NANOCOLOR® PC-Software

for

Spectrophotometer

Version 4 (October 2010)

Ref. 4.1

Manual

Dear Customer,

Thank you for choosing to purchase our photometer ***NANOCOLOR***® .Spectrophotometers You are now in possession of a powerful spectrophotometer for use in the wavelength range of 190 nm to 1100 nm. The ***NANOCOLOR***® PC Software for Spectrophotometers has been developed to facilitate working with your new device and enable optimum use of all the photometer's functionalities.

Please read these software instructions carefully.

The ***NANOCOLOR***® **PC** Software for Spectrophotometers essentially performs the following tasks:

- Reading out the ***NANOCOLOR***® test data from the photometer memory
- Online saving of ***NANOCOLOR***® test data
- Exporting ***NANOCOLOR***® test data
- Creating special photometric methods
- Complete control of the photometer
- Management and analysis of wavelength scans
- Testing the photometer
- Performing special applications (e. g. colour measurement)
- Control the ***NANOCOLOR***® Autosampler AS 53

If you are already familiar with other photometers in the ***NANOCOLOR***® series or have experience with the ***NANOCOLOR***® data export software, several of the functions here will also be familiar. Since the facilities of the ***NANOCOLOR***® Spectrophotometers far exceed the older ***NANOCOLOR***® photometer version, the software is also considerably more powerful. The software manual is divided into three sections. Part 1 describes the "classic" ***NANOCOLOR***® software functions; Part 2 describes the creation of wavelength scans and control of the photometer. In Part 3, the software settings are explained.

To perform the special applications please refer to the following manual:

Instructions for Colour Measurement, Software Manual Addendum I

Instructions for Brewery Analysis, Software Manual Addendum II

Instructions for Enzymatic Tests, Software Manual Addendum III

***NANOCOLOR*®** Autosampler AS 53, Software Manual Addendum IV

This manual uses a few typographical conventions in the description of the ***NANOCOLOR*®** PC Software for Spectrophotometers program and how it is used in conjunction with your photometer. These conventions are described below.

Keys or buttons that should be pressed on the computer keyboard or the photometer touchscreen, for example the **[ENTER]** key, are printed in bold face upper-case letters and enclosed in square brackets.

Program or data directories and file names are printed in *italics*, e.g. the default program directory, *c:\programs\macherey_nagel\uvvis*.

Other softwares that are required for operating the ***NANOCOLOR*®** PC Software for Spectrophotometers program or that can be used in conjunction with the software, for example, MS EXCEL, are printed in upper case letters.

Buttons, screen keys, option buttons and text entry boxes in the ***NANOCOLOR*®** PC Software for Spectrophotometers program, such as for example, the *Open* button, are printed in italics and surrounded by a box frame. The majority of the software buttons are shown graphically.

In this case, the relevant button is displayed as a picture such as:  .

Menu commands, such as ***Scan/Open*** in the ***NANOCOLOR*® PC Software for Spectrophotometers** program are printed in italics and with a grey background.

Important notices are identified by a yellow triangle. 

1 Installation

1.1 System Requirements

The ***NANOCOLOR*®** PC Software for Spectrophotometers program requires **at least** a Pentium 4 / Athlon XP processor, 100 MB free space on the hard disk, 256 MB RAM (512 MB or more is recommended) running under Windows® XP or higher. The Microsoft **.NET Framework V 2.0** program must be installed on the system, including all service packs. The VGA resolution should be at least 1024 * 768 pixels. The computer requires one free serial RS-232 port or one free USB port.

For creating MS EXCEL worksheets, ***Microsoft OFFICE 2003/2007/(2010*** or higher is also required. A DVD ROM drive is required for installing the program. OpenOffice is supported from version 3.2.

OFFICE 2003/2007/2010/OPENOFFICE **are not part of this software!**

No support can be guaranteed when the program is installed on other operating systems!

When using a USB port, the USB driver <u>and</u> the Virtual-Com driver supplied must be installed.

Administrator access rights are required for the installation.

1.2 Installing the Software

Insert the installation DVD in the drive on your computer and open the file *setup.exe*. By default, the ***NANOCOLOR*®** PC Software for Spectrophotometers software is installed in the program directory on your computer (usually *C:\Programs*) in the sub-directory *MACHEREY-NAGEL\uvvis*. To ensure problem-free support, MACHEREY-NAGEL strongly recommends that the default installation directory is not changed.

With the installation and during the initial start of the software, the following sub-directories are created in the installation directory:

uvvis\beer	Contains the calibration files of brewery analysis measurements
uvvis\bio	Contains bio analysis measurements
uvvis\cielab	Contains the files *cielab.xml, cielabref.xml, color.txt, custom_radiation.txt, hazen.xml, iod.xml, munsell.xml, pheur.xml* and *us_color.xml*. Necessary for color measurement
uvvis\database	Contains the XML database for photometer memory data
uvvis\enzymes	Contains the file uvtest.xml holding all the names and test data of the enzymatic tests
uvvis\contract	Contains the software licence agreement as an RTF file and the legal disclaimer as a TXT file in various languages
uvvis\errorlog	Contains the files *error.log* and *error.crp* where information is saved regarding any possible software errors (see also Chapter 7.9)
uvvis\examples	Contains the example scan files *example_1.cdf* up to *example_5.pdf* (see also 4.1)
uvvis\guid	Contains the XML database for used guids
uvvis\ini	Contains the initialisation files *ini.xml* and *internet.ini*
uvvis\methods	Contains the XML database for user defined methods
uvvis\language	Contains the file *mn_uvvis_language.xml* that comprises all languages included in the software (see also Chapter 7.7) and the LANGUAGE_TOOL program
uvvis\logos	Standard storage for company logos (protocol layout)
uvvis\manual	Contains the file *uvvis_manual_de.pdf*, i.e. this manual in PDF format
uvvis\nanocheck	After installation, this sub-directory is empty and is required for saving the NANOCHECK LOT data (see also Chapter 6.2.5)
uvvis\originals	After installation, this sub-directory is empty and is required for saving the original files (see Chapter 3.7)

uvvis\syslog Contains the files *sys.log*, *sys.crp* and *environment.log* where technical information on your computer system is saved for possible future support purposes (see also Chapter 7.9)

1.3 Installing the USB Driver

If you intend to connect the ***NANOCOLOR*®** Spectrophotometer to the computer via a USB port, two driver files must be installed on the PC.
To install the drivers, proceed as follows:

With the ***NANOCOLOR*®** Spectrophotometer switched off, connect the instrument to a free USB port on your computer. Switch on the ***NANOCOLOR*®** Spectrophotometer.

Wait until WINDOWS® has detected the new hardware and requests the driver DVD.
If WINDOWS® does not recognise the new hardware automatically, check whether automatic hardware detection has been disabled in the BIOS of your computer or inform your system administrator.

Insert the ***NANOCOLOR*®** PC Software for Spectrophotometers **DVD** supplied into your DVD-ROM drive and start driver installation by clicking *OK*.

WINDOWS® first installs the USB chip driver. Then Windows® once more reports detection of the new hardware and the installation process starts again. WINDOWS® now installs the Virtual COM Port driver.

If your PC cannot detect new hardware automatically, proceed as follows:

With the ***NANOCOLOR*®** Spectrophotometer switched off, connect the instrument to a free USB port on your computer. Switch on the ***NANOCOLOR*®** Spectrophotometer.

Open the WINDOWS® Control Panel:
Start/Settings/Control Panel .

Insert the ***NANOCOLOR*®** PC Software for Spectrophotometers **DVD** supplied into your DVD-ROM drive.

In the Control Panel, click on ***Add Hardware*** and then on ***Next***.

When the Add Hardware Wizard requests the path for the driver data, enter your DVD drive letter.

1.4 Operating Instructions

The ***NANOCOLOR*®** PC Software for Spectrophotometers has been developed to conform to all regulations of “Good Laboratory Practice” (GLP). This requires an unambiguous assignment of user rights in order to prevent, for example, the unintentional deletion of information. Consequently, some of the functions described in this manual can **only** be executed if the user has **administrator access rights**.
All functions that are inhibited for normal users are identified in this manual by a red “X” at the right hand edge of the text. X

Do not confuse this symbol with the Cancel button  for open windows in the program.

If functions in this software do not appear to operate correctly, please consult your system administrator.

2 GLP and FDA 21 CFR Part 11 Compliance

The ***NANOCOLOR***® PC Software for Spectrophotometers software complies with GLP and 21 CFR Part 11 by virtue of the following measures:

- LOG File
All changes to the software settings and deletions are logged together with details of the date, time and user ID.

- RIGHTS
Security-relevant changes and deletions can only be made by the system administrator.

- PRINTER OUTPUT
All printouts are marked with the date, user name, software version and device serial number and can be signed with a WINDOWS® guid.

- ORIGINAL DATA
All original data is stored in binary-coded non-editable files with checksum information.

- SYSTEM DATA
All system settings and the LOG file are encrypted and include a checksum.

- RECOGNITION OF MANIPULATION
The software automatically recognises whether measured values or system data have been manipulated (checksums). There are two different security settings available: **LOW** (manipulation warning message, the user can open the relevant file if technically possible), **HIGH** (manipulated files cannot be opened).

- EXCHANGEABILITY OF DATA
NANOCOLOR® test data can be output in MS EXCEL, OPENOFFICE, SDF or XML format, scan data can be saved as netCDF/ANDI files. The data complies with the **ASTM E 1947 -98 (2004)** and **ASTM E 1948 -98 (2004)** standards.

- SECURE DATA TRANSFER
Data transfer is performed via a bi-directional protocol with checks of the transmitted/received information.

In order to achieve full compliance with 21 CFR Part 11, the software must be installed on a certified computer with an NTFS file system. Access to the software must be conducted via Windows® authorisation rights.

The security setting must be set to HIGH.

3 Part 1: *NANOCOLOR*® Photometer Functions

The first part of this manual describes the functions for saving, management and the export of ***NANOCOLOR***® test data.

3.1 *NANOCOLOR*® Cuvette Tests

This chapter deals with the ***NANOCOLOR***® tube tests and standard tests.

3.1.1 Reading out the Photometer Memory

To read out the memory of your photometer, connect the instrument to your PC via the RS-232 port or a USB port and switch the instrument on. Start the ***NANOCOLOR***® PC Software for Spectrophotometers. Fig. 1 shows the start screen of the software.

When using this software (with the exception of online measurements), the photometer operating controls cannot be used via the photometer display. This PC mode of the photometer can be cancelled via the *System/Disconnect* software menu, by clicking the USB RS232 button, or by switching off the photometer.

The photometer switches to PC mode.

If a cuvette is present in the photometer before the instrument is connected to the computer, PC mode cannot be set.
Remove the cuvette from the photometer before connection to the computer!

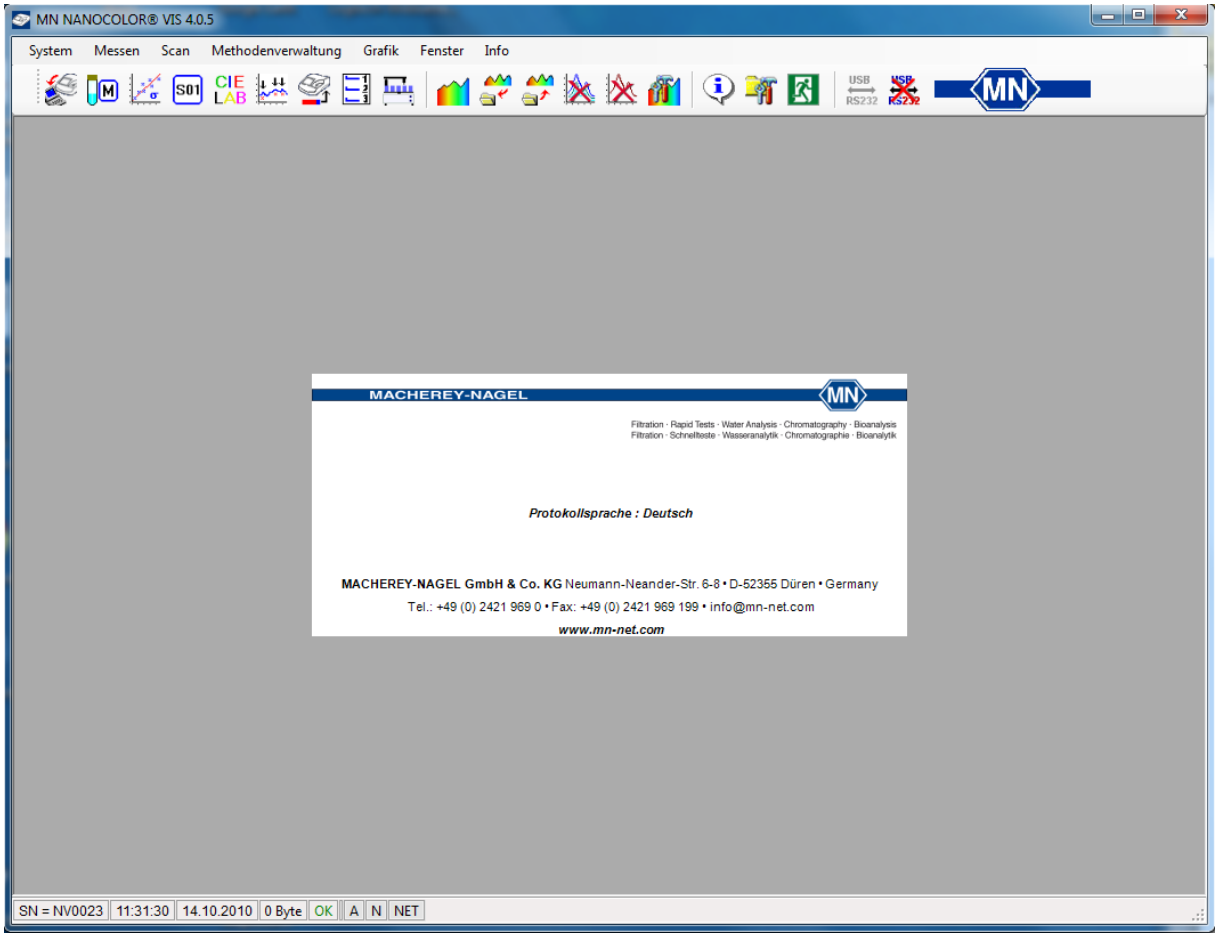


Fig. 1: Start screen

Click on the menu command *Measure/Read photometer memory*. The menu selection is shown in Fig. 2.

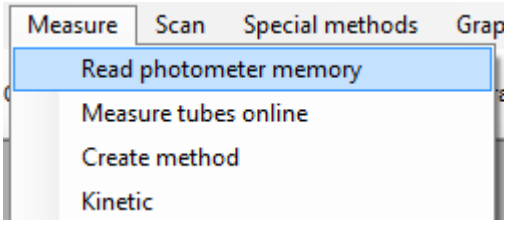


Fig. 2: Menu command Measure/Read photometer memory

Alternatively, click on the first button in the icon bar  . The software opens the data window and fills the table with the data from the photometer. Depending on the port being used and the number of data records, this process can last for a minute or more. Fig. 3 shows the data window with some measurements.

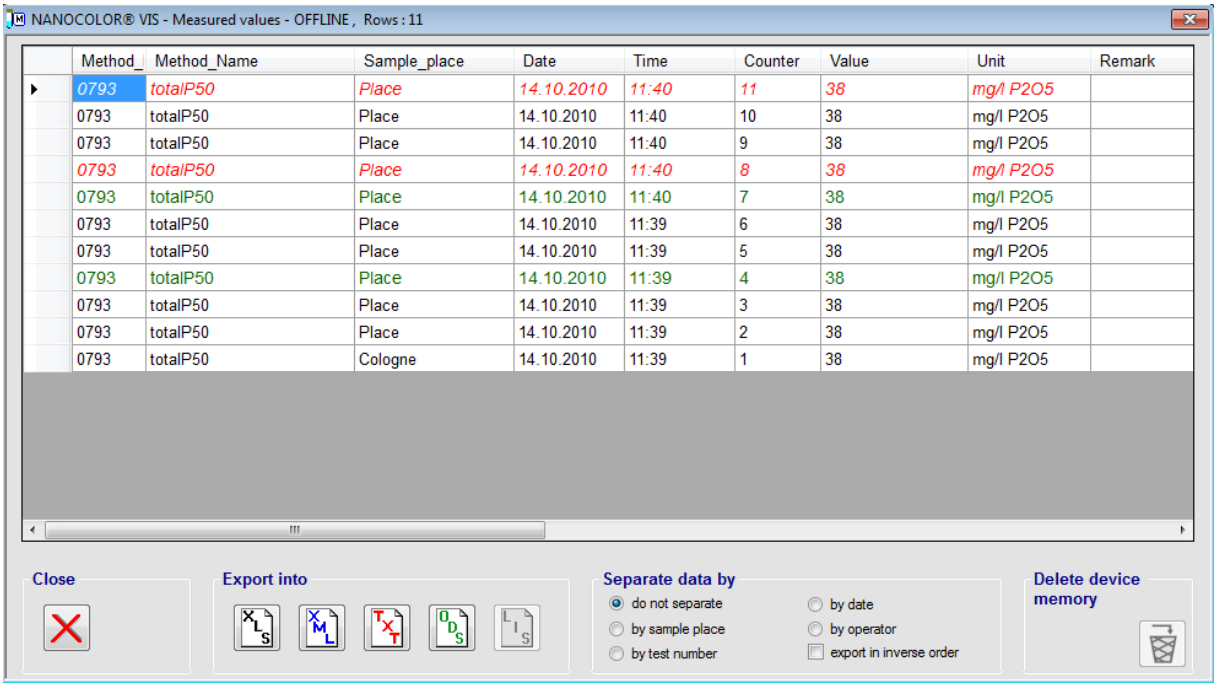


Fig. 3: Data window with measured values

The table shown is intended only as a summary. The data here cannot be edited. The table can be sorted by clicking on the column titles. The window allows you to save the measured values in one of four possible formats in order to process the data in other software products, or for archiving.

Some of the rows in figure 3 are marked in red or green color. These measurements are marked as IQC-measurements in the photometer, see chapter 3.15 IQC chart 4, page 50.

In the lower area of the window, there are five buttons that can be used to initiate the corresponding export function (see 3.1.1.1).

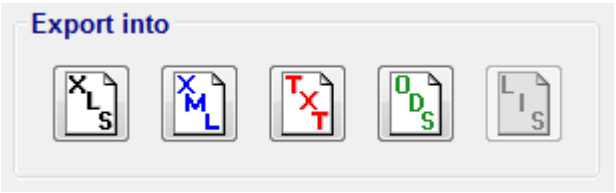


Fig. 4: Buttons for data export

With the options under the heading *Separate data by* the data can be split into individual tables. The measured values can be split into individual tables, according to: *by sample place*, *by test number*, *by date* and *by operator*.

These options are not relevant when the export is to a text file.

3.1.1.1 Export to MICROSOFT EXCEL,  Button

To use this function, Microsoft® EXCEL 2000 or higher must be installed on your computer. This button exports the data to an MS EXCEL worksheet. The software opens a file selection window (Fig. 5).
Select an appropriate file path and in the *File name:* box enter a valid name for your EXCEL file and then click the *Save* button.

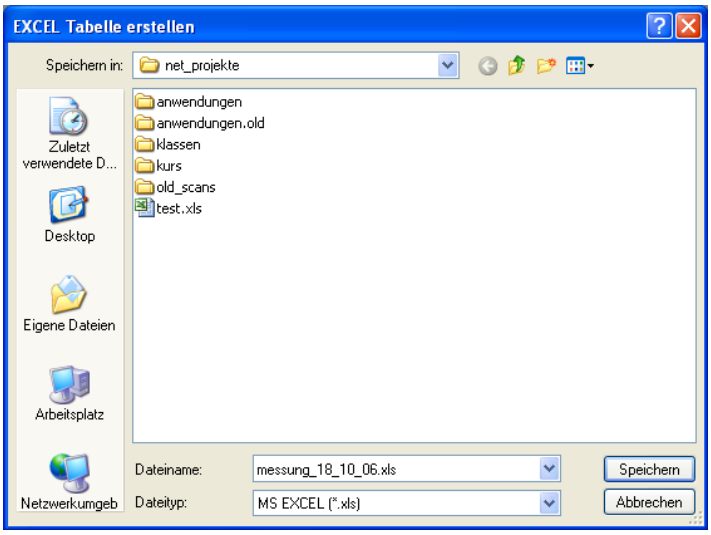
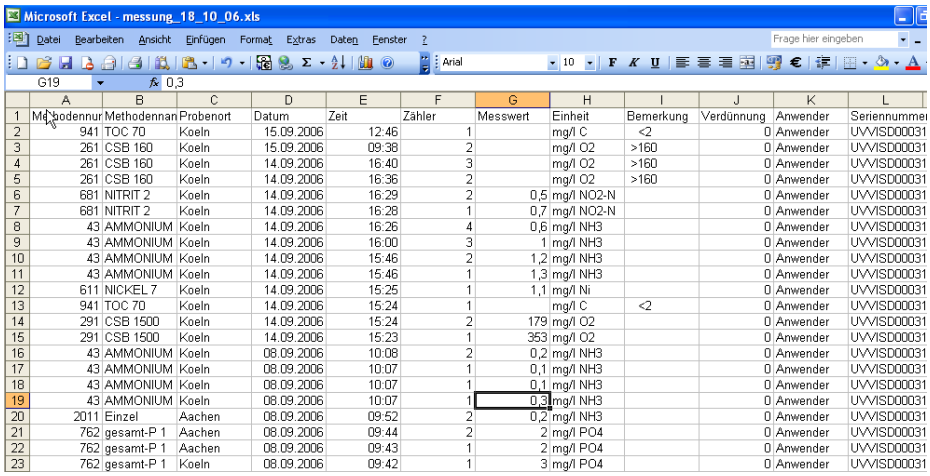


Fig. 5: File selection window "Create EXCEL Worksheet"

The ***NANOCOLOR®*** PC Software for Spectrophotometers opens MICROSOFT EXCEL and fills the worksheet with the measured values. The MS EXCEL worksheet is automatically saved with the selected file name. Fig. 6 shows the relevant view in the MS EXCEL worksheet.



	A	B	C	D	E	F	G	H	I	J	K	L
1	Methodennur	Methodennan	Probenort	Datum	Zeit	Zähler	Messwert	Einheit	Bemerkung	Verdünnung	Anwender	Seriennummer
2	941	TOC 70	Koeln	15.09.2006	12:46	1		mg/l C	<2	0	Anwender	UVVISD00031
3	261	CSB 160	Koeln	15.09.2006	09:38	2		mg/l O2	>160	0	Anwender	UVVISD00031
4	261	CSB 160	Koeln	14.09.2006	16:40	3		mg/l O2	>160	0	Anwender	UVVISD00031
5	261	CSB 160	Koeln	14.09.2006	16:36	2		mg/l O2	>160	0	Anwender	UVVISD00031
6	681	NITRIT 2	Koeln	14.09.2006	16:29	2	0,5	mg/l NO2-N		0	Anwender	UVVISD00031
7	681	NITRIT 2	Koeln	14.09.2006	16:28	1	0,7	mg/l NO2-N		0	Anwender	UVVISD00031
8	43	AMMONIUM	Koeln	14.09.2006	16:26	4	0,6	mg/l NH3		0	Anwender	UVVISD00031
9	43	AMMONIUM	Koeln	14.09.2006	16:00	3	1	mg/l NH3		0	Anwender	UVVISD00031
10	43	AMMONIUM	Koeln	14.09.2006	15:46	2	1,2	mg/l NH3		0	Anwender	UVVISD00031
11	43	AMMONIUM	Koeln	14.09.2006	15:46	1	1,3	mg/l NH3		0	Anwender	UVVISD00031
12	611	NICKEL 7	Koeln	14.09.2006	15:25	1	1,1	mg/l Ni		0	Anwender	UVVISD00031
13	941	TOC 70	Koeln	14.09.2006	15:24	1		mg/l C	<2	0	Anwender	UVVISD00031
14	291	CSB 1500	Koeln	14.09.2006	15:24	2	179	mg/l O2		0	Anwender	UVVISD00031
15	291	CSB 1500	Koeln	14.09.2006	15:23	1	353	mg/l O2		0	Anwender	UVVISD00031
16	43	AMMONIUM	Koeln	08.09.2006	10:08	2	0,2	mg/l NH3		0	Anwender	UVVISD00031
17	43	AMMONIUM	Koeln	08.09.2006	10:07	1	0,1	mg/l NH3		0	Anwender	UVVISD00031
18	43	AMMONIUM	Koeln	08.09.2006	10:07	1	0,1	mg/l NH3		0	Anwender	UVVISD00031
19	43	AMMONIUM	Koeln	08.09.2006	10:07	1	0,3	mg/l NH3		0	Anwender	UVVISD00031
20	2011	Einzel	Aachen	08.09.2006	09:52	2	0,2	mg/l NH3		0	Anwender	UVVISD00031
21	762	gesamt-P 1	Aachen	08.09.2006	09:44	2	2	mg/l PO4		0	Anwender	UVVISD00031
22	762	gesamt-P 1	Aachen	08.09.2006	09:43	1	2	mg/l PO4		0	Anwender	UVVISD00031
23	762	gesamt-P 1	Koeln	08.09.2006	09:42	1	3	mg/l PO4		0	Anwender	UVVISD00031

Fig. 6: Measured values in MS EXCEL

If one of the options from *Separate data by* has been selected, an individual table is created for each sample place, ***NANOCOLOR***® test number, user and date. The tables are automatically labelled with the selected criteria.

If MS EXCEL is already opened or the selected file already contains data, the new data is added after a blank line.

If the values measured are outside the measurement range (< or > symbol), the values are shown in the *Remark* column.

User name, sample place and dilution are output if they have been entered in the photometer during the measurement. If no user name is entered, the Windows® logon name is output.

To differentiate between measurements from various photometers, the serial number of the instrument in use is output with the data.

As soon as the data has been successfully saved, the  button under *Delete device memory* at the lower right hand edge of the data window is enabled (Fig. 7).

Fig. 7: Button to delete the photometer memory

Using this button, the memory in your photometer can be deleted and the memory prepared for future measurements. The button with the red X at the lower left edge closes the data window.

The deletion process cannot be reversed (i.e. there is no Undo function)! ONLY delete the data in the photometer once you have successfully saved / exported it.

3.1.1.2 Export in the Database Format XML,  Button

The second export button is used to create an XML database. The software opens a data selection window (Fig.5). Select an appropriate file path and in the *File name:* box, enter a valid name for your XML database and then click the button *Save*.

The XML database can be read by all database programs, regardless of the operating system being used. A description of the XML structure can be found in the appendix under Chapter 7.2. Any questions regarding import to an existing database system should be addressed to your database administrator.

If one of the options from *Separate data by* has been selected, an individual table for each selection is created in the XML file. When importing into a database system, the measured values are automatically assigned to the various tables.

3.1.1.3 Export in the TEXT Format,  Button

The third export button is used to create a formatted text file with semicolons as separators. The software opens a data selection window (Fig.5). Select an appropriate file path and in the *File name:* box enter a valid name for your TEXT file and then click the button *Save*. Details of the file format used can be found in the appendix, **Chapter 7.1**.

3.1.1.4 Export to OPENOFFICE SCALC,  Button

To use this function, OPENOFFICE, Version 3.2 or higher must be installed on your computer. This button exports the data to an OPENOFFICE SCALC table. The software opens a file selection window (Fig. 5). Select an appropriate file path and in the *File name:* box, enter a valid name for your ***SCALC*** file and then click the button *Save*.

The ***NANOCOLOR***® PC Software for Spectrophotometers opens OPENOFFICE SCALC and fills the table with the measured values. The OpenOffice SCALC table is automatically saved with the selected file name.

If one of the options from *Separate data by* has been selected, an individual table is created for each sample place, ***NANOCOLOR***® test number, user and date. The tables are automatically labelled with the selected criteria.

If OPENOFFICE SCALC is already opened or the selected file already contains data, the new data is added after a blank line.

3.1.1.5 Export to a Laboratory Information System,  Button

The last export button sends the data records to a laboratory information system. This button is active only if the “send” function is activated in the laboratory information system configurator. Please see chapter 7.3.6, page 108.

3.1.2 Online Measurements

To complete measurements online, connect the photometer to your PC via the RS-232 port or a USB port and switch the instrument on. Start the ***NANOCOLOR*®** PC Software for Spectrophotometers.

Click on the menu command *Measure/Measure tubes online*. The correct menu selection is shown in Fig. 2. If the photometer is in PC mode, after the menu selection, the instrument is set to the normal working mode.

Alternatively, click on the second button in the icon bar  . The software opens the data window with an empty table and waits for the data.

Complete your measurements. The measured values are transferred to the PC when the cuvette is removed. Before removing the cuvette, you can enter your settings for the sample place, the dilution and the user ID on the photometer display.

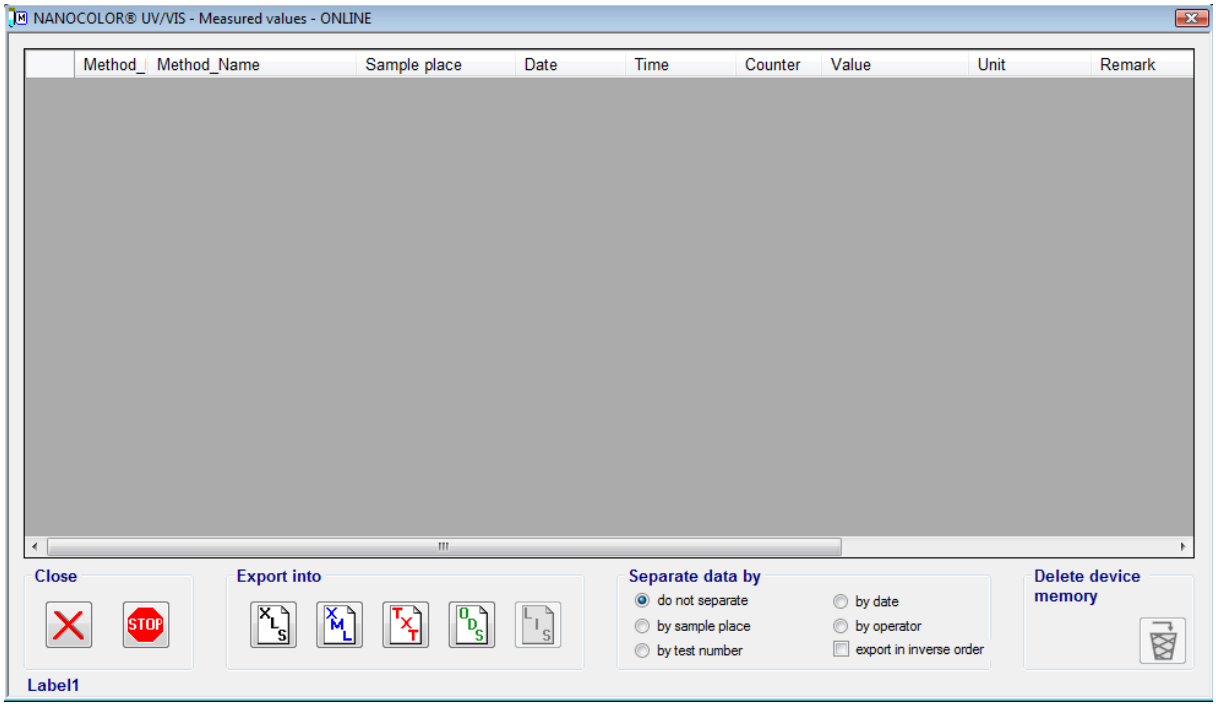


Fig. 8: Online measurement, waiting for data input

In online mode, the  button is shown at the lower left of the data window. This button allows you to quit online mode without closing the data window.

