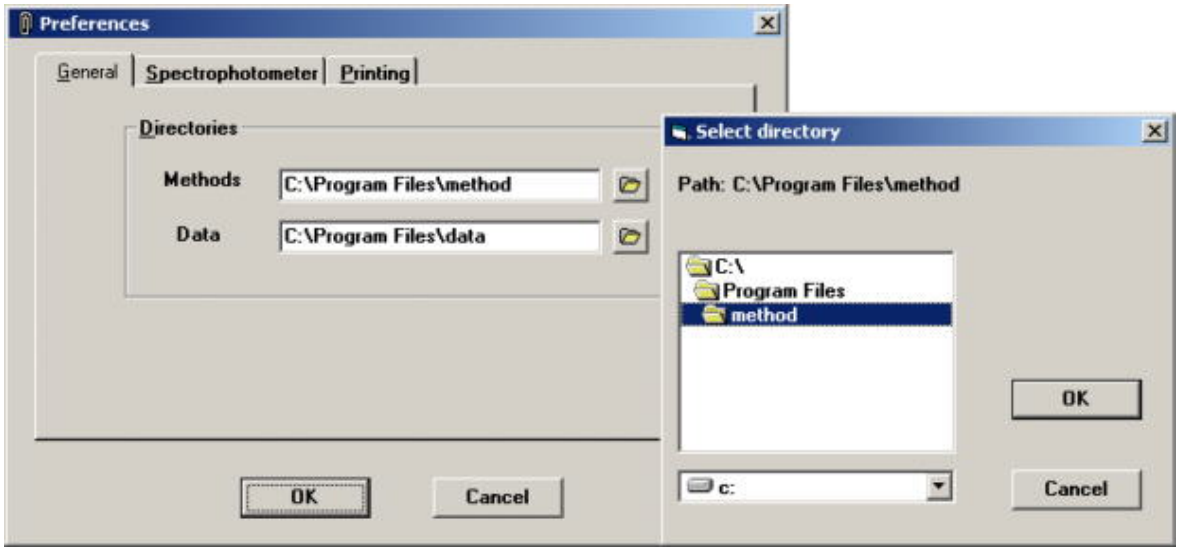
- **Preferences**
- **Manual Control**

Preferences Command

The **Preferences** command is used to define basic settings of the software, the spectrophotometer and printing. The settings are grouped through tabs.

The **General** tab allows you to select the data and method directory:
The software stores data/results and methods in 2 different directories, which can be located at any position in the directory tree. You can directly edit the directory names or open the directory selection window with the directory symbol behind the entry field. Double-click on the required directory and confirm your selection with the **OK** button.

General Functions



Preferences *window*

The **Spectrophotometer** tab allows you to define special settings related to the instrument. In particular, you can select the COM interface. The setting will only be valid with the next software start.
Caution: If you change the COM interface without correspondingly changing the socket into which the cable is inserted, communication between the PC and the instrument will be interrupted. Other settings are instrument specific. You will find details of the specific instruments in the *appendix*.

The **Printing** tab allows you to define printing parameters as the print color handling and the footnote.
The Printing **Mode** defines if the diagram printout is in color or in black. The basic setting **Automatic** applies the printer configuration. The settings **Color** and **Black** override the printer settings. The setting **Black** transforms the colors of the curves to different dashes and prints respectively.

To define the footnote contents, enter text into the **Footnote** parameter field. This text is printed with the current date and time on the left and the page designation **Page n of m** on the right side of the bottom line of the page.

***Note*:** All applications share the modifications to the settings.

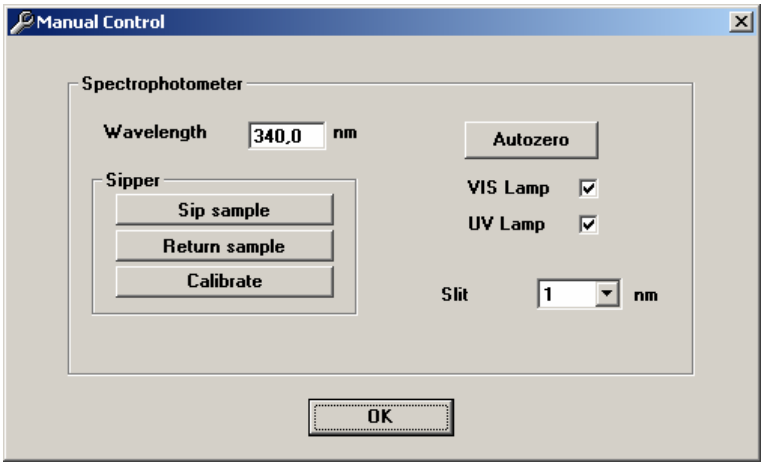
Manual Control Command

This command allows you to drive the instrument to a specific **Wavelength**, to **Autozero** at the selected wavelength and to read the ordinate value from the live display. No further measurements, evaluations or printouts are possible.

Note *When using a sipper, the **Autozero** is function is performed without pumping the blank solution. Therefore, start the sipper manually before the measurement.*

Note *The spectrophotometer will only execute the wavelength drive or move the cell changer when the entry has been finalized. This is done by pressing the **OK** button or by addressing another entry field.*

If an accessory is attached, the control options of this accessory are additionally presented, e.g. driving the cell changer to a required position or actuating the sipper pump.



Manual control window with sipper and variable slit

Additionally the window allows selecting the status of the **VIS Lamp**, **UV Lamp** and the **Slit**, if the instrument has these options. The lamp settings apply to all application modules. Some spectrophotometer models automatically actuate lamps, which are switched-off when required.

The attached sipper can be operated in forward and backward direction. After starting the sipper, the appropriate button is labeled with **Stop** in red colour. Press the button again to stop the sipper. The sipper sampling volume can be calibrated as described in section *Cell changer and Sipper Operation*.

The installed cell changer can be driven to any cell position.

The **Autosampler** button (VISIONlite auto only) opens a separate autosampler window to directly control the autosampler.

3.1.4 Help Menu

The **Help** menu follows the standard Windows conventions.

The **Contents** command is used to open a comprehensive help system. You operate this help system according to standard Windows conventions.
Also use the **F1** function key to open the help system with a page that corresponds to the currently active window.

The **Info** command displays information about the software version and copyrights.

3.2 Graphics Functions

(Scan and Rate only)

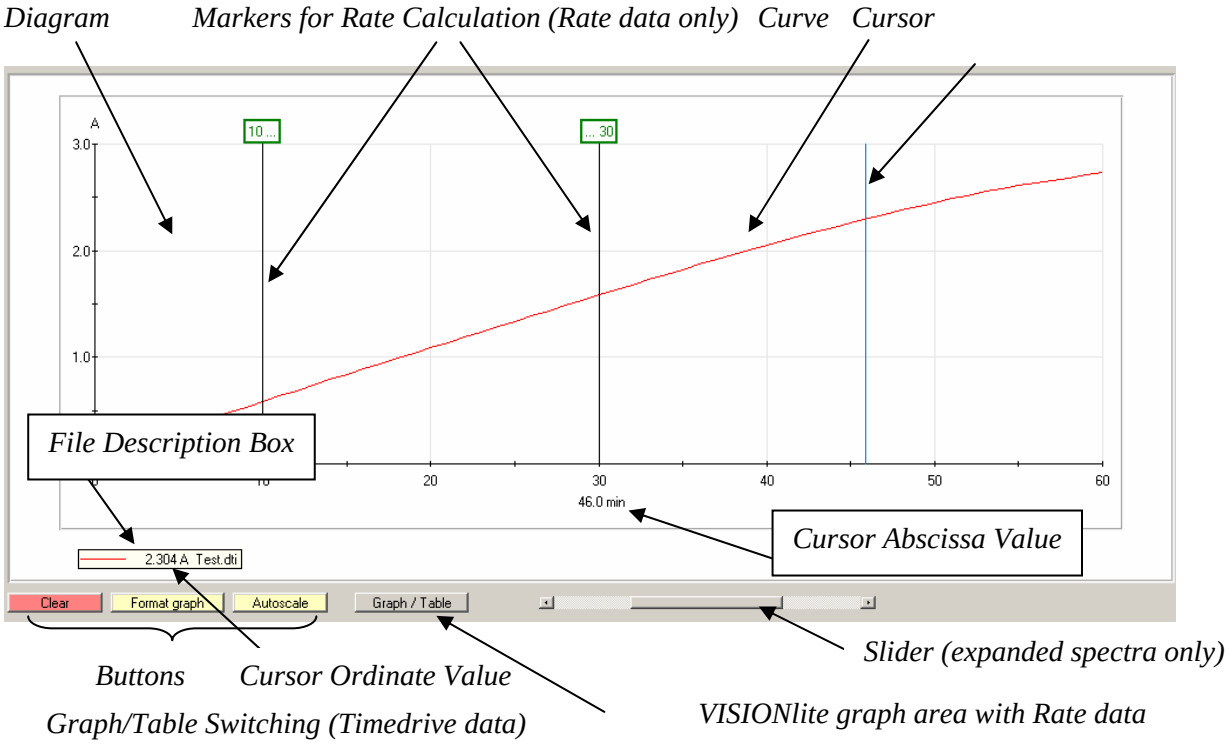
The graph area displays data from scan and Rate measurements diagrammatically. The Quant mode also uses the graph area for displaying the calibration curve, but then the user has no options to modify the presentation. Only the cursor function is active.

The data can have been freshly collected and/or loaded from hard disk via the **Load Spectrum/Data Set** command.

3.2.1 Graphic Space

The graph area consists of:

- The diagram with automatic cursor and possible marks originating from evaluation functions and peak picking,
- The file description box,
- The graphics buttons and scrolling bars (slider).



By modifying the program window size you can modify the graph area size.

The split of the graph and the results area can be varied at the upper margin of the results area. Place the mouse pointer on the edge. Thus, the mouse pointer will be displayed as a two-headed arrow. Then move the margin while pressing the left mouse key.

Note *Sizes and positions of the file description box and the diagram area are automatically optimized. This may result in a clipping of these elements, when curves are added/deleted or when the cursor is activated/deactivated.*

3.2.2 Diagram

The diagram is annotated on the x-axis (abscissa) with either wavelength (nm) or time (min) and on the y-axis (ordinate) with absorbance (A) or transmission (%T) for example. If curves with different ordinate modes are overlaid, the right Y-axis will be used to scale the curves with the second ordinate independently. If the curves displayed in the diagram are defined with more than two ordinate modes, the right Y-axis will be labelled with ***.

Colors are used for overlaid curves in a fixed order. Up to 20 curves can be displayed. If further data files are loaded or measured, the latest will overwrite the oldest existing file.
The scaling is according to the method or according to the data for recalled data files and can be modified by the graph tools (detail expansion and graphic buttons).

Expansion of Details
You can expand any area within the diagram: Position the mouse pointer at any corner point of the area to be expanded. Then hold the Shift key and drag the mouse pointer with pressed left mouse button over the area to be expanded. The area will be framed. After releasing the mouse button the framed area will be expanded to the full size of the diagram. If you release the Shift key first, the frame will be removed.
***Note*:** Take care not to use the Ctrl key instead of the Shift key accidentally.
As an alternative you may also use expansion via cursor only (see below).
A slider becomes available after expansion (see below). To return to the default expansion, use the **Autoscale** button.

3.2.3 Cursor
Whenever you move the mouse pointer into the diagram a blue vertical cursor line is added to the diagram, which follows the mouse movement. The current abscissa position of the cursor is displayed below the abscissa axis. At the same time the corresponding ordinate value is added to the file description box. The ordinate value is taken from the nearest measured data point.
For spectra, the cursor can also be used to insert single spectrum values to the result area, see *Read Cursor Mode (Scan application only)*.

Expansion of Details via Cursor (spectra only)
An expansion of an abscissa section can be selected via cursor:
Position the mouse pointer at the left or right edge of the area to be expanded. Then move the mouse pointer with pressed left mouse button over the area to be expanded. The area will be shown in inverse colors. After releasing the mouse button the highlighted area will be expanded to the full size of the diagram with unmodified ordinate scaling.
A slider becomes available after expansion (see below).
To return to the default expansion, use the **Autoscale** button.

Note: The expansion via cursor will not be executed if a range of 3 data points or less has been selected.

Range Selection for Evaluation (only Rate application)
For the Rate application the described cursor entry mode is used to define the abscissa (time) range for the enzyme rate calculation.

3.2.4 File Description Box
The file description box lists all curves currently displayed in the diagram with:
- Color of the curve,
- Current cursor ordinate value (if the cursor mode is active)
- Filename
  The two-headed arrow in front of a name indicates that this dataset is assigned to the right axis of the diagram.
- File description

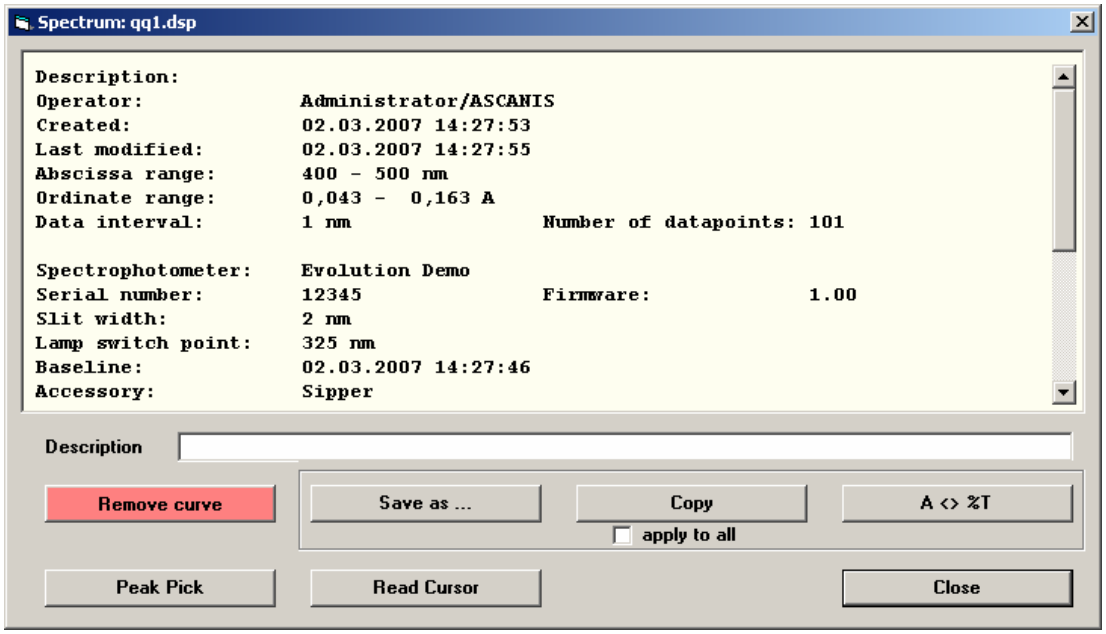
If the mouse pointer is moved into the file description box it changes into a hand symbol. This is to draw your attention to the option to click on a filename. This will open the file information Window (see below).