The buttons for data export are shown at the lower edge of the data window. Their functions are explained in Chapter 3.1.1.

3.2 Calibrating Special Methods

With the ***NANOCOLOR***® PC Software for Spectrophotometers, you can create your own special methods. All you require is a series of cuvettes each with a specified test concentration. After measuring the cuvettes, all relevant statistical parameters are automatically measured and displayed. If required, a measurement log can be printed and the method can be saved in the photometer by clicking a button.

3.2.1 Measuring Special Method Data

To create a special method, click on the menu function *Measure/Create method*. The photometer is in PC mode. Fig. 2 shows the menu selection required. Alternatively, click on the  button. A Method window is opened, see Fig. 9.

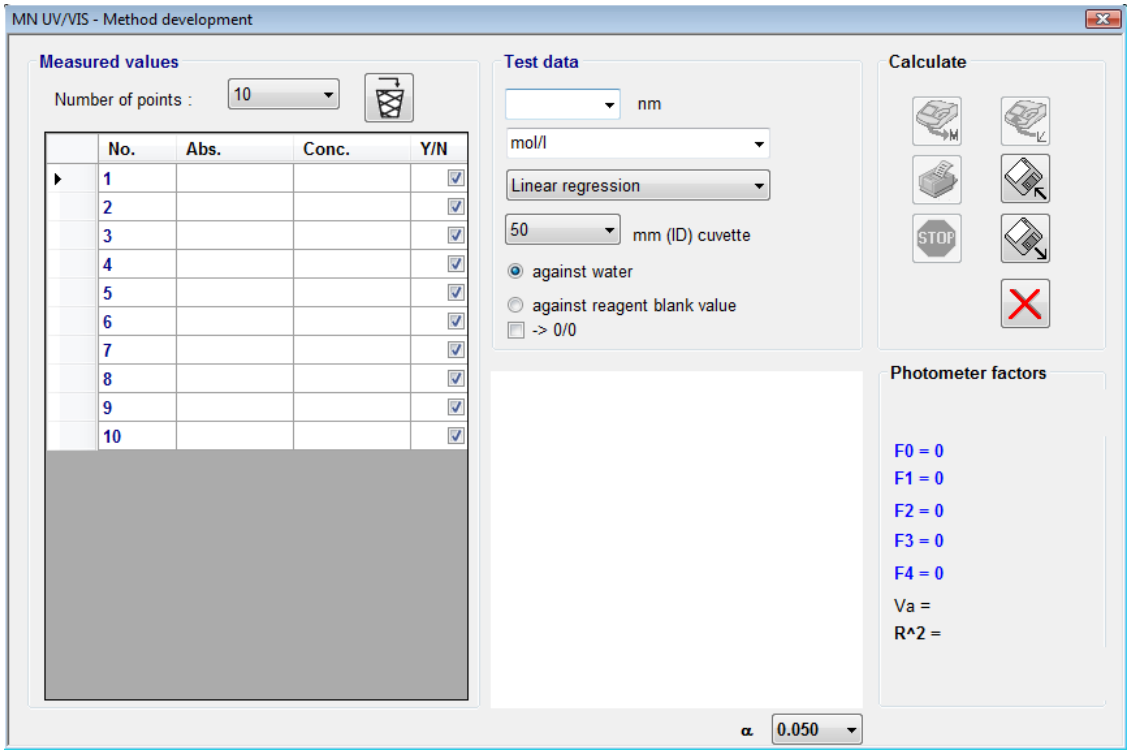


Fig. 9: Method development window

In the *Number of points* list, enter the number of measurement points. We recommend that at least 10 concentrations are measured.

In the *Test data* box, see Fig. 10, enter the required wavelength. If the required wavelength is not included in the drop-down list, the value can be directly entered in the text box (smallest permissible value 190 nm, largest value 1100 nm, no decimal places).

In the second box, select the concentration unit you are using. If the required unit is not included in the drop-down list, the value can be directly entered in the text box. Please also read Chapter 7.8 in the appendix! In the third box, the type of regression to be calculated is selected.

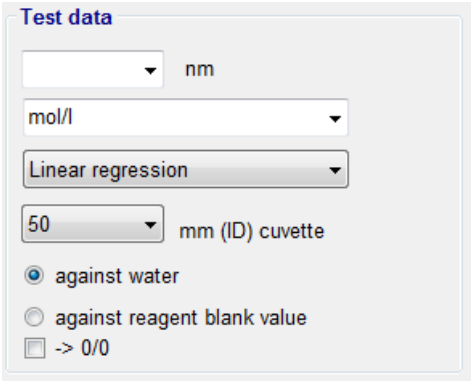


Fig. 10: Measured value settings

The type of cuvette used in your method must be entered in the fourth box. If a cuvette other than the type specified here is subsequently inserted, an error message will be displayed by the ***NANOCOLOR***® PC Software for Spectrophotometers or, in the case of standalone measurements, on the photometer.

In the lower section of the field, you must specify whether the measurements in your method are to be made against water or against a reagent blank value.

If you measure absorbance values, the photometer requests a blank solution. Set the type of blank solution used via the *against water* and *against reagent blank value* option buttons. If the option *against reagent blank value* is selected, the program assumes that all regression curves must pass through zero. If you want to force the curve passing through zero while measuring against water, activate the function *→ 0/0* .

Now, click in the first (upper) field of the *Conc.* column and enter the concentration set for the first cuvette (see Fig. 11).

	No.	Abs.	Conc.	Y/N
✎	1		10	☑
	2			☑
	3			☑
	4			☑
	5			☑
	6			☑
	7			☑
	8			☑
	9			☑
	10			☑

Fig. 11: Entry for the concentration, cuvette 1

Next, click in the second field of the *Conc.* column or press the **[↓]** or **[ENTER]** key on your PC keyboard and enter the concentration set for the second cuvette (see Fig. 12).

	No.	Abs.	Conc.	Y/N
	1		10	☑
✎	2		20	☑
	3			☑
	4			☑
	5			☑
	6			☑
	7			☑
	8			☑
	9			☑
	10			☑

Fig. 12: Entry for the concentration, cuvette 2

Finally, click in the third field of the *Conc.* column or press the **[↓]** or **[ENTER]** key on your PC keyboard. Entries are made automatically in the subsequent fields with equidistant values, as shown in Fig. 13.

	No.	Abs.	Conc.	Y/N
	1		10	☑
	2		20	☑
▶	3		30	☑
	4		40	☑
	5		50	☑
	6		60	☑
	7		70	☑
	8		80	☑
	9		90	☑
	10		100	☑

Fig. 13: Automatic column entries

If you are working with non-equidistant concentration steps, simply overwrite the value in the third field. Subsequent fields are then automatically deleted.

The complete table can be deleted with the  button.

Now, in the *Calculate* area of the window, click on the  button. The photometer expects a blank solution (see Fig. 14).

Fig. 14: Message: Insert blank cuvette

Insert the cuvette with the blank solution in the photometer. If a cuvette is not detected in the photometer within 3 minutes after the message box (Fig. 14) is displayed, the measurement is aborted. After the blank-value measurement, the photometer requests that the blank cuvette be removed, see Fig. 15.

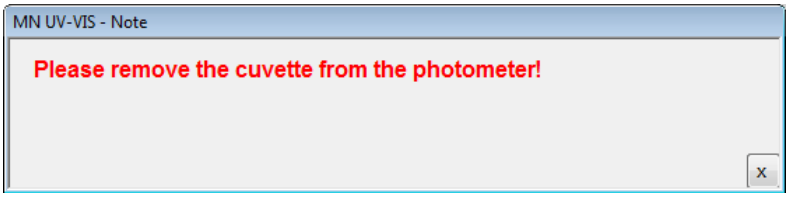


Fig. 15: Message: Remove blank cuvette

Remove the blank cuvette from the photometer. If a cuvette is detected in the photometer 3 minutes after the message (Fig. 15) is displayed, the measurement is aborted.

The photometer now requests the first measurement (sample) cuvette be inserted (see Fig. 16).

Fig. 16: Message: Insert sample cuvette 1

Insert the cuvette with the **first** sample in the photometer. If a cuvette is not detected in the photometer within 3 minutes after the message box (Fig. 16) is displayed, the measurement is aborted. Allow the photometer one to two seconds to measure the cuvette. **Wait** until the absorbance measured is shown in the table (see Fig. 17).

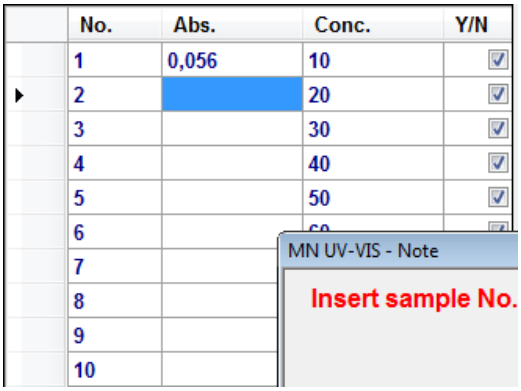


Fig. 17: Acceptance of the first value measured

Now insert all cuvettes, one after the other in the correct sequence, in the photometer. When the last cuvette is removed from the photometer, all static parameters are calculated and displayed graphically, as shown in Fig. 18.

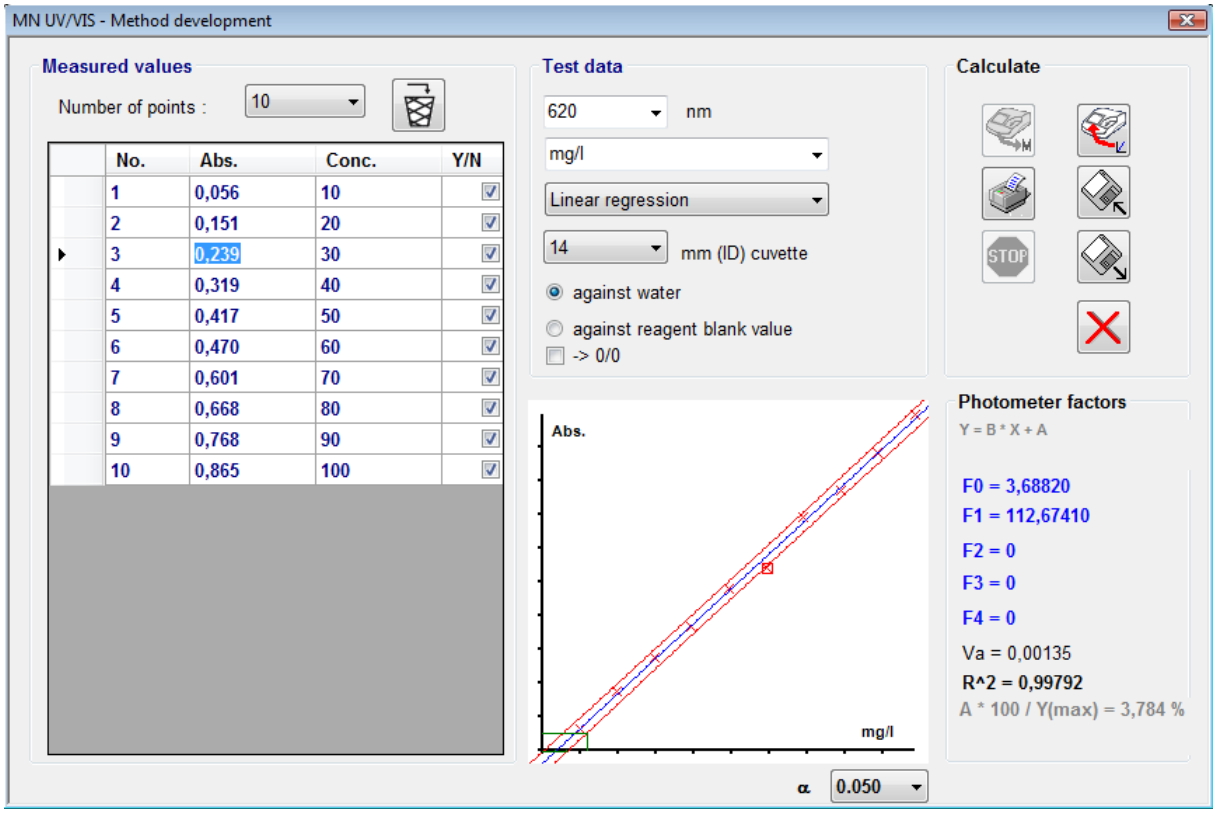


Fig. 18: Photometer factors and graphic display

Confidence intervals are indicated as red hyperbolas if linear or quadratic functions are calculated. Decision limit, detection limit and determination limit are displayed as green markers.

Please read chapter 8.9, page 132, "Outlier elimination and multiple measurements".

3.2.2 Change Regression Type and Print the Log

A measurement log of your calibration can be printed via the  button. To cancel the measurement prematurely, click the  button. If it is seen from the curve that an incorrect regression has been selected, a different type of regression can be selected from the list. The parameters are immediately converted automatically, as shown in Fig. 19.

The measurement points of the calibration can also be saved in a file, if for example, it is not possible to print at the present time. For this, click on the  button and in

the selection box, enter an appropriate file name. To re-load a saved measurement, click on the  button.

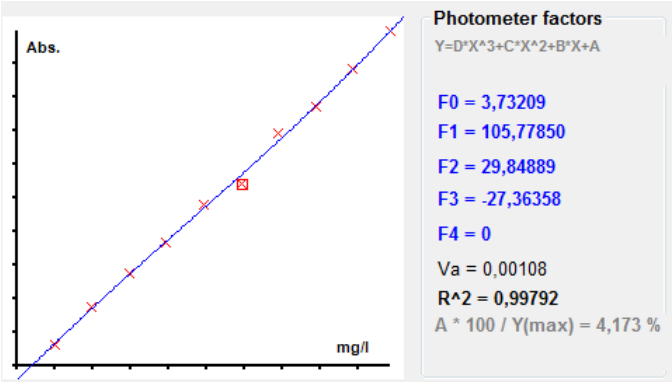


Fig. 19: Switch-over to cubic regression

The factors F0 to F4 are the photometer factors, not the parameters of the displayed curve. The curve parameters A0 to A4 are printed with the measurement protocol.

3.2.3 Saving Special Methods in the Photometer

If you wish to save the calculated methods in your photometer, simply click on the  button. The software opens the “Save Method” window as shown in Fig. 20. A few parameters must be specified here, that the photometer requires for measurement and calculation.

Using the menu command *Graphic/Copy to clipboard*, the curve can be copied to the Windows® clipboard and used in other applications if required.

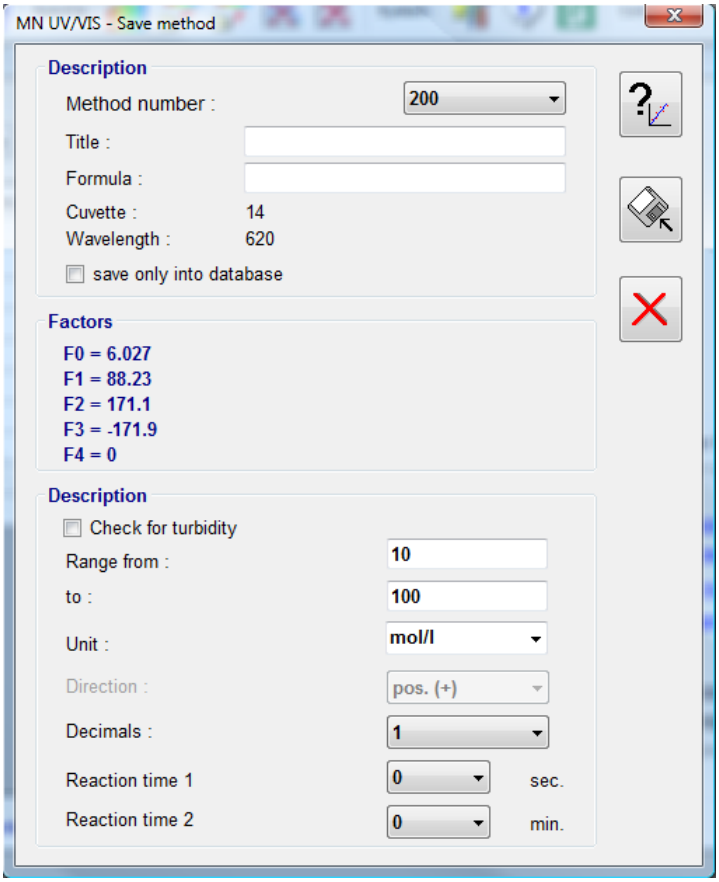


Fig. 20: "Save Method" window

From the *Method number* list box, select the number of the special method to be called in the ***NANOCOLOR***® Spectrophotometer. The photometer can store up to 100 special methods; the method numbers 200 to 299 are reserved for this purpose.

Read also the Chapter Special Method Management, in the second part of the manual, Chapter 5!

Enter a name or title in the *Title* text box. There are 12 characters available for the title, which is then displayed on the photometer.

In the *Formula* text box, you can enter a detailed description of the measured parameter, for example, NH4 or NH4-N etc. There are 10 characters available here. If your new method is not to be stored in the photometer, but to be saved to your PC database only, then press the button *Save only into database*.

In the *Range from* and *to* text boxes, the measurement range of your test can be entered, e.g. from 10 to 150. The first and last values from the table of

measurements from figure 18 are automatically entered in the boxes. **Do not** include any units in these boxes. The units that were specified in the method window are entered in the *Unit* list box. You can however, use up to 10 optional characters.

With the software version 2.0 the option *Direction* is not used any more.

Specify the number of decimal places the photometer should display in the *Decimals* list box. Values between 0 and 3 are allowed.

If your special method requires a reaction time, enter period of time in the list boxes *Reaction time 1* in seconds (0 to 59) and *Reaction time 2* in minutes. If a reaction time is not required, enter zero (0).

Refer to the operating Instructions for your photometer for more information on the method parameters.

If the method number has already been assigned, the method cannot be saved!

Using the  button, a check can be made whether the method number that you wish to assign to the special method is already in use in the photometer. When you click on the button, the next available method number is shown in the list box in green, see Fig. 21. If the method number is already assigned, the *Method number* list box is shown in red.

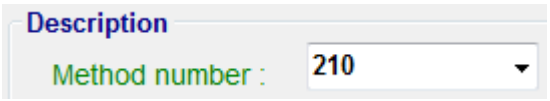


Fig. 21: Method number 210 is still available

Using the menu command *Graphic/Copy to clipboard*, the curve can be copied to the Windows® clipboard if required and used in other applications.

3.3 Kinetics

To create a kinetic with a fixed wavelength, select the menu function *Measure/Kinetic*. The photometer is in PC mode. Fig. 22 shows the window where the basic data can be specified.

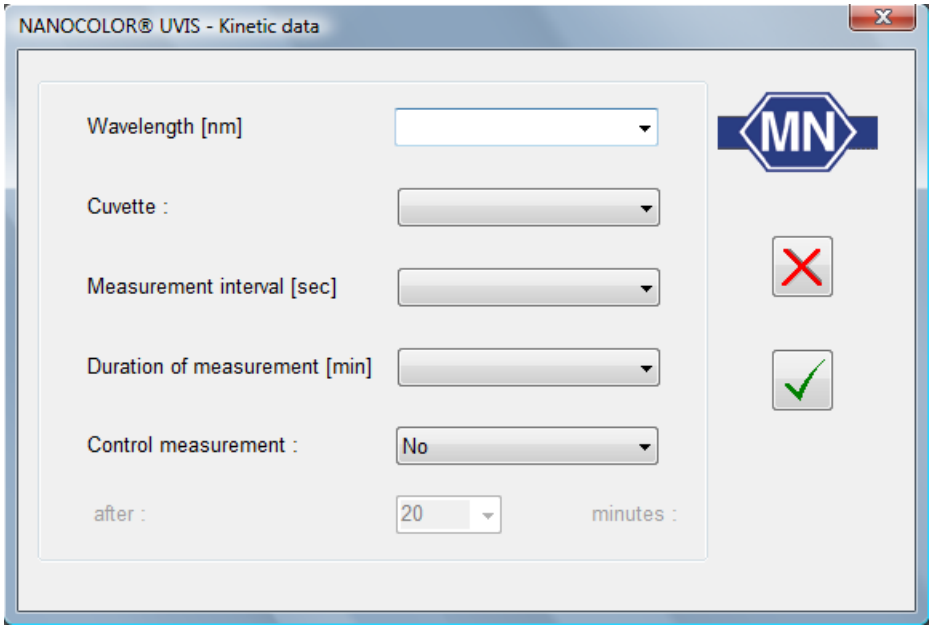


Fig. 22: Kinetic, Basic settings

Enter the wavelength required in the *Wavelength [nm]* list box. If the value required is not included in the drop-down list, the value can be entered directly. In the next list box, select the cuvette size required. In the *Measurement interval [sec]* list box, specify how many seconds should elapse before a new measurement is initiated. The *Duration of measurement [min]* list box specifies for how many minutes the kinetic should be measured. In the example above, a measurement is initiated every 10 seconds over a period of 2 minutes. Thus, 12+1 measurement points are stored. Click on the button with the red “X” to cancel the action or, click on the button with the green check-mark to start the kinetic measurement.

If the *Control measurement* list box is set to “Yes”, the software performs a final measurement after the last kinetic measurement. The waiting time in minutes after which a control measurement should be made can be specified in the *after* list box.

The software now opens the kinetic measurement progress bar, as shown in Fig. 23.

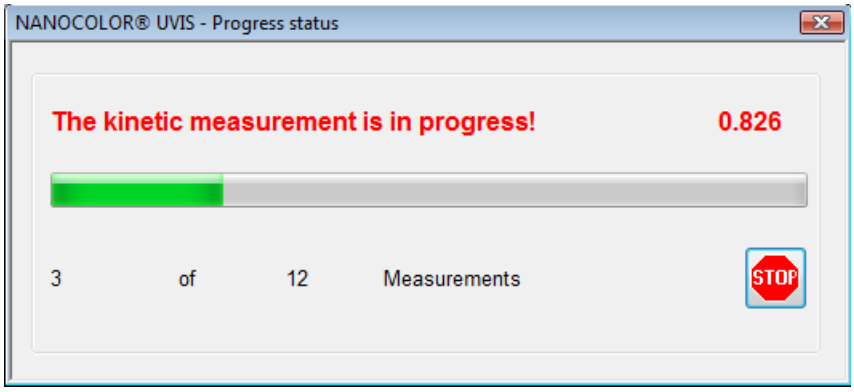


Fig. 23: Kinetic measurement progress status

After the last measurement, the software opens the kinetic window. A description of the kinetic functions can be found in Chapter **4.3.1.8**.

3.4 Bio-analysis Functions

An alternative automated methode for several samples is described in the appendix, chapter 8.10.

The bio-analysis functions simplify the determination of RNA-, DNA- and protein concentrations. Click on the menu command *Measure/Bio analysis*. The photometer is in PC mode. The method determines the concentration according to the formula "C = Absorbance x Factor". Commonly-used factors are pre-defined and displayed according to the settings in the list box *Sample* in the *Factor* field, but these can replaced with your own factors (see Fig. 24).

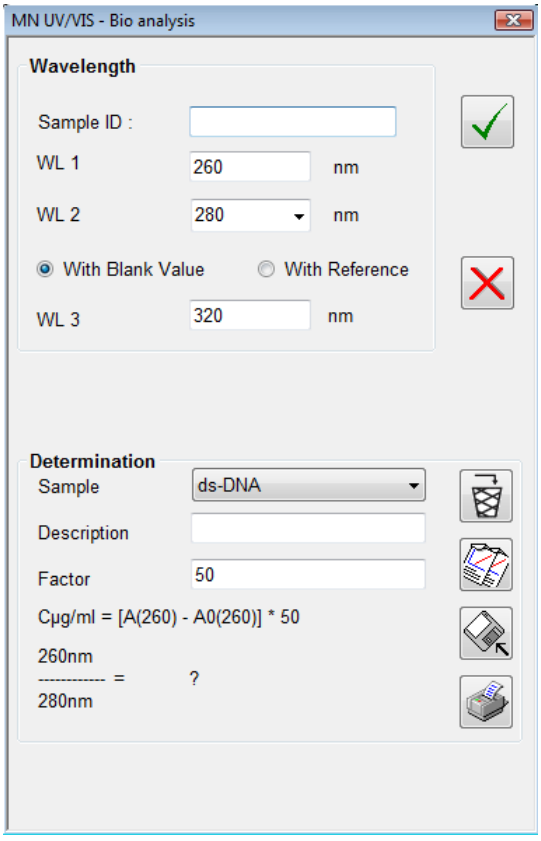


Fig. 24: Bio analysis functions

The wavelengths are set automatically when a sample is selected from the *Sample* list box. If your wavelengths deviate from the default values, enter your specified test wavelength in the *WL 1* text box. If the wavelength required is not included in the drop-down list, the value can be entered in the box. The wavelength 2 box *WL 2* is used for the purity test. Select a wavelength or enter the wavelength required in the box.

There are two methods of measurement available. The methods can be selected via the *With reference* and *With blank value* option buttons.

When the *With blank value* option is selected, a cuvette with a blank sample must be available. The absorbance is correctly measured with the values I / I_0 at the *WL 1* wavelength.
If a blank sample is not available, the *With reference* method can be selected as an alternative. This assumes that for example, DNA and RNA do not exhibit any absorption at 340 nm and the level of the blank value can be determined from the absorption at 340 nm. This reference wavelength is specified in the box *WL3* and can be altered.

When all parameters have been set, click on the  button and the photometer starts the measurement. The result is shown in the text line below the *Factor* box.
The measurement log is copied to the clipboard by clicking the  button and a click on the  button starts the printout. To save the measurement log as TXT file, click on the  button. A file selection window is opened where the file name and save location can be specified.
Using the buttons  and  , the results of the measurements can be written straight to an Excel or Open Office spreadsheet.
The last button  deletes all entries in this window.

Literature references for all the predefined bio-tests can be found in the appendix, Chapter 7.5.

For biological measurements, 10 mm quartz cuvettes are required!

This method can not be performed with the *NANOCOLOR*® *VIS* photometer.

3.5 Standard Photometric Methods

The standard photometric methods, absorbance, transmission, factor and spectral absorption coefficient (SAC value) can be found under the menu item *Measure/Basic functions*. When this menu item is selected, the standard method window is opened, see Fig. 25:

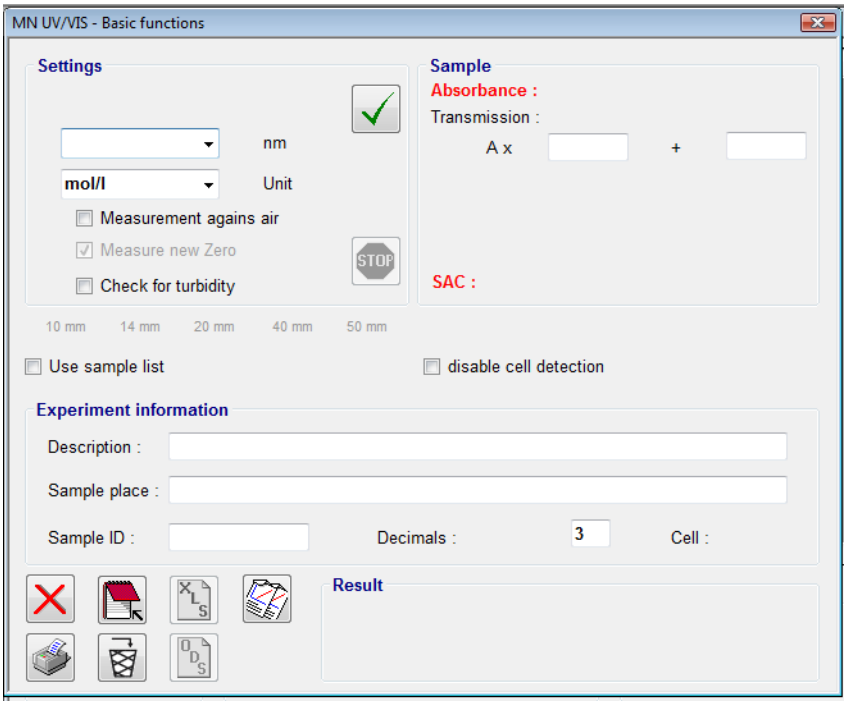


Fig. 25: Basic function window

To start the normal absorbance measurement, enter the wavelength you wish to measure in the list box *nm* under *Settings*. In the second list box, enter the units used in your measurement. If a measurement log is to be created or printed, the text boxes in the *Experiment information* area of the window, must be completed. If a factor measurement is to be completed, i.e. multiply the absorbance by a factor, in the text box *E ** in the *Sample* area of the window, enter the factor required. Any possible additive element, can be entered in the second text box *+*. The factor can be entered or changed after the measurement. Click on the  button to start the measurement. If the option *Measurement against air* is not enabled, a message is displayed requesting that a BLANK cuvette be inserted. After the blank value measurement, the software asks for the sample to be inserted. Following the message, once the cuvette has been removed from the photometer, the measured values are displayed, see Fig. 26.

If the option *check for turbidity* is set, the software performs a nephelometric turbidity measurement before the absorbance measurement. If the turbidity value is higher than a threshold value (see chapter 6.1 “General functions”) the software displays a warning message (available with photometer firmware 2.1). You can set the warning threshold in the settings menu (see chapter 6.1) according to your own requirements.