Note: According to Windows conventions, the mouse pointer does not change its shape, if a menu or a selection list is open.

3.2.5 File Information Window (only Scan and Rate applications)

Clicking on a filename in the file description box opens the file information window. The window lists:

- Sample description
- Operator
- Date of measurement and date of last modification. The date of measurement corresponds to the beginning of the measurement. The date of modification corresponds to the end of the measurement. Additionally, modifications can be for example **A<>%T** transformations or editing the sample description. If no date of modification is given, the file has not yet been modified.
- Abscissa and ordinate range for spectra and the measurement wavelength for Rate data, data interval and number of data points
- Cycle number and measurement time after start (for repetitive scans only)
- Type of spectrophotometer with serial number and firmware revision
- Spectrophotometer settings
- Date the associated autozero/baseline/ was performed
- Accessories in use and sample location (cell changer, autosampler)
- Software Version
- The **Audit Trail** section documents all changes of data (A<>%T) and filenames for the spectra/dataset.



File information window

In addition, the file information window offers the following functions:

- **Description** Entry field for editing the sample description.
- **Remove Curve** Deletes the selected curve from the list of displayed files.
- **Peak Pick** (spectra only) Starts a peak pick routine for the selected data file. Parameters for peak picking are taken from the current parameter settings. If neither **Maxima** nor **Minima** is selected, an error message is given. The numerical value for the peak threshold is used, even if it is assigned to another ordinate mode.

The detected extrema are numbered in the graph and are listed in the results area.

- **Read Cursor** (spectra only) — Starts the Read Cursor mode. This will allow transferring the ordinate and abscissa data of the spectrum at current cursor position to the results space (see below).
- **Save As** — Opens a **File Save as** window to store the selected data file as an *.dsp* or *.dti* file.
  Additionally, the file formats csv and JCAMP-DX (spectra only) are available. With the definition of an extra data format via a spectrum converter (see *appendix*, *Data Formats*), the additional format will be shown in the list and can be selected as well.
  If you selected the **Apply to all** option, all spectra/datasets will be offered for storage subsequently.
  The data directory defined in the **Preferences** command is used as the default directory.
- **Copy** — Copies the spectra/datasets as listed numerical values to the clipboard.
  If you selected the **Apply to all** option, a list containing the data of all spectra/datasets in the graphic area will be generated. Make sure, that all datasets in the graph are shown with the same ordinate range and data interval.
- **A<>%T** — Transforms the selected data file from absorbance (A) to Transmission (%T) and vice versa. Accordingly, spectra in ordinate mode %R will be transformed to log(1/R).
  If you selected the **Apply to all** option, all spectra in the graph will be transformed.
- **Close** — Closes the file information window.

3.2.6 Graph Tools

The following tools are used with the graph:

- **Clear** button,
- **Format Graph** button,
- **Autoscale** button,
- **Graph/Table** button (Rate data only)
- Expansion slider (only expanded spectra of the Scan application).

Clear button

The red **Clear** button deletes all curves and results in the diagram. If you want to delete only single curves, use the individual file information window.

Format Graph button

This button opens a window with a schematic coordinate system. You can enter the desired ordinate and abscissa minimum and maximum values. You can step from one field to the next with the Tab key.

Autoscale button

The **Autoscale** button resets the scaling to default values, which are abscissa and ordinate ranges that fully display all the curves. The ordinate range is automatically selected so that all data points are displayed with suitable scaling and ordinate labels.

During scanning the **Autoscale** button only operates in the y-direction to show the continuation of the curve.

Graph/Table button (Rate data only)

The **Graph/Table** button is available in the Rate application only. It allows switching from the standard graphical presentation of kinetic data to a tabular listing of all curves present. No further action is possible with the tabular data. Note that the time range and the data interval of several files must be compatible to have a full table.

Text Positioning Area (only Scan data)
Use the positioning area **Text** to generate a label within the curve area in the graphic. Click in the positioning area and drag the area to the desired position, holding the left mouse button pressed. After you have released the mouse button an entry dialog is displayed, where you can enter the required text. You can enter multi-line texts and position the whole text area later.

Note *The position of the text is assigned to the scaling of the diagram. Therefore, the text will change its absolute position, when changing the scaling of the graph.*

Note *If all datasets are deleted from the diagram, the text labels will be cleared as well.*

Expansion slider (expanded spectra only)
The slider is available in the Scan application only. It is active if an abscissa expansion has been performed with either option. The slider allows you to move the expanded section without changing the ordinate range and depicts the position of the section in respect to the full abscissa range.

3.2.7 Read Cursor Mode (Scan application only)

The **Read Cursor** mode allows transferring the ordinate and abscissa data of a spectrum at the current cursor position to the results space. The mode is only applicable for spectra. One spectrum can be handled at a time. The mode is accessed from the file information window of the spectrum in question.

After selecting the mode, the information window is closed and the cursor is placed into the graph area. The mouse pointer takes the form of a cross. If the cursor is intermittently moved out of the significant graph area or if any other action is performed, the **Read Cursor** mode will be deactivated instantly and the mouse pointer is displayed in its normal shape.

Once the **Read Cursor** mode is active, a click on the left mouse button will generate a **Read Cursor** table in the **Results** space, containing the x,y-values at the current cursor position. Further lines can be added to the table with every click on the left mouse button.

Note *Make sure however that the mouse is not moved between pressing and releasing the left mouse key – if so the action will be interpreted as a scale expansion.*

The **Read Cursor** mode transfers the spectrum name and description, the cursor ordinate position and the spectrum abscissa. Any number of **Read Cursor** actions can be performed. To terminate the **Read Cursor** mode just move the cursor out of the graph.

Note: **Read Cursor** tables will add to existing peak-pick tables of the same spectrum and **Read Cursor** tables of other spectra. This allows generating combined peak and read tables as well as read tables for multiple spectra. However, new peak-pick tables or a new **Read Cursor** table of the same spectrum will clear the **Results** space.

3.3 Results Space

The results space is in the right section of the software window and is used to present alphanumerical results. The varying usage of the results space is:

- Scan: This space lists the optional peak tables and cursor readings. It is only shown if a peak-pick or the Read Cursor mode has been done.
- Rate: This space lists the optional enzyme activity evaluation results. The space is always shown.
- Fixed: This space is the standard output space for results. As no graphics are generated, the results space covers the full output space.
- Quant /Calibration mode: This space is used for presenting the standards table.
- Quant /Samples mode: This space is the standard output space for results.

The split of the graph and the results area can be varied at the upper margin of the results area: Move the mouse pointer onto this margin, and with the mouse pointer being displayed as a two-headed arrow, move the margin with the left mouse key pressing.
If necessary, use the window slider to show hidden results.

After clicking on the text, you can use the Ctrl+C key combination to transfer the text of the result space to the clipboard. If you mark the text partially (with pressed left mouse button) only the marked text is transferred. From there you can paste the information to other software.

Store the results in a tabular form with the **Export Results** command (not available in the Scan application).

Refer to the section *Printing and* Print Preview for further information on printing the results.

Typical result files *demo.r*** are supplied with the install set where applicable.

Results Number Format

With the Rate, Fixed and Quant applications, the number format of the results can be defined as a method parameter. Select a number of output digits before the decimal, which is appropriate for the highest expected result value. This assures that all figures are output in line. However if one of the figures should have more digits before the decimal the figure is shown correctly with a shifted decimal position. The number of output digits after the decimals should follow the expected or possible accuracy of the results. The figures are rounded to the selected number of output digits. The table below shows the output of figures with some selected examples:

Selected number of digits before decimal	Selected number of digits after decimal	Value	Output
2	3	11.254	11.254
2	2	11.254	11..25
2	1	11.254	11.3
2	0	11.254	11
2	4	11.254	11.2540
1	3	11.254	11.254
3	3	11.254	11.254
2	3	-11.254	-11.254

Figures with too many digits may be truncated in the middle with dots if the available space is insufficient.

3.4 Printing and Print Preview

3.4.1 Printing Procedures

Use the **Print** button of the operating toolbar or the **Print** command of the **File** menu to print the diagram and/or results. Configure the printer with the **Printer Setup** command.

Note *The main purpose of the **Printer Setup** command is to switch printers. Some printer drivers will not observe the selected print parameters. Settings should preferably be selected in the print window or in the system settings.*

You can check the page(s) to be printed beforehand with the **Print Preview** function and then start the printout from this window.

When the printing configuration is set to the standard **Automatic** mode (see *Preferences Command*), the printout of the diagram will be in color with a color printer, and in monochrome with a monochrome printer. With the monochrome printer, the colors of the curves are automatically transformed into different dashes. If the printing mode should be set deliberately to color or monochrome, this can be done with the **Preferences/Printing** command.

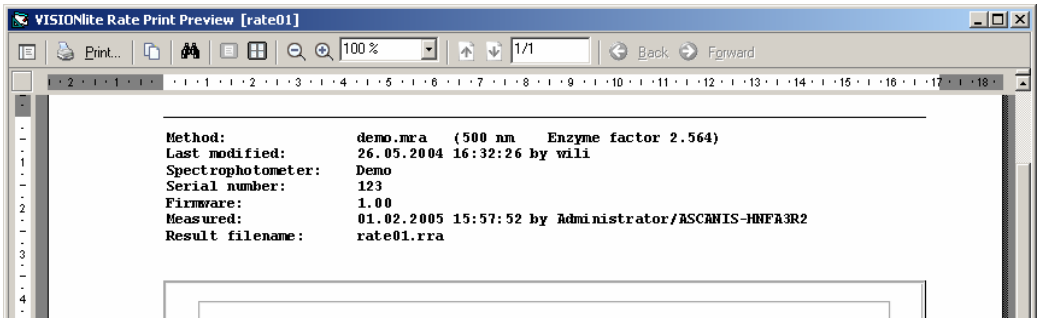
Printouts are extended with a header and a footnote. The header contains general measurement information. The footnote contains

- date and time of printing,
- the footnote text as defined in the **Preferences** command (as a default this is the software designation),
- the page number (Page n of m).

3.4.2 Print Preview Window

The print preview window is used to check a report before printing. It is also used with the **Load Results** command to show the respective contents.

In this window, you can swap pages, modify the size of the presentation, and actuate the printout.



Print Preview window

The header title in the **Print Preview** window displays the name of the evaluated spectrum, or the name of the first spectrum of a series of spectra, respectively.
Note: The **Print Preview** window shows only the results generated in the current series of measurements, even if the current results are appended to existing results.

The operating buttons in the toolbar are separated by vertical lines and arranged from left to right.

- **Table of Contents** Opens a column at the left-hand side of the screen to show the report contents. You cannot make changes.

- **Print** Prints the current report. In addition, you can select and configure the printer, define the number of copies to be printed, or print to file.
- **Copy** Copies the current report page to the clipboard. After that, you can insert it in documents of other application software via the **Paste** command.
- **Find** Opens the **Find** dialog that allows you to search for specific words or phrases in the report. The phrase is entered at the **Find what** parameter in the **Find** dialogue box.
- **Single Page/ Multiple Pages** These buttons allow you to change between a single page presentation and a multiple page presentation. When you select **Multiple Pages,** additional buttons are presented to select the number of pages to be displayed (see figure above). These buttons are only active if there is more than one page in the report.
- **Zoom Out /Zoom In** These buttons allow you to change the scale of the presentation. The selected scale is displayed in the selection box. Alternately, in the selection box, you can also select a value directly or enter a new value.
- **Previous Page/ Next Page** If there is more than one page in the report, you can jump from page to page with these buttons. The page being displayed is shown in the textbox.
- **Page Number textbox** This box shows the number of the currently active page. To jump to a specific page, enter the number of the designated page and press the ENTER key at the keyboard.
- **Back/Forward** These buttons are only active if there is more than one page in the report and you have already jumped pages. These buttons allows you to jump directly to the previous page or following page, respectively.
- **Close** Close the window to return to basic software.

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4 Performing Measurements

4.1 Starting a Measurement

To perform a measurement, use the **Measure Samples** button or the equivalent menu command to start the measurement. A sample information window is displayed.

If an autozero/baseline run has not yet been executed under method conditions, you will be requested to insert a blank sample for the first measurement. In the cell changer mode the first position is reserved for the blank.