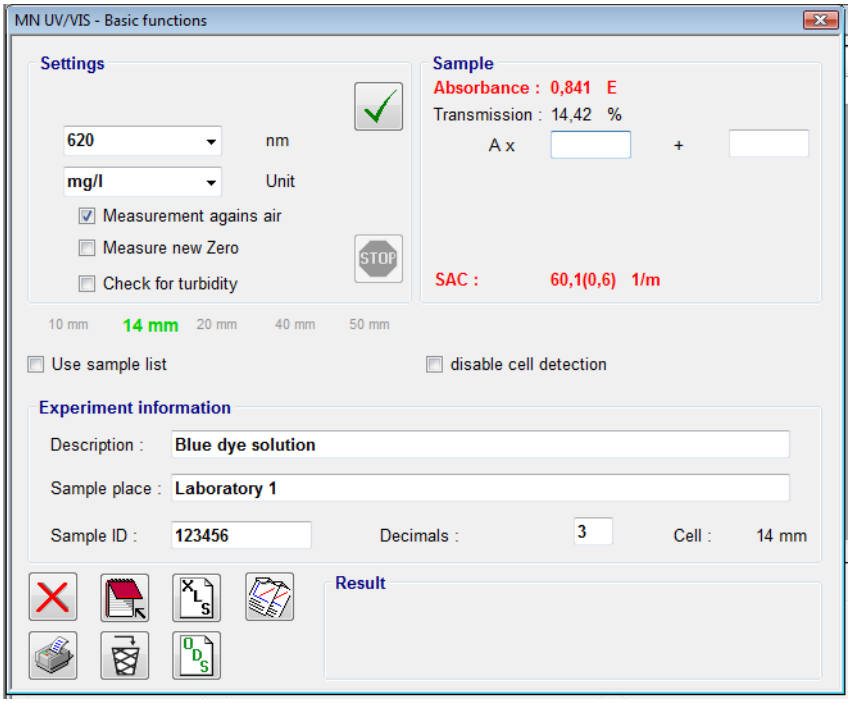


Fig. 26: Basic function window with measured values

If a factor has been entered or is entered now, the result is displayed, as shown in Fig. 27.

A measurement log in text format can be saved to your PC using the  button. To print the log, click on the  button.

A copy of the log can be copied to the clipboard using the  button.

Using the buttons  and  , the results of the measurements can be written straight to an Excel or Open Office spreadsheet.

The last button  deletes all entries.

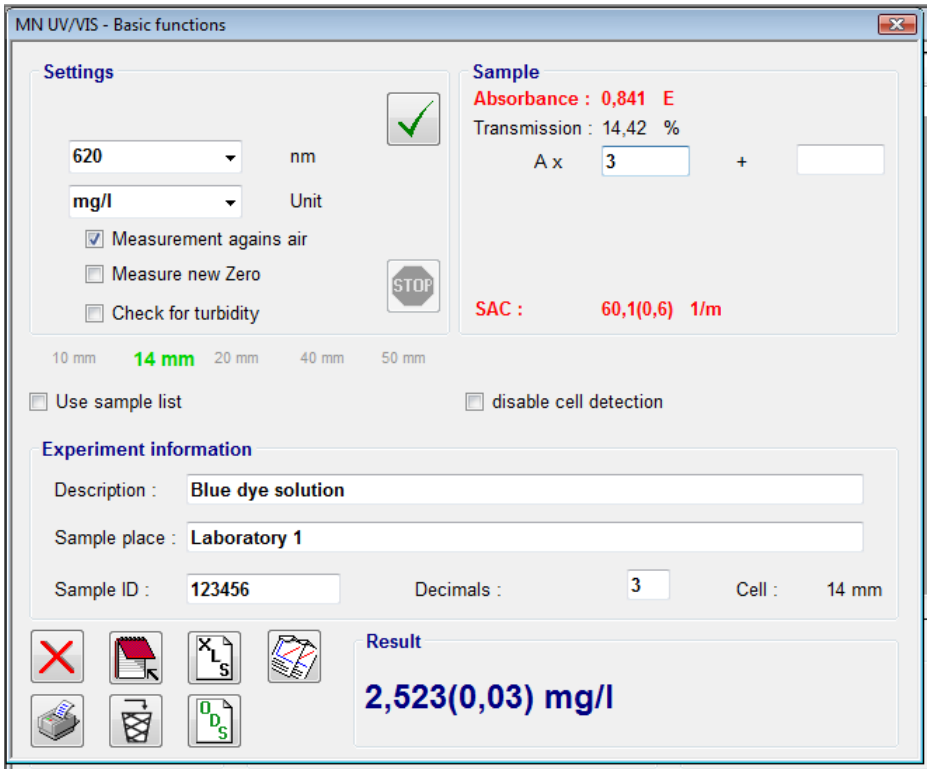


Fig. 27: Measurement with factor

3.6 Special methods

Under this menu item, you can find the same functions as in the standard photometric functions, chapter 3.5. In contrast to the standard functions, the special methods also offer you polynomial calculations up to the 4th degree.

If you have already set up special methods in accordance with chapter 3.2 or chapter 5 then these methods will be displayed in the options list at the top of the *Settings* window area, see Fig. 28. If you select a method from this list, the stored values will be entered into all subsequent fields.

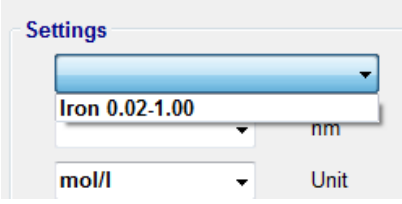


Fig. 28 :Selecting special methods

3.7 Multi-wavelength Measurement

The ***NANOCOLOR*®** PC Software for Spectrophotometers can perform multi-wavelength measurements. For this purpose, several (up to 6) wavelengths are started one after the other – it may well be the same wavelength – and absorbances and transmissions are determined. The measured values can be assigned individual factors and calculated using various mathematical formulae. Fig. 29 shows the window for multi-wavelength measurements.

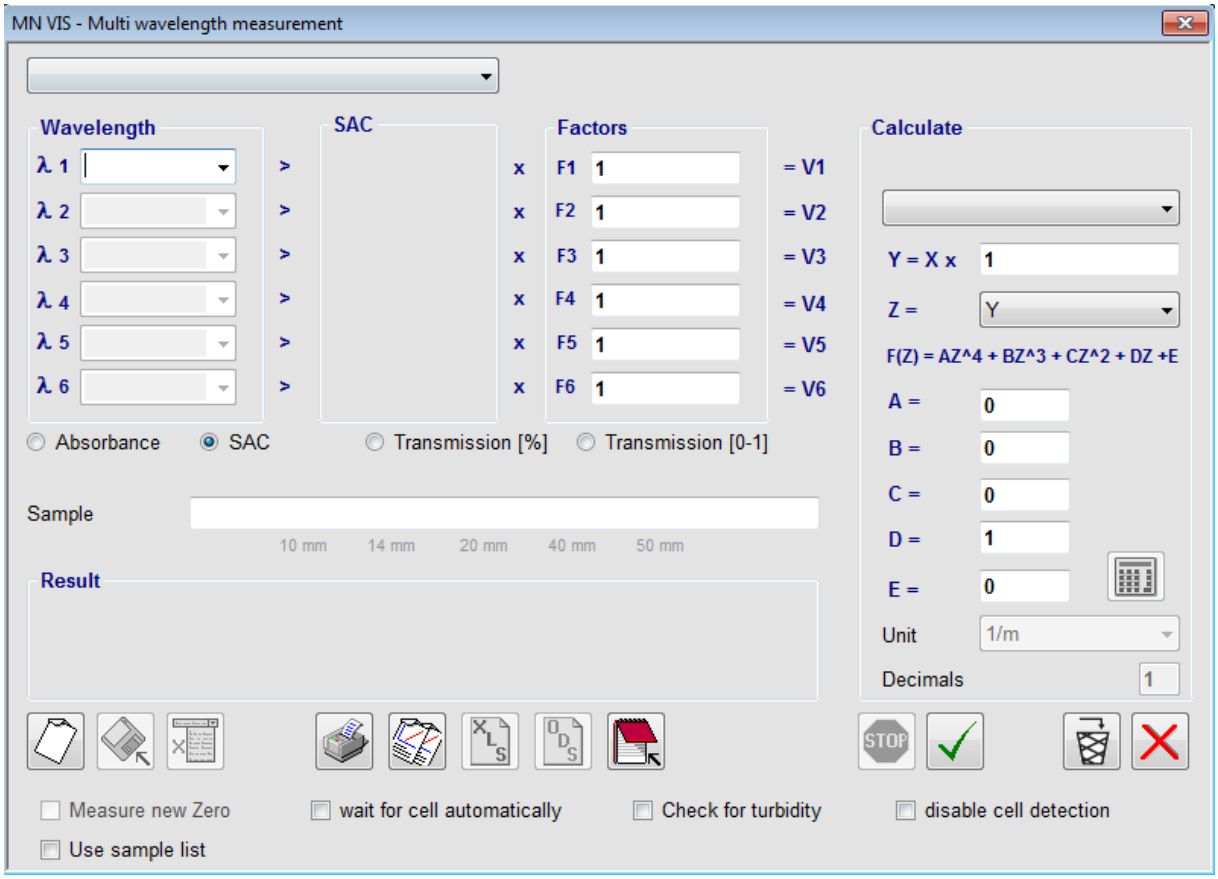


Fig. 29: Multi-wavelength window

At the left of the window is the *Wavelength* area. This shows 6 list boxes, *λ1* to *λ6*, with possible wavelengths. If the wavelength required is not included in a list, the value required can be entered directly in the text box. Ensure that all lists you do not require display a “0”.

In Fig. 29, the *Absorbance* field (depending on the setting) is empty. The values are shown here after a measurement has been completed. In the third area, *Factors* an individual factor can be specified for each wavelength, for multiplication with the measured value. Below these three areas are the option buttons, *Absorbance*, *SAC* , *Transmission [%]* and *Transmission [0-1]*. These buttons

are used to specify whether absorbance, spectral absorbance coeffcient or transmission should be included in the calculations. Here, [%] indicates the transmission from 0 to 100% and [0-1], the transmission as a fraction between 0 and 1.

At the right hand side of the window is the *Calculate* area. Here, you can specify how the individual measured values are to be calculated. Please enter a sample name in the text box Sample, which is in the centre of the window area at the bottom.

In the following example, a measurement is made with 4 wavelengths, see Fig. 30.

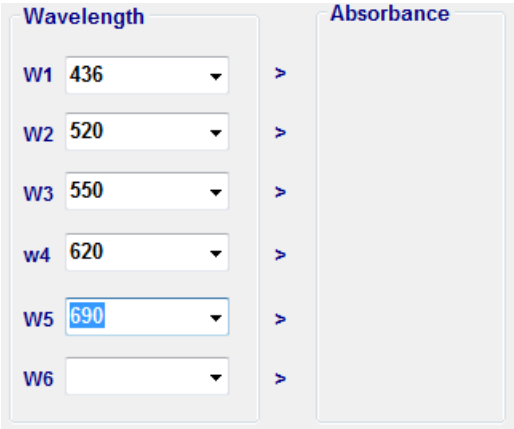


Fig. 30: Settings for 4 wavelengths

First, set the required wavelengths; up to 6 can be specified. Wavelengths that do not require evaluation must be set to “0”. The arrow symbol “>” points to the corresponding absorbance, which has not yet been measured here. If the measured values are to be multiplied by a factor before the actual calculation, complete the entries in the corresponding text boxes in the *Factors* area of the window, see Fig. 31.

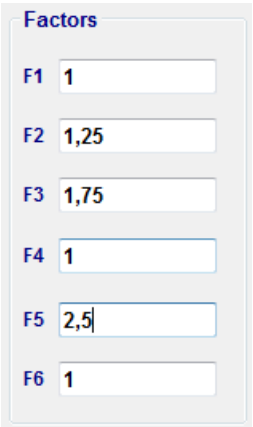


Fig. 31: Entry of individual factors

If a factor is not required for the absorbance, a “1” must be entered in the box. The product of absorbance 1 and factor 1 (F1) is known as V1. Now, in the list box at the top of the *Calculate* area of the window, select the formula to be used for the 4

values V1 to V4 from the drop-down list, see Fig. 32. The contents of this list are dependent on the number of wavelengths used.

If an essential formula is not included in the list, please contact MACHEREY-NAGEL.

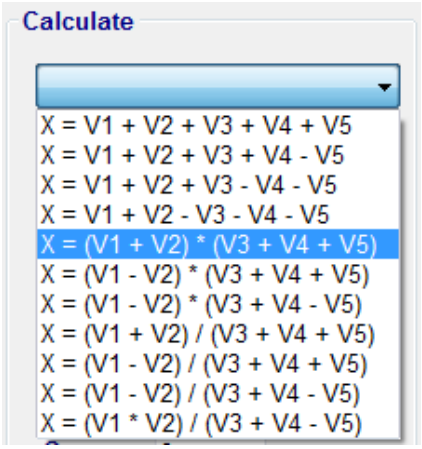


Fig. 32: Formula selection

In the example, X = (V1 + V2) / (v3 + V4) is selected. Below the formula selection, it is possible to multiply the calculated value “X” by a factor and then apply a mathematical operation to the result, see Fig. 33.

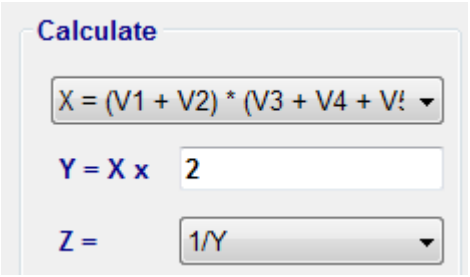


Fig. 33: Common factor and mathematical operation

In the example, the result of the formula from Fig. 31 is multiplied by the factor “2” (value “Y”). Using the result, the reciprocal value (value “Z”) is calculated.

Furthermore, it is possible to evaluate this calculated value as polynomial up to the 4th degree, see Fig. 34.

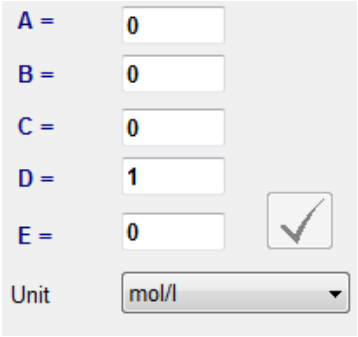


Fig. 34 : Polynomial evaluation

Thus, the function

$$F(z) = A \times Z^4 + B \times Z^3 + C \times Z^2 + D \times Z + E$$

is calculated from the value “Z”. **Without a polynomial evaluation, the factors A to C and E must display the value “0” and factor D the value “1”.**

Below the polynomial boxes, a unit can be selected from the *Unit* list included in the measurement log.

When all settings have been completed, the measurement can be started by clicking the  button at the lower left of the window. The software asks for a blank cuvette to be inserted in the photometer. After measuring the zero value, the software requests you to remove the cuvette and insert the sample cuvette in the photometer. After the sample has been measured, you will be requested to remove the cuvette. The measured values are then displayed as shown in Fig. 35.

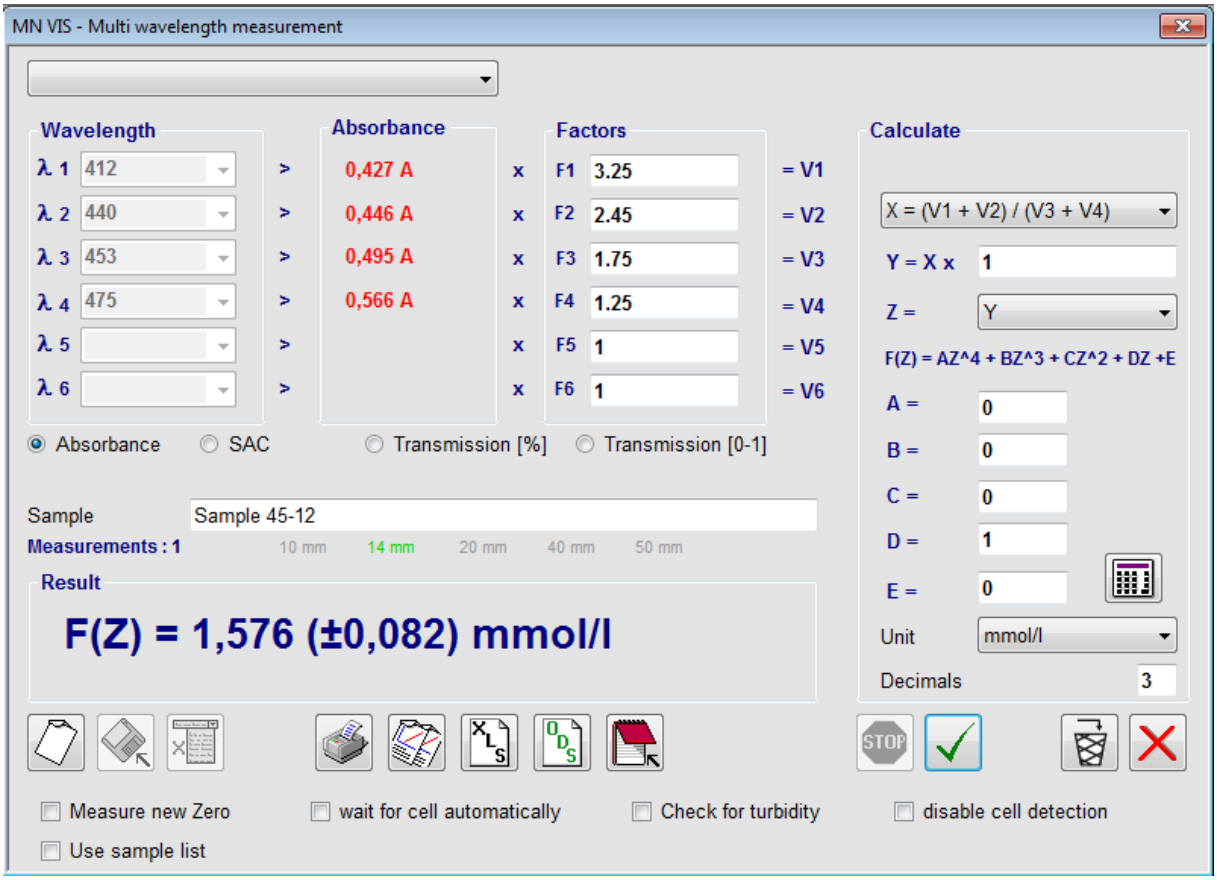


Fig. 35 : Results of the measurement

The absorbance values are displayed, the result including errors, is calculated and displayed at the lower left. A detailed measurement log is also created. This log can be copied to the clipboard with the  button or printed by clicking the  button. After a measurement has been completed, the  button at the right of the *Calculate* window area is still enabled. This allows the result to be re-calculated (not measured). This allows you to modify the factors/formulae and perform a new calculation with the previously-measured values.

After a measurement, a second sample can be measured without performing a second zero calibration. If a new zero measurement is required, the *Measure new zero* option button at the lower left of the window must be enabled before the second measurement. The cuvette size of your ZERO measurement is displayed in green below the text box for the sample name.

Next to the button *Measure new ZERO value* , you can see the functions *Automatically wait for cuvette* and *Check for turbidity* . If you have enabled the first function *Automatically wait for cuvette* , then you can remove the cuvette from the photometer subsequent to the measurement of an absorbance. The multi-wavelength measurement is continued as soon as the cuvette is reinserted. In this way, you are

able to perform measurements where reagents need to be added to the sample in-between measurements.

If the option *check for turbidity* is set the software performs a nephelometric turbidity measurement before the absorption measurement. If the turbidity value is higher than a threshold value the software displays a warning message (available with UVVIS firmware 2.1). You can set the threshold value in the settings menu (see chapter 7. 3.1) according to your requirements.

If you wish to save the method used to your computer, then click on the button  . At the top right-hand edge of the window a text box will appear. Please enter the name under which you want to save the method. Then click the button  and your method will be saved. In order to open stored methods, please click on the button  . At the top left-hand edge, a drop-down menu will appear from which you can select the multi-wavelength measurement you require. Once you have selected a stored method, the button  will become active. If you no longer require the method, you can enable this function. Once you close the window, the method will be deleted. This function requires administrators rights.

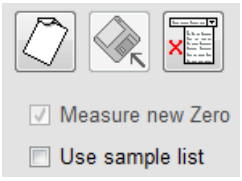


Fig. 36 : Delete stored multi-wavelength measurements

3.8 Colour measurement, button CIE LAB

Beginning with version 3.0 of the ***NANOCOLOR*®** PC Software for Spectrophotometers MACHEREY-NAGEL offers the option “colour measurement”. The colour measurement is described in an extra manual, please read “**Addendum I: Colour measurement**”.

3.9 Brewery analysis

Beginning with version 3.0 of the ***NANOCOLOR*®** PC Software for Spectrophotometers MACHEREY-NAGEL offers the option “Brewery analysis”. This measurement option contains all photometric tests according MEBAK. The brewery analysis options are described in an extra manual, please read “**Addendum II: Brewery analysis**”.

3.10 Enzymatic tests

Beginning with version 3.0 of the ***NANOCOLOR*®** PC Software for Spectrophotometers MACHEREY-NAGEL offers the option of measuring enzymatic tests. The enzymatic tests options are described in an extra manual, please read “**Addendum III: Enzymatic tests**”.

3.11 Ainsidine index

Beginning with version 3.0.8 of the ***NANOCOLOR*®** PC Software for Spectrophotometers MACHEREY-NAGEL offers the measurement of the anisidine index. The anisidine index is a dimension for the content of a,b-unsatturated aldehydes (2-alkenales) in animal and plant fats and oils. It is determined according DGF C VI 6e (05).

Enter in the text box *V [ml]* the volume of the diluted fat sample. As a standard 25 ml are preset. Enter the sample amount in g into the text box *m [g]* . Enter the reference concentration of anisidine into the text box *Q [g/ml]* , As a standard 0.01 g//ml are preset.

Enter the sample name or ID into the text box *Description:* . Alternatively create a sample list according chapter 7.2, page 98 and activate the switch *Use sample list* . Figure 37 shows the Anisidine Index window.

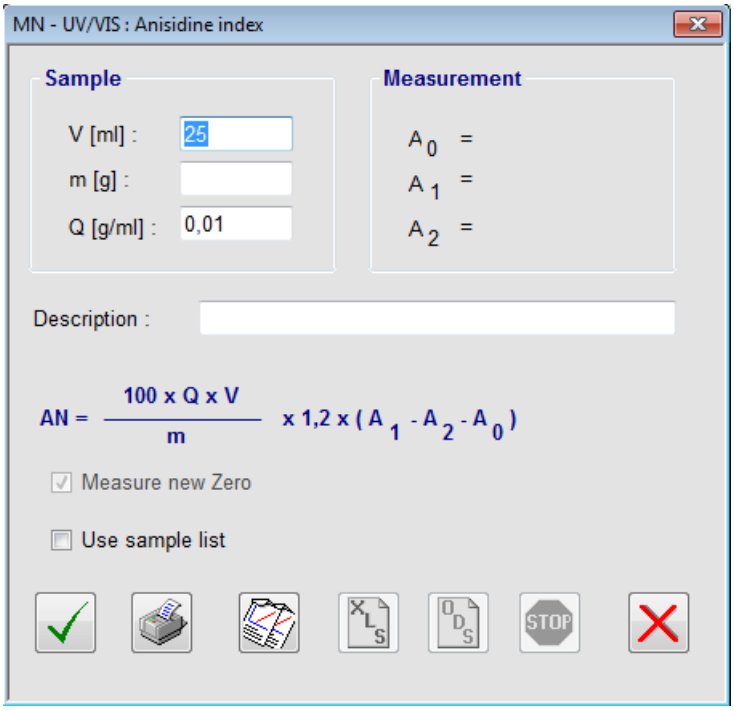


Fig. 37 : Anisidine Index window

Now click on the button  and start the measurement. The absorbances of the three test solutions are displayed in the upper right edge of the window, see figure 38:

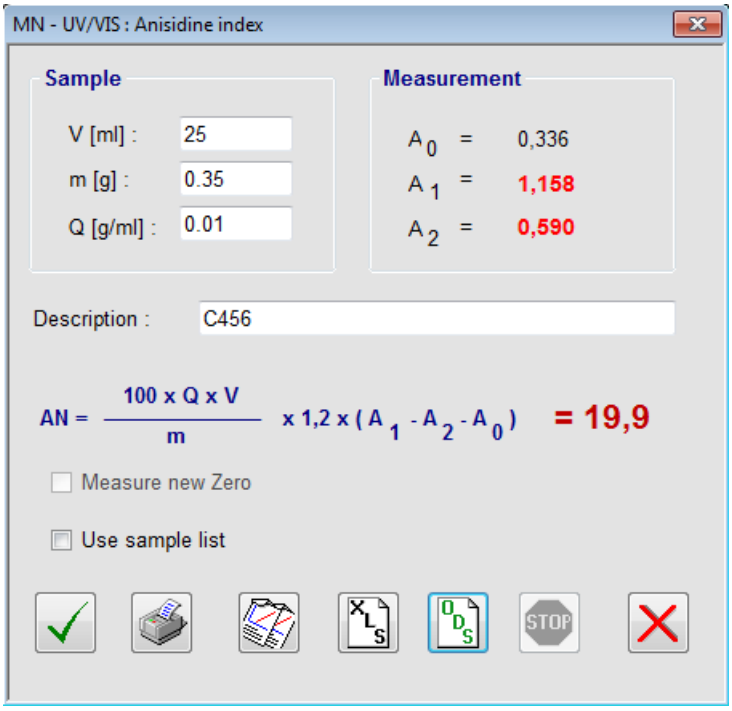


Fig. 38 : Anisidine Index window with absorbances and result

The button  copies the measure protocoli nto the WINDOWS® clipboard and the button  prints the protocol. With the buttons  and  it is possible to export the results directly to **MS EXCEL** or ***OpenOffice***

3.12 Import from USB stick, button 

The ***NANOCOLOR***® Spectrophotometers offer the option to save the photometer memory on an USB memory stick. The photometers write the data into the subfolder *UVIS\MEMORY* and the file name is *date_of_day.TXT* . You can read these data into the ***NANOCOLOR***® PC Software for Spectrophotometers. Use the menu function *Import from USB stick* .The software opens a file-open-dialog. Select the file from your USB stick and cklick OK.

3.13 Original Files

The strict regulations of GLP and 21 CFR Part 11 state that all data measured using an instrument and saved on a computer via PC software must be stored in a form that prevents any accidental or intentional manipulation. The software must also be able to automatically detect any manipulation of measured values. To this end, the MACHEREY-NAGEL ***NANOCOLOR***® PC Software for Spectrophotometers writes all exported *NANOCOLOR*® test data to a protected, binary-coded file, the original file. This original file cannot be read or edited without causing damage, unless the appropriate software is used (see Chapter 2). An original file is created, even if the exported data is **not** saved in one of the suggested formats. As default, the directory used for saving is:

c:\Programs\MACHEREY-NAGEL\uvvis\originals

It is possible to specify a different path for saving the original files via the Settings window, for example to save the original files to an external server.

Administrator access rights are required to allow the use of functions that work in conjunction with original files.

3.13.1 Reading Original Files

Regulation 21 CFR Part 11, specifies that original files may be read at any time by authorised persons or testers. If you wish to see what is contained in the original files, click on the menu command *Measure/Show original file*. A file selection window is opened (see Fig. 39).

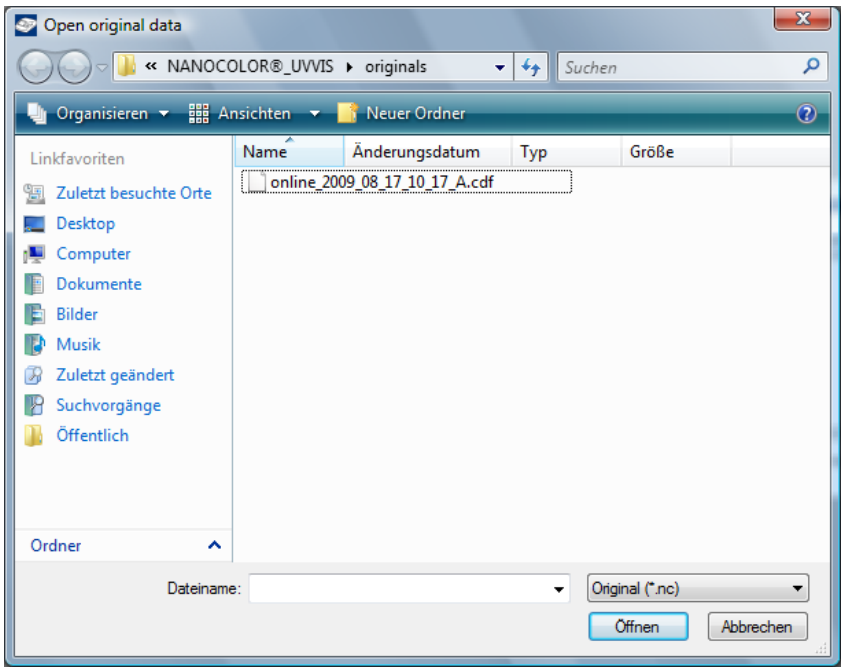


Fig. 39: Selection of original files

Switch to the directory where the original files are saved. By default, the original files are saved in the directory:

c:\Programs\MACHEREY-NAGEL\uvvis\originals

As will be seen, the file name is automatically formed and consists of type_year_month_day_hours_minutes_letter.cdf. The letter, usually an “A”, represents a counter in case more than one process is completed within one minute. For the type, the terms *online*, *offline*, *standard*, *multi*, *bio brewery, devicetest, enzymatic* or *kinetic* can be displayed, depending on the type of measurement completed.

Select a file and click on the *Open* button. A window displaying the contents of the original file opens, see Fig. 40.

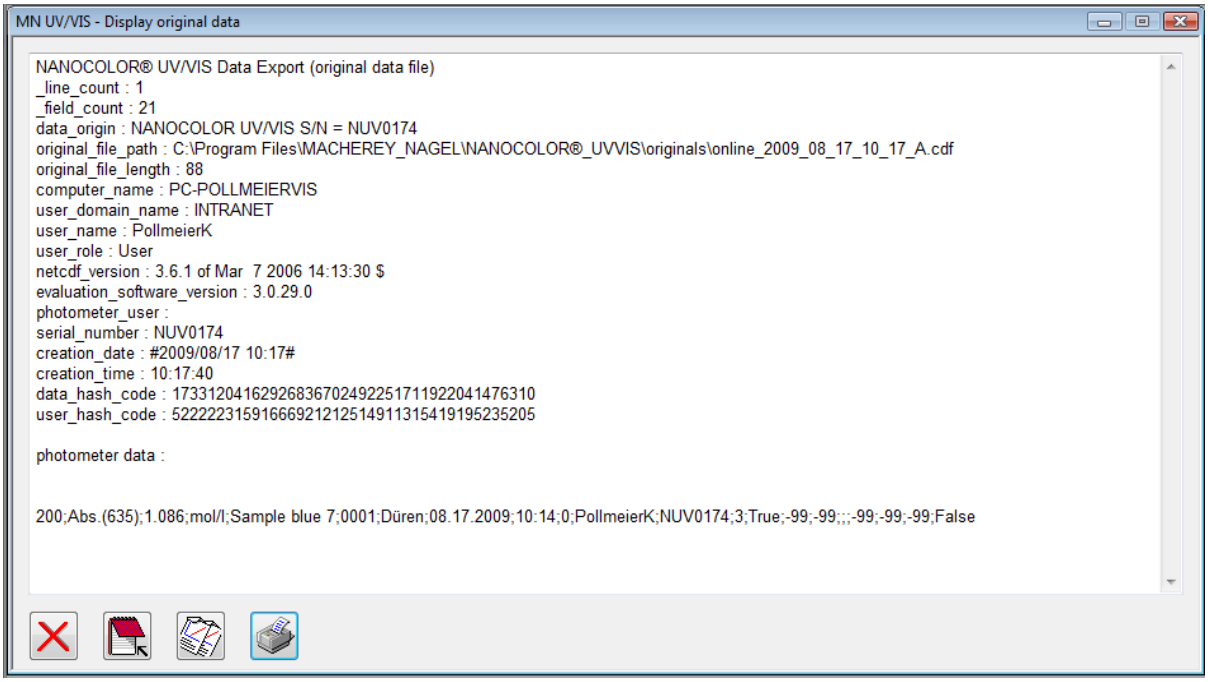


Fig. 40: Display of original files

A printout of the original file can be created by clicking on the  button. The text window is copied to the clipboard with a click on the  button. The button with the red X closes the window.

3.13.2 Restoring from Original File

If exported measured values are lost, it is possible to recover these from the original files as EXCEL worksheets or in any other available export format. By default, the original files are saved in the directory

c:\Programs\MACHEREY-NAGEL\uvvis\originals

Click on the menu command *Measure/Extract from original data*. The file selection window is opened, as shown in Fig. 37. Select the original file to be restored and click on *Open*. If the file is not damaged, the data table window shown in Fig. 3 is opened and filled with the measured values. The data from the original file can now be exported in the formats as MS EXCEL, XML, OPENOFFICE or as text.

Only the original types *offline* and *online* can be converted to spreadsheets!

3.14 Autosampler, button 

Since version V 3.0 of the ***NANOCOLOR***® PC Software for Spectrophotometers MACHEREY-NAGEL offers measurements with the ***NANOCOLOR***® Autosampler AS 53. For measurements with the autosampler please refer to the manual addendum "***NANOCOLOR***® Autosampler AS 53, Software Manual Addendum IV".

3.15 IQC Chart 4

The ***NANOCOLOR***® Spectrophotometers give the operator the option to measure standard measurements (internal quality control) according German DWA-A 704 including all neccessary information on the photometers screen. The ***NANOCOLOR***® PC Software for Spectrophotometers is able to extract this information and to create all IQC documentation. The German DWA-AG IG-4.3 recommends to perform a standard test, individual-related every 10th measurement of one parameter, but at least one standard measurment per month and parameter. How to set your regulations into the photometer please refer to the photometer manual.

If you use IQC standard measurements the ***NANOCOLOR***® PC Software for Spectrophotometers is able to create the DWA-A 704 IQC chart No. 4 automatically.The software stores IQC data in a special database. This database is updated every photometer memory read-out. Using this test data you can create individual-related and parameter-related IQC charts just by a mouse click.

A requirement for the creation of an IQC chart 4 is that the IQC function in the photometer is activated and that all photometer operators perform IQC standard measurements!

To create an IQC chart 4 every operator must be registered with his name in the photometer and all operators must perform standard measurements regularly for all parameters. These measurements must be marke das IQC measurement in the photometer (see photometer manual). For all IQC measurements you need the LOT code of the used standard, the LOT code of the used test, nominal value of the standards and the allowed tolerence. During every data export from photometer

memory to the ***NANOCOLOR***® PC Software for Spectrophotometers these data are evaluated by the IQC function.

As chapter 3.1.1 **Reading out the Photometer Memory**, page 15 shows, some data rows may be marked red or green. These data rows contain IQC standard measurements. Green marked rows fit the IQC standard test were as red marked result lay outside the confidence interval of the used standard.

Clicking the menu item *Measure/IQC Chart 4* opens the IQC window, see figure 41.

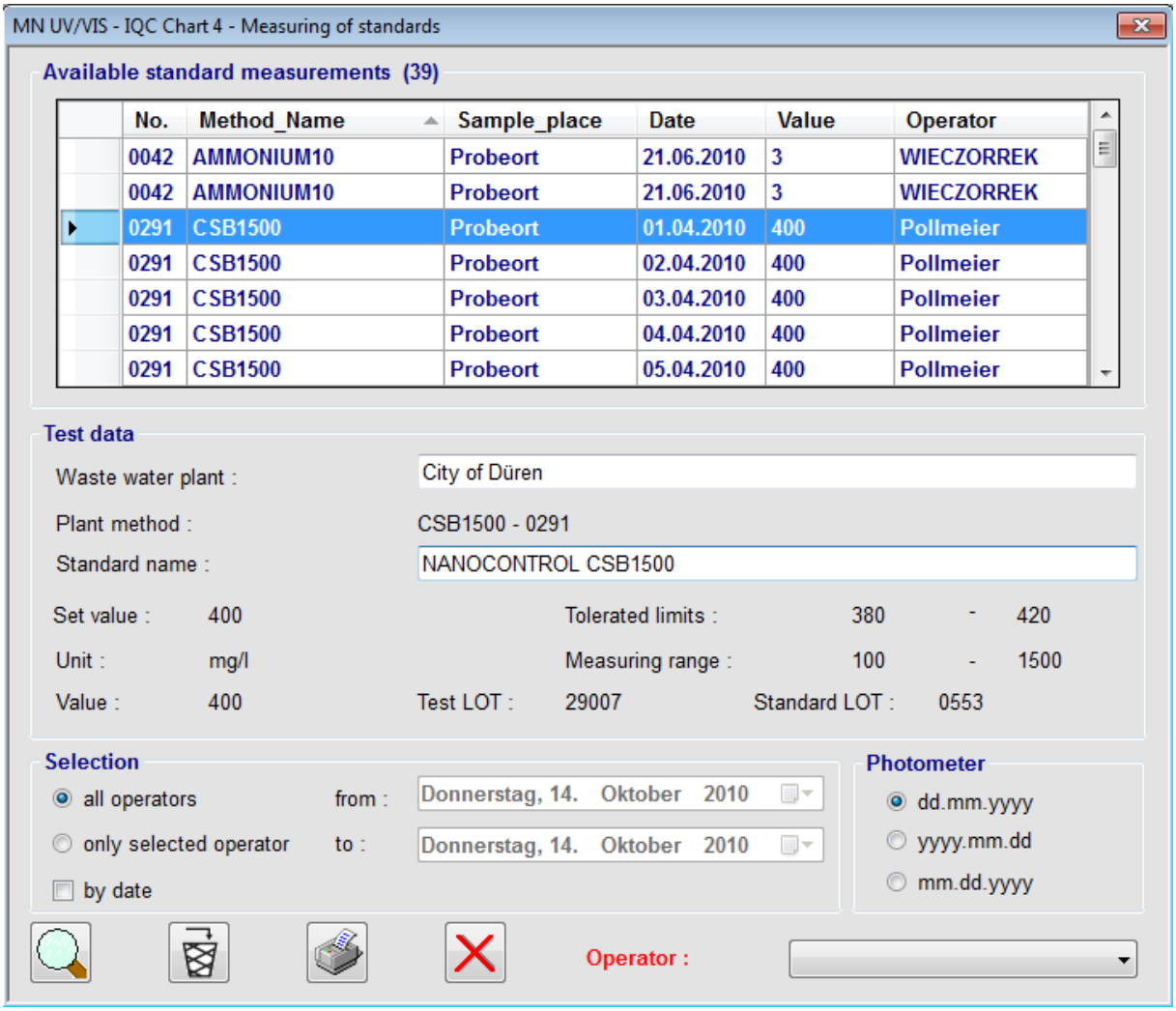


Fig. 41 : IQC Chart 4 window

The table in the upper part of the window shows all standard measurements stored in your database. Attention: If you never have read out the photometer memory or if you never have performed an IQC measurement this table will be ampty!

You can sort the table by clicking the column headers. Select one entry by clicking in the table or – alternatively – select one operator from the list *Operator* . If an operator is selected, the table will display only measurements performed by the

selected operator. As figure 41 indicates all parameters of the selected test are displayed in the frame *Test data* .

Fill out the text boxes *Waste water plant :* and *Standard name :* . The options in the frame *Selection* allows to create a IQC chart for all users performing this test (not DWA conform) or to create a chart just for the selected operator. If you select *only selected operator* you must select an operator from the list. With the two options *from:* and *to:* you can select measurements within a given time period.

The button  deletes your selection and the button  creates an IQC chart 4 in the WINDOWS print preview. The printer button  prints the chart directly. If more than 25 measurements are selected, several pages may be print. Figure 42 shows the resulting IQC chart 4:

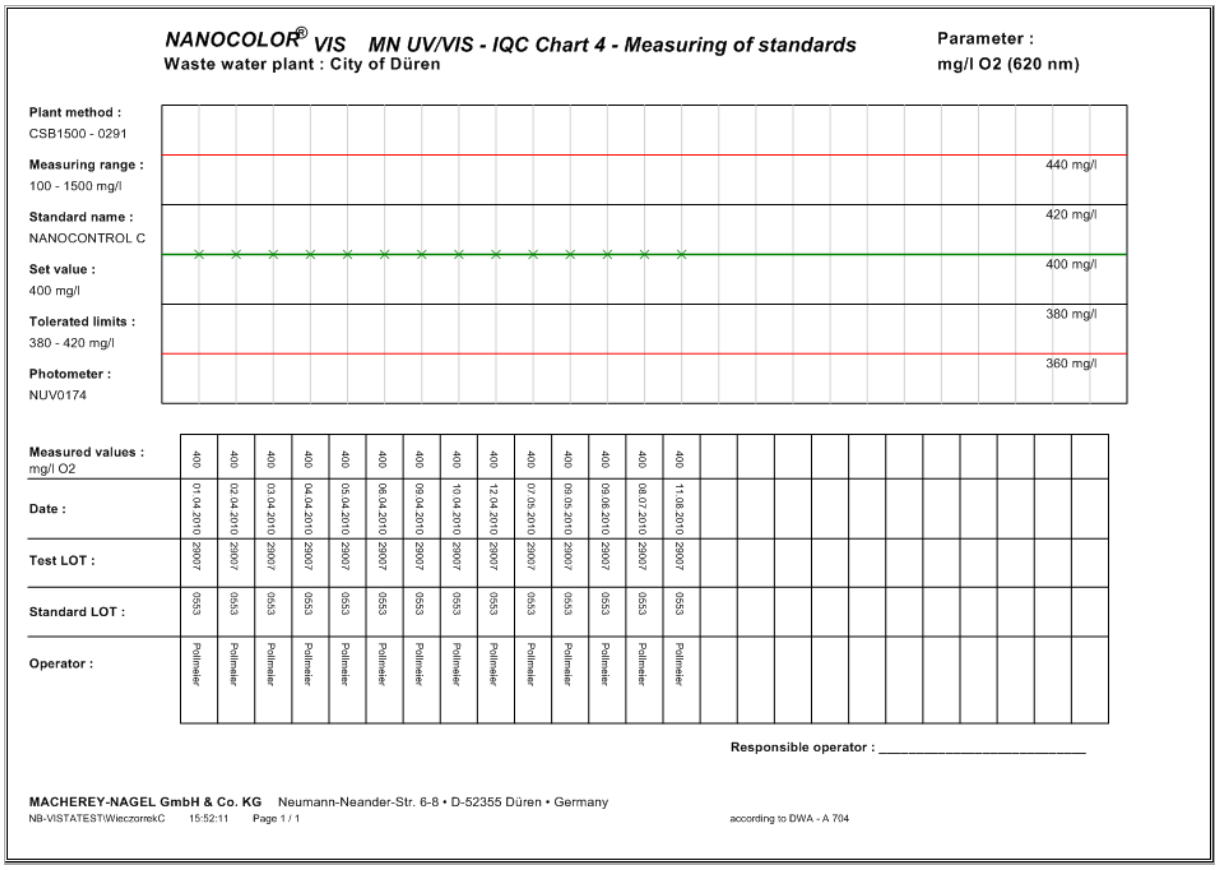


Fig. 42 : IQC Chart 4

It is important to use the same date format in the frame *Photometer* as is selected in the photometers settings!

4 Part 2: Scan Functions

The ***NANOCOLOR*®** PC Software for Spectrophotometers incorporates various functions for creating, processing, measuring and saving wavelength scans. The following Chapter 4.1 describes the method of operation of the software and shows the individual elements of the scan window.

To understand the processing philosophy of this software, Chapter 4.1 should be read very carefully!

A description of individual functions follows later in Chapter 4.2.

4.1 Working with Wavelength Scans

Before the scan window can be opened, a scan must be created or a previously-saved scan opened. The ***NANOCOLOR*®** PC Software for Spectrophotometers includes a few example files. Click on the menu command *Scan/Open scan* or the  button.

From the file selection window, select the file *example_1.cdf* from the directory *example* in the installation directory of the ***NANOCOLOR*®** PC Software for Spectrophotometers. The scan window is opened and the scan in drawn as a red line, as shown in Fig. 43. Note that this scan includes text labelling.

The colour of the current scan can be changed in the *System/Settings* menu.

A scan file (*.nc) can contain more than one scan.

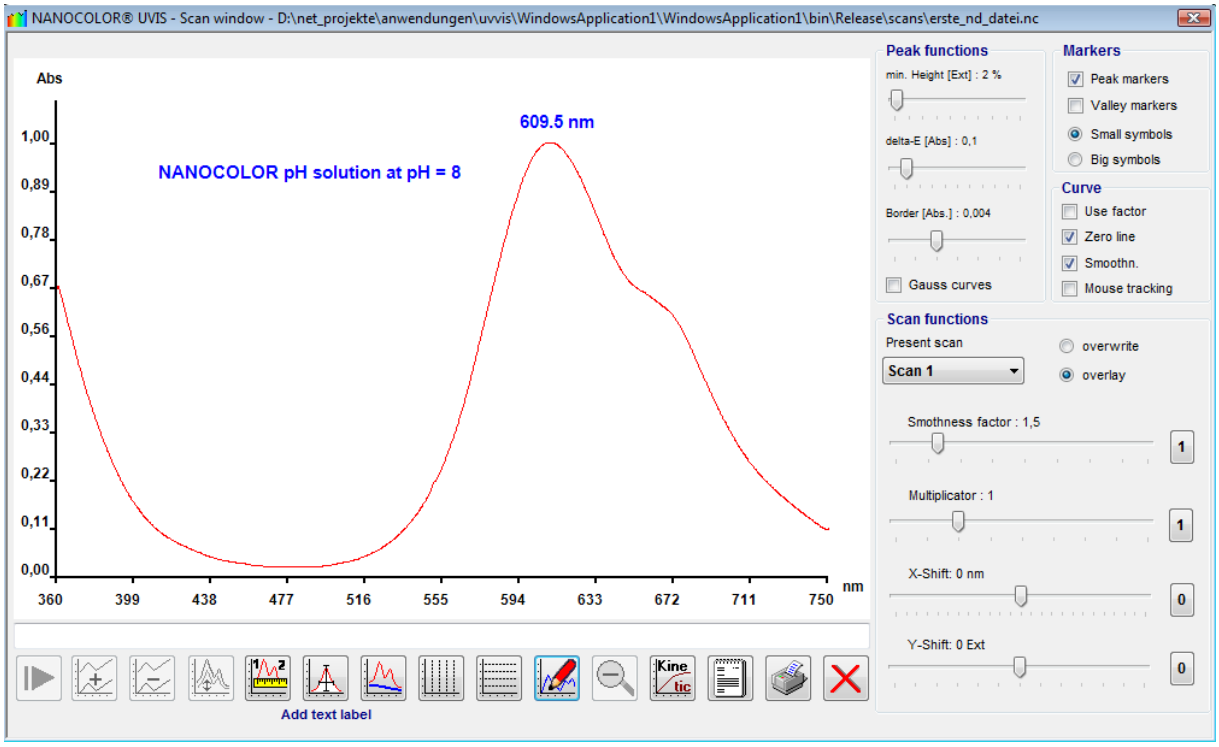


Fig. 43 Scan display

The ***NANOCOLOR***® PC Software for Spectrophotometers can display several scans simultaneously. The number of scans is restricted only by the size of the memory in your computer. Each further scan that is opened or created is managed in the same workspace as the example scan shown above. The form of the display depends on the properties of the scan window. The status line in the lower part of the main window provides information on what is displayed in the scan window (Fig. 44).

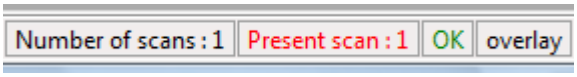


Fig. 44 Status line with scan information

The first box shows “Number of scans: 1”. The *example_1.nc* file contains only one scan. Therefore, the “Present scan:” is also set to “1”. Now, open the file *example_2.nc*. The software now asks whether the present (current) scan should be held or deleted, see Fig. 45.

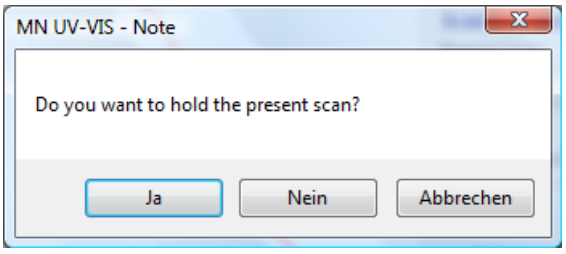


Fig. 45: Processing mode, new scan

If the question is answered with the *Yes* button, all scans from the *example_2.cdf* file are loaded to the same environment as the scan from the first example file.
If the question is answered with the *No* button, the first scan is closed and the *example_2.cdf* file is loaded to a new, empty workspace.

If this first scan is a newly created scan that has not yet been saved, the scan will be discarded when a second scan is opened or created and the question has been answered with "No". The data from the first scan is then lost!

Now, click on the *Yes* button. A second scan is drawn in the scan window. The first scan is now displayed as a green line, the second as a red line (Fig. 41). The ***NANOCOLOR*®** PC Software for Spectrophotometers automatically assigns a different colour to each scan opened: the first in green, the second in turquoise, etc. The currently active scan, the "present scan" is always displayed in red. Therefore, the second scan shown in Fig. 46 is displayed in red and not turquoise. Any action performed on a scan, e.g. integration, measurement, labelling, etc., **always refers only to the present (current) scan**. After opening or creating a new scan, the last scan automatically becomes the present scan. Therefore, the labelling is no longer shown since the second scan contains no labels.

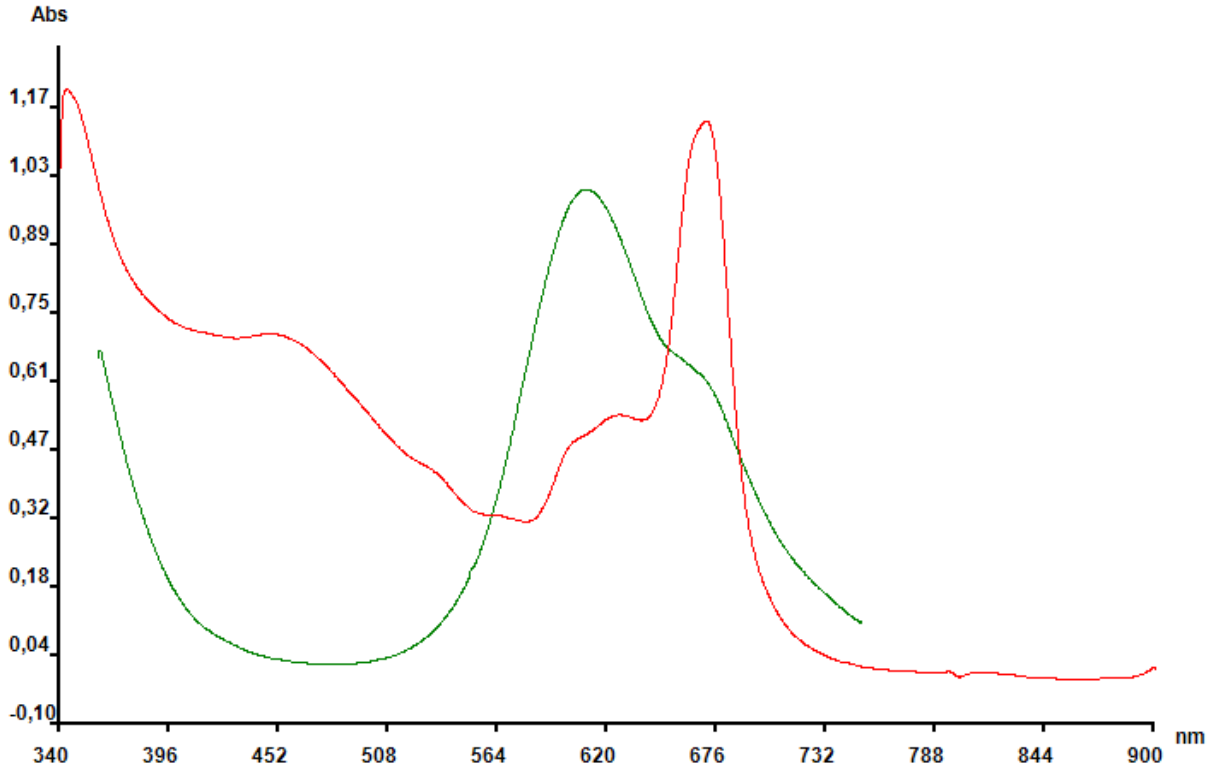


Fig. 46: A second scan is opened

The status line in the main window (Fig. 47) shows the new scan.

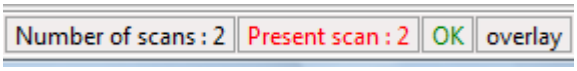


Fig. 47: Information in the status line

To change the current scan, select a different number from the drop-down selection list *Present scan* in the *Scan functions* area of the window, as shown in Fig. 48.

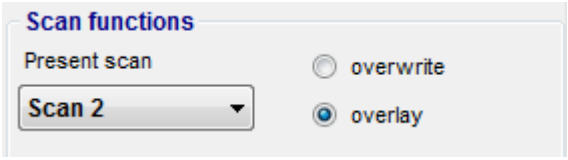


Fig. 48: Drop-down list, Present scan

Set this list to Scan 1 again. Fig. 49 shows the changes in the screen area: the first scan is now displayed in red (with labelling) and the second scan is now turquoise.

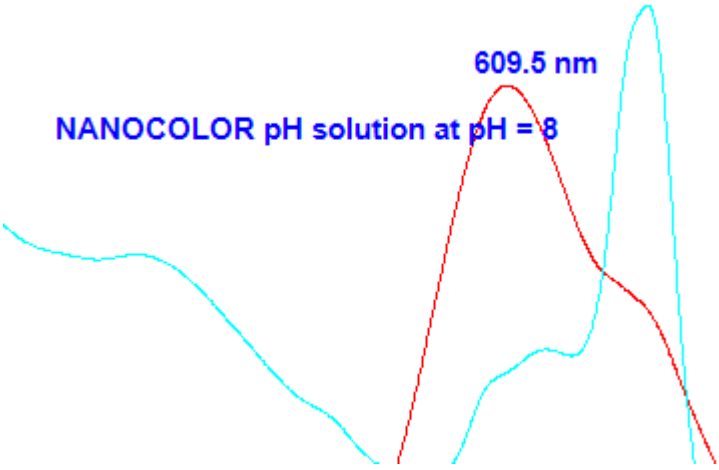


Fig. 49: Changed current scan (detail of screen)

As can be seen from Fig. 46, the size of the scan, i.e. the wavelength range, has no significance. The software adjusts the scale of the graphics so that all scans can be displayed on the screen without any loss of detail, provided the function *automatic scale* has not been disabled in the Settings.

It can also be seen from Figs. 47 and 48that the scan window is in overlay mode. This means that all scans shown in one workspace are drawn on the same graphic co-ordinates. In the *Scan functions* window area (Fig. 43), there are two option buttons: *overlay* and *overwrite*. In overwrite mode, only one scan is drawn in the graphic. Individual scans can be paged through by selecting the scan from the drop-down selection list *Present scan* in the *Scan functions* area of the window.

Selected areas of a scan can be enlarged if required. Drag a frame with the mouse as shown in Fig. 50.

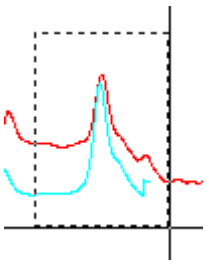


Fig. 50: Zoom frame marked with the mouse

As shown in Fig. 51, all scans in the workspace are shown enlarged when the window is set to overlay mode.

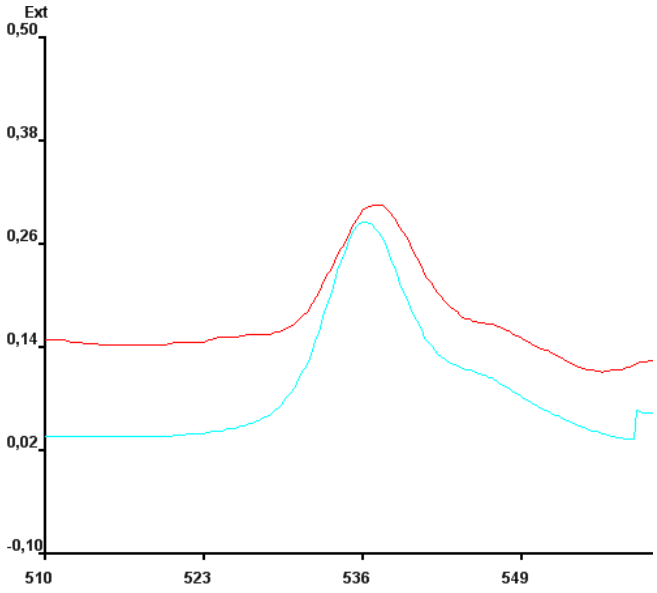


Fig. 51: Enlarged scan section

To exit zoom mode, simply click on the graphic or the zoom-out button  . When the mouse is moved over the scan, the current mouse position is indicated in nanometres, absorbance and transmission in the status line of the main window (see Fig. 52).

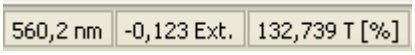


Fig. 52: Mouse position information in the status line

The current mouse position is also indicated in the graphic as cross hairs (Fig. 53), provided the function has not been disabled in the Settings.

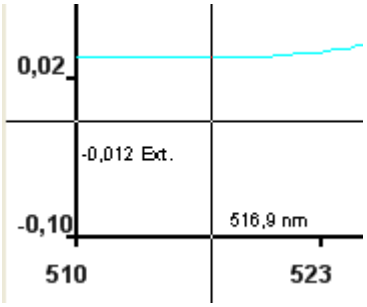


Fig. 53: Mouse position information in the graphic

Individual functions are described in more detail in the following chapter.

4.2 Functions of the Scan and Graphic Menus

This chapter describes the functions in the menu of the main window and the buttons in the toolbar at the top of the window.

4.2.1 Menu Function *Scan/Measure spectrum*,  Button

The menu command *Scan/Measure spectrum* starts a scan process.

This function is completed only when a *NANOCOLOR*® Spectrophotometer is connected to the computer and switched on!

The software opens the experiment window, where further details of the sample must be entered, see Fig. 49. The data in the *Scan Information* area of the window is used for documenting the scan and is included in the scan file when the scan is saved.

The boxes in the *Scan range* function area must be completed by the user. In the *from wavelength [nm]* and *to wavelength [nm]* boxes, enter the range that is to be scanned. If the *Measurement against air* box is enabled, a zero measurement without a blank cuvette is completed. **If a blank cuvette or a blind value is to be taken into account, this box must not be enabled**.

The options under *List of scans* are only enabled if a scan is already present in the workspace. You can then specify whether the new scan is to be added to the workspace or whether a new empty workspace is to be created, i.e. all old scans are to be closed.

If the security setting is set to **high**, the text boxes *Title*, *Sample ID* and *Description* **must** be completed. Compulsory fields are identified by a red asterisk * after the entry box. In the *Cuvette* text box, enter the type of cuvette being used.

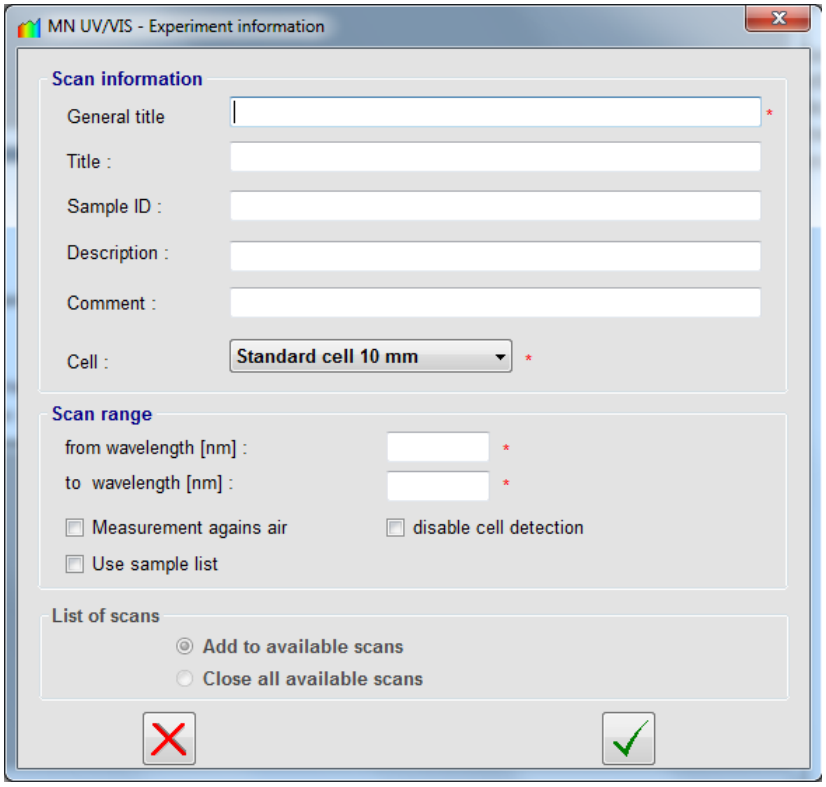


Fig. 49: Experiment window, basic scan data

The  button closes the experiment window without initiating the measurement.