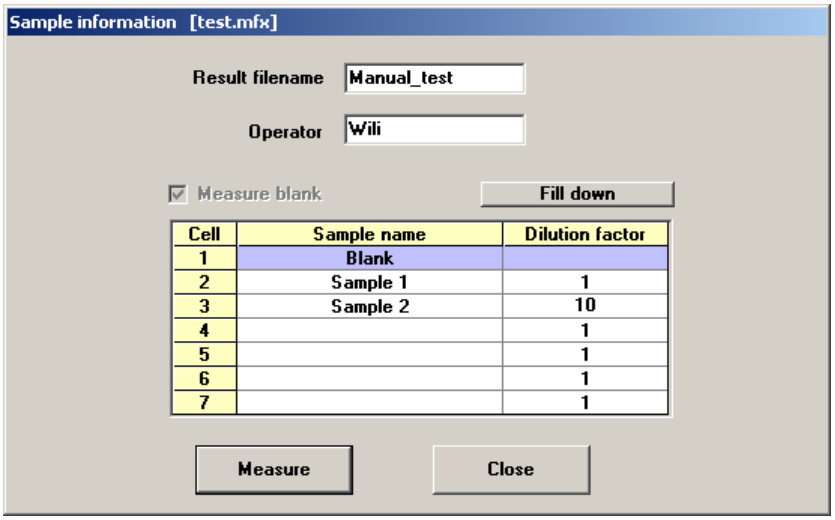
Alternatively, you can insert the blank sample beforehand and manually start the baseline/autozero run with the **Autozero/Baseline** button in the toolbar or the equivalent menu command (not in sipper and autosampler operation).

Important: The software demands a new autozero/baseline run, if a new measurement uses wavelengths outside the previous range. When only instrument settings like the slit or the lamp change have been modified, a new autozero/baseline run is recommended for high precision measurements.

Note *In the configuration file you can disable the demand to perform an autozero/baseline run, see section* Software Configuration.

4.1 Sample Information Window

You define the next sample or sample group (cell changer mode) to be measured in the **Sample Information** window. The window provides some entry tools and a baseline/autozero run can be defined (see below).



Fixed application sample information window in 7-cell changer mode

Details in the sample information window depend on the application and the attached accessory. The table contains the following entry fields:

- **Result filename** (obligatory, if applicable; not applicable in Scan and Rate without enzyme rate calculation)
- **Operator** (displays automatically the recent entry or the Windows user name of the currently logged-on user)
- **Sample name** (obligatory; automatic incrementation of numbers, if the last digit of the sample name is a number)
- **Description** (only Scan, optional)
- **Dilution Factor** (not Scan; otherwise with 1 as a default)

The column width can be varied by dragging the separating line with the mouse to the desired position.

Note *The result filename and all sample names within the Scan and Rate applications are used as Windows filenames. Thus the Windows conventions must be fulfilled: up to 215 characters (including spaces) are allowed. Furthermore, you may not use the following characters: \ / : * ? " < > |. Additionally, avoid using extensively long filenames, because most software applications do not permit of extremely long filenames.*

Note *Principally, there is no restriction on the length of the entries. Remember however that the space for the sample name and sample description is limited in the report output. When space is insufficient, both entries will be truncated in the middle.*

Note *The automatic incrementing of sample names can be disabled in the configuration file, see section Software Configuration.*

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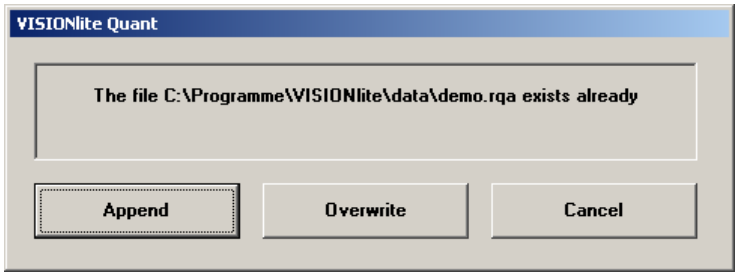
Note *In the configuration file you can pre-select to take the description and dilution factor of the recently performed measurement series, see section Software Configuration.*

The **Result filename** is used for saving the results of the measurement to disk. It is requested in the Quant and Fixed applications, and also in the Rate application if an enzyme rate calculation is defined. The result file contains the complete alphanumerical result data of the measurement series. When an already existing filename is entered a query is given to append or overwrite the results. ***Important:*** If you accept overwriting at this point and enter the same filename for another measurement series again, it is assumed that the existing file should again be overwritten: no further overwrite query will be shown.



Result file overwrite query

All applications offer the entry of the **Operator** name. As a default, the Windows user name is used if available. Once the name has been modified, it will be applied as the new default for all applications.

Sample name and sample characteristics (**Description** or **Dilution Factor**) are entered within a sample line or table (cell changer mode). Click on the **Sample list** button to setup a sample list (only present with the first sample of a measurement series). As a result, a sample table is shown for parameter entry (see below). In autosampler operation, always a sample list is applied to enter the sample data.

Note *Right-clicking on an entry box will display a popup menu holding editing tools.*

Start the measurement by clicking on the **Measure** button or press the Enter key on your keyboard.

The Quant application offers an additional **Re-measure** function (not in autosampler operation). Selecting this function shows the **Sample information** window with the sample data of the recently measured sample for repeating the measurement.

Click on the **Close** button to terminate the measurement.

In cell changer mode and in sipper mode the layout of the window is somewhat different; see section *Cell changer and sipper Operations*.

When measuring a batch of samples, it is possible to enter the sample data of the sample batch in a sample list before starting the measurement of the first sample. Clicking on the **Sample List** button (only present in the **Sample information** window with the first sample of the batch) will open a sample table. See section *Using a Sample List* for further information on sample lists.

Entry Tools in the Sample Information Window

In entry mode (dotted cell border) and in editing mode (flashing vertical entry cursor) you can display several popup menus by clicking the right mouse button. You can use the menu commands to **Delete** entries or **Paste** text type data from the clipboard, see *Using a Sample List*. By using the **File open** command to load a sample list, the current sample table will be extended with the new data.
With multi-line sample information windows (sample list or cell changer mode), you can use the **Fill down** button to transfer the contents of the active cell to all cells beneath. With a number in the rightmost digits of the sample names, the number will be incremented.
Highlight the required entry and, optionally, the area below by clicking on it. Then use the **Fill down** button to copy the highlighted data to the cells below.

The right mouse button opens a pop-up menu with the commands **Fill down** (only with table) and **Delete**. Use the **Fill down** command as described above.

Note *Use the **Delete** command to clear the entry in the active cell or copy and paste the entry of an empty cell.*

Autozero/Baseline in the Sample Information Window

If no baseline/autozero run has been performed under the current method conditions, you will be requested in the sample information window to do this.

The (first) **Sample name** field is designated as **Blank**. You cannot modify this entry (as shown by the blue field). Insert the cell containing the blank into the instrument and start the run.

***Note*:** In the configuration file you can disable the demand to perform an autozero/baseline run, see section *Software Configuration*.

The software requires a new autozero/baseline as well, if relevant method settings were modified or another method is selected for measurements outside the wavelength range of the existing /baseline readings. If instrument parameters (e.g. slit width, or lamp change-over) were modified, a new autozero/baseline should be performed for accurate sample measurement.

If a baseline/autozero run has already been performed or an appropriate data file is available, it is still possible to repeat this measurement – and also at any time within a series of samples – by activating the **Sample information** window **Measure Blank** selection box above the table.
Once this option has been activated, it is also the default setting for the next series of measurements.

Note *In the Rate application with a cell changer an autozero will be performed with each measurement cycle, if the autozero has not been executed beforehand. The blank is expected always in cell position 1 (or **B** with some spectrophotometer models).*

Note *In autosampler operation, the autozero/baseline sample is measured in the beginning and is taken from the position defined in the rack definition file.*

4.2 Measurement Procedure

After starting the run, the **Stop** button is presented so that you can cancel the run at any time. All readings will be stored automatically during measurement; thus preventing any loss of data, if the measurement is terminated during a run.
During the run the results are shown in the graphics and/or results area according to the selected application and the selected data evaluation.

After completion of the data transfer, the **Sample information** window is shown with the data of the next sample/sample batch. If a sample list is used, the data of the next pre-defined sample will be displayed. You may change these entries. Proceed as described above or click on the **Close** button to cancel the measurement series.

Note *Right-clicking on the sample information will open a popup menu. Use the menu commands to **Insert** additional samples into the list or to **Delete** samples from the list (see Using a Sample List). There is no possibility to interfere with an autosampler measurement series.*

The Quant application offers the additional **Remeasure** option to repeat the measurement of the previous sample in case an error has occurred. Clicking on the **Remeasure** button recalls the entries of the previous sample and allows repeating this measurement.

4.2.1 End of Measurement

After the run, the sample information window for the next sample/sample group is presented. Proceed as described above, or stop the measurement series with the **Close** button.

Thereafter you can start another series of measurements or perform the following tasks (depending on the specific application), for example:

- Use the graphic functions. Among other things you can re-run the peak pick function or export a spectrum.
- **Export** results as a file for spreadsheet software.
- Printout results.
- Use the **Recalculate** button to re-run the enzyme rate calculation with modified parameters.

After measuring all pre-defined samples of a sample list or a series of replicates, the **Sample information** window will not be shown again.

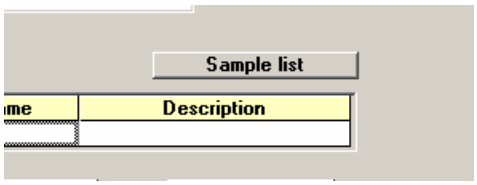
If you plan another measurement with similar sample(s), you start the measurement again with the **Measure Samples** button. If you are using a different solvent or cell type or change the parameter settings, you should perform a new autozero/baseline run.

If you plan another measurement with a new method, select the new method or modify the parameter settings accordingly.

If you want to use another application, use the **Change Application** command in the **File** menu to open the required application.

4.3 Using a Sample List

Normally, the software demands the sample data for each of the samples directly before the start of the measurement. As an alternative the **Sample information** window provides the **Sample list** button with the first sample for all applications. Click on this button to enter the sample data for a complete sample batch before starting the first measurement. Alternatively, you can import an already existing sample list. Use this feature for measuring sample batches e.g. when using a sipper. A sample list is standard for autosampler operation.



When a sample list has already been established with the same name as the method, this sample list will be loaded automatically.

After starting a method, the **Sample information** window offers the button **Sample list**. Clicking on this button will extend the sample table to 1000 lines.

Note: In cell changer mode the sample table has a number of lines equivalent to the number of cell changer positions. The **Sample list** button is not available.

Sample data are entered line by line without empty lines. Missing entries for sample names will cause the software to give a request for the missing data. In the Scan and Rate application, sample names must be different for each sample as the sample names are used for the automatic storage of spectra/datasets.

Clicking on the table with the right mouse key will open the table context menu, holding the following commands:

- **Delete**
- **Delete line**
- **Insert line**
- **Open file**
- **Save as**
- **Copy from clipboard**

Note: This menu can also be opened during the measurement of the samples (not autosampler operation). It allows adding additional samples or deleting samples.

4.3.1 Editing a Sample List

Sample information entry to the table is done similar to a spreadsheet program. After clicking at a cell a new entry or overwriting the existing entry is possible. After an entry, the edit mode is activated. This mode is recognised with a blinking vertical entry cursor. To edit an existing entry, double-click on the requested cell and then set the entry cursor to the required position.

Right-clicking at a cell in the edit mode will open a standard edit context menu, which allows to reverse the action or to copy/paste texts.

The **Delete** command in the table context menu deletes all entries in the area marked previously. To mark an area, move the mouse over the required area with the left mouse key pressed.

The **Delete line** and **Insert line** commands of the table context menu will add or deletes a single line at the marked position.

4.3.2 Fill down Function

You can use the **Fill down** button to transfer the contents of the active cell to all cells beneath. Sample names are automatically incremented, when the last character is a figure.

Click on the relevant cell in the table to highlight the required entry. Then click on the **Fill down** button to copy the marked entry to the cells below. Alternatively highlight the required cell entry and an area below. The **Fill down** function then will only fill the marked area.

Note *Use the **Delete** command in the context menu of the sample table, to delete unnecessary entries. It is also possible to use the **Fill down** function to transfer an empty cell.*

4.3.3 Storing and Importing a Sample List

Sample lists can be handled as a separate file for repeated use. Sample list files use the file extension .lxx (xx is the identifier of the assigned application) and they are stored in the current method directory. Sample list files are text files, which can be generated by the VISIONlite software or externally (for instance by a LIM system).

After completing a sample list table, you can use the table context menu command **Save as** to store the sample list with a selectable filename. We recommend using the pre-selected filename extension (see above). For the next measurement series, the sample list can be loaded with the **File open** command. The software will enter the loaded sample information beginning with the first line.

When the user selects the name of the current method as the sample list filename, the sample list will be loaded automatically with the next use of the method.

Sample list files can also be generated by external software (e.g. MS Windows Editor/Notepad). As a data separator, use semicolon, tab or carriage return. The first line holds the results filename. This optional entry in the first line will be displayed in the **Sample information** window in the entry box **Result filename**. When storing the sample list file, make sure to use the proper method directory path and the proper file extension.

It is possible to save and open sample lists with any filename extension you like. Sample list files with the filename extension .lst are taken automatically by the software. With this extension, a sample list can be used for different applications. After using a different extension, this new extension will be pre-selected in the **File open** window for sample lists.

Alternatively, to loading a sample list file, it is also possible to paste sample information via the clipboard. Use the **Copy from clipboard** command in the table context menu, if a properly formatted sample list has been copied to the clipboard beforehand. The information will be pasted to the sample data table beginning with the active cell. Thus it is possible to combine sample lists from different sources.

5 Cell Changer and Sipper Operation

Cell changer and sipper are spectrophotometer accessories for increasing the sample throughput substantially.

Using a cell changer, you load the system with a batch of samples before starting the measurement. During the measurement the cell changer moves the required sample automatically in the sample beam of the spectrophotometer. This type of measurement procedure is advisable for time-consuming analysis, e.g. rate measurements.
Note: In the configuration file (see section *Software Configuration*) you can reduce the number of active cell positions for all subsequent measurements.

In sipper operation the sipper pump transports the sample in the flow cell in the sample beam. Then the measurement starts automatically. Therefore, there is no need to change and clean the cells. This operation mode is advisable for high sample throughput in routine analysis with short measurement times, e.g. quantitative measurements with the Quant application.

5.1 Autozero/Baseline Function

With the cell changer, the autozero/baseline correction is always performed on cell position 1 (or **B** with some spectrophotometer models).

Note *For Rate measurements, the software will perform an autozero with the autozero sample on cell position 1/B at each measurement cycle, when an autozero has not been done beforehand. This allows for compensating for blank reactions.*

With sipper operation, the **Autozero/Baseline** button in the tool bar is inactive. An autozero/baseline run is only possible with a sample measurement or – after aspiration of a blank sample – at a selected wavelength using the **Manual Control** function.

5.2 Manual Control Command

Use the **Manual Control** command to move the attached cell changer to a specified position.

Via the **Manual Control** command you can start the attached sipper for forward/reverse pumping. After activating the sipper, the respective button changes to **Stop**. Press this button again, to stop the sipper.

5.2.1 Sipper Volume Calibration

The sipper volume can be calibrated. Start the function via the **Calibrate** function of the **Options**/**Manual Control** window and enter the desired sample volume for a single sample aspiration. The system will use a corresponding aspiration time, which is based on the existing sipper volume calibration factor.

Provide the liquid to be aspirated in a graduated cylinder and initiate the sample aspiration several times (max. 20) with the **Sip sample** button. After pressing **OK** enter the total volume, which has actually been aspirated. The sipper volume calibration factor is calculated and transferred to the spectrophotometer.

Note *Whenever the pump tube deteriorates or has been changed, a repeated sipper volume calibration is recommended.*

Note *The software generated sipper volume calibration factor will not apply for a stand-alone operation of the spectrophotometer.*

5.3 Accessory Window

If a sipper is attached, the control options of the sipper are available in each application via the additional **Accessory** button in the **Spectrophotometer** group. Click on the **Accessory** button to display the **Accessory** window and set up the **Pump time**, **Air gap**, and **Waiting time** for the sipper operation.

During the selected **Pump time** the sample is aspirated. Ensure that the pumped sample volume sufficiently fills the flow cell without wasting sample.

Via the **Air gap** parameter, a small amount of air is pumped after each sample for separating the samples and to generate a cleaning effect to the sample system. If an **Air gap** has been defined, the software gives an acoustic signal after the aspiration of the sample, to cause the operator to remove the sample. After 2 seconds the sipper pump is started again to aspirate air.

The **Waiting time** is the time delay after aspiration and before the measurement. This parameter allows the sample liquid to settle and to remove streaks/schlieren, especially with long-path cells.

When an autosampler is attached (VISIONlite auto software), further parameters are shown (see manual supplement).

All entries in this window are saved with the method.

For cell changer operation, this window is not available.

5.4 Sample Information Window

Depending on the attached accessory, there are several operating functions in the **Sample information** window.

In cell changer mode the sample table holds a line for each cell position in the cell changer. Beginning with the first line in the sample table, samples will be measured up to the first empty sample name entry field.

If you press the sipper button at the spectrophotometer, the sipping and subsequent measurements starts immediately. Alternatively, you can use the additional buttons **Sip & Measure** or **Measure** in the **Sample information** window to start the measurement. Pressing the **Sip Sample** button will start the sipper in forward direction without starting the measurement.

Note *If you unintentionally started the sipper before entering the sample data, an error message is given after the sample aspiration. After completion of the sample data entry, you can start the measurement without further sipper action, using the **Measure** button.*

6 Scan Application

The scan application is used to record sample spectra in a selectable wavelength range. The maximum wavelength range is determined by the working range of the spectrophotometer. An optional peak pick is available and it is possible to read single data via the graphics functions.

The recorded spectra are automatically saved to disk with the selected sample name as the filename and the extension *.dsp* in the data directory. The function **Save Spectrum** is used to store the spectra displayed in the graph in a different format and/or directory.

For later inspection or evaluation, spectra can be loaded into the graph via the **Load Spectrum** command in the **File** menu.

The parameters are summarized in the following groups:

- **Scan**
- **Spectrophotometer** including the buttons **Advanced Parameters** and **Accessory** (if applicable)
- **Graph**
- **Peak Pick**

The **Advanced Options** button allows defining additional non-standard parameters.

Note *If the active method is "write-protected", you can not change the parameters and all group titles are grayed out.*

6.1 Scan Parameter Group

The entry of **Start** and **End** wavelengths (nm) defines the spectral range to be scanned. The ordering is arbitrary.

Additionally you enter the **interval** (nm) as the data point distance. The maximal entry is 10 nm; the minimal entry depends on the instrument model and the selected scan speed (see *appendix*).

The wavelength range can automatically be adjusted by the system, if the scan range is not a multiple of the data interval.

6.2 Spectrophotometer Parameter Group

In the **Measurement mode** selection box you select whether data are recorded (and listed) in …

A	absorbance,
%T	% transmission
%R	% reflectance
%Ra	Absolute reflectance by square root calculation when using a VW accessory
f(R)	Kubelka-Munk transformation for quantitative reflectance measurements

%R and f(R) use the selected **Reflectance correction** files (see **Advanced Parameters**).

An additional entry field allows selecting the spectrometer **Scan speed** (nm/min). This parameter determines the time required for the spectrum. For small data intervals, the system may automatically select a compatible lower scan speed.

6.2.1 Advanced Parameters

The **Spectrophotometer** parameter group holds an additional button **Advanced Parameters**. This button provides access to additional instrument and measurement parameters.

The **Advanced Parameters** window holds the following parameters:

- Selection of the **Reflectance correction** files, see *appendix*.
  These files are necessary for transforming %R and f(R) mode readings online. The entry box is only active in the measurement modes %R and f(R).
- **Slit** – the slit width setting in nm (only for spectrophotometer with adjustable slit width).
- **Lamp change-**– Wavelength position for switching from UV to Vis Lamp (if applicable).

6.2.2 Accessory

With a sipper or a sipper/autosampler is connected to the instrument, you can setup the sipper or sipper/autosampler parameters via the **Accessory** window (see section *Cell Changer and Sipper Operation* and the *VISIONlite auto manual supplement*). The selected settings in this window will be saved with the method.

6.3 Graph Parameter Group

You define the graph scaling via the **Y-axis minimum** and **Y-axis maximum** entry boxes.
Note: Data exceeding the selected range are nevertheless recorded. During and after the measurement you can still adjust the ordinate scaling.

The selection buttons **Single/Overlay** define whether the data are overlaid in the graph or if the graph is cleared for each new curve.

6.4 Peak Pick Parameter Group

In this parameter group, you define if and how a peak pick is executed.

If neither of the **Maxima** or **Minima** control boxes is activated, no peak pick will be performed. You can select one or both options to detect a number of the maxima/minima of the spectra and list the respective values to a list in the results space.

The **Threshold** entry box defines the minimum height for an extremum to be detected as such. In this way, very small peaks due to noise can be eliminated. The value is given in respect to the selected ordinate mode.

Note *The **Peak Pick** button of the File Information window allows performing an offline peak detection. You can perform this peak picking after varying the respective parameters.*

Note *The peak list cannot be stored as a separate result file but is attached to the report.*

6.5 Advanced Options

The **Advanced Options** button will open the **Advanced Options** window. The Scan application **Advanced Options** window allows defining

- The parameter **Start Delay: Wait time** (seconds) is a time delay after triggering off the measurement by pressing the **Measure** button (see below) and before the actual start of the data acquisition. The count-down of the delay time is shown in a separate window. Use this parameter for equilibration of the sample before the measurement.
- **Repetitive measurement**
  The parameters **Repetitive Measurement**: **Total run time** and **Interval time** reflect the timing for automatically repeated measurement cycles for repetitive sample measurement. The **Interval time** is the time delay between two subsequent scan starts (seconds). The **Total run time** defines the number of measurement cycles. You can de-activate this feature by setting the **Total run time** to “0” (zero). If the **Time interval** is selected lower than feasible, the lowest possible time interval will be used automatically. Repetitive measurements can

generate a large number of spectra. The filenames of the spectra series are generated by adding an incremented number to the selected filename: *_001, *_002, etc. The file information window displays the number of the current cycle and, for replicates, the measurement time. After completion of the measurements, the **Sample information** window is not displayed again. This is because repetitive measurements are usually not performed in routine analysis.

All parameters are stored with the method.

6.6 Measurement and Data Handling

(For further information see section *Performing Measurements*.)
To run a measurement, select a stored method or enter new parameters.
***Note*:** If the active method is "write-protected", you can not change the parameters and all group titles are grayed out.

If you want to use the selected parameter settings for repeated use, save the method via the **Save Method** command in the **File** menu.

If a suitable baseline/autozero run has not yet been performed, perform a run in advance via the **Baseline** function (not in sipper and autosampler operation). Alternately you will be requested to perform a baseline run in the **Sample Information** window.
Important: The software demands a new autozero/baseline run, if a new measurement uses wavelengths outside the previous range. When only instrument settings like the slit or the lamp change have been modified, a new autozero/baseline run is recommended for high precision measurements.

Start the measurement via the **Measure Samples** button. Enter the sample data to the **Sample Information** window. Sample data for the following samples are entered just before measurement (not in autosampler operation). Alternatively, use the **Sample list** button to enter the sample data for an entire measurement series, and therefore generate a sample list. If a sample list already exists with the same name as the active method, the sample list will be loaded automatically and the data will be entered in the sample table.

Press the **Measure** button to start the measurement. To cancel the spectrum measurement, press the **Stop** button. This also cancels a sample list measurement sequence. The spectrum will be displayed with the wavelength range measured up to this moment; it is not however saved to disk automatically. If required, the recorded part of the spectrum can be saved via the **Save spectrum** command.

After completion the spectrum is stored to disk. A new **Sample Information** window is presented (not with repetitive measurements or sample lists) to define the next sample/sample list (or samples in cell changer mode). If you are using a sample list, the data of the next sample will be shown in the **Sample Information** window.

Terminate the series of measurements with the **Close** button in the **Sample Information** window.

After completing the series of measurements you may:
- Adapt the diagram axes and use the cursor to investigate spectral details,
- Use the **Print** button to print the diagram with results (if applicable),
- Use the **Print Preview** function to inspect the report, to print it or to copy it to the clipboard,

- Click on a spectrum filename in the file description box to open the **Spectrum Information** window. Among other things you can start a new peak pick, export the spectrum in a .csv data file or use the **Read Cursor** function to read spectrum data,
- Use the **Save Spectrum** command to store the spectrum with a new name or in a different format (.csv or JCAMP-DX) or directory,
- Highlight the results partly or completely and copy the highlighted text to the clipboard by simultaneously pressing the Ctrl+C keys. Thereafter, you can paste the text into other programs,

7 Rate Application

The Rate application is used to record time-dependent changes of the sample absorption at a specific wavelength. From this curve the sample enzyme activity can optionally be calculated via the slope of the curve. The slope is calculated by a linear least-squares fit.

The recorded data sets are automatically saved to disk with the selected sample name and the extension *.dti* in the data directory. The optional enzyme activity (rate) results are stored under the selected result filename with the extension *.rra* as a result text file. For later inspection or evaluation, data sets can be loaded into the graph by the **Load Data Set** command in the **File** menu. Existing rate results can be presented with the **Load results** command in the **File** menu.

The parameters are summarized in the following groups:

- **Rate**
- **Spectrophotometer** including the buttons **Advanced Parameters** and **Accessory** (if applicable)
- **Graph**
- **Rate calculation**

The **Advanced Options** button allows defining additional non-standard parameters.

Note *If the active method is "write-protected", you can not change the parameters and all group titles are grayed out.*

7.1 Rate Parameter Group

The general parameters you enter are the **Measurement time** (min), the **Data interval** (s) and the measurement **Wavelength** (nm). A reference wavelength can be defined additionally, see section *Advanced Options*. Measurement time and data interval determine the number of data points to be recorded.

The minimum data interval is determined by the spectrophotometer. For long-term measurements, the data interval must be selected so that not more than 100 000 data points are generated in a run.

Note *If the selected data interval is too small to measure all samples in cell changer mode, the lowest possible data interval will be used instead. This new interval will be stored with the method. It is also applied for subsequent measurement even if the measurement could be faster.*

Note *The collected data points are saved to disk every 10 minutes. If data storage is very slow, e.g. to floppy disk, exceeding of the data interval might cancel the run. If so, the selected data interval should take into account some seconds for disk operations.*

7.2 Spectrophotometer Parameter Group

In the **Measurement mode** selection box you select whether data are recorded in

A	absorbance,
%T	% transmission
%R	% reflectance
%Ra	Absolute reflectance by square root calculation when using a VW accessory
f(R)	Kubelka-Munk transformation for quantitative reflectance measurements

%R and f(R) use the selected **Reflectance correction** files (see **Advanced Parameters**).

It is important to select A (absorbance) for valid rate calculations.

The **Integration time** is a measurement parameter to reduce the signal noise. Increasing the integration time will further suppress signal noise, but might smoothen out possible fast signal changes.

7.2.1 Advanced Parameters

The **Spectrophotometer** group holds an additional button **Advanced Parameters**. This button provides access to additional instrument and measurement parameters.

- Selection of the **Reflectance correction** files, see *appendix*
  These files are necessary for the online transformation of readings in %R and f(R) ordinate mode. Therefore, this entry box is only active in the ordinate modes %R and f(R).
- **Slit** – the slit width setting in nm (only spectrophotometer with adjustable slit).
- **Lamp change -** Wavelength position at which the spectrophotometer switches from UV to Vis Lamp

7.2.2 Accessory

With a sipper or a sipper/autosampler is connected to the instrument, you can setup the sipper or sipper/autosampler parameters via the **Accessory** window (see section *Cell Changer and Sipper Operation* and the *VISIONlite auto manual supplement*). The selected settings in this window will be saved with the method.

7.3 Graph Parameter Group

You define the graph scaling via the **Y-axis minimum** and **Y-axis maximum** entry boxes.