Now, click on the  button to start the measurement. The software then asks for a blank cuvette, see Fig. 50. Insert the blank cuvette in the photometer. If a different type of cuvette is used than the one specified in the *Cuvette* entry box, an error message is displayed ("Incorrect Cuvette"). If a correct cuvette is not inserted in the photometer within 2 minutes of the error message being displayed (see Fig. 55), the measurement is aborted.

Fig. 55: Insert blank cuvette message

Alternatively, when the function *Measurement against air* is enabled, a message is displayed requesting you to wait until the air measurement has been completed.

After the measurement with a blank cuvette, the software requests that the cuvette be removed, see Fig. 56.

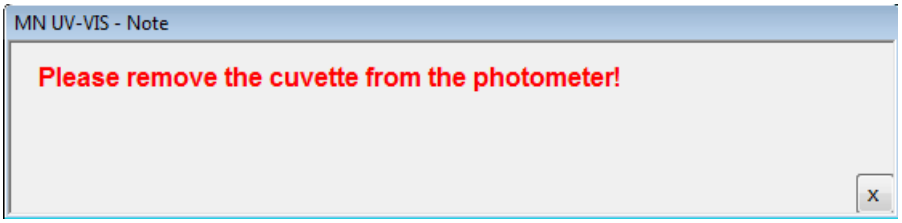


Fig. 56: Remove blank cuvette message

Remove the blank cuvette from the photometer. If a cuvette is still present in the photometer 2 minutes after the display of this message (Fig. 56), the measurement is aborted. The software then requests the sample cuvette (Fig. 57).

Fig. 57: Insert sample cuvette message

Insert the cuvette with your sample in the photometer. If a correct cuvette is not inserted in the photometer within 2 minutes of the error message display (Fig. 56), the measurement is aborted. During the data transfer, the volume of data transferred is shown in the status line of the software, as shown in Fig 58.

Fig. 58: Bytes transferred

After data transfer is completed, the scan window is opened and the scan graphic is displayed.

4.2.2 Menu Function Scan/Measure blank

This function is used to repeat a blank value measurement of the scan. Insert the blank cuvette in the photometer and click on the menu command *Scan/Measure blank*. The photometer scans the range previously specified in the *Scan/Measure spectrum* function and measures the blank value again.

This function can also be used to compare the same wavelength range for several samples, but with different blank solutions, see also Chapter 4.2.3. This function is enabled only when a scan has already been created.

4.2.3 Menu Function *Scan/New scan*,  Button

This function allows you to repeat the same scan that was specified before with the function *Scan/Perform scan*. Insert the cuvette in the photometer that contains your sample and click on the menu command *Scan/New scan*. The photometer starts the scan again.

It is also possible to insert a different sample and scan through the same wavelength range, to compare two different scans. If a different zero measurement is required for the second sample, read through Chapter 4.2.2. This function is enabled only when a scan has already been created.

4.2.4 Menu Function *Scan/Open scan*,  Button

The menu command *Scan/Open scan* opens one or more scans previously saved in a cdf file, as described in Chapter 4.1. A file selection window opens. Select the required file and click on the *Open* button.

4.2.5 Menu Function *Scan/Save scan*,  Button

The menu command *Scan/Save scan* saves **all scans in the current workspace** in one nc file. A click on the menu command opens a file selection window, see Fig. 59.

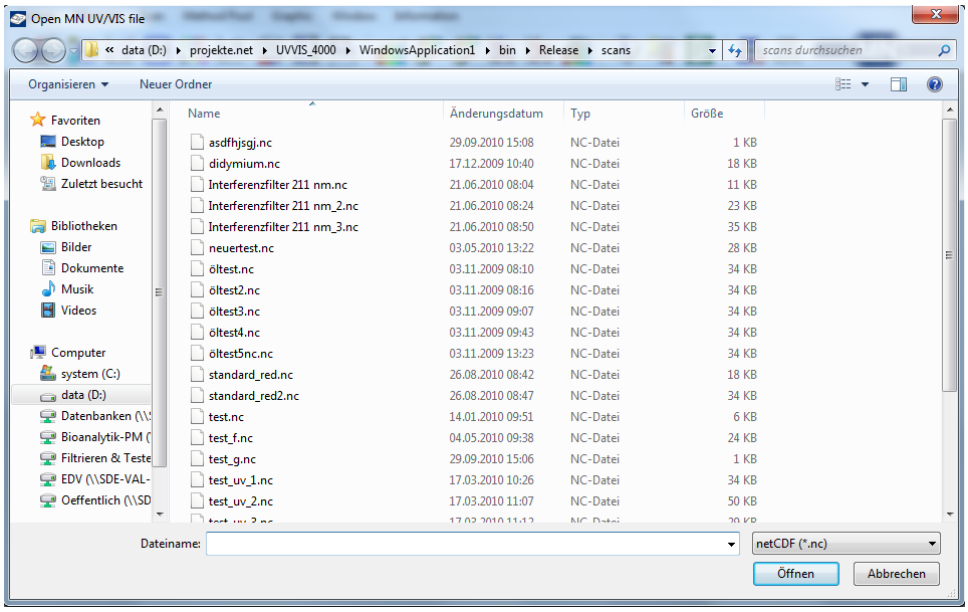


Fig. 59: Save scan, select file

Select a valid file path and a file name. It is **not** possible to overwrite an existing file. Click on the *Save* button. The file selection closes and the experiment window opens, see Fig. 60. For each scan here, a title must be assigned together with a sample name, sample number and details of the company. If more than one scan has been created, the other scans can be labelled by selecting the scan number from the drop-down list *Data for scan No*.

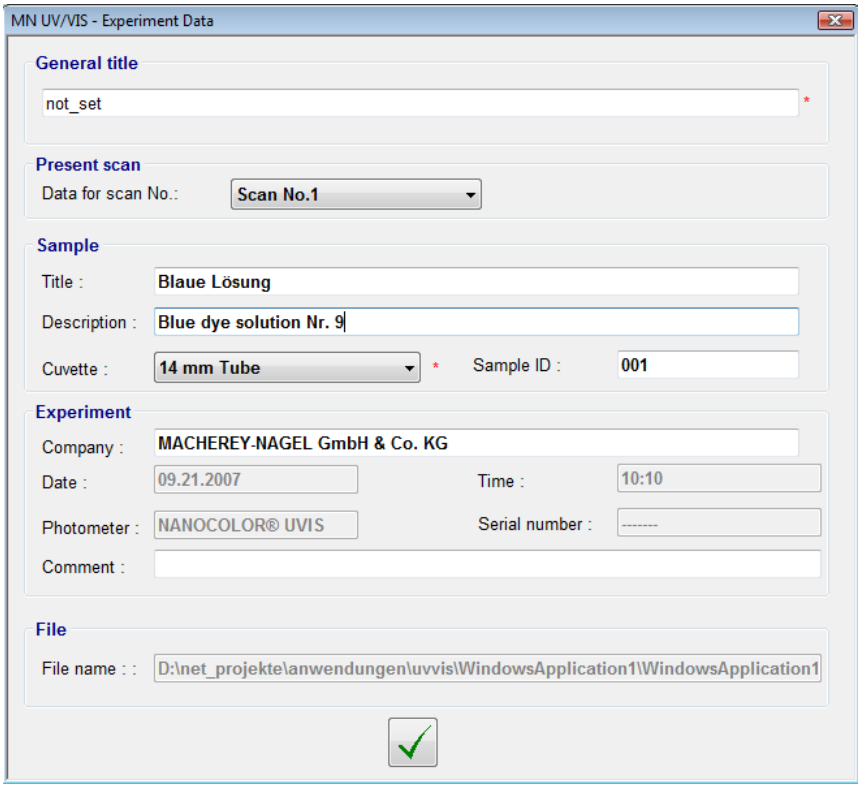


Fig. 60: Saving a scan

The boxes *Title*, *Description*, *Cuvette*, *Sample ID* and *Company* must be completed if the security setting is set to **high**. Compulsory fields are identified with a red asterisk * to the right of the box.

Further information on the nc format used can be found in the appendix, Chapter 8.4.

4.2.6 Menu Function *Scan/Print scan*

The menu command *Scan/Print scan* prints all scans in the current workspace together with all supplementary information.

4.2.7 Menu Function *Scan/Scan Methods*

To allow this function to be used, a photometer must be **connected and switched on**. With the menu function *Scan/Scan Methods*, regularly occurring work can be saved in the form of independent, standalone methods. Scan Methods is a subsection of the photometer special methods. Refer to Chapter 5.

4.2.8 Menu Function *Scan/Export scan*

The ***NANOCOLOR***® PC Software for Spectrophotometers provides an export function for the scan information in various other formats. As shown in Fig. 61, a choice can be made between four formats:

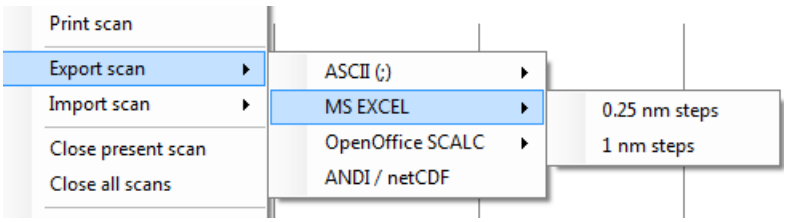


Fig. 61: Export formats

All available export formats provide scan resolutions of 0.25 or 1 nm.

In contrast to the *Scan/Save scan* function, the export function applies only to the current scan. Only one scan is transferred!

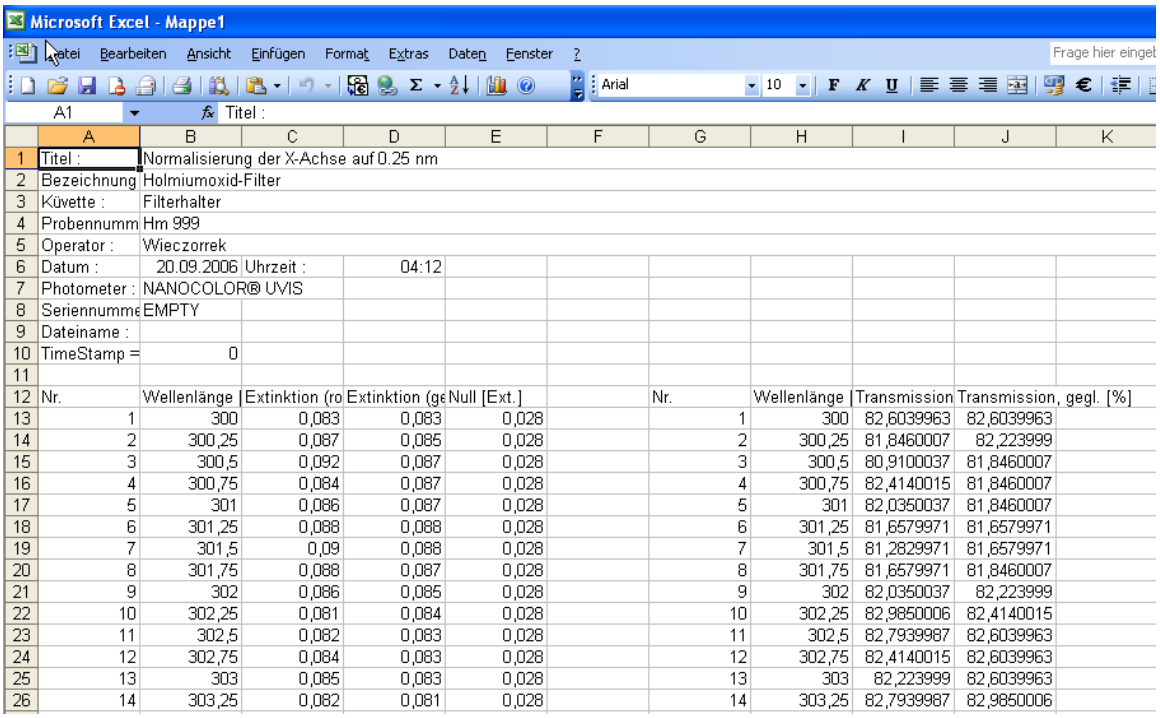
4.2.8.1 Menu Function Scan/Export scan/ASCII (;)

The menu function *Scan/Export scan/ASCII (;)* creates a formatted text file with semicolons as separators (SDF format), containing all relevant scan data. In the data header, the supplementary information is in the form of a list. This is followed by the data tables. An exact description of the format can be found in the appendix, **Chapter 7.3**.

4.2.8.2 Menu Function Scan/Export scan/MS EXCEL

The menu function *Scan/Export scan/MS EXCEL* creates a Microsoft EXCEL spreadsheet. The spreadsheet header contains all the additional information such as user, computer name, sample name etc. The table shown below contains the following data fields: item number, wavelength, measured absorbance, smoothed absorbance, ZERO line absorbance. Further to the right you will find a second table with the subsequent data fields: item number, wavelength, measured transmission, smoothed transmission, ZERO line transmission. The transmission is shown in %.

Fig. 62 shows an example created from the file *example_3.cdf*. The creation of very extensive EXCEL tables requires a considerable amount of time. Keep an eye on the progress bar in the status line of the main window (see Fig. 63).



Microsoft Excel - Mappe1

Datei Bearbeiten Ansicht Einfügen Format Extras Daten Fenster ?

A1 Titel :

	A	B	C	D	E	F	G	H	I	J	K
1	Titel :	Normalisierung der X-Achse auf 0.25 nm									
2	Bezeichnung	Holmiumoxid-Filter									
3	Küvette :	Filterhalter									
4	Probennumm	Hm 999									
5	Operator :	Wieczorrek									
6	Datum :	20.09.2006	Uhrzeit :	04:12							
7	Photometer :	NANOCOLOR® UVIS									
8	Seriennumme	EMPTY									
9	Dateiname :										
10	TimeStamp =	0									
11											
12	Nr.	Wellenlänge	Extinktion (ro	Extinktion (ge	Null [Ext.]		Nr.	Wellenlänge	Transmission	Transmission, gegl. [%]	
13	1	300	0,083	0,083	0,028		1	300	82,6039963	82,6039963	
14	2	300,25	0,087	0,085	0,028		2	300,25	81,8460007	82,223999	
15	3	300,5	0,092	0,087	0,028		3	300,5	80,9100037	81,8460007	
16	4	300,75	0,084	0,087	0,028		4	300,75	82,4140015	81,8460007	
17	5	301	0,086	0,087	0,028		5	301	82,0350037	81,8460007	
18	6	301,25	0,088	0,088	0,028		6	301,25	81,6579971	81,6579971	
19	7	301,5	0,09	0,088	0,028		7	301,5	81,2829971	81,6579971	
20	8	301,75	0,088	0,087	0,028		8	301,75	81,6579971	81,8460007	
21	9	302	0,086	0,085	0,028		9	302	82,0350037	82,223999	
22	10	302,25	0,081	0,084	0,028		10	302,25	82,9850006	82,4140015	
23	11	302,5	0,082	0,083	0,028		11	302,5	82,7939987	82,6039963	
24	12	302,75	0,084	0,083	0,028		12	302,75	82,4140015	82,6039963	
25	13	303	0,085	0,083	0,028		13	303	82,223999	82,6039963	
26	14	303,25	0,082	0,081	0,028		14	303,25	82,7939987	82,9850006	

Fig. 62: Export in EXCEL format

Fig. 63: Progress bar in the status line

4.2.8.3 Menu Function Scan/Export scan/OpenOffice SCALC

The menu function *Scan/Export scan/OpenOffice SCALC* operates in exactly the same way as the function *Scan/Export scan/MS EXCEL*, chapter 4.2.8.2, except that the spreadsheet is created in the OPENOFFICE program SCALC. The creation of very extensive SCALC tables requires a considerable amount of time. Keep an eye on the progress bar in the status line of the main window (see Fig. 58).

4.2.8.4 Menu Function Scan/Export scan/ANDI / netCDF

The menu function *Scans/Export scan/ANDI / netCDF* exports the current scan in the ANDI format (ANDI = Analytical Data Interchange). This format is defined in the **standards ASTM E 1947 – 98 (2004)** and **ASTM E 1948 – 98 (2004)** and is used as

a comprehensive data format platform for chromatographic and spectroscopic data analysis. Further information can be found in the appendix, **Chapter 8.4**.

4.2.9 Menu Function *Scan/Import scan*

This option enables you to import wavelength scans from other sources. With the recent version the import of scans saved in ANDI format or in the SECUMAM –SCN file format is possible.

4.2.10 Menu Function *Scan/Close present scan*,  Button

The menu function *Scans/Close present scan* in the main window closes the current scan and clears the scan from the workspace, regardless of whether the scan window is visible or not. The last remaining scan then becomes the current scan. Saved scans are not deleted. If the scan has not been saved, a warning message is displayed.

4.2.11 Menu Function *Scan/Close all scans*,  Button

The menu function *Scan/Close all scans* closes all scans and creates a new blank workspace, irrespective of whether the scan window is visible or not. Stored scans are not deleted. If a scan has not been saved, a warning message is displayed.

4.2.12 Menu Function *Scan/Delete labels*

The menu function *Scan/Delete labels* deletes all labels and captions created via the  function from the current scan.

4.2.13 Menu Function Scan/Delete all labels

The menu function *Scan/Delete all labels* deletes all labels and captions created via the  function from all scans in the current workspace.

4.2.14 Menu Function Scan/Delete Zero-line

The menu function *Scan/Delete Zero-Line* deletes the ZERO line of the current scan, provided that a ZERO line has been specified.

4.2.15 Menu Function *Scan/Derivative*

The menu function *Scan/Derivative* calculates the first derivative of the scan function. The first derivateive is added as new scan automatically and is set to the present scan, see figure 64:

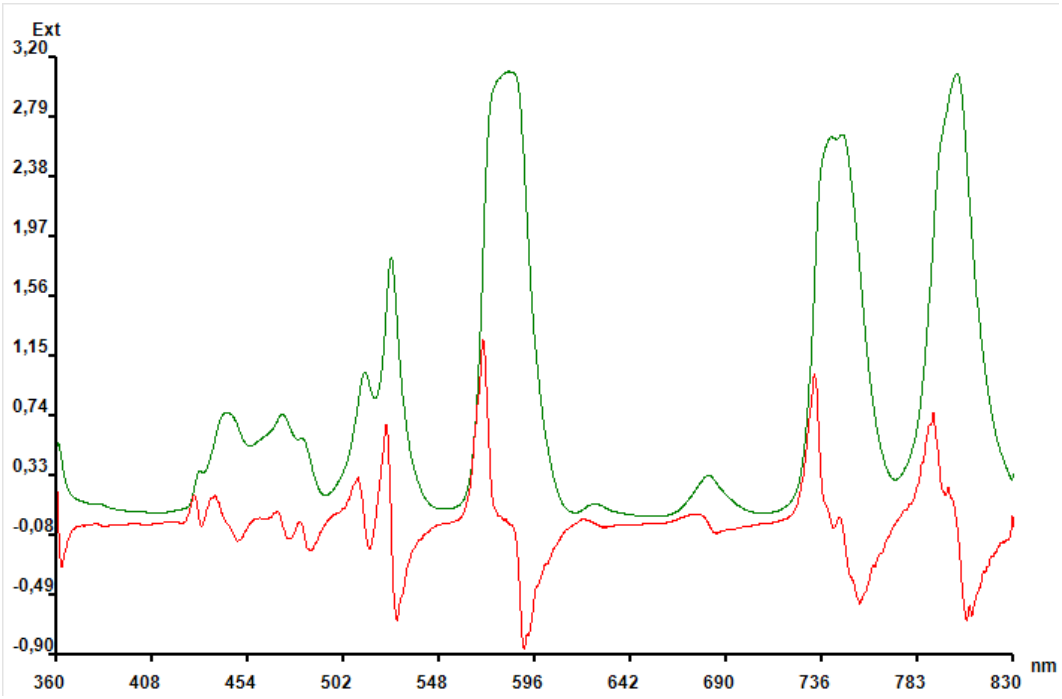


Fig. 64 : Caclculation of the first derivative of a scan

As the first derivative becomes the present scan you can create the second, third and so on derivative just by clicking the menu function again.

4.2.16 Menu Function Scan/Bioanalysis scan

The bio analysis scan function is a quick and convenious method to evaluate several biological scans at the same time. This function is explained in detail in chapter 8.10.

4.3 Functions in the Scan Window

The functions to edit scans are available as buttons at the lower edge of the scan window and as scroll bars and option buttons at the right of the scan window.

4.3.1 Buttons

The more important functions for editing a scan are positioned at the lower edge of the scan window in the form graphic buttons.

4.3.1.1 Add Scans,  Button

The **Add scans** function creates a new scan from two or more scans by adding together the measured values. The button is not enabled when only one scan is present in the workspace. The scans must also have an identical scan range, i.e. the same starting and ending wavelength. Otherwise, the function is aborted. Fig. 65 shows the drawing area of the scan window with two different scans.

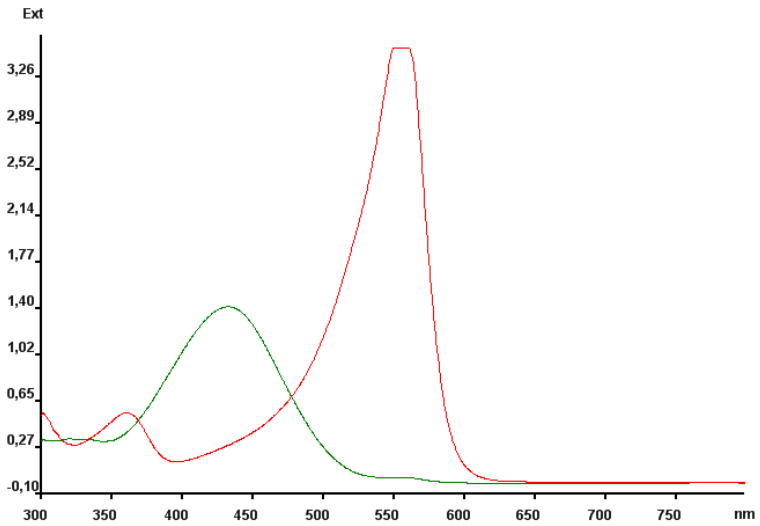


Fig. 65: Two different scans in the drawing area, before addition

By clicking the  button, a third scan is calculated by adding the absorbance values and then displayed in the drawing area. Figure 66 shows the calculated scan in red.

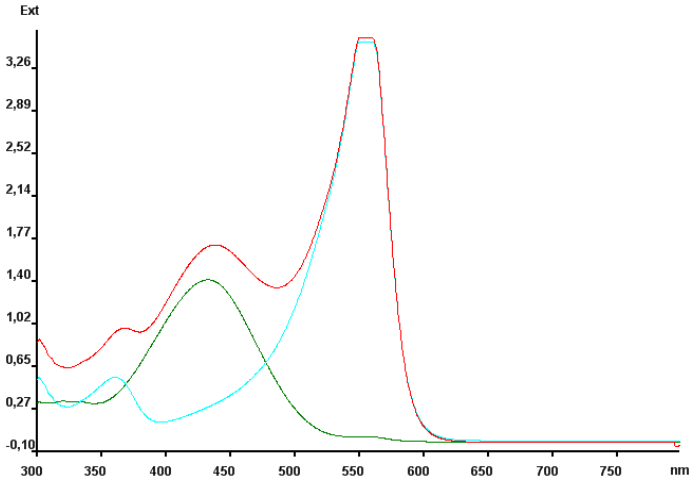


Fig. 66: Addition of two scans

4.3.1.2 Subtract Scans,  Button

The function **Subtract scans** creates a new scan from two scans, where the measured values of the second scan are subtracted from the first. The button is not enabled when there are less or more than two scans present in the workspace. The scans must also have an identical scan range, i.e. the same starting and ending wavelength. Otherwise, the function is aborted. Fig 67 shows the drawing area of the scan window with two different scans.

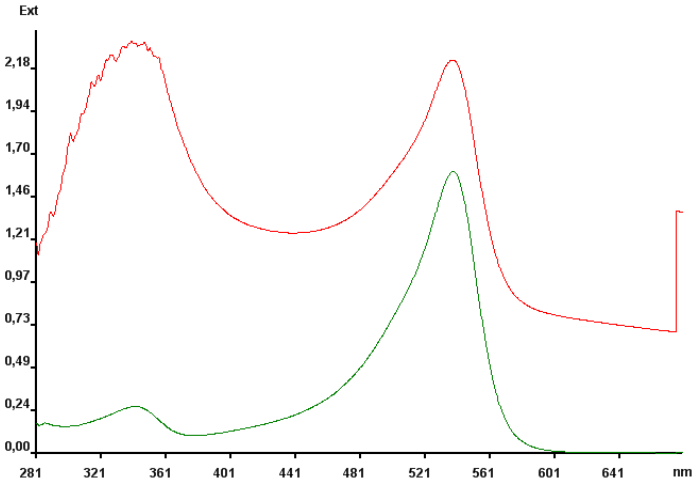


Fig. 67: Two different scans in the drawing area, before subtraction

By clicking the  button, a third scan is calculated by subtracting the absorbance values and then displayed in the drawing area. Figure 68 shows the calculated scan in red.

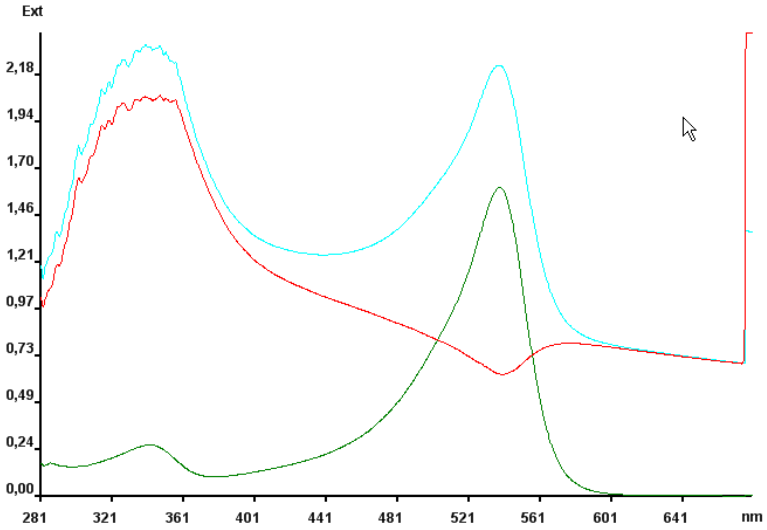


Fig. 68: Subtraction of two scans

4.3.1.3 Measuring the Peak Factor,  Button

This function measures the factor of the difference in amplitude of two or more coincident peaks in two or more scans. At the position where the peaks coincide, the factor is calculated from the absorbance of the active scan divided by the absorbance of the other scan. The button is not enabled when only one scan is displayed. An example is shown in Fig. 69.

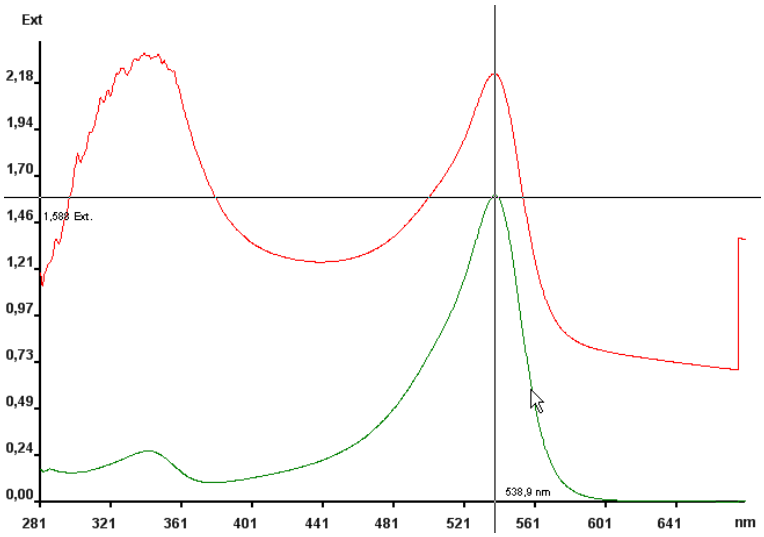


Fig. 69: Peak factor, selection of the reference point

Click on the  button. If you wish to cancel the calculation prematurely, click on the  button a second time or simply move the mouse pointer outside the area of the scan graph. Now, click inside the scan area with the left mouse button at the position where the amplitude of the peaks are to be compared, as shown in Fig. 69. Fig. 70 shows the result of the operation after clicking on the peaks at 530 nm: the peaks of both scans are drawn with the same amplitude. In order to arrive at the result shown below, you have to make the lower green scan from Figure 69 the active scan.

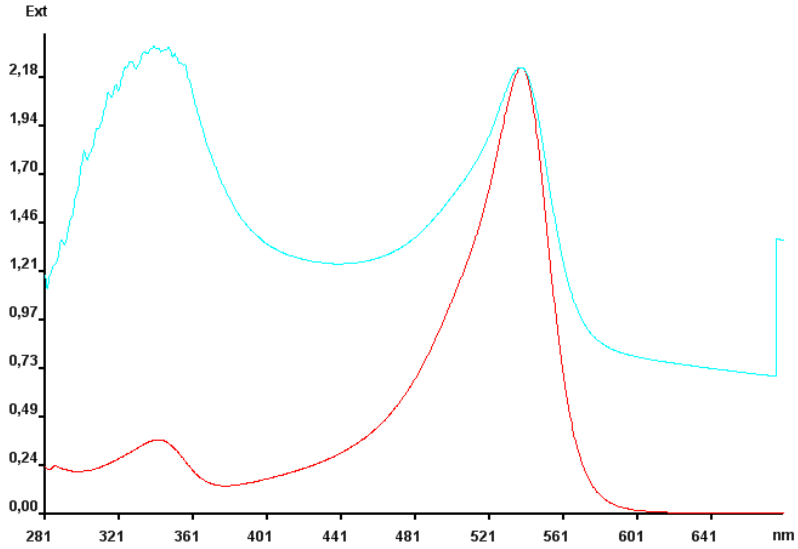


Fig. 70: Measuring the peak factor

The peak factor measured by this method is shown in the status line of the main window, see Fig. 71. This information always applies only to the current scan.

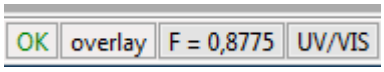


Fig. 71: Display of the peak factor

The factor F = 1,3959 means that the absorbance values of the lower (turquoise) scan in Fig. 69 must be multiplied by 1,3959 to give the same absorbance value as that of the red scan shown in Fig. 69 at the selected position.

4.3.1.4 Scan Analysis,  Button

A click on this button starts the analysis of the current scan. Here, a search is made for the peaks, the half-width values and integrals are measured. The results are

written in a log and can then be printed. Fig. 72 shows the graph after a scan analysis and Fig. 73 shows the log window. After a successful analysis, the log window can be opened by clicking the  button or via the menu function *Window/Open protocol window*. Via the settings window in the *System/Settings* menu, you can set a log window to open automatically after each analysis.

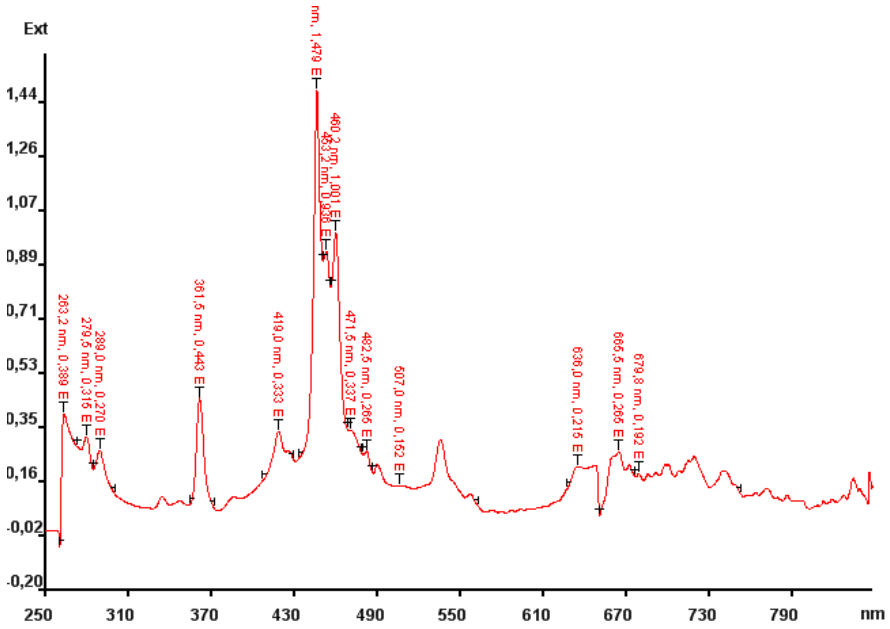


Fig. 72: Scan analysis

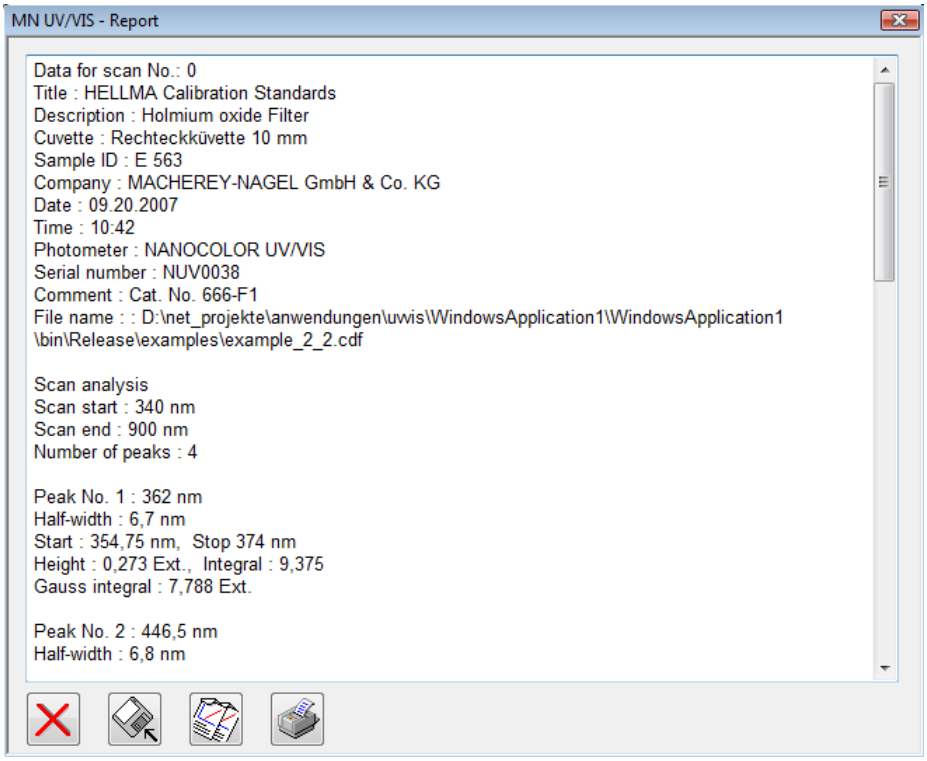


Fig. 73: Log data of scan analysis

The log can be saved as a text file via the  button and printed via the  button. The third button  , closes the log window. The fourth button  copies the contents of the log window to the clipboard.

4.3.1.5 Integration of Individual Peaks,  Button

A click on the  button sets the software to single peak integration mode.

To exit this mode, click again on the  button or move the mouse pointer outside the area of the scan graph. Using this function, individual peaks can be integrated that are inside a mouse frame. With the left hand mouse button pressed, drag a frame around the peak required for measurement. The exact height of the peak is not of critical importance, but the start and end points of the integration must be determined on the wavelength axis. Fig. 74 shows how the frame is dragged and Fig. 75 shows the result of the function.

Manual peak integration is also possible in zoom mode.

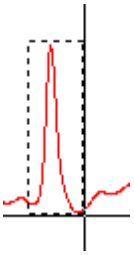


Fig. 64: Integration of individual peaks in a mouse frame

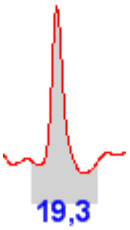


Fig. 75: Display of the integral

Provided that a ZERO line has not been specified in the scan, integration is made up to absorbance = 0. When a ZERO line is inserted manually, the integral is

automatically corrected. The value 19,3 is given by the sum of all absorbance values that lie between the selected start and end points.

4.3.1.6 Inserting a ZERO Line,  Button

A click on the  button sets the software to ZERO line mode. To exit this mode, click the button again or simply move the mouse pointer outside the area of the scan graph.

An individual ZERO line can be assigned to each scan in the current (active) workspace. In this software, ZERO lines are formed by defined sections of straight lines. To insert a ZERO line in the scan, the intersection points, i.e. the transition points between the sections of straight lines, must be set in the graph by clicking the mouse at the position required. The intersection points that have been set are marked in the graph by small squares. The first and last intersection point is always Absorbance = 0; they are at the left and right edges of the graph.

By clicking the  button, the intersection points can be marked in the graph using the mouse. A second click on the  button causes the lines to be drawn in the graph. Fig. 76 shows an example of the operation.

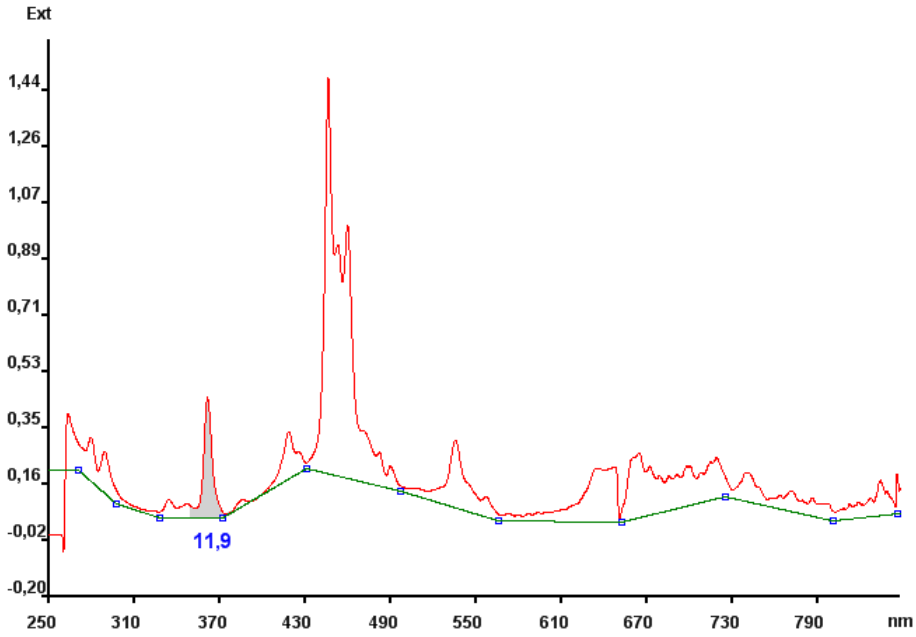
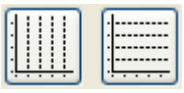


Fig. 76: Inserting a ZERO line

A comparison of the manual peak integrals with Fig. 75 shows that the manual integrals are automatically adapted.

4.3.1.7 Grid Lines,  Buttons

The two buttons for the X and Y grids insert corresponding grid lines in the graph. An example is shown in Fig. 77.

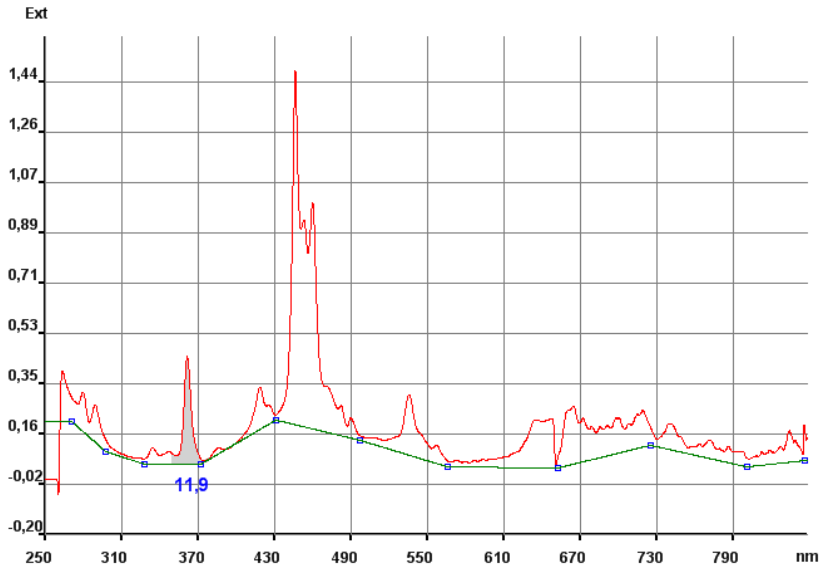


Fig. 77: Grid lines

4.3.1.8 Labelling,  Button

This function is used to insert labels and titles in the graph. Each scan can be individually labelled. The labelling is always shown for the current scan. After a click on the  button, the graph is set to labelling mode. If the labelling is to be cancelled, click on the button a second time, or move the mouse pointer outside the area of the graph. Now, click the **right** mouse button on the graph at the position where the label is to be inserted. A text entry window is opened, see Fig. 78.

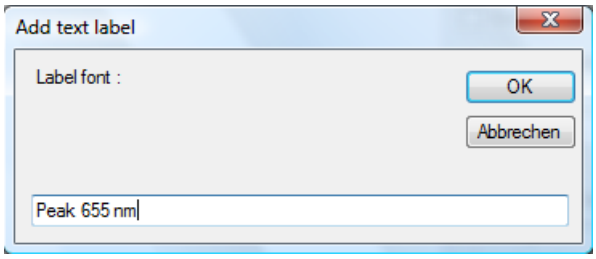


Fig. 78: Entering label texts

If the button *Mouse tracking* is enabled, then wavelength and absorbance (or transmission) are set as standard text. After clicking on the *OK* button, the text is inserted in the graph. If the position of the insertion is not exactly where it should be, the text can be dragged with the mouse to the position required (provided that the graph is still in labelling mode). The manually inserted integrals can also be moved in the same way, with the mouse pointer. After all texts have been inserted, labelling mode can be closed by clicking again on the  button. The labelling mode can be opened at any time. A double click on a label text opens a correction window, where the text can be corrected or changed, see Fig. 79.

View depends on Windows® version!!!

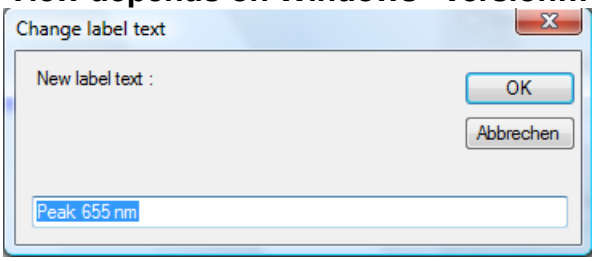


Fig. 79: Label correction

The font type, colour and size can be specified in the *System/Settings* menu. Fig. 80 shows a scan with labels.

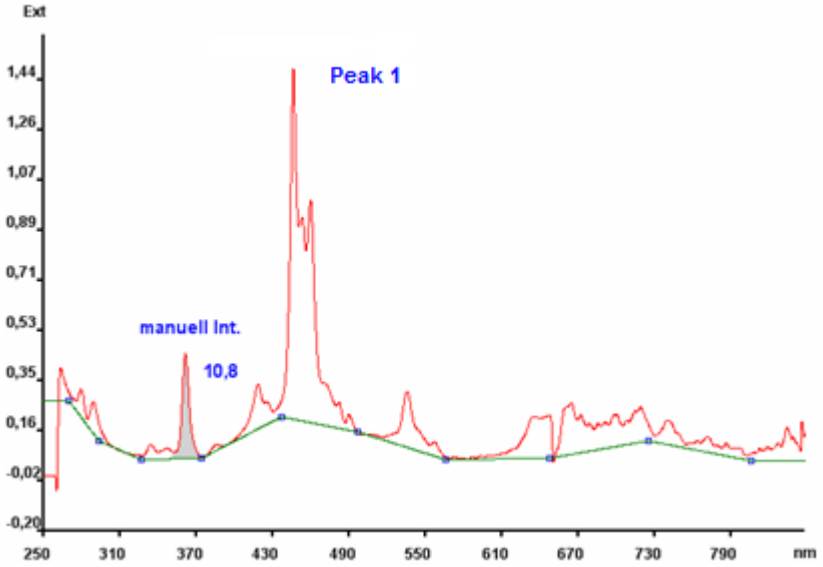


Fig. 80: Scan with labels

4.3.1.9 Zoom Out,  Button

If the graph is in zoom mode (after previously dragging a mouse frame), zoom mode can be closed by clicking the  button. Or, click anywhere in the zoomed graphic.

4.3.1.10 Evaluate Scan Kinetic,  Button

This button is not enabled when less than 4 scans are open. To create a scan kinetic, a corresponding scan method under the menu item *Scan/Scan methods* must be created and executed. **Refer also to Chapter 5**. If a scan kinetic has been executed, a graph similar to Fig. 81, for example, is displayed.

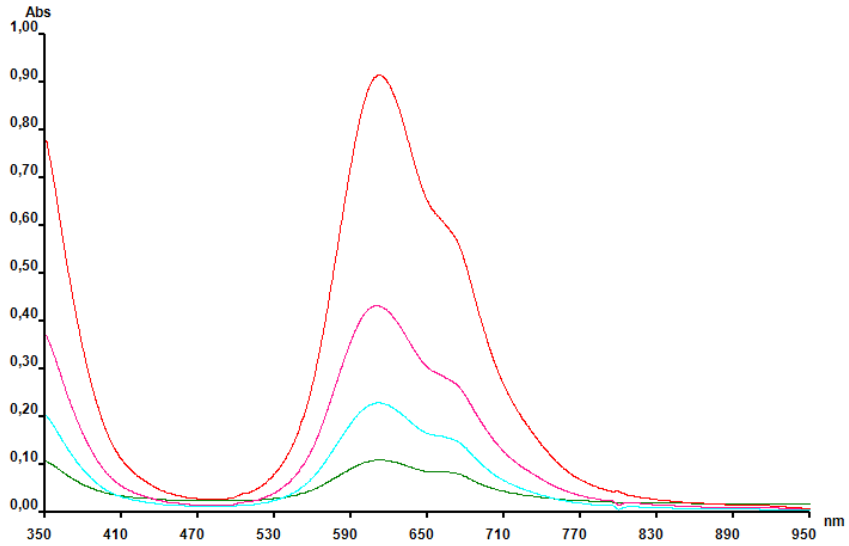


Fig. 81: Scan Kinetic

After clicking on the  button, the position at which a kinetic is to be measured can be specified by clicking the left mouse button within the graph area. The position is usually the top of a peak. After the click, kinetic mode of the scan window is closed and the kinetic window is opened, as shown in Fig. 82.

To close kinetic mode without calculating the kinetic, you can click on the  button again, provided no start position has yet been specified.

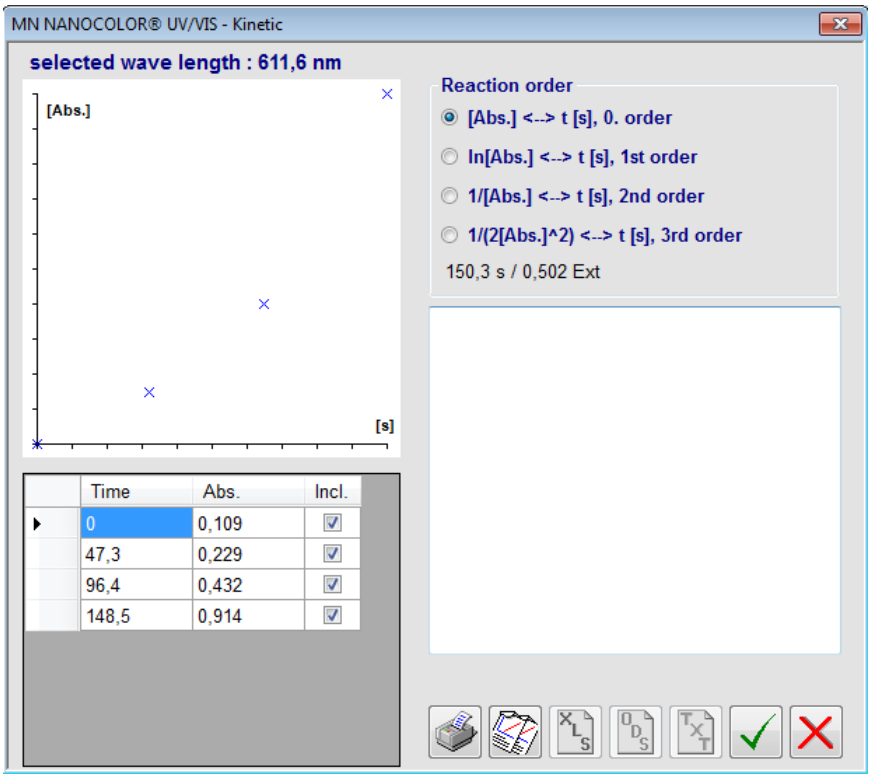


Fig.82: Kinetic window

The chart in the upper left section of window shows the distribution of the measuring points. The measured values are listed in the table below the chart.
If individual measurement points are to be excluded from the calculation, the green check-mark in the third column of the table can be removed. Alternatively, by dragging a mouse frame in the chart, the number of points for calculation can be specified, as shown in Figs. 83 and 84.

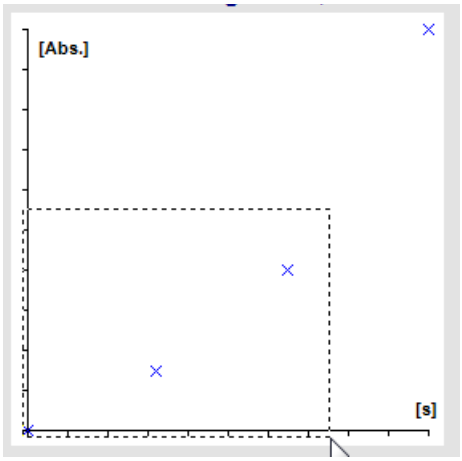


Fig. 83: Marking measurement points with the mouse

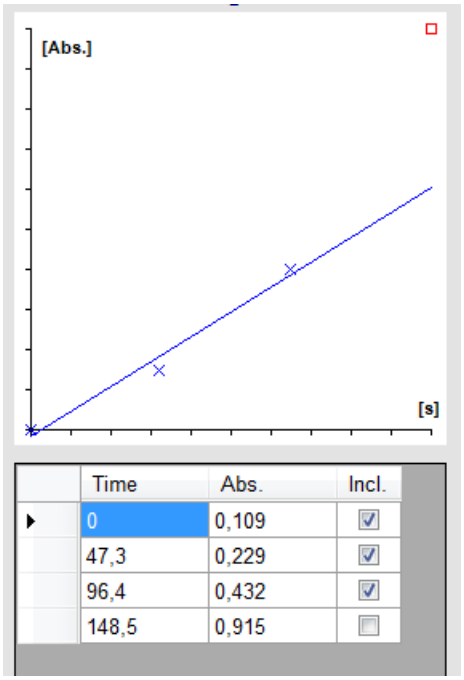


Fig. 84: Unmarked measurement points are removed from the table

Measurement points that are marked for calculation are identified as a blue cross on the chart and as a green check-mark in the table. Unmarked points do not show a check-mark in the table and are displayed on the chart as small red squares. After dragging with the mouse, the calculations are started, automatically. If the marking has been made in the table with the mouse, the  button must be pressed. The types of evaluation are shown in the *Reaction Order* section of the window (Fig. 85). A click on one of the option buttons starts a new calculation of the table and the chart is redrawn accordingly.

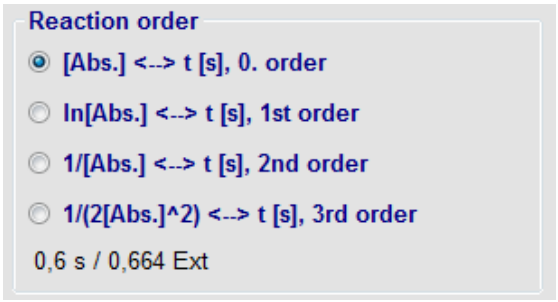
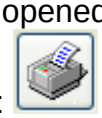


Fig. 85: Reaction Order window section

The  button starts a printout of the log; the  button closes the kinetic window.

The scan kinetic and the simple kinetic functions do not provide any direct statement of the order of reaction or the reaction time constant. The user is responsible for assigning the measured values to one of the four standard evaluations!

4.3.1.11 Open Log Window,  Button

If a scan is analysed using the Automatic Analysis function  , the log window can be opened at any time with the  function. There are 2 buttons in the log window:  for printing the measurement log and  for saving the log as a text file.

4.3.1.12 Print Log,  Button

A calculated log, including the scan graph, can be printed at any time by clicking the  function. The log window does not necessarily need to be opened.

4.3.1.13 Close Scan Window,  Button

The button with the red “X” closes the scan window. Provided the workspace has not been overwritten by a new or saved scan, the scan window can be opened at any time with the menu function *Window/Open scan window*. The scans are not lost when the scan window is closed.

4.3.2 Functions that influence the Scan Analysis

Automatic analysis of a scan is influenced by several factors. The settings for these factors can be changed at the right hand edge of the scan window. This chapter describes individual settings, sorted according to the relevant area of the window.

4.3.2.1 *Peak functions* Window Area

Depending on the settings of these parameters, some peaks may not be found or too many peaks may be detected during the automatic peak search. This parameter should be changed in small steps, until the result of an analysis is satisfactory. Fig. 86 shows the *Peak functions* window area.

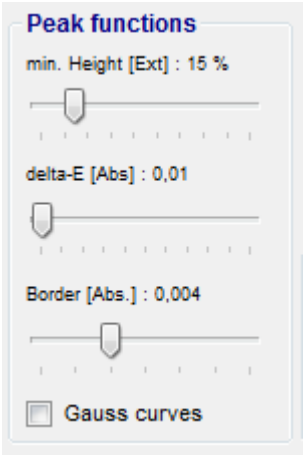


Fig. 86: Peak functions window area

The first slider control *min. Height [Abs]:* specifies the minimum absorbance value that can be detected as a peak from the local maximum values. In the above example, peaks will not be displayed the height of which is less than 15% of the absorbance of the highest peak in the scan.

The slider control *delta-E [Abs]:* describes the difference in absorbance between peaks and the cuspidal (or stationary) points on the curve, regardless of the absolute amplitude. In the above example, the so-called shoulders at the peak edges are interpreted as independent peaks if the amplitude of the local maximum is greater than 0.01 Abs. above the amplitude of the peak.

The slider control *Border [Abs.]:* describes the gradient of the curve at the start and end of the peak. The number 0.004 describes the difference in amplitude between two scan steps (approx. 1.2 nm). This setting directly influences the base width of a peak.

When the *Gauss curves* option button is enabled, an approximation is calculated using a Gaussian distribution curve. To approximate asymmetric peaks, the two halves of a bell (or normal) curve with different parameters are also calculated. The bell curves are automatically drawn in the graph.

Each time a change is made to one of these slider controls, the graph is re-drawn, showing the immediate effects of any change to the parameters.

4.3.2.2 Automatic Markers

In an automatic scan analysis, the peaks and valleys, together with their starting and end points, are identified by small black markers, see Fig. 87. The size of these markers can be changed or the insertion of these markers can be suppressed. The functions required are in the *Markers* window area, shown in Fig. 88.

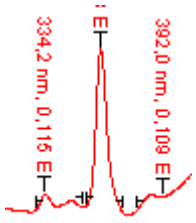


Fig. 87: Peak markers

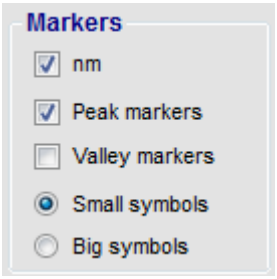


Fig. 88: Markers window area

A check-mark in the *nm* option forces the insertion of the wavelength.

A check-mark in the *Peak Markers* option forces the insertion of peak markings.

A check-mark in the *Valley Markers* option forces the insertion of valley markings.

The *Small symbols* and *Big symbols* option buttons are used to change the size of the markings.

4.3.2.3 Curve Drawing

Fig. 89 shows the *Curve* windows area.

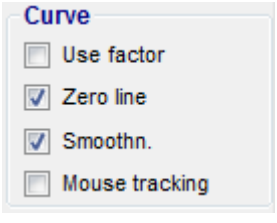


Fig. 89: Curve window area

The *Use factor* button causes the absorbance of the curves to be enlarged or reduced by a scaling factor, when this factor has been specified by the peak factor function  (see Chapter 4.3.1.3).

With the *Zero line* button, a calculated ZERO line can be suppressed, i.e. the ZERO line is not drawn in the graph. If this function is enabled, a calculated ZERO line is also output.

The *Smooth* function specifies whether raw data or rounded values are used as a basis for the scan calculation and output. When the function is enabled, the measured raw data is subjected to a mathematical smoothing, whereby the amount of the smoothing can be specified (see Chapter 4.3.2.4). Further information on smoothing can be found in the appendix, Chapter 8.6.

The *Mouse tracking* option offers an easy method of examining a curve more precisely. When the function is enabled, the movement of the mouse in the absorbance axis/Y-axis is restricted as soon as the mouse is moved on the graph. The mouse can then only move over the nanometre axis. The position on the absorbance axis is automatically taken from the measured values on the curve, so that the mouse then moves directly on the curve. If the mouse has a scroll wheel, each click of the wheel causes the mouse to move 1 pixel width. If several scans are displayed on the screen, this function is enabled with the current scan.

4.3.2.4 *Scan functions* Window Area

All the settings in the *Scan functions* window area (Fig. 90) are only effective on the current scan. At the upper left is the *Present scan* list box. This is used to specify the current scan when several scans are available. To change the current scan, select its number from this drop-down list.

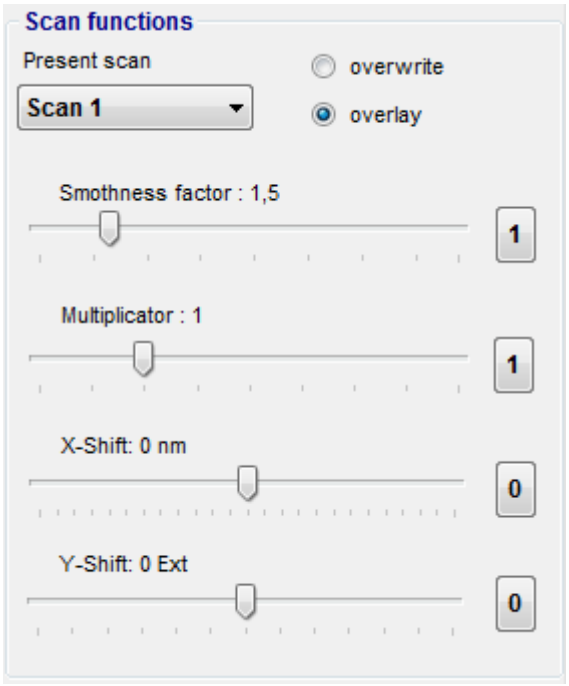


Fig. 90: Scan functions window area

At the right of the present scan, are the *overwrite* and *overlay* option buttons.
If the Overlay option is enabled, all scans are drawn in one graph; if Overwrite is enabled, each scan is drawn in its own graph.

The slider control *Smoothness factor* is used to specify the amount of smoothing of the measured values. Small values result in a low level of smoothing, large values produce a greater level of smoothing. Up to a factor of around 1.8, there are no significant changes to the measured values. A smoothing factor greater than 2 produces noticeable changes to the data.

With the *Multiplicator* slider, a factor can be set for each individual scan. The measured values are multiplied by this factor before the graph is drawn. In this way, it is quite straightforward to compare various scans. **The data measured is not changed when this control is used**. Also, refer to Chapter 4.3.1.3.

The *X-Shift* and *Y-Shift* slider controls move the current scan in the graph and ease the task of comparing the scans. **When this slider is used, the measured values are not changed**.

4.3.3 Scan pre-settings

The scan functions operate with several pre-settings. These settings can be changed by clicking the menu command *System/Settings/Scan-Settings* see chapter 7.3.2, or by clicking the button  from the icon area, see figure 91.