Note *Data exceeding the selected range are nevertheless recorded. Scaling of the axes can be optimized during or after the measurement.*

7.4 Rate Calculation Parameter Group

To calculate the enzyme activity via a zero order rate calculation, the slope of the curve must be determined in the linear section of the curve. The slope is then multiplied by the **Enzyme factor** to obtain the enzyme activity value.

If an enzyme activity evaluation is requested, you enter the enzyme specific **Enzyme factor** – otherwise the entry field is left blank. The factor is entered as a negative value if absorbance decreases during the run. The linear range of the curve is selected via the **From** and **To** (min) entry fields or via the cursor (see below).
To define the output of results the number of **Output digits** before and after the decimals can be selected. Additionally you enter an enzyme **Unit** or select it from the list of units.

Note *Changing the unit will not change the calculation accordingly. Instead the enzyme factor must be adapted.*

To re-calculate the enzyme activity after the measurement, modify the **Rate calculation** settings and click on the **Recalculate** button. You can alternatively enter the values for the **From** and **To** entry fields by dragging the cursor with pressed left mouse key from the desired start to the end time of evaluation.

7.5 Advanced Options

The **Advanced Options** button will open the **Advanced Options** window. The Rate application **Advanced Options** window allows defining

- **Reference wavelength**
  The option **Reference Wavelength** allows defining a second wavelength to be read with each measurement cycle. Readings from the reference wavelength are subtracted online from the readings at the measurement wavelength and are not visible to the user. The data set description will contain the information reference value: xxx. The last reference wavelength reading will be inserted to allow an estimate of the reference level.
- The **Wait time** defines the start delay as the time between pressing the **Measure** button and the actual start of data recording. The countdown of the start delay is shown in a dedicated window. Use the **Wait time** for instance to equilibrate the sample before the data acquisition.
- In the parameter group **Result limits** the **High limit** and **Low limit** values can be optionally entered to mark enzyme activity results, which exceed or which fall below of the given entries. The numbers use the unit as entered with the **Rate Calculation** Parameter group. The corresponding **Output text** is applied for marking the results in question.
- With the **Output Options** you define, which of the statistical parameters (Intercept, Correlation coefficient, Residual) calculated for the linear lest-squares slope determination is output. Mark the parameters of interest.

All parameters are stored with the method.

7.6 Measurement and Data Handling

(For further information see section *Performing Measurements*.)
To run a measurement, select a stored method or enter new parameters.

Note *If the active method is "write-protected", you can not change the parameters and all group titles are grayed out.*

If you want to use the selected parameter settings for repeated use, save the method with the **Save Method** command.

If a suitable autozero run has not yet been performed, perform a run in advance via the **Autozero** function (not in sipper and autosampler operation). Alternately you will be requested to perform an autozero run in the **Sample Information** window.
Important: The software demands a new autozero/baseline run, if a new measurement uses wavelengths outside the previous range. When only instrument settings like the slit or the lamp change have been modified, a new autozero/baseline run is recommended for high precision measurements.

Note *With a cell changer, the first position of the cell changer is reserved for the autozero sample, if an adequate autozero has not yet been done beforehand. With this autozero mode, the software will perform an autozero with the autozero sample at each measurement cycle. This allows compensating for blank reactions.*

Start the measurement with the **Measure Samples** button. Enter the sample data to the **Sample Information** window and press the **Measure** button. Sample data for the following samples are entered just before measurement (not in autosampler operation). Alternatively, use the **Sample list** button to enter the sample data for an entire measurement series and therefore generate a sample list. If a sample list already exists under the same name as the active method, the sample list will be automatically loaded and the sample data will be entered in the sample table.

Note: If you select an existing result filename an overwrite query will be issued. If you accept overwriting at this point and select the same filename again, no further overwrite query will be shown.

Rate Application

The data acquisition is started by clicking on the **Measure** button. You may cancel the measurement by clicking on the **Stop** button at any time. After that, the already measured data will be displayed and stored to disk. The current measurement series will not be cancelled.

Note *During measurement all menu and button functions (except for the **Autoscale** button) are locked in order to maintain correct measurement timing.*

Note *The collected data points are saved to disk every 10 minutes to prevent total data loss in case of system failure.*

After each measurement, the data file and the result file are stored to disk. A new **Sample Information** window is presented to define the next sample (or samples in cell changer mode). Enter the sample data for the next sample/sample batch. Using a sample list, the sample data of the next sample in the sample list will be entered automatically. After completion of the measurement series, the **Sample information** window will not be shown again.

Terminate the series of measurements with the **Close** button in the **Sample Information** window.

Note: Enzyme activity is calculated for up to 200 runs recorded in a series. If a data set is loaded from disk, however, only the data shown in the graph are evaluated.

After completing the series of measurements you may:

- Adapt the diagram axes and use the cursor to investigate curve details (see *Graphics Functions*),
- Use **Table/Graph** toggle button to switch to a tabular display of data,
- Use the **Print Preview** function to inspect the report,
- Use the **Print** button or the **Print** menu command to print the diagram with results (if applicable),
- Recalculate the enzyme activity: modify the **Rate calculation** settings and click on the **Recalculate** button. Alternatively, define the new working range by moving the cursor with the left mouse button pressed. The results space is cleared and rewritten.
- Click on a curve filename to open the File Information window. Among other things you can copy or export the data set to other software (see *Graphics Functions*),
- Use the **Save data set** command to store the dataset with a new name or in a different format or directory,
- Highlight the results partly or completely and copy the highlighted text to the clipboard by simultaneously pressing the Ctrl+C keys. Paste the text into other programs.
- Export the optional results with the **Export results** command for use with other software. ***Note:*** The results table of the Rate application contains the parameters of regression in three additional data columns.

8 Fixed Application

The Fixed application records sample absorption data at single wavelengths. The data can be processed by means of simple functions (difference or ratio).
The recorded data and results are automatically saved to disk under the selected result filename with the extension *.rfx* as a text file in the data directory.
For later inspection or evaluation, data sets can be loaded into the **Print Preview** window via the **Load Results** command in the **File** menu.

The parameters are summarized in the following groups:

- **Test**
- **Spectrophotometer** with additional buttons: **Advanced Parameters** and **Accessory** (if applicable)

The **Advanced Options** button allows defining additional non-standard parameters.
***Note*:** If the active method is "write-protected", you cannot change the parameters and all group titles are grayed out.

8.1 Test Parameter Group

In this parameter group you select the measurement **Mode,** enter the measurement wavelengths into a table and define the unit and format of calculated results and the type of calculation.

The **Mode** options are:

- **Wavelength(s)** — no calculation
- **Wavelength difference** — f1 • WL1 – f2 • WL2
- **Wavelength difference with reference** (wavelength) — f1 • (WL1 –RefWL) – f2 • (WL2 –RefWL)
- **Wavelength ratio** — f1 • WL1 / WL2
- **Wavelength ratio with reference** (wavelength) — f1 • (WL1 –RefWL) / (WL2 –RefWL)
- **Wavelength(s) with factor** — fx • WLx

If no calculations are required, set the **Mode** to **Wavelength(s**). In this mode, you can enter up to 31 single wavelengths (less for some instruments), which are measured automatically in sequence.

Accordingly the **Mode Wavelength(s) with factor** measures at up to 31 wavelengths and will multiply each reading with a specific factor.

With the **Wavelength difference** and **Wavelength ratio** modes, you can enter 2 or 3 wavelengths into the table, which are designated as **WL1, WL2** and **RefWL** (reference wavelength). In addition, you can enter **factors f1** and **f2**, which extend the difference or ratio calculations as listed above. If no factors are required, enter a value of 1.

Output Digits and Unit

To define the output of results the number of **Output digits** before and after the decimals can be selected (see section *Results space*). You can enter the **Unit** or select it from the list of units.

Note *Changing the unit will not change the calculation accordingly. Instead, the factor(s) must be adapted.*

8.2 Spectrophotometer Parameter Group

In the **Measurement mode** selection box you select the following ordinate modes

A absorbance,

%T	% transmission
%R	% reflectance
%Ra	Absolute reflectance by square root calculation when using a VW accessory
f(R)	Kubelka-Munk transformation for quantitative reflectance measurements

%R and f(R) use the selected **Reflectance correction** files (see **Advanced Parameters**).

The **Integration time** is a measurement parameter to reduce the signal noise. Increasing the integration time will further suppress signal noise, but might smoothen out possible fast signal changes.

8.2.1 Advanced Parameters

The **Spectrophotometer** group holds an additional **Advanced Parameters** button to access extra spectrophotometer and measurement parameters:

- Selection of the **Reflectance correction** files, see *appendix*
  These files are necessary for online transformation of readings in %R and f(R) ordinate mode. Therefore, this entry box is only active in the ordinate modes %R and f(R).
- **Slit** – the slit width setting in nm (only spectrophotometers with adjustable slit)
- **Lamp change** – Wavelength position for the spectrophotometer to switch from UV to Vis Lamp

8.2.2 Accessory

With a sipper or a sipper/autosampler is connected to the instrument, you can setup the sipper or sipper/autosampler parameters via the **Accessory** window (see section *Cell Changer and Sipper Operation* and the *VISIONlite auto manual supplement*). The selected settings in this window will be saved with the method.

8.3 Advanced Options

The **Advanced Options** button will open the **Advanced Options** Window. The Fixed application **Advanced Options** window allows defining the following parameters.

- The **Wait time** defines the start delay as the time interval between pressing the **Measure** button (see below) and the actual start of data recording. The countdown of the start delay is shown in a dedicated window. Use the **Wait time** for instance to equilibrate the sample before the data acquisition.
- Parameters for repetitive measurement are the **Total run time** (minutes) and the **Interval time** (seconds) between consecutive measurement starts. The **Total run time** reflects the number of replicates. Entering „0“ for the **Total run time** deselects repetitive measurement. If the **Interval time** is selected lower than feasible, the lowest possible time interval will be used.
  The repetitive measurement option will add the measurement time to the sample name. Since repetitive measurement experiments generally are non-routine measurements, no new **Sample Information** window is shown after all measurements have been performed.
- In the parameter group **Result limits** the **High limit** and **Low limit** values can be optionally entered to mark results, which exceed or which fall below of the given entries. The numbers use the unit as entered with the **Test** group. The corresponding **Output text** is applied for marking the results in question.
  The **Result limits** option is not applicable for the Mode **Wavelength(s)**. For **Wavelength(s) with Factor** all results (reading x factor) will be checked against the entered limits. If one of the products is outside the limits, the appropriate output text is added.

All parameters are stored with the method.

8.4 Measurement and Data Handling

(For further information see section *Performing Measurements*.)
To run a measurement, select a stored method or enter new parameters.
***Note*:** If the active method is "write-protected", you can not change the parameters and all group titles are grayed out.

If you want to use the selected parameter settings for repeated use, save the method via the **Save Method** command.

If a suitable autozero run has not yet been performed, perform a run in advance via the **Autozero** function (not in sipper and autosampler operation). Alternately you will be requested to perform an autozero run in the Sample Information window.
Important: The software demands a new background correction/autozero, if a new measurement uses wavelengths other than the previous ones. When only instrument settings like the slit or the lamp change have been modified, a new background correction/autozero is recommended for high precision measurements.

Start the measurement via the **Measure Samples** button. Enter the sample data to the Sample Information window. Sample data for the following samples are entered just before measurement (not in autosampler operation). Alternatively, use the **Sample list** button to enter the sample data for an entire measurement sequence and generate a sample list. You may enter a dilution factor for each sample (not in **Wavelength(s)** mode). If a sample list already exists with the same name as the active method, the sample data will be automatically entered.

Note *The **Dilution Factor** entry is not applicable to the **Wavelength(s) Mode**.*

Note *If you select an existing result filename you will be asked, whether to add the new results to the existing file or overwrite the existing file. If you accept overwriting at this point and select the same filename again, no further overwrite query will be shown.*

Note *The results files will hold the results max. 1000 measurements.*

When pressing the **Measure** button the data recording will start. You can cancel the measurement by clicking on the **Stop** button. The report for the samples measured so far will be saved to disk and the measurement series will be terminated.

After each measurement, the result file is stored to disk. A new **Sample Information** window is presented (not with repetitive measurements or sample lists) to define the next sample (or samples in cell changer mode). When analysing a sample list, the sample data of the next sample will be entered in the data fields.

After completion of a sample list or repetitive measurement, the **Sample information** window is not displayed again.

Terminate the series of measurements with the **Close** button in the **Sample Information** window.

After completing the series of measurements you may:

- Use the **Print** function to print the results,
- Use the **Print Preview** function to inspect the report,
- Highlight the results partly or completely and copy the highlighted text to the clipboard by simultaneously pressing the Ctrl+C keys. Thereafter, paste the text into other programs,
- Export the results with the **Export results** command for use with other software.

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9 Quant Application

The Quant application is used to photometrically quantify sample components according to Beers law. The procedure includes the calibration of the analysis, either by entering a known calibration factor/linear curve parameters or by measuring a series of reference samples (**Standards)** to generate a calibration function. Once the calibration function has been generated, it can be used for repeated measurements (e.g. if the method is loaded again afterwards) – an optional re-calibration is still possible.

The calibration function can be stored in the method and re-loaded again with the method. Alternatively, the method can be stored without a calibration so that a new calibration must be performed for each batch of samples.

The default method *Test.mqa* does not yet include a calibration. Alternatively the method *TestCalibrated.mqa* can be used to exercise with a method including a calibration.

Normally, starting the Quant application or loading a Quant method opens the method in the **Samples Mode**. All entry fields are inactive in this mode. In order to create a new method, switch to the **Calibration Mode** (see below).
A method without an included calibration (see below) always starts in **Calibration Mode**.

The recorded readings and results are automatically saved to disk under the selected result filename with the extension *.rqa* as a text file in the data directory. For later inspection or evaluation, results can be loaded into the **Print Preview** window via the **Load Results** command in the **File** menu.

The parameters are summarized in the following groups:

- **Calibration** with **Reference** mode
- **Spectrophotometer** with additional buttons **Advanced Parameters** and **Accessory** (if applicable)

The **Advanced Options** button allows defining additional non-standard parameters.

Note *If the active method is "write-protected", you can not change the parameters and all group titles are grayed out.*

9.1 Samples and Calibration Mode

The Quant application uses two modes:

- **Calibration mode:** You use this mode to enter all basic parameters and the calibration data. You can either enter the calibration factor (if known) or measure a series of standards to determine the calibration function. Once calibration has been performed, switch to the sample mode with the **Go to Samples Mode** button.
- **Samples Mode:** You use this mode to measure the samples and generate the concentration results. The measurement and calibration parameters cannot be modified. However, it is possible to switch back to the calibration mode by clicking on the **Go to Calibration Mode** button.

9.2 Calibration Parameter Group

The basic settings are defined in the **Calibration parameter** group:

- **Curve type**, i.e. **calibration factor** entry, linear **curve parameters** entry or one of 4 calibration functions,
- **Measurement wavelength**,
- **Reference mode** with **Reference wavelength 1**/**2** and **Factor** (if applicable)

- Number of **Output digits** before and after the decimals for displaying the samples concentration results (see *section Results space*),
- **Unit** of standards and samples (as text). You can enter the **Unit** or select it from the list of units.
- Numerical value(s) of the **Calibration factor** or **Curve parameters**, if applicable.

Curve Type

If you select the **Curve Type** *Factor entry* and enter the calibration factor (F = c / Abs; c = F • Abs), no further calibration measurements are required and the **Measure Standards** button is inactive (grayed out). The same applies when you select the **Curve Type** *Curve parameters entry* and enter the values to the **Slope** and **Intercept** entry fields (Abs = Slope • c + Intercept).

With these options, the **Measure Standards** button is inactive and you can immediately switch to the **Samples Mode**.

Alternately, select one of the 4 calibration functions:

- **Linear through Zero** (at least 1 reference sample/standard)
- **Linear with Intercept** (at least 2 reference samples/standards)
- **Quadratic through Zero** (at least 2 reference samples/standards)
- **Quadratic with Intercept** (at least 3 reference samples/standards)

Quadratic calibration functions will be advantageous, if the calibration seems non-linear.

If you select a calibration function, the standards table opens up in the results space and you must set up a calibration (see below).

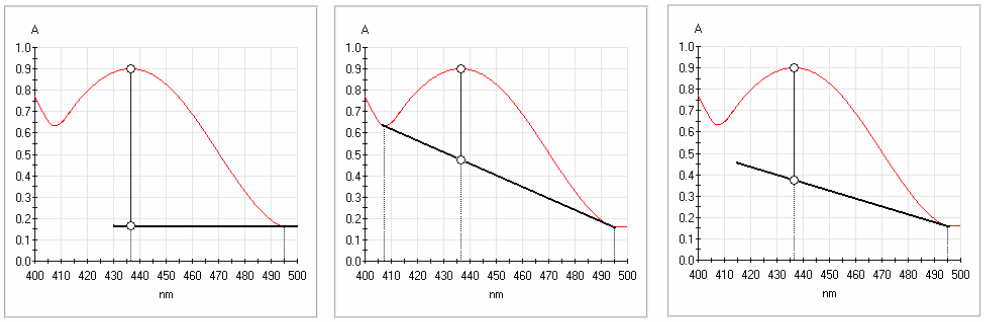
Measurement Wavelength and Reference Mode

Enter the desired analytical wavelength. This is typically the wavelength of the peak maximum.

Using the selection box **Reference mode**, 1 or 2 reference wavelength(s) can be defined besides the measurement wavelength to determine peak net-heights. Select one of the following options:

- **No Reference wavelength**
- **Single Reference wavelength**
- **Two Reference wavelengths**
- **Reference wavelength with Factor**

The **Reference wavelength**(s) serve to correct the reading at the measurement wavelength for an underlying matrix absorbance.
With a **Single reference wavelength** the reading at the selected wavelength is subtracted from the reading at the measurement wavelength.



Schematics of the 1- and 2 reference wavelength(s) subtraction mode and the reference wavelength with factor mode

With **Two reference wavelengths**, a linear connection is drawn between the two readings. Its value at the measurement wavelength is subtracted from the reading at the measurement wavelength.

In the reference mode **Reference wavelength with factor,** the absorbance value at the reference wavelength is multiplied with the entered factor and subtracted from the reference wavelength. Use this type of calculation, for correcting a linear background with known slope.

The readings at the reference wavelengths are taken in the same way as performed for Standards (reference samples) and unknown samples.

Output Format
To define the output of concentration results the number of **Output digits** before and after the decimals can be selected. You can enter the **Unit** or select it from the list of units. The selected **Unit** is also applied for the reference samples (standards).
Note: Changing the unit will not change the calculation accordingly. Instead, the calibration data must be adapted.

9.3 Spectrophotometer Parameter Group

The **Integration time** is a measurement parameter to reduce the signal noise. Increasing the integration time will further suppress signal noise, but might smoothen out possible fast signal changes.

All measurements are performed in absorbance.

9.3.1 Advanced Parameters

The additional **Advanced Parameters** button will open the **Advanced Parameters** window, holding further instrument and measurement parameters. All parameters are stored with the method.

The window holds the following parameters:

- Selection of the **Reflectance correction** files, see *appendix*
  These files are necessary for online transformation of readings in %R and f(R) ordinate mode. Therefore, the entry box is only active in the ordinate modes %R and f(R).
- **Slit** – the slit width setting in nm (only for spectrophotometer types with adjustable slit).

Lamp change – Wavelength position for the spectrophotometer to switches from UV to Vis lamp

9.3.2 Accessory

With a sipper or a sipper/autosampler is connected to the instrument, you can setup the sipper or sipper/autosampler parameters via the **Accessory** window (see section *Cell Changer and Sipper Operation* and the *VISIONlite auto manual supplement*). The selected settings in this window will be saved with the method.

9.4 Advanced Options

The **Advanced Options** button will open the **Advanced Options** Window. The Quant application **Advanced Options** window allows defining the following parameters:

- **Start Delay: Wait time**
  The **Wait time** defines the start delay as the time interval between pressing the **Measure** button (see below) and the actual start of data recording. The countdown of the start delay is shown in a dedicated window. Use the **Wait time** for instance to equilibrate the sample before the data acquisition.

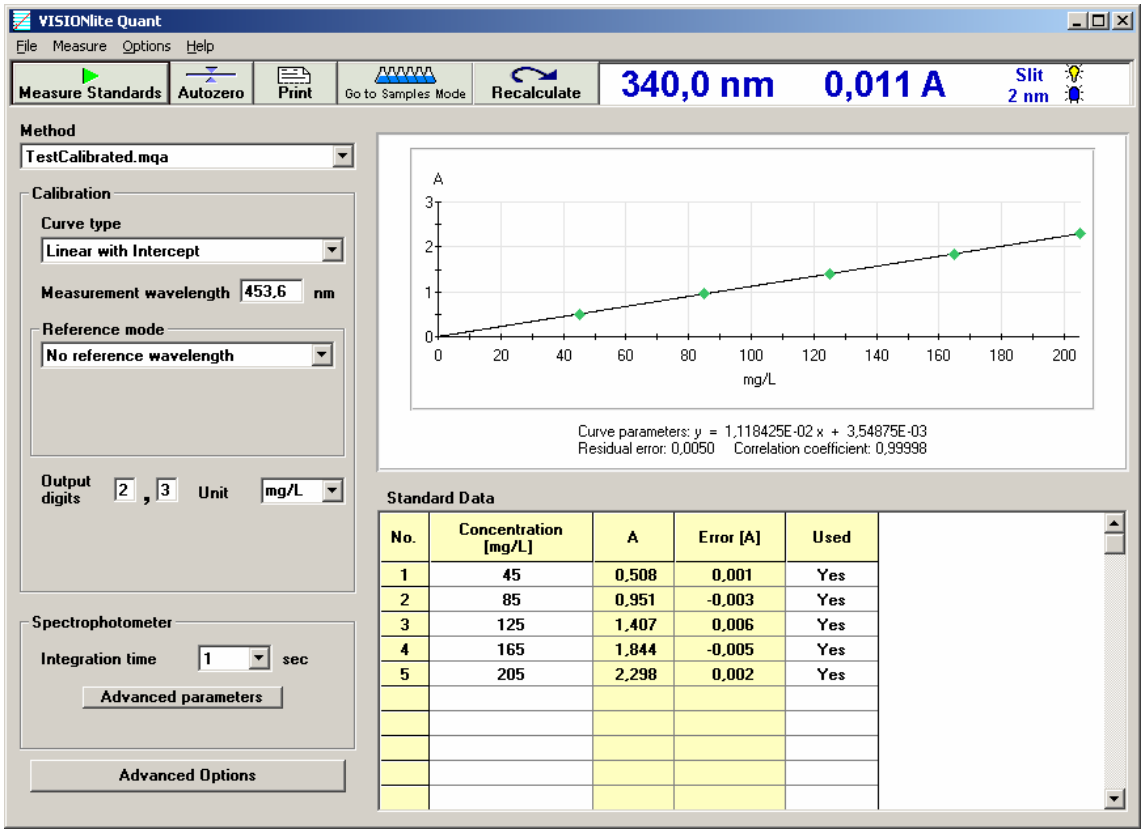
- **Correlation coefficient: Minimum** (only for autosampler operation with VISIONlite auto)
- **Result Limits: Limits, Output text**.
  High limit and **Low limit** values can be optionally entered to mark results, which exceed or which fall below of the given entries. The numbers use the unit as entered with the Calibration parameter group. The corresponding **Output text** is applied for marking the results in question.

All parameters are stored with the method.

9.5 Setting up a Calibration

If you select a calibration function, you must first measure a series of reference samples (standards) of known component concentration.

A standards table is opened in the results space in which you define the standards. You can make your entries in the white columns of the table. The yellow columns are filled by the system. Enter the concentration of your standards row-by-row. For multiple measurements of a standard enter the concentration repeatedly.



Quant Application in Calibration Mode

After you have entered all standards in the table, start the measurement of the standards with the **Measure Standards** button. You can modify the standard concentrations before the actual measurement in the **Sample information** window.

If there is no valid autozero value available, you can perform an autozero before measuring the standards. Otherwise, in the first **Sample information** window you will be prompted to perform an autozero.

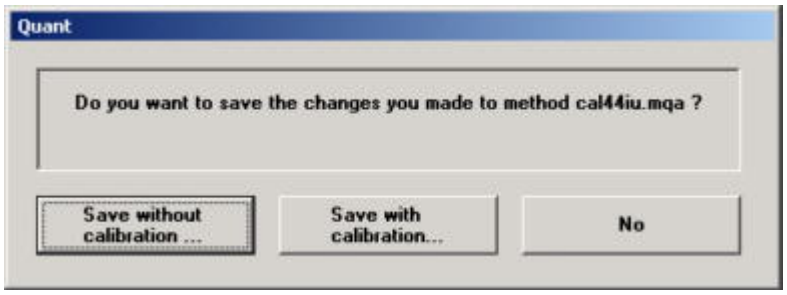
After all standards have been measured the software automatically calculates the calibration function by a least-squares regression algorithm. The calibration function is presented in the graph window together with the data points of the measured standards (green diamonds). The cursor and the graph expansion option are available to review the curve.

In addition the following coefficients are given:

- The mathematical function of the calibration curve with its calibration coefficients.
- The residual error of the curve fit. The value should be near zero (typically <0.01).
- The correlation coefficient of the fit. The value should be close to 1 (typically >0.99).

Important: If you change the measurement wavelength during or after calibration all calibration data will be erased.

If the calibration is satisfactory (otherwise see section *Recalculate a Calibration*), click the **Go to Samples Mode** button to proceed to the measurement of the unknown samples. The following window will appear (not for write-protected methods):



Result file overwrite query

- Press **Save without calibration** to store the method without calibration. This implies that with the next use of the method the calibration must be repeated.
- Press **Save with calibration** to store the method with the new calibration. This is equivalent to the **Save Method** menu command. A file selection window is displayed.
  With the calibration included in the method there is no need to measure the reference samples (standards) again for the next use of the method. Therefore the software will directly enter the samples mode in this case.
- Press **No** to advance to the samples mode without saving the method.

If the calibration is not yet satisfactory, you can recalculate the calibration with modified settings with the **Recalculate** button (see below).

9.5.1 Recalculate a Calibration

If the calibration is not yet satisfactory, you have the following options to re-run the calibration:

- Repeat measurements of all standards. To do so click on the **Measure Standards** button.
- Correct an obviously erroneous concentration value and start a recalculation by clicking on the **Recalculate** button.
- Remove an obviously erroneous standard from the standard set. To do so, click on the respective entry in the **Used** column. A selection box opens. Select **No** to deactivate the standard. Then start a recalculation by clicking on the **Recalculate** button. Eliminated standards remain in the table, are marked by a red cross in the graph, and are not included in the calculation. They can be re-activated as above by selecting **Yes**.
- Select another calibration function and re-calculate the calibration.
- Add a standard to the table. However, in this case all measured absorbance values are cleared and you must re-measure all standards.

- The last standard in the list can be deleted: Click on the concentration value and then open the **Delete** button with the right mouse key. Clicking on the button will remove the standard without query.

These options can be repeated as often as required. Verify the quality of the calibration via the statistics data of the calibration function.

After successful termination of the calibration procedure, click on the **Go to Samples Mode** button (see above) to measure the unknown samples.

9.5.2 Calibration in Autosampler Operation

Autosampler operation allows doing the calibration in a single run together with the samples measurement; see the VISIONlite auto manual supplement.

9.5.3 Protected Methods

If you are using a protected method (see section *Methods Concept*), the calibration mode is not available. Therefore you can only use a pre-defined calibration. If a re-calibration is necessary for a protected method, the required number of reference samples (**Standards**) must be entered each with zero concentration (0.0), stored to disk and set write-protected. Selecting this method for measurement will open the method in the calibration mode for measuring the pre-defined number of reference samples (**Standards**).