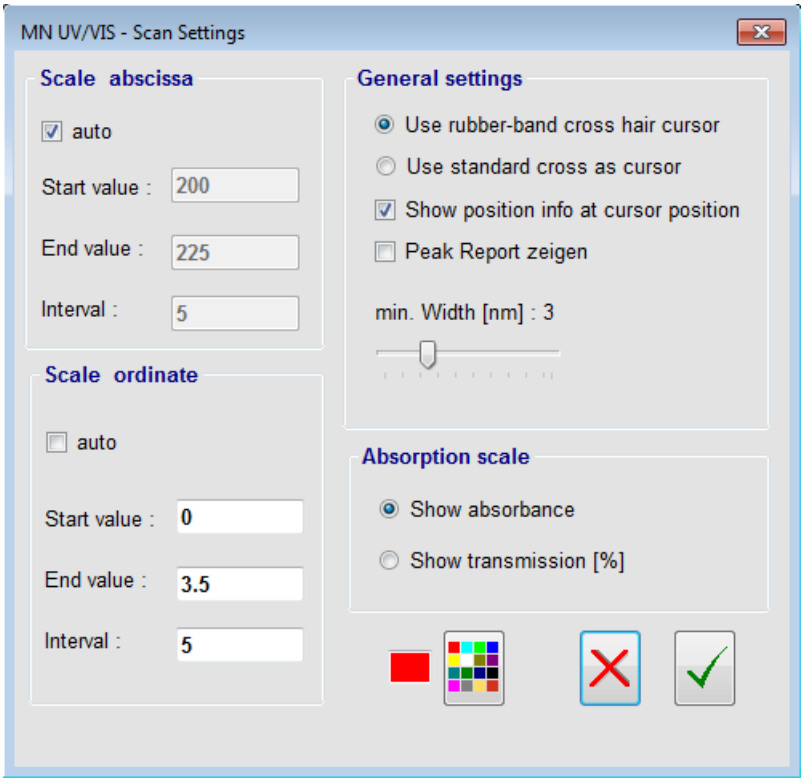


Fig. 91 : Scan setting window

Use the scan settings to scale the axes and to select transmission mode or absorption mode. With the button  you change the colour for the present scan. User with red-green visual impairment usually have problems to recognize the present scan if it is printed in red. With the option *min. Width [nm] :* you set the minimum width of a peak to be recognized by the software. If necessary – if you find ghost-peaks/spikes on your scan, you may select a different value.

All other options are self-descriptive or see chapter 7.3.2.

You can change the most important options directly through a context menu by clicking the scan graphic with the right mouse button, see figure 92:

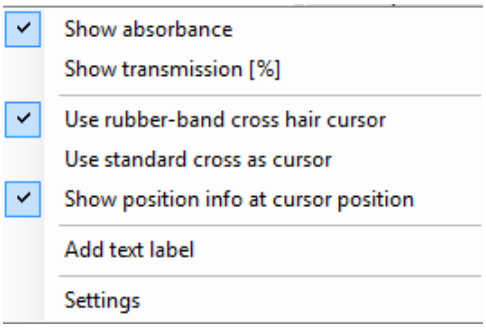


Fig. 92 : Context menu "Scan settings"

5 Photometer Special Methods

Special methods are methods of measurement that are defined by the user.
In the ***NANOCOLOR*®** photometers, a differentiation is made between 5 different special methods:

- Scan methods
- Scan kinetic
- Single wavelength measurements
- Kinetic
- Multi-wavelength measurements

All special methods can be saved under a separate method number in the photometer. The ***NANOCOLOR*®** Spectrophotometers can manage up to 100 special methods; the method numbers 200 to 299 are reserved in the photometer for this purpose.

All special methods can also be saved under a method number in a database on your PC. The database can manage as many special methods as required.

With a scan kinetic, a scan method is repeated at specified intervals. Scan kinetics cannot be saved in the photometer.

Single wavelength measurements define linear or polynomial measurement methods.

Kinetic methods repeatedly use a single wavelength measurement at specified intervals.

Multi-wavelength measurement methods evaluate samples at several different wavelengths

All special methods can be managed via the menu functions *Special methods* or *Scan/Scan methods* . Figure 93 shows the window *Method management* . The special methods shown are only examples and are not contained in your PC / photometer. If you have not yet established a connection to your photometer, please click on the button  .

The special methods window shows two tables. On the left, the *PC Database* table lists methods that are saved in your PC in a special database. You may create and manage as many special methods as you require on your PC. After a new installation of the ***NANOCOLOR*®** Spectrophotometer Software, this table is empty. At the right hand side, the *Photometer* table lists the special methods that are already saved in the photometer. If you have not yet saved any special methods in the photometer, this table is also empty.

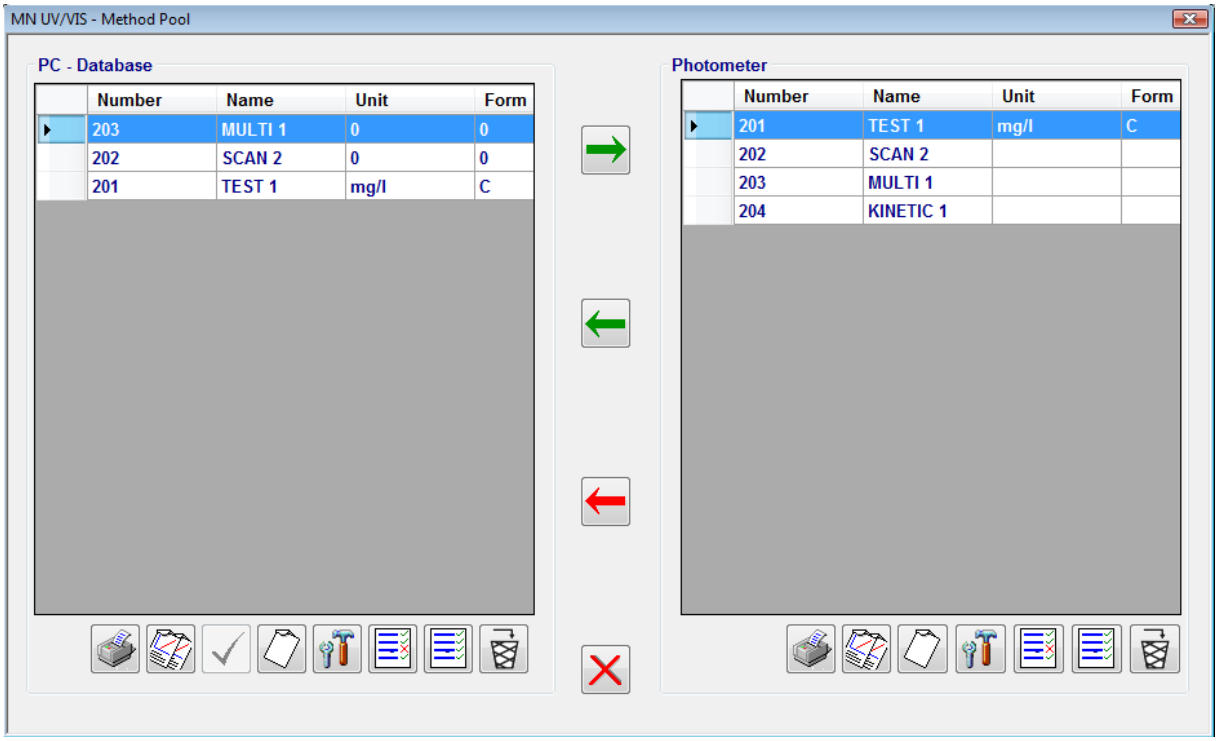


Fig. 93: Method management window

Below the tables, are the functions for managing the special methods.

5.1 Functions in the *PC Database* Table

From right to left, the following functions are available:

The  button deletes all special methods from the PC database. This operation cannot be reversed! After this function, the table is empty. **Special methods that are saved in the photometer are not deleted**.

All methods in the table can be selected with the  button.

The  function deletes selected methods from the PC database. If no method or more than one method is selected, this function has no effect. **A special method with the same name in the photometer is not deleted by this function**.

To edit a selected special method, click on the  button. Creating and editing special methods is explained in Chapter 5.4. **Changes made to PC special methods are not automatically transferred to the photometer**.

A new special method can be created in the database with the  function. Creating and editing special methods is explained in Chapter 5.4. **Newly created methods are not automatically transferred to the photometer**.

A selected special method can be executed in the PC from the PC database via the  button.

The method data can be printed with the  function or copied to the windows clipboard using the  function.

By clicking the right mouse button on the PC database table a context menu opens which provides additional options, see figure 94:

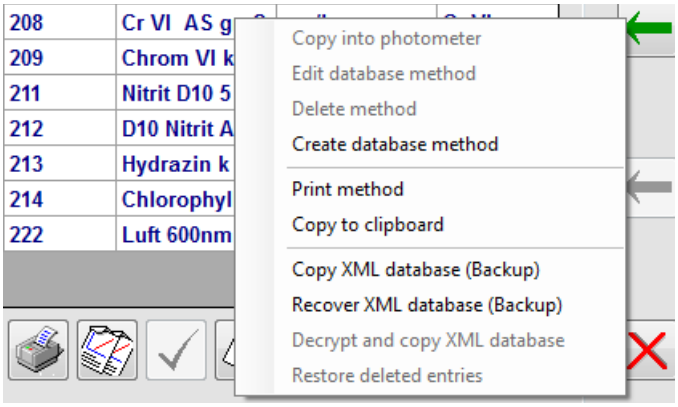


Fig. 94 : PC database context menu

In addition to the functions available by buttons you find the following options:

Copy to clipboard copies the data of the selected method into the Windows® clipboard.

Copy XML database (Backup) creates a backup-copy of your method database. A folder-browser dialog opens where you can select the backup folder for your copy.

Recover XML database (Backup) recovers a damaged or lost database from your copy database (if present).

Decrypt and copy XML database creates a readable XML file of your method data that can be imported into ***EXCEL / ACCESS / OpenOffice***.

This option is for documentation purposes only. The readable XML file can not be read by the *NANOCOLOR*® Spectrophotometer Software.

If you delete a method, the method is only marked as “deleted” and deleted methods are invisible. If you delete a method by mistake, it is always possible to restore the deleted method. Use the option *Restore deleted entries* to undelete your method.

To restore a single method is not possible, this option always restores ALL deleted database entries!

5.2 Functions in the *Photometer* Table

The functions in the photometer special methods apply fully analogously to those in the PC special methods in Chapter 5.1. Changes, deletions and new creations are made only in the photometer. These functions have no effect on the PC database.

The  button **is not** included in the *Photometer* table. Photometer special methods must be started in the photometer and cannot be started from the PC. The button  copies the selected method data to the clipboard.

The button  prints the selected method data using your printer.

5.3 Exchanging Methods between PC and Photometer

There are several functions available for exchanging methods between the PC and the photometer. Before moving methods between the PC and the photometer, ensure that the following points are fully understood:

- each special method has been assigned a method number

- all special method numbers are within the range 200 to 299

- each method number can only be used once in the photometer

- **up to 100** special methods can be saved in the photometer

There are several buttons between the two tables in the special methods window.

The  button copies all marked special methods from the PC database to the photometer (i.e. from the left table to the right table). If no special methods are selected, the button has no effect. In the following cases, a method is not copied to the photometer: a) the method is a scan kinetic and b) the method number already exists in the photometer. Existing methods are **not** overwritten. **This function does not delete any data**.

The opposite action is performed with the  button. All marked special methods are copied from the *Photometer* table to the PC database. Methods are **also copied** if the method number already exists in the PC database. Under some circumstances, double entries may appear in the PC database, which must be corrected manually. **This function does not delete any data**.

The second way to transfer special methods from the photometer to the database, is with the  button. This copies all marked special methods from the *Photometer* table to the PC database. Methods are **also** copied if the method number already exists in the PC database. Under some circumstances, double entries may appear in the PC database, which must be corrected manually. **In contrast to the previous function, all marked special methods in the photometer are deleted!**

The  button closes the special method window.

5.4 Create / Edit Special Methods

The method create/edit window is shown in Figs. 95 to 99. The appearance of the window depends on the type of special method.

5.4.1 Single Wavelength Measurement

Fig. 95 shows the “Wavelength” method type, i.e. a measurement at one wavelength.

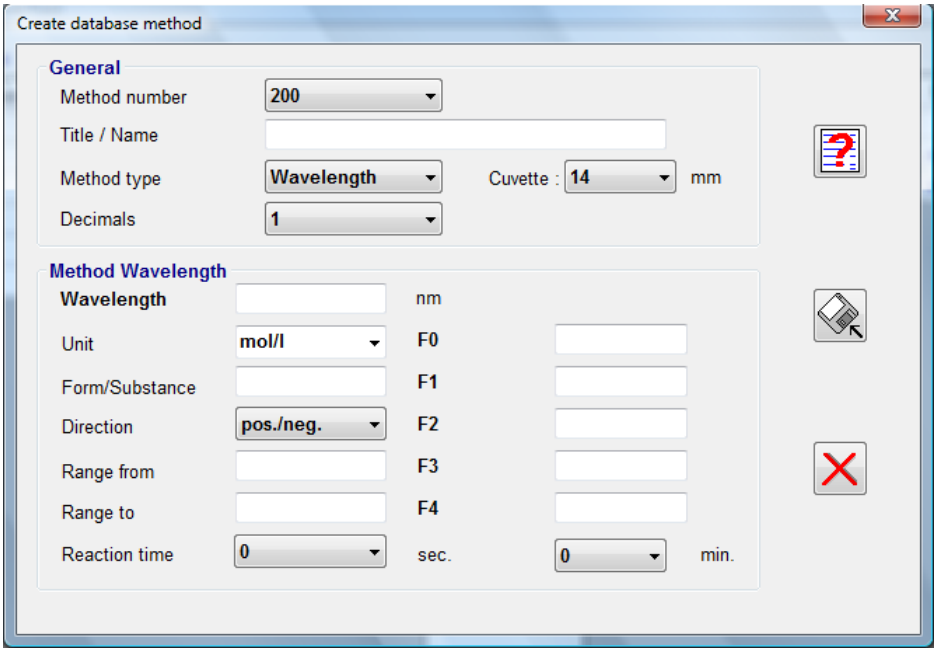


Fig. 95: Special method "Create single wavelength measurement"

In the upper section of the window, general details of the special method are entered. The method number is selected from the *Method number* drop-down list. The name (max. 12 characters) that is to be displayed in the photometer is entered in the *Title / Name* text box. The *Method type* list box must indicate "Wavelength". Set all parameters according to your method. The meaning of individual parameters can be found in the photometer manual. Refer also to Chapter 8.8 in the appendix. The  button can be used to check that all parameters are correct and that the method number is still available. The result of a check is shown as text at the lower edge of the window. Incorrect results are shown in red letters.

The new/changed method is saved with the  button.

5.4.2 Multi-wavelength Measurement

Fig. 96 shows the “Multi-wavelength” method type, i.e. a measurement at more than one wavelength.

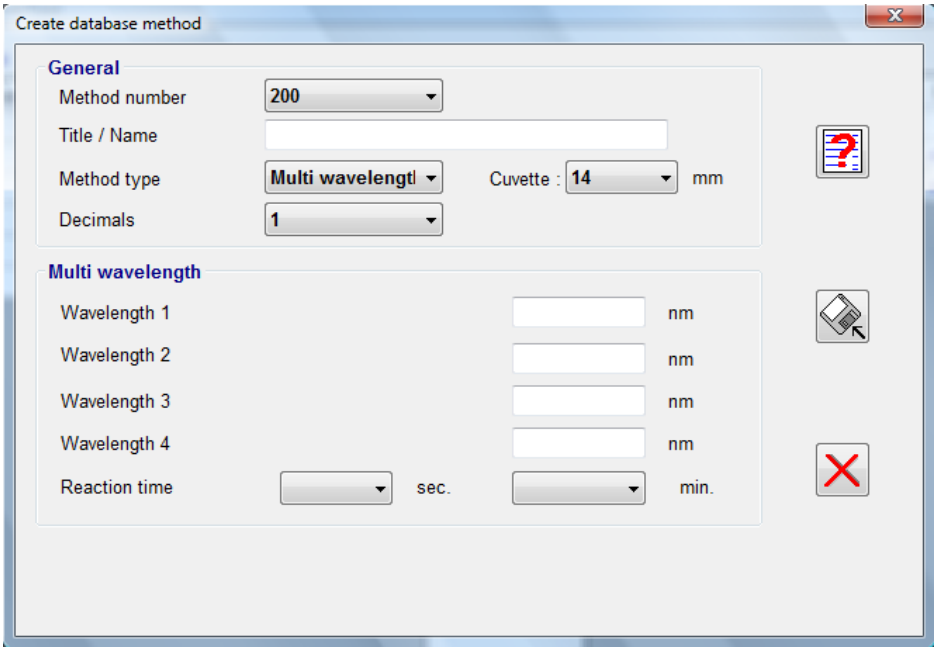


Fig. 96: Special method “Create multi-wavelength measurement“

In the upper section of the window, general details of the special method are entered. The method number is selected from the drop-down list *Method number*. The name (max. 12 characters) that is to be displayed in the photometer is entered in the *Title / Name* text box. The *Method type* list box must indicate “Multi-wavelength”. Set all parameters according to your method. The meaning of individual parameters can be found in the photometer manual. Refer also to Chapter 8.8 in the appendix.

The  button can be used to check that all parameters are correct and that the method number is still available. The result of a check is shown as text at the lower edge of the window. Incorrect results are shown in red letters.

The new/changed method is saved with the  button.

5.4.3 Kinetic

Fig. 97 shows the “Kinetic” method type, i.e. a single wavelength kinetic measurement.

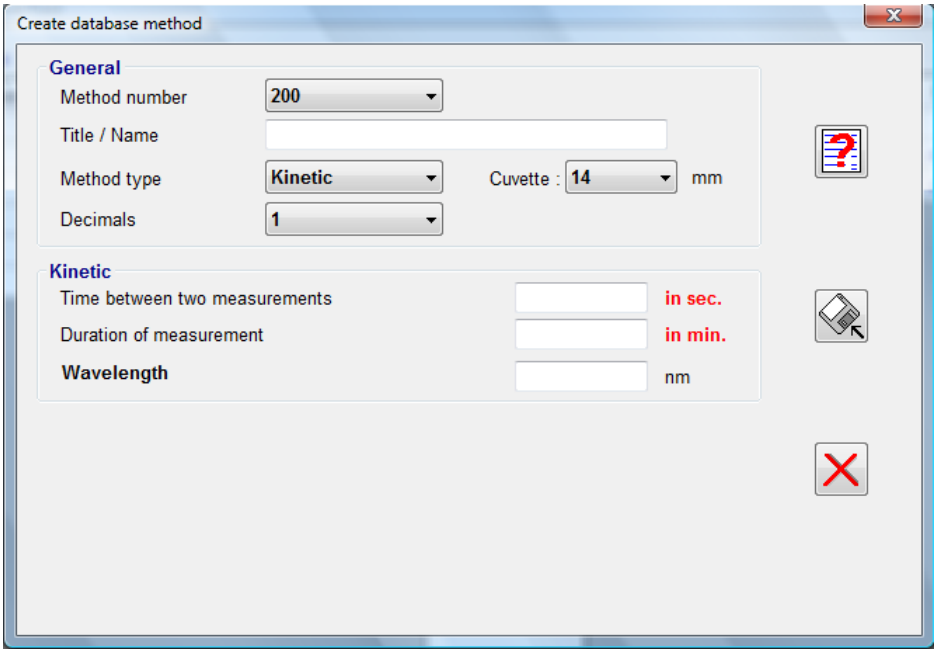


Fig. 97: Edit special method, kinetic

In the upper section of the window, general details of the special method are entered. The method number is selected from the drop-down list *Method number*. The name (max. 12 characters) that is to be displayed in the photometer is entered in the *Title / Name* text box. The *Method type* list box must indicate “Kinetic”.

Set all parameters according to your method in the second box Kinetic. The meaning of individual parameters can be found in the photometer manual. The button can be used to check that all parameters are correct and that the method number is still available. The result of a check is shown as text at the lower edge of the window. Incorrect results are shown in red letters. The new / changed method is saved with the button.

5.4.4 Wavelength Scan

Fig. 98 shows the “Scan” method type, i.e. a wavelength is scanned.

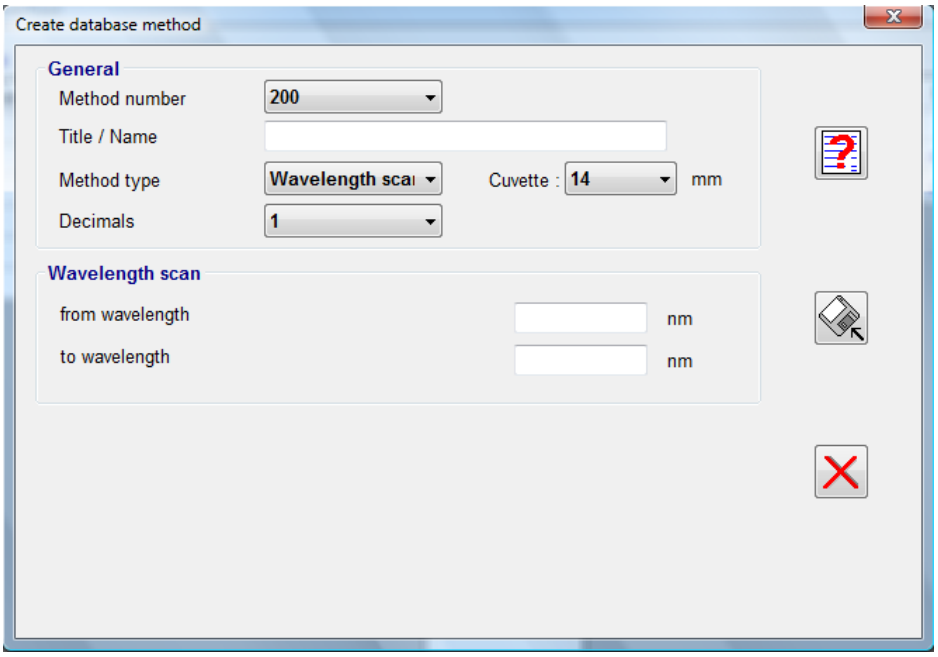


Fig. 98: Special method “Create wavelength scan”

In the upper section of the window, general details of the special method are entered. The method number is selected from the drop-down list *Method number*. The name (max. 12 characters) that is to be displayed in the photometer is entered in the *Title / Name* text box. The *Method type* list box must indicate “Wavelength scan” to perform scanned wavelength measurements. Set all parameters according to your method. The meaning of individual parameters can be found in the photometer manual. The  button can be used to check that all parameters are correct and that the method number is still available. The result of a check is shown as text at the lower edge of the window. Incorrect results are shown in red letters. The new/changed method is saved with the  button.

5.4.5 Scan Kinetic

Fig. 99 shows the “Scan-Kinetic” method type, i.e. a repeated wavelength scan.

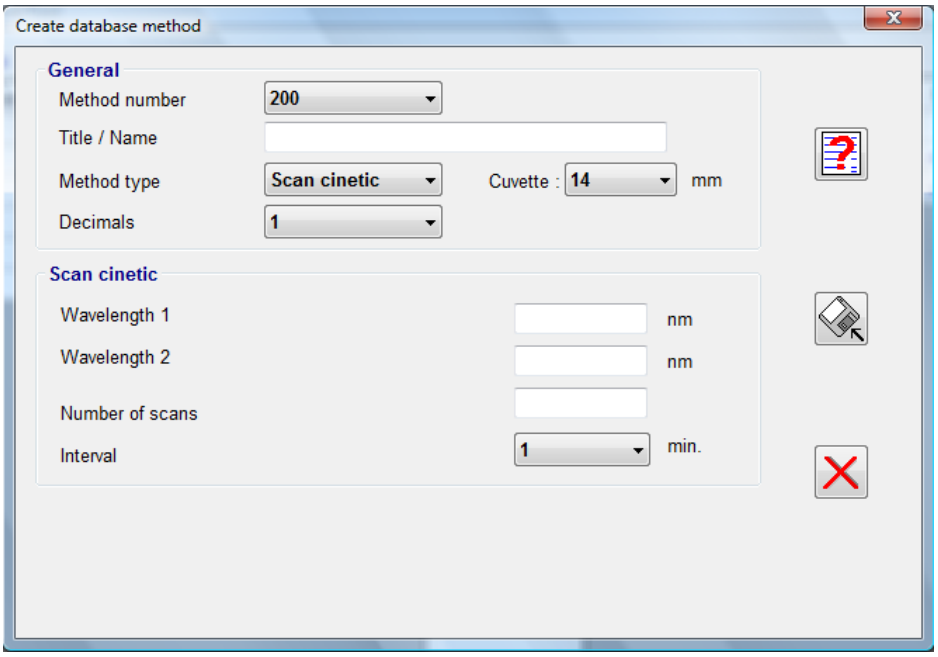


Fig. 99: Edit special method, scan kinetic

In the upper section of the window, general details of the special method are entered. The method number is selected from the drop-down list *Method number*. The name (max. 12 characters) that is to be displayed in the photometer is entered in the *Title / Name* text box. The *Method type* list box must indicate “Scan-kinetic” to perform a scan kinetic. Set all parameters according to your method.

Select the size of the cuvette from the *Cuvette* list box. The sizes available are: 10 mm, 20 mm (both rectangular cuvettes), 14 mm (test tubes) and 50 mm (rectangular cuvettes).

In the *Wavelength 1* text box, enter the starting wavelength of the scan in nm and enter the end wavelength in nm in the *Wavelength 2* text box.

Enter the number of scans required in the *Number of scans* text box.

Select the time interval between scans, from the *Interval* list box.

The time interval here is the waiting time of the computer program. Depending on the size of the scan range, the actual interval is extended by the time required for scanning!

The  button can be used to check that all parameters are correct and that the method number is still available. The result of a check is shown as text at the lower edge of the window. Incorrect results are shown in red letters.

The new/changed method is saved with the  button.

6 Graphic options

6.1 Menu function Graphic/Copy to clipboard

The menu command Graphic/Copy to clipboard copies the graphic of the active window to the Windows® clipboard. This may be a scan, a calibration curve, a kinetic graph or a chromatographic chart.

6.2 Menu function Graphic/Save to file

This function allows you to store the current graph, i.e. a scan, a calibration curve, a kinetic graph or a chromatographic chart as a graphics file. A file selection window opens and you will need to enter the save location and the name. You can save the graphic in the following formats: JPG, GIF, BMP, TIF and WMF.

7 Part 3: System Menu and Software Settings

Beginning with version 4.0 of the software the menu structure was changed. Figure 100 shows the new system menu.

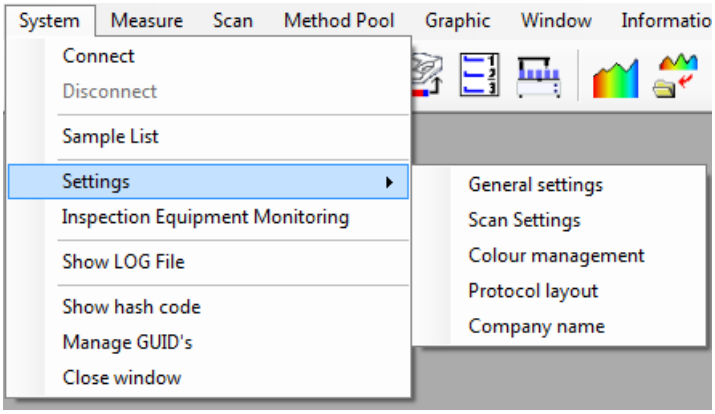


Fig. 100: System menu

7.1 Connect / Disconnect

The *Connect* function searches for a connected photometer and establishes the connection. Alternatively, click on the 

button.

Depending on the functions used in the software, the operating elements on the photometer display cannot be used. This PC mode of the photometer is cancelled via the software menu *System/Disconnect* or by switching off the photometer.

To disconnect the photometer, click on the *Disconnect* function. Alternatively, click on the 

button.

7.2 Sample list, button 

The option Sample list creates a list of sample names to be measured. Most functions of this software like Colourmeasurement, Multi-Wavelength-Measurment, Scans etc. can work with sample lists. Figure 101 shows the sample list window:

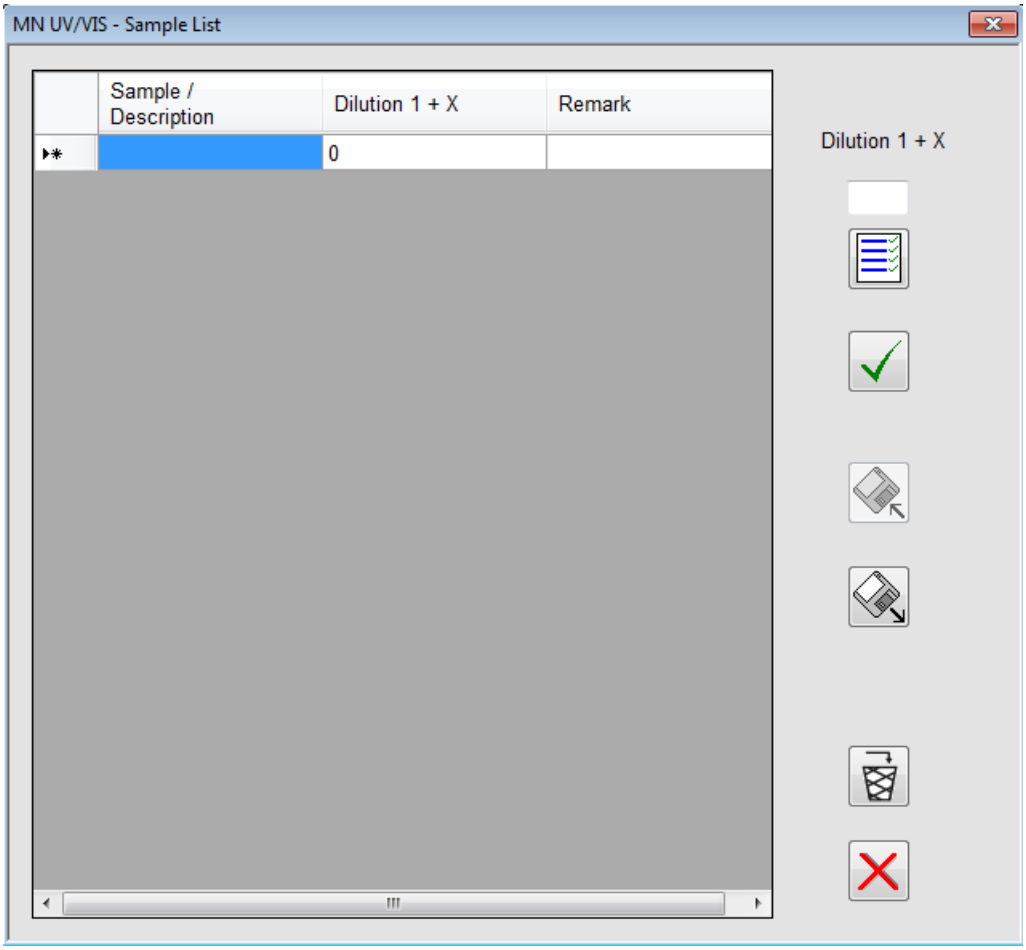


Fig. 101 : Sample list window

To create a sample list you need a unambiguous sample ID or sample name for each sample you want to measure. The column *Sample/Description* holds your sample ID's. If your sample come with a BAR code registration you can easily fill the sample list with a BAR code reader. Just set the focus in the first field of the column *Sample/Description* and read all necessary codes.