Start the data acquisition via the **Measure Standards** button and enter the concentration of the reference samples (**Standards**) in the **Sample information** window. There are some restrictions for entering data in the standards table. A belated modification of the calibration data is not possible, but you can re-measure the reference samples (**Standards**) without changing the concentration values.

When the **Measure sample** button was pressed once, the calibration mode is no longer accessible.

9.6 Measurement and Data Handling

(For further information see section *Performing Measurements*.)
To run a measurement, use the just calibrated method, select a stored method in the **Method** selection box or enter new parameters by changing to the calibration mode.

Note *If the active method is "write-protected", you can not change the parameters and all group titles are grayed out.*

If you want to use the selected parameter settings for repeated use, save the method via the **Save Method** command.

If a suitable autozero has not yet been performed, perform a run in advance via the **Autozero** function (not in sipper and autosampler operation). Alternately you will be requested to perform an autozero in the **Sample Information** Window.
Important: The software demands a new autozero, if a new measurement uses wavelengths other than the previous ones. When only instrument settings like the slit or the lamp change have been modified, a new autozero is recommended for high precision measurements.

If the calibration is not required or already existent, start the measurement via the **Measure Samples** button. Enter the sample data to the **Sample Information** window. Otherwise you have to set up a calibration beforehand as described above.
Sample data for the following samples are entered just before measurement (not in autosampler operation). Alternatively, use the **Sample list** button to enter the sample data for an entire measurement series and generate a sample list. If a sample list already exists with the same name as

the active method, the sample data will be automatically entered.

Note *If you select an existing result filename an overwrite query will be issued, whether to add the new results to the existing data file or overwrite the existing data file. If you accept overwriting at this point and select the same filename again, no further overwrite query will be shown.*

Note *The results files will hold the results max. 1000 measurements.*

Click on the **Measure** button to start the data acquisition. You can cancel the measurement by clicking on the **Stop** button. The report for the samples measured so far will be saved to disk and the measurement series will be terminated.

After each measurement, the result file is stored to disk. A new **Sample Information** window is presented to define the next sample (or samples in cell changer mode). Enter the sample data for the next sample/sample batch. When analysing a sample list, the sample data of the next sample will be entered in the data fields.
If a sample list or replicates have been measured, the **Sample information** window will not be shown again after measuring all pre-defined samples.

Terminate the series of measurements with the **Close** button in the **Sample Information** window.

The **Remeasure** option will load the sample entries of the previous sample to repeat the measurement.

After the series of measurements you can:

- Use the **Print** function to print the results together with calibration graph,
- Use the **Print Preview** function to inspect the report,
- Highlight the results partly or completely and copy the highlighted text to the clipboard by simultaneously pressing the Ctrl+C keys. Paste the text into other programs,
- Export the results via the **Export results** command for use with other software.
  Note: The results table contains the remarks for the sample (e. g. **Working range exceeded**) in an additional column.

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10 Advanced Options for Software Configuration

The software can be configured in various ways. Some of these options like print format, data and method directory are selected with the user interface. These settings are automatically stored with the software configuration file.
The user can modify other settings by editing the configuration file a*scapp.ini* or by extending the program call.

Note *Before modifying the configuration file make sure to have a copy. Also, close the software before editing the configuration file. Otherwise, the software will overwrite the modified file when closed.*

Adding additional Applications to the Start Routine

By editing the software configuration file ascapp.ini with the Windows Notepad text editor it is possible to extend the list of accessible applications and to open a specific method when selecting the application. Extend the *Configuration* block with a line such as

Application5=Name|Fixed.exe /method|.

This will add an entry *Name* to the list of applications in the start routine. Clicking on this item will start the Fixed application with the given method opened.

Define Storage and Export Format

The configuration file may hold information about the permanent data storage and export format, see the appendix section *Data Import und Export*.

Increased Number of Decimals with the Graph and Live Display Ordinate Values

When it is required to have 5 instead of 4 relevant figures displayed for the ordinate values with the cursor or peak-pick functions, add the parameter **|ordplus:1|** to the “ConfigOptions” line in the [Configuration] section of the configuration file. Transmittance data (%T) will then be displayed with 2 decimals, absorbance data with 4 decimals.

Keep Sample Information from Previous Sample

Within a series of samples, the sample description must be entered for each sample. To keep the sample information from the previous sample, use the **|KeepSampleInfo:1|** setting. Add the parameter to the “ConfigOptions” line in the [Configuration] section of the configuration file. The sample description can be modified nevertheless.

Switch off Automatic File Increment

Within a series of samples (not sample list) a sample name ending with a digit will automatically be incremented with the next sample. When this is unwanted, use the **|NameIncrement:0|** setting. Add the parameter to the “ConfigOptions” line in the [Configuration] section of the configuration file. With this parameter the sample name entry box will be empty for the next sample.

Export Format for Rate Datasets

The abscissa values (time scale) in Rate datasets will be given in decimal numbers. If you wish to output the time values in time format hh:mm:ss (corresponding to former versions of the software), then in the configuration file in the [Configuration] group add the option **Exportformat:8 t**o the “ConfigOptions” line. If this option is already included, then add the new value to the existing value.

Switch off mandatory Baseline Correction

Usually a background correction run must be performed after the software has been started or after extension of the wavelength range. This is good laboratory practice and it is required for accurate and reliable results. When measurements should become possible without a prior background correction run, e.g. to check the instrument, use the **|NoBlank:1|** setting. Add the parameter to the

"ConfigOptions" line in the [Configuration] section f the configuration file. If set, the background correction run is not requested mandatory.

Number of Cell Changer Positions

When a cell changer is detected with the instrument, the sample information window offers as many sample lines, as positions are available with the cell changer. It is possible to reduce the number of used cell positions with the **/MaxCellx** parameter. x is the number of active cell changer positions. A call of the software can be extended by adding the name of a method, separated by the "/" character. The command is accessible via Properties/Link/Target. For example the command

c:\program files\VL MaterialsCalc\VL_MaterialsCalc.exe /MaxCell2

will deactivate the cell changer and will offer only cell position 2 for measurements.

With x=1 the cell changer is driven to position 1 for each measurement. A sample list can be entered. For x=0 the cell changer remains on its current position.

Main Window Header

If an additional information text is to be placed in the title bar of the main window, e.g. the instrument identification number, this information is added to the command line of the start menu command or desktop icon in square brackets. The command is accessible via Properties/Link/Target. Use for example:

C:\Program Files\ VISIONlite \ VISIONlite.exe [Instrument 20 A]

Software Start

A call of the software can be extended by adding the name of a method, separated by the "/" character. The command is accessible via Properties/Link/Target. For example the command

c:\Program Files\VISIONlite\VISIONlite.exe /test1.msc

will start the software with the method test1 and will proceed to the sample information entry. The desktop may include several software icons with different definitions.

The following, additional options are available for routine operation:

/export	Generates a csv-spectrum export file, if at least one sample has been measured.
/print	Prints the report automatically, if at least one sample has been measured.
/terminate	The software is closed automatically after closing the sample series.

For example the call

c:\Program Files\VISIONlite\VISIONlite.exe /test1.msc /print /terminate

will start the method test1, configure the spectrophotometer and open the sample information window. After the sample series, the report is printed and the software is closed.

Appendix
Notes for specific Instrument Models

Information for Specific Spectrophotometer Models
GENESYS 6, GENESYS 10 Vis/ 10 Bio/ 10 UV/ 10 UV*scanning*, BioMate 3, Evolution 60

These spectrophotometers are non-recording and recording single-beam and split-beam instruments. Instruments with an older firmware must be updated. Please contact you sales representative if the software shows a respective error message.

Caution: The instrument keyboard cannot be switched off. As long as the instrument is under PC control the instrument keyboard should not be used. Otherwise the system may crash and erroneous data may result.
Caution: If the instrument does not display the main screen (but e.g. the utility screen) the software may partly malfunction.

Note *When initializing the instrument perform a wavelength calibration at 270 nm. If an absorbing sample or a plastic cuvette is left in the sample position the calibration may not work.*

Error Messages
When you start the software and the instrument is not connected to the proper COM interface or is not yet switched on or is already in the initialization phase, the error message will be No response from instrument on device comx.

Display and Standby
When the software controls the instrument, the instrument display shows the message
Instrument is in Remote Mode
Press ESC to cancel.

During spectrum scanning the software live display and the graph remain blank since the data are transferred to the software only when the scan is finished. The software messages the scan or the baseline correction run with an appropriate window. After the end of the scan the graph is displayed and the live display is reactivated.

If the instrument is configured with a lamp saving standby time and if no measurements are done for 5 minutes, the software will quit this communication. After the selected standby time the instrument itself then will go into the standby mode and will shut down the lamp.

Autozero/Baseline
An autozero/baseline run is required whenever the scan range or the data interval is changed. An autozero/baseline run is also required even if the data interval is a multiple of the previous value, or if the scan range is a restricted section of the previous range. Even though some instruments accept this, GENESYS 6 and compatible instruments return an error message.

Specific Parameters
The scan speed of GENESYS 6 is selected in three levels. With a 1 nm data interval, these levels correspond to:

- Slow approx. 100 nm/min
- Medium approx. 200 nm/min
- Fast approx. 400 nm/min

The baseline correction run is always done with the lowest possible scan speed.
The other models of this group do not offer a choice of scan speeds.

A scan must cover at least 5 data points. The scan range cannot exceed 101 data points for the models GENESYS 10 and compatibles.

Cell Changer
GENESYS 6 and compatibles are equipped with a 6-cell changer. The first position of the cell changer **B** is reserved solely for the blank and cannot be used for sample measurements. An autozero is always performed at this position.

An alternative cell holder (Auto-3) is offered to permit the use of long-path cells. You can define use of this holder in the **Preferences** command. This setting is valid for all applications.

Note *After installing or removing the cell changer it is necessary to restart the software so that the new configuration can be detected.*

GENESYS 20, Helios Epsilon

GENESYS 20 and compatibles are non-scanning, single-beam spectrophotometers. With the VISION*lite* software scanning and multi-wavelength measurements are made possible. However some limitations apply:

- Scanning data should only be used to detect peak positions. The wavelength range should be limited to 100 nm.
- When performing **Fixed** and **Rate** measurements, an autozero should be done immediately before the measurement is started.

Note *The instrument and the software displays may display deviating values. This is because the instrument display presents uncorrected values, whereas the software display presents corrected readings if possible.*

Error Messages
When you start the software an error message **Instrument offline** will be displayed, if the instrument has not finalized its initialization.

Autozero/Baseline
As non-scanning instruments, they do not perform an automatic baseline scan or store baseline correction data. Therefore, the software must drive the instrument to each data collection point individually. The scan speed that can be attained is therefore only approx. 40 nm/min at a 1 nm data interval. Smaller intervals are not reasonable since the spectral bandpass (8 nm) is distinctly larger.

For baseline correction the software actuates an autozero at the scan start wavelength and then collects the correction data. With a larger scan range, it may be possible that the dynamic range is exceeded. If so the entire correction run is repeated. Possible instrument error messages due to low signals are ignored.

During the baseline run only the wavelength is shown in the live display.

Scanning
Data can become unreliable at larger signal differences in the scan range. Therefore, it is recommended to scan only smaller ranges, i.e. 100 nm.

Evolution 100/300/600, UV 1/3xx/5xx, Helios Alpha/ Beta/ Gamma/ Delta, BioMate 5, AquaMate

Evolution 100/300/600, UV 1, Helios Alpha and BioMate 5 are scanning double-beam instruments for the UV/Vis or Vis ranges. The others are single-beam instruments. They can be equipped with a 7/8-cell changer (not double 7-cell changer with Evolution 300/600) and/or a sipper (Smart, Mini or Multi Sipper).

Important: For establishing communication between software and instrument it is necessary to switch the instrument to REMOTE mode. You do this by pressing the REMOTE key at the spectrophotometer (right-hand key under the software-defined keys). If the instrument is not in REMOTE mode, the software will present the error message **No response from instrument**. During REMOTE mode the keyboard is locked and the instrument's LC display only indicates REMOTE mode operation. To switch back to normal instrument operation, first exit the software and then press the HOME key.

Note *After installing or removing an accessory it is necessary to restart the software so that the new configuration is detected.*

Note *If the cell changer is not detected, it might be that the instrument is configured such that the cell changer is not active.*

Note *If a measurement has been interrupted, an immediate start of a new measurement will return an instrument error. This error reports that the instrument is still busy.*

Caution: During measurements, the sample compartment cover must not be opened. Otherwise the communication will be interrupted with an error message.

Error Messages

When starting the software during initialization the error message:

Instrument is initializing please wait is presented.

Specific Parameters

Evolution 100 and compatibles allow a number of further settings compared to the other models. For measurements at fixed wavelengths (Rate, Fixed and Quant applications) an integration time can be entered. This entry determines the time over which the signal is averaged to improve its signal-to-noise ratio. High integration times however increase the measurement time. Especially when using the cell changer the measurement time is substantially increased.

The **Options/Manual Control** command allows you to switch the light sources with Evolution 100 and compatibles, which user two light sources. An entry in any one application applies to all other applications. If, however, a specific light source is required for a measurement, the necessary source is switched on automatically. The software's live display continuously shows the current lamp status.

Scan speeds can be selected up to 3800 nm/min. If a fast scan speed has been selected together with a smaller data interval, an appropriate slower scan speed is used automatically.

A scan speed of **0** nm/min can also be selected. This setting corresponds to the *IntelliScan* mode, where the scan speed is automatically optimized to maintain a constant noise ratio.

Some other models compatible to the Evolution 100 include a selectable spectral bandpass. The **Slit** setting can be selected in the **Advanced Parameters** window.

The Fixed mode allows you to enter up to 20 single wavelengths.

Sipper Accessory for Evolution 100 and Compatibles

The instrument series can be equipped with a sipper accessory, which includes a peristaltic pump and a flow-through cell. The sipper increases the sample throughput by automated sample aspiration.

Note *When the instrument is equipped with both a cell changer and a sipper, the software will give priority to the sipper. When the cell changer is going to be used, disconnect the sipper cable.*

RS-232C Cable PC-Spectrophotometer

GENESYS 6/10/20, Helios Epsilon, BioMate 3, Evolution 60	1:1, 9pin female/9 pin female, P. No 335942000
Evolution 100/300/600, UV1/ 3xx/ 5xx, Helios Alpha/ Beta/ Gamma/ Delta, BioMate 5, AquaMate	Null Modem, 9-pin female/9-pin female, P.No. 4013 172 82111

Data Formats and Data Import/Export

File Extensions

Various file types are generated and used by the software. The three-character filename extensions allow you to distinguish the various file types. The filenames can be of any length. All data and results files are contained in the data directory, while all method files are contained in the methods directory.

Scan method file	xx.msc
Rate method file	xx.mra
Fixed method file	xx.mfx
Quant method file	xx.mqa
Scan data file (spectrum)	xx.dsp
Rate data file (kinetic data set)	xx.dti
Rate result file	xx.rra
Fixed result file	xx.rfx
Quant result file	xx.rqa
Export file (Scan and Rate data sets; Rate/Fixed/Quant results)	xx.csv
JCAMP spectrum file	xx.dx
Scan sample list file	xx.lsc
Rate sample list file	xx.lra
Fixed sample list file	xx.lfx
Quant sample list file	xx.lqa
General sample list file	xx.lst
Rack definition file (autosampler operation with VISIONlite auto only)	xx.rck

The software registers itself with its method and data file types (not result files). Thus it is possible to start the application modules with a double click on a method or a data file. Links to these files can be placed on the desktop for an immediate start of the application modules with a standard method or data file. Because of Windows settings this is not possible for Scan methods.

Save and Open Spectra and Datasets

Usually, spectra and Rate datasets are stored to disk in the specific format of the software with the filename extensions .dsp or .dti.

Use the **Export** feature in the Info window to store data in MS Excel® compatible .csv data format. Additionally, it is possible to open spectra via the **Load Spectrum** command and thereafter save the spectra via the **Save Spectrum as** command in JCAMP-DX or .csv data format. The **Load Spectrum** command accepts simple data lists too.

Additionally, it is possible to store spectra automatically in .csv or .dx data format (see below).

Note *It is possible, that JCAMP spectra from different data sources can not be loaded, because incompatible file format versions or compression algorithms are used.*

Note *All information on the instrument parameters are lost, if a dataset is stored in .csv or .dx data format. Only the VISIONlite data format (.dsp) will retain this information.*

Data Format

Each spectral file or Rate data file includes only one spectrum/dataset. The corresponding .dsp and .dti files are text files with a file header holding some general parameters: instrument settings, date/time of measurement, spectrophotometer details etc. The section holding the readings follows the header.
The results files (extensions: .rra, .rfx and .rqa) are text files as well.

Format of csv-Files

The separator for the data values is the list separator as defined in your Windows regional settings in the control panel. If undefined, elements of the table are separated by a semicolon. The csv-files as generated by the Export functions are Microsoft Excel® compatible text files. The decimal separator as well follows the regional settings.
The first line of spectra .csv files holds basic information on the spectrum. The second line holds the column header of the table. You can save the files without this file header. To do this, in the configuration file add *ExportFormat:2*| to the line *ConfigOptions* in the *[Configuration]* group.

csv-files generated by the Rate application include all statistical parameters (regression data). csv-files generated by the Quant application have an additional error column, which holds information like “working range exceeded”. The **Advanced options** low/high limit messages are not included with the csv-files.

JCAMP Data File Format

The JCAMP text format used by the software consists of a header and a data table. The header contains various general information designated by a leading ##, like

##TITLE= Azorubin, 0.9384mg/100ml
##JCAMP-DX= 4.24
##DATA TYPE= UV/VIS SPECTRUM

As far as possible, this information is taken over in the assigned spectrum information. Other parameters are taken from the **Audit Trail** block of the spectrum.

The data section follows after the factors for abscissa and ordinate values and the internal format definition. Each line includes an abscissa value and the following ordinate values. For instance:

```
##XFACTOR= 1.00
##YFACTOR= 0.00000011920928955078
##XYDATA= (X++(Y..Y))
  700.000000   11743   11743   11743   11743   11743
  695.000000   11743   11743   11743   11743   11743
  690.000000   11743   11743   11743   11743   11743
.....
##END=
```

In the configuration file you can pre-set the number of ordinate values per line. This pre-selection will be used, when storing spectra in JCAMP-DX data format. The default setting is 1 ordinate value per line. To modify this value, in the configuration file *ascapp.ini* in the *[Configuration]* section add |*DXColumns:*n| to the *ConfigOptions* parameter, where n is the required number of ordinate values per line. The entry must be separated from the other entries by the pipe character “|” and also end with this character.

The format may contain a series of spectra. According to JCAMP 4.24 definitions, this is defined by the line

##BLOCKS=n (n: number of following, complete datasets)

at the beginning of the file. UVWinLab does not support this option.

Automatically Saving and Reading Spectra in .csv or JCAMP Format

Besides the special VISIONlite file format, the **Export** and **Save as** commands in the file information window can store spectra in the .csv or JCAMP format.

When spectra generally are to be stored as .csv or JCAMP files, the configuration file *ascapp.ini* in the *[Configuration]* section has to be modified: Add the line

SP_EXT=.xyz (xyz = „csv“ or „dx“)

The JCAMP format is according to the JCAMP-DX 4.24 definitions and can be imported by other software packages. It is possible to read JCAMP datasets via the **Load Spectrum** or **Load Dataset** commands of the software as well.

Note *When saving spectra in the csv- or in the dx-format, information about the spectrum like instrument parameters is lost. This information is only maintained with the proprietary dsp-format.*

Pre-Selecting Filename Extensions for Exporting Spectra/Datasets

JCAMP and .csv files are normally stored to disk, using the characteristic filename extensions .dx or .csv. Alternatively, other extensions can be pre-selected in the configuration file. To modify this default, in the configuration file *ascapp.ini* in the *[Configuration]* section set the option *JCAMPEXT:.xxx* or *EXPORTEXT:.xxx* in the *ConfigOptions* entry. Thus, .xxx will be used permanently as filename extension for exporting spectra/datasets. The entry must be separated from the other entries by the pipe character “|”.

Using an External Converter for Spectra

It is possible to use further data formats within the software. For that, external routines for spectrum conversion can be added. These must be executable program files, stored in the program directory of the software under the file name SPC_CONV_xxx_yyy_zzz.exe, where xxx, yyy and zzz have the following meaning:

xxx the required process: R (Read); W (Write/Save as); S (Save/automatic storage) (multiple entries are possible)
yyy the name of the external file type
zzz the existing or requested filename extension

You find the routine *SPC_CONV_W_UVDM_SP.exe* as an example in the directory **\Tools** on the program CD. This routine provides the UVDM format as an additional data format for data storage via the **Save as** command. Contact ***ascanis***, if you intend to use this feature for other data formats.

Import of Data Files

VISIONlite can import different spectrum file formats.

Appendix

Software/source	File extension
OptLab-SPX spectra	.dsp
OptLab-SPX Export spectral data	.csv
JCAMP spectrum generator, format as UVWinLab (version 2.0-3.0)	.sp, .dx, .jcm
Various sources, e.g.. MS Excel, tabular format (see below)	Any, e.g. .txt

Import of tabular formats

When importing tabular data OptLab-SPX requires data with the following structure:

- Up to 3 header lines with the sample description and abscissa/ordinate units
- Table of abscissa/ordinate data pairs

Such a text format is typical for ASCII data storage in the txt or csv-format of Microsoft Excel.

If the last of the header lines contains the list separation character (see below), the information before and after that character is interpreted as the name of the abscissa and ordinate units, e.g. nm;%T. The other header lines are interpreted as sample description. Header lines are recognized by the fact that the first character is not a figure.

The list separation character is expected to be as set in the Windows configurations. Does this standard list separator character not appear, a comma, tab or space is searched. The list separator is recognized by occurring only once in a data line.

The decimal character also is expected to be a set in the Windows settings. If numbers are not recognized other decimal characters are used.

When a user repeatedly imports data file with its specific file extension, it is possible to include this file extension in the list of default extensions offered by the Load Spectrum command. This is defined by adding the following line to the Configuration parameter group in the ascapp.ini file:

```
[Configuration]
SP_EXT = .xyz                (.xyz:  specific file extension)
```

As an example for the import of tabular data, spectra generated by the Dataprint function of the Shimadzu® UVProbe software.

```
"Storage 151148 5GI205 - RawData -
D:\Applikationen\Hilfe\File_040622_R.spc"
"Wavelength nm.","R%"
190.00,6.758
192.00,6.671
194.00,6.532
196.00,6.421
```

and so on.

Handling of Reflectance Measurements

In order to measure reflectance data, the spectrophotometer must be equipped with a dedicated reflectance accessory like an integrating sphere or a specular reflectance accessory. In this case, the **Measurement Mode** is selected as one of the reflectance modes. The software will still read %T data from the spectrophotometer, but will correct the readings online and will apply the selected ordinate mode.

For the available reflectance measurement modes, the software performs the following calculations:

- **%R** correction for the white standard and the dark value
- **%Ra** calculates the square root (no white and dark value correction)
- **f(R)** corrected for the white standard and the dark value and transformation according to the Kubelka-Munk function

The white standard correction is necessary since the baseline run assumes that the white standard reflects 100 %. However, in practice a white standard typically has an absolute reflectance value in the range of 90 – 99 %R. Therefore all untreated readings are too high. This discrepancy is taken into account by simply multiplying the raw data by the absolute reflectance data of the white standard. The spectrum used for this calculation must contain the absolute white standard reflectance data for the whole wavelength range covered by the sample spectrum. These absolute white standard reflectance data cannot be measured on the system. Vendors of white standard materials typically will supply these data with the material.

The necessity for the dark correction is based on the fact that due to the construction of the spectrometer a small portion of the measurement light will be detected without striking the sample. Thus all samples will display slightly too high reflectance values. The dark correction values can be identified by measuring an empty sample position after baseline correction. With proper instrument alignment, dark correction readings should not be higher than a few %R.
White and dark correction procedures are especially important if absolute sample reflectance values must be generated, for example for color evaluations and if very high or very low reflecting samples are to be examined.
The described corrections are irrelevant if only qualitative measurements are performed, e.g. to compare two samples or to determine absorbance band positions.

The software includes example system files for white and dark correction. The files are csv-files (comma separated values) as created by Microsoft Excel ® resident in the software's program directory:

- White correction files xxx.*R100.csv*
- Dark correction files xxx.*R0.csv*

The following sets of reflectance correction spectra are installed:

- xxx = Null (200–2000 nm/ 10 nm data interval)
  with a value of 100 %R for the white correction and with a value of 0 %R for the dark correction. Obviously this set does not modify the raw data.
- xxx = Spectralon (200–2500 nm/ 5 nm data interval)
  with a typical reflectance curve of Spectralon ® for the white correction and 0,2 %R for the dark correction.
- xxx = BASO4 (380-780 nm/ 1 nm data interval)
  with a typical reflectance curve of $BaSO_4$ white reflective material for the white correction and 0,2 %R for the dark correction.
- xxx = Al mirr (250-2500 nm/ 10 nm data interval)
  with a typical reflectance spectrum of a front-surface aluminum mirror with a thin MgF_2 coating and 0,2 %R for the dark correction.

The default setting for the %R mode is no correction by applying the *Null* set of reflectance correction spectra.

If you want to use the white and dark correction of reflectance measurements, you must adapt these files according to the specific spectrometer and white standard in use or create new files following the scheme. Use the Windows Editor or MS Excel to edit the data in the files. See the appendix for further information about the format of csv files.

- Enter the absolute reflectance data of your white standard to the xxx.R100.csv file.
- Enter the dark value readings as measured with an empty reflectance sample position into the xxx.R0.csv file. You also may use the measured spectrum directly by storing it as a csv file with the appropriate name to the program directory.

The set of white and dark correction files will appear in the **Reflectance Correction** selection box (Advanced Options window), if defined properly. Select the correction set as required for a method.

Note *The files used for white and dark correction must cover the total wavelength range of the sample spectra. If not, it is not possible to record the data in %R mode.*
The data interval is correspondingly interpolated.

Note *For every corrected spectrum, the software registers when and with which data the reflectance correction was performed. You can inspect this information in the* File Information Window *under* Audit trail*. If you create your own xxx.R0 and xxx.R100 files, you should include a detailed commentary in the spectrum description so that the correction is traceable.*

Problem Solving

Error Messages

Error messages generally are self-explanatory and given in English. The software or operation system can generate the following messages:

Error message: Instrument offline.
Instrument not switched on; not in remote mode or at initialization. Cable to PC loose.

Error message: No response from instrument on COMx
Probably, the instrument interface is not configured correctly for PC remote control

Error message: Path/File access error.
File probably write-protected.

Error message: Internal error retrieving device control block for the port (cmdsend).
PC probably was in stand-by mode before.

Error message: Error accessing the spectrophotometer via com port n: port already open.
Probably another application module already has communication with spectrophotometer.

Precision of calculations

The software performs all calculations with high precision with data as recorded from the spectrophotometer. For presentation data and results are shown as rounded figures with a limited number of decimals. In some cases, therefore there may be deviation of the results shown to those results calculated with the presented data. The number of decimals for the results may be defined in the method.
Note: Data are generally presented with 4 significant figures, that is 3 figures after the decimal for A (absorbance) data and 1 figure for %T (%transmission) data. It can however be selected to show all data with an additional figure (see section *Software configuration*).

Appendix

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