As an option you can give every sample an individual dilution factor and a remark. Remarks are printed into the measurement protocol. For absorption, SAC, transmission, multi-wavelength, standard-methods and special-methods the dilution factor is used to calculate the result. For scans and colour measurements the dilution factor is only mentioned in the protocol but not used for result calculation. Figure 102 shows a completed sample list.

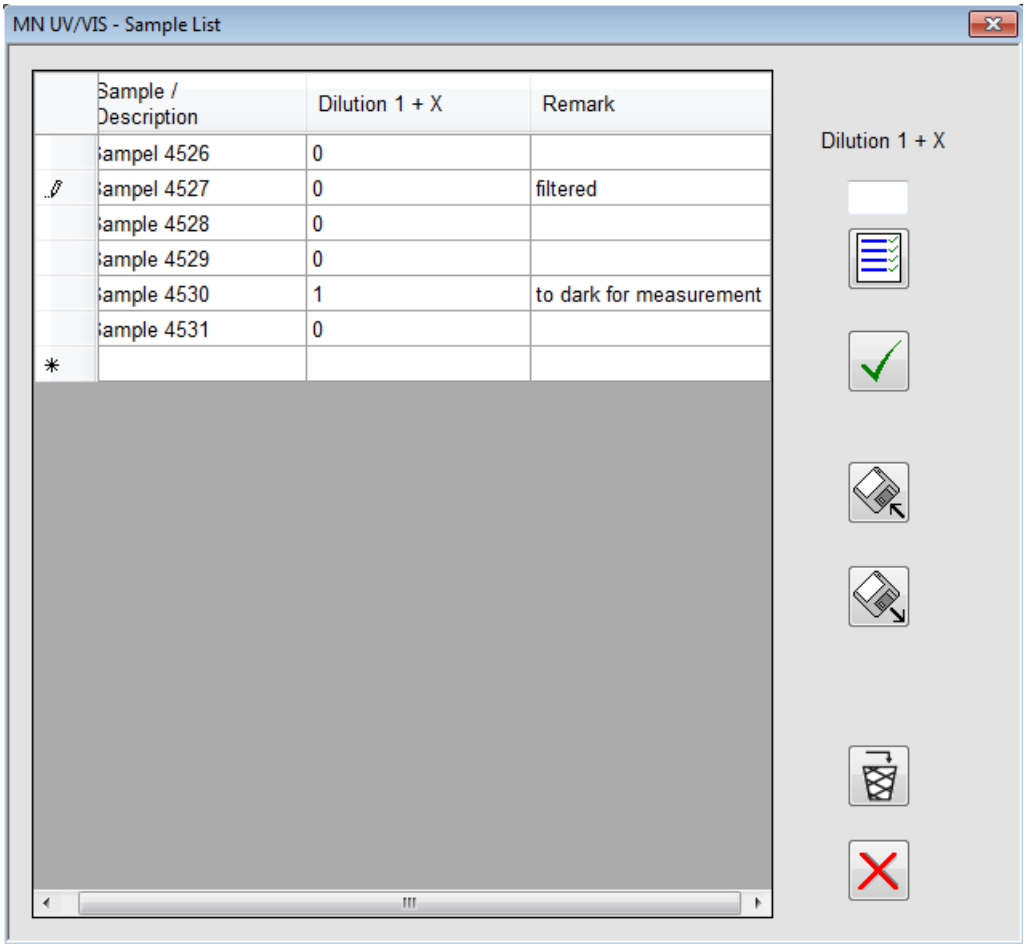


Fig. 102 : Filled sample list

If all samples are diluted the same way you can enter all dilution factors by using the text box *Dilution 1 +X* and clicking the button . Each field of the column *Dilution 1+x* is filled automatically.

If you need the same sample list regularly you can save the list using the button . As a standard sample lists are saved into the subfolder *sample_list* of the installation folder of the software. The button opens saved sample lists. To clear the present table use the button . After completing your sample list you can activate it with the button . The last button closes the window without activating the sample list.

Sample names or ID's that are measured are removed from the list automatically. After measuring a sample list without interruption the sample list is always ampty!

7.3 System settings

7.3.1 General settings

The *System/Settings/General Settings* function or the  button open the settings window of the ***NANOCOLOR*® Spectrophotometer Software**. This window is shown in Fig. 103.

Some functions in the Settings window are greyed out if you do not have administrator access rights. In this manual, greyed out functions are identified by the X symbol.

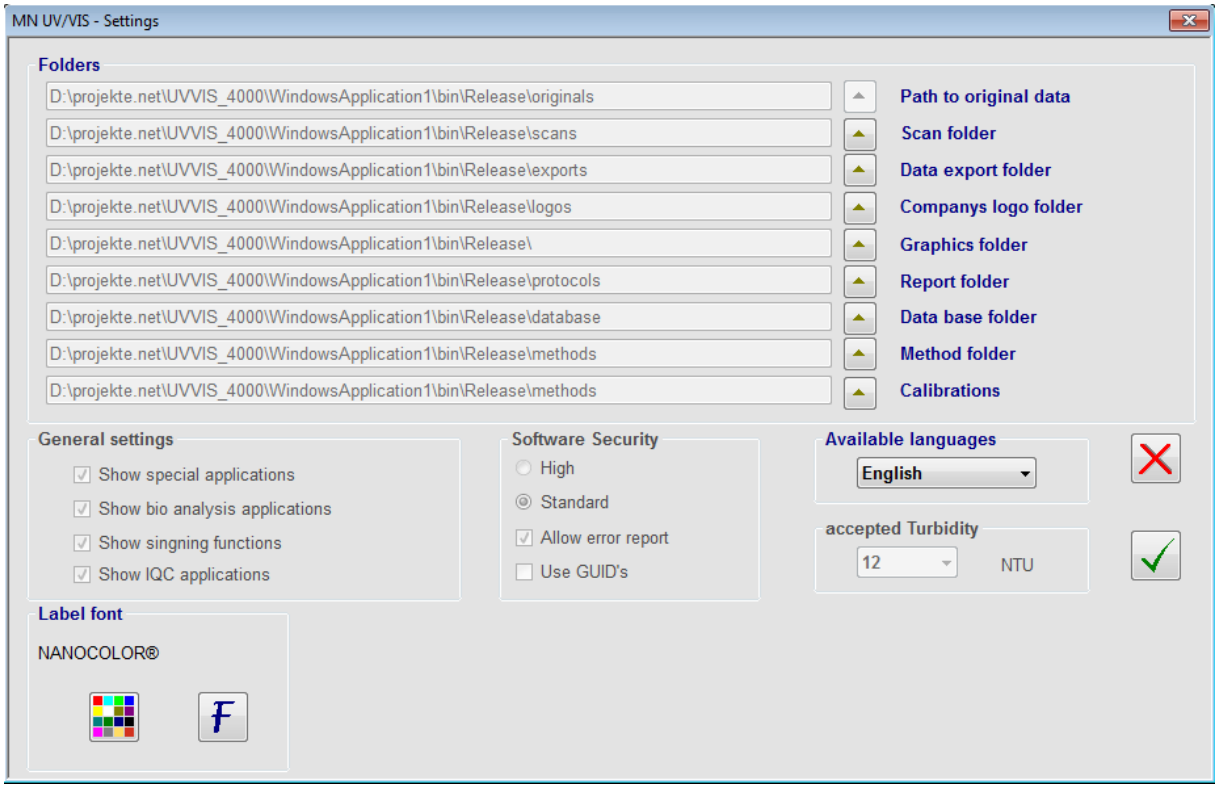


Fig. 103: Settings window

In the upper part of the window you see the area *Folders* where you can set the folders to store your measurements, protocols and original files. As a standard, special subfolders of the installation folder are pre-set but you can select your own folders.

The area *General settings* determins which functions the software presents through its menus. If you never perform colour measurements or brewery anaylsis, switch the corresponding options off to simplify the software menu structure.

In the *Software security* function area, the level of security for the software can be specified (see also Chapter 2, GLP and FDA 21 CFR Part 11 Conformity). If the setting **High** has been selected, the software will not open damaged or manipulated files. This includes saved scans, photometer export files and the configuration file in the software. Some information boxes that can record the data of a scan, are also changed to compulsory fields that must be completed.

The **Normal** setting allows damaged or manipulated files to be opened (where it is technically still possible), after a warning prompt is accepted. The number of compulsory fields is also less when a scan is created.

The third option *Allow error reports* enabled the user to send an error report to MACHEREY-NAGEL. The error report transmits the log files *error.log*, *sys.log* and *environment.log* through the SMPT protocol. If the local policies does not allow such a transmission, this option must be switched off. Please read chapter **7.5 Show Log files.**

The last security option is the *Use GUID's* button. If this option is enabled, a GUID is calculated for each document to be printed and is included in the printout (see Chapter 7.7). GUIDs are stored in a database and can be used to determine the origin of a printed document.

The box for selecting the language used in the software is located to the right of the colour setting for the present scan. Details on how other languages can be included can be found in the appendix. At the upper right of the settings window is the list box *Available languages*. Select your preferred language for the software here.

Some photometric measurements offered by this software allow an automatic turbidity check. The threshold value for the warning message in case of turbidity can be set in NTU in the frame *accepted Turbidity* below the language selection.

The functions for setting the colour and font of the graph labelling are located below the function for setting the colour of the present scan. The  button is used to specify the colour and the  button opens a font selection window.

When all settings have been completed, the data must be saved by clicking on the  button.

The  button closes the settings window without saving.

7.3.2 Scan settings, button

Figure 104 shows the scan settings window.

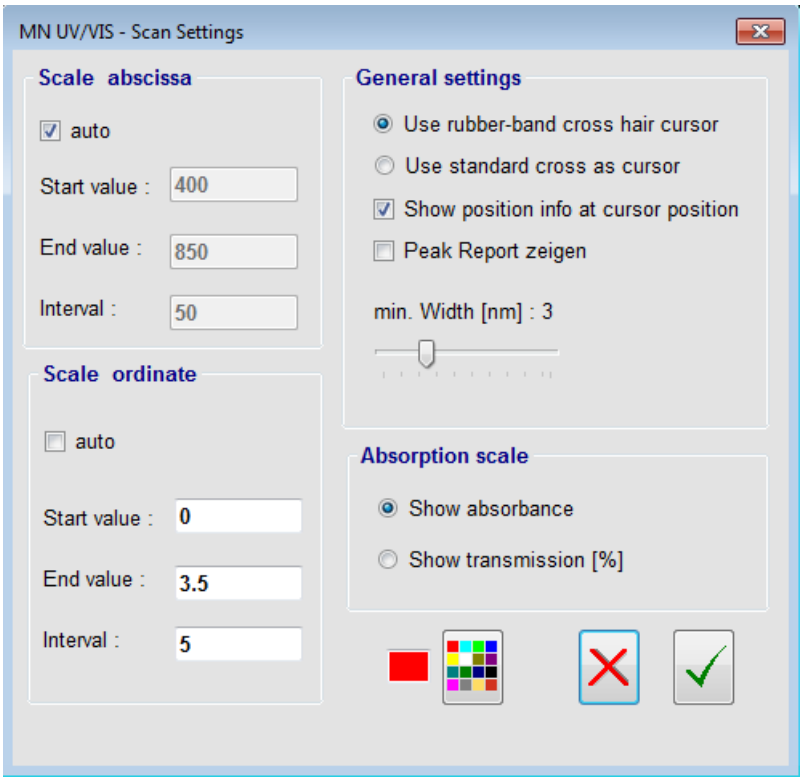


Fig. 104 : Scan settings window

In the left part of the window you find the areas *Scale abscissa* and *Scale ordinate* .
Both scaling functions offer the *auto* option. This option is set as standard to ensure that all scan fit the window correctly.
For manual scaling set the parameters *Start value* , *End value* and *Interval* for each axe as necessary.

The area *General settings* contains some important settings. The options *Use rubber-band cross hair cursor* and *Use standard cross as cursor* determine the shape of the cursor displayed on the scan graphic.

If the option *Show position info at cursor position* is enabled, the mouse cursor displays wavelength and absorption or transmission values. Independent from this setting wavelength and absorption/transmission information is always displayed in the status bar at the bottom of the scan window.

With the button  you change the colour for the present scan. User with red-green visual impairment usually have problems to recognize the present scan if it is printed in red. With the option *min. Width [nm] :* you set the minimum width of a peak to be recognized by the software. If necessary – if you find ghost-peaks/spikes on your scan, you may select a different value.

The area *Absorption scale* gives the possibility to switch between transmission and absorption scales.

Save your settings by clicking the button  . The button  closes the window without saving any changes.

The main functions can be reached easily by context menu, see chapter 4.3.3.

7.3.3 Colour management

This menu item holds the general settings for the colour measurement. Please read “Addendum I : Colour measurement“ of the software manual.

7.3.4 User defined printouts,

menu command *System/Protocol layout*

Frequently, the ***NANOCOLOR*®** Spectrophotometer Software may ask you or require you to create a protocol printout after carrying out a measurement. By default, the MACHEREY-NAGEL logo will be printed in the header and the MACHEREY-NAGEL company address in the footer. Figure 105 shows how a protocol is created in principle. The view will always be shown in the background of the software’s main window.

Fig. 105 : Display of the protocol design

In the area at the top, you can see the graphic used for the logo. Below, you can see “Protocol language : German”. This shows that the protocol is being created in German. In the area at the bottom of the picture, you can see the company address. There are three lines available, with the first line consisting of two parts which are printed in two different typefaces.

Below the menu function *System/Protocol layout* , you can set up your own header and footer. Figure 106 shows you the address settings window. If you run a contract laboratory, for example, you can create client-specific protocols, as you can store an infinite number of addresses and logos.

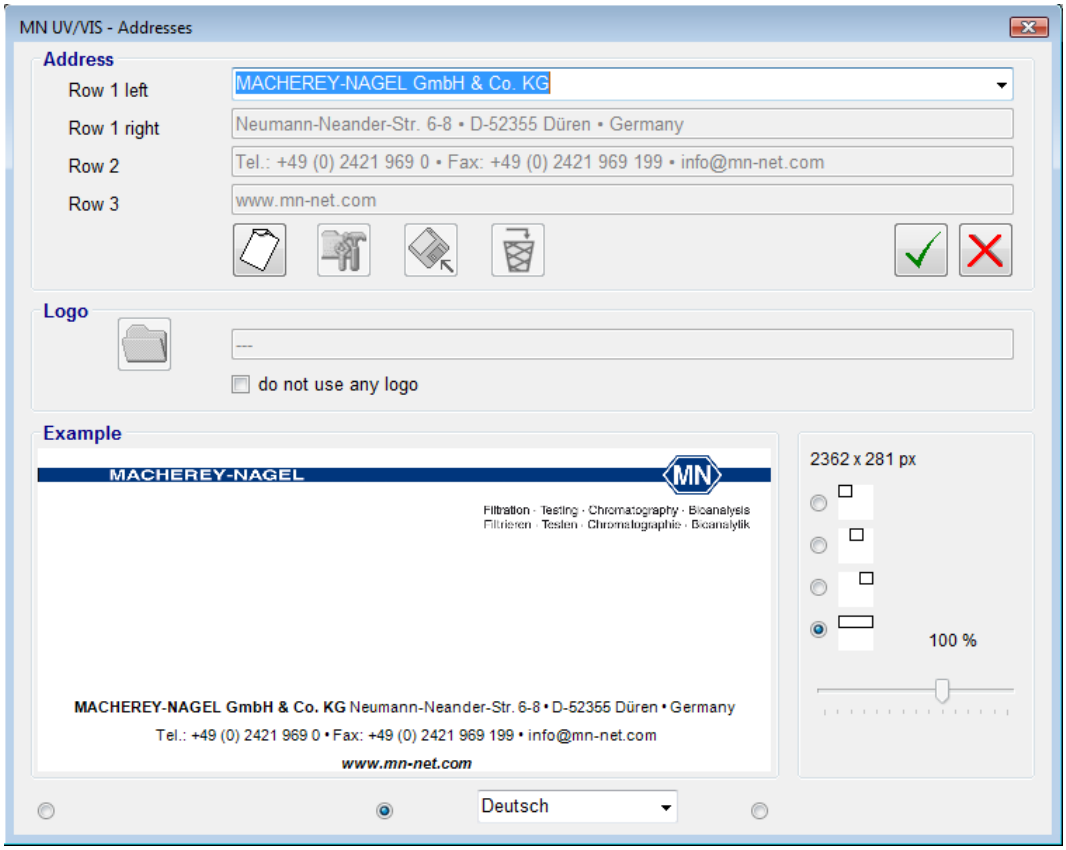


Fig. 106 : The address settings window

The first text field *Row 1 left* is designed as a drop-down menu. If you have already saved addresses, then you will be able to select these stored addresses from this list. When the software is initially installed, it will only contain the address of MACHEREY-NAGEL. This entry can neither be deleted nor edited.

To create a new address, click on the button  . To edit an existing address, select this address from the drop-down menu *Row 1 left* and click on the button  . The content of this first row is displayed in **bold** on the left-hand side of the first line of text in the footer, and should generally contain the company name. The

text of the second row appears in normal typeface on the right-hand side of the first line of text, and may contain the address. The third text box *Row 2* contains the second line of text in the footer. It is displayed in normal typeface and is intended for telephone and fax numbers. The fourth text box *Row 3* is used for the third line of text in the footer, and appears in ***bold italics***.

Once all the text boxes have been completed with the relevant data, click the button  to load up a graphic (GIF, JPG) to use as a logo. If the protocols are to be printed without a logo, then enable the option *No logo* .

To delete an address, select the address and click on the button  .

Once all the text has been entered and a graphic has been chosen, all these elements may be positioned individually as shown below in figure 107.

Fig. 107 : Positioning the text elements

In the top right-hand corner, there are four option buttons represented by an icon in the shape of a small box. These determine the position of the logo. The first three are for either left, centre or right alignment. The fourth option resizes the logo across the entire breadth of the page. Below these four buttons is a slide adjuster. If the option “Resize across the entire page“ is **not** enabled, then the size of the logo can be adjusted in %.

Below the protocol example, there are three more option buttons, one each in the left-hand corner, the middle and the right-hand corner. Use these to adjust the alignment of the lines of text in the footer. In the example shown, the text is aligned flush right. In the list box, you can also set the output language of the protocol. It is possible to print protocols in a different language than the one set in the software itself.

Ensure that logo graphics are stored in such a way that the software can always find them. If the graphics file is moved or deleted, then it can no longer be used for printing the protocol.

Once you have completed all the settings, save your individual design by clicking on the button  . Your settings are now saved in the database. If this design is to be used for printing the protocol, then you also need to click on the button  .

7.3.5 Company name

In some cases, depending on the software installation on your PC, the ***NANOCOLOR*®** PC Software for Spectrophotometer may not detect the name of your company from the Windows® settings. In this case, the name “MACHEREY-NAGEL” is output in the protocols as the name of the company by default. A different name for the company can be entered via the *System/Company name* function.

7.3.6 LIMS configurator

In principle, there are two ways of sending measurement data from the photometer to the laboratory information system:

a) Direct
Connect the photometer to the LIMS using the serial interface. After each measurement, the data is transmitted every time the cuvette is removed. It is not possible, however, to alter the data format. Your LIMS must be able to accept the MACHEREY-NAGEL data format.

b) Indirect via the PC
Connect your photometer to your PC using an RS-232 or USB interface. Then connect your LIMS to the same PC using another RS-232 interface. The ***NANOCOLOR*®** Spectrophotometer Software is now able to send the photometer data on to the LIMS.

The advantage of this option is that you can control the data as well as the format in which they are transmitted.

Now click on the menu command *System/LIMS configurator* . The configuration window opens as shown in Figure 108.

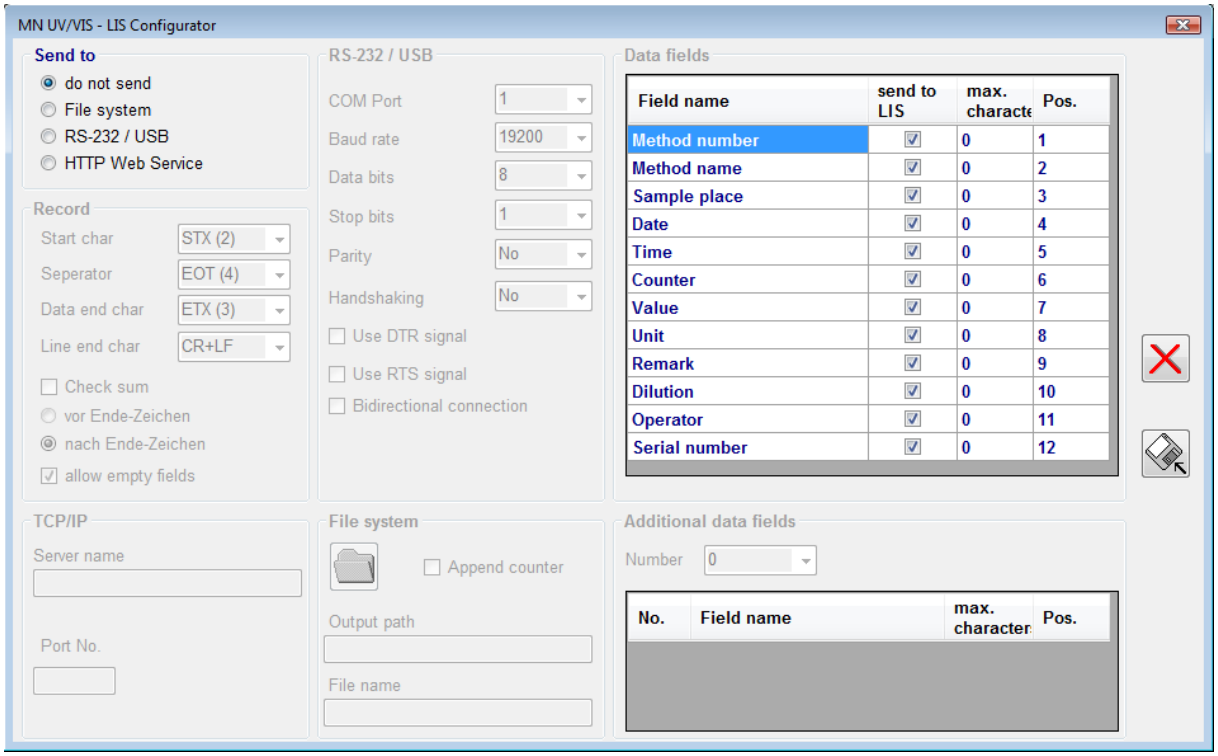


Fig. 108 : LIMS Configurator

In the left upper part of the windows you see the frame *Send to* . Select here the way of data transmission. The first option *do not send* deactivates the LIMS function. The second option *File system* sends the data as text file. If you activate this option the frame *File system* is activated, see figure 109.

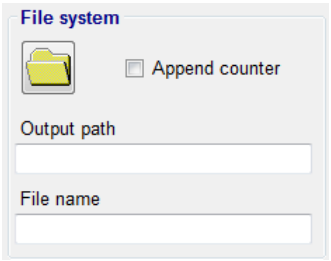


Fig. 109: Send records as file

With the button  , you can open a file selection window. Set the file to which you want the data to be written. Enter the name of the text file to be created by the software in the cell *File name* . If you enable the function *Append counter* , then a counter is attached automatically to the file name, e.g. *sample.txt* becomes *sample_3.txt*.

The function *File system* requires that your LIMS is able to collect and process files automatically.

The third function in the frame *Send to* , *RS-232/USB* , sends the measurement data via a serial interface or a virtual USB adapter. If the function is enabled, the frame *RS-232 / USB* is activated as shown in Figure 110.

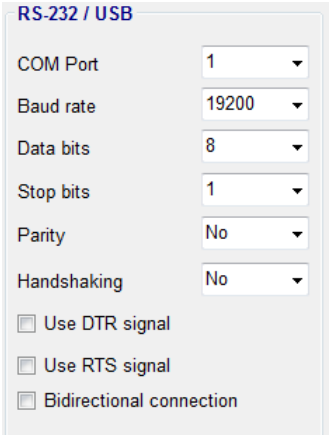


Fig. 110 : Send data via a serial interface

The options of this frame are self-descriptive. Set all parameters that are required by your LIMS.

Finally, data may be sent by setting up the option *HTTP web service* . The data is sent via the PC network in TCP/IP format. If you enable this option, the frame *TCP/IP* is activated as shown in Figure 111.

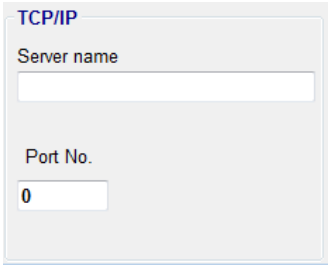


Fig. 111 : Send data via TCP/IP

Enter the correct name of the server on which the LIMS-TCP/IP Listener runs into the box *Server name* . Alternatively, you can enter the IP address. Enter the number of the TCP/IP port on which the Listener is listening into the box *Port no.* . Using these settings, you are able to send the measurement data via the internet, provided you instruct your Firewall that the ***NANOCOLOR*®** Spectrophotometer Software is permitted to send via the internet. **This function requires that your LIMS is capable of reading data via a TCP/IP port.**

Having determined how you are going to send the data, you now have to configure the data record yourself. The most important settings are made using the frame *Data record* . Figure 112 shows the frame *Data record* .

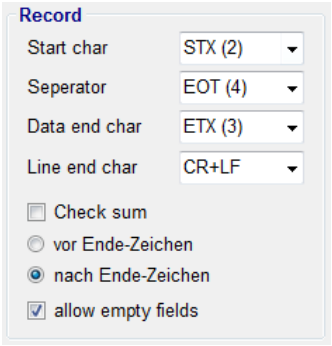


Fig. 112 : Configurating a data record

In general, a data record looks like this:

```
<startcharacter>datafield_1<separator>datafield_2<endcharacter
><checksum><lineend>
```

Some of the elements in <> brackets may be absent. Usually, STX = ASCII 2 is used as a start character and ETX = ASCII 3 is used as end character. To mark the end of the record line either CR = ASCII 13 or CR+LF = ASCII 13 + ASCII 10 is used as line end character. As separator, the semicolon (;) is commonly used, but its use as separator then prohibits its use within the data fields.

Simplified, a data record may look like this:

STXdatafield_1;datafield_2;datafield_3ETXCR

Which characters you have to use depends on your LIMS system. Using the options in the frame *Data record* , it is possible to set the most common formats.

As there are several methods of check-sum calculation in use, the *NANOCOLOR*® Spectrophotometer Software will probably have to be re-programmed, if you have to use a check-sum. Please contact us, if this is the case.

You will find an overview of the data fields provided by the photometer on the right-hand side of the window as shown in Figure 113.

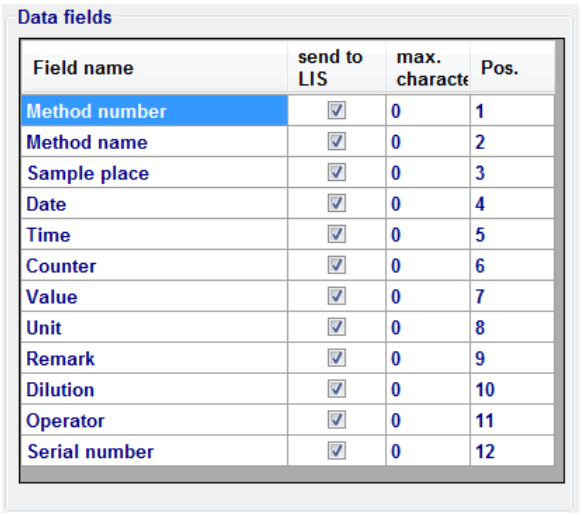


Fig. 113 : Data fields of the photometer

The first column displays the field name. If the checkmark in column two is activated, the data field is sent to the LIMS. If you do not need one of the data fields provided, just deactivate the option (depends on your LIMS setting). Usually, the number of characters for each data field is limited. In this case, write the allowed number of character for each field in column 3. A ZERO (0) means no character limitation. Data fields marked with a ZERO (0) may hold any number of characters. If the data field is marked with a number greater than ZERO (0), the software will shorten the field content to the allowed number of characters, if the photometer is sending more characters than allowed. The last column determines the position of the data field within the LIMS data record. The order in Figure 113 is the original order sent by the ***NANOCOLOR***® Spectrophotometer.

In some cases, it is necessary to integrate additional data fields into the data record. For example, if you wish to add the job number to each measurement, an extra field has to be inserted. Than proceed as follows:

In the lower right-hand area of the window, you see the frame *Extra data fields* as shown in Figure 114.

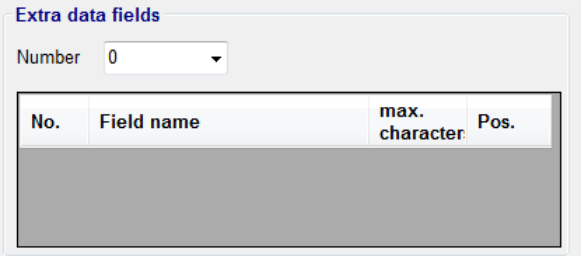


Fig. 114 : Extra data fields frame

In the drop-down menu *Number* , enter the number of extra fields required. Create a unique name for the data field in the column *Field name*. As in the table with the fields of the photometer, you can now enter the maximum number of characters and the position within the data record as shown in Figure 115.

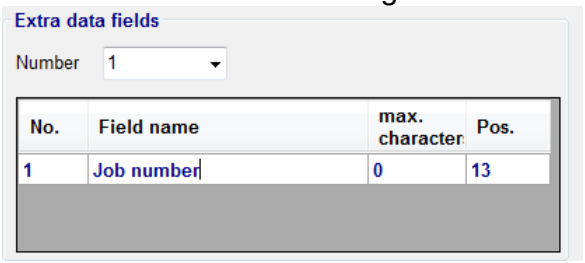


Fig. 115 : Definition of an additional data field

Once you have defined an extra data field and saved the settings using the button  , the software will open a text box after every cuvette measurement (and for each data export line), asking for the extra information to be entered. This can also be done by a barcode reader that has been correctly connected.

7.4 Monitoring of Measuring and Testing Equipment

(German worksheet DWA-A 704)

The menu function *Inspection Equipment Monitoring* is used for monitoring the measurement and test instruments according to worksheet DWA A 704, IQK-card 9. There are tests available for checking the performance of the halogen and deuterium lamps, the wavelength accuracy, the degree of scattered light, the photometric accuracy (*NANOCHECK*), and for determining the signal/noise ratio.
The function area is shown in Fig. 116.

MACHEREY-NAGEL recommends that all tests be run at six-monthly intervals.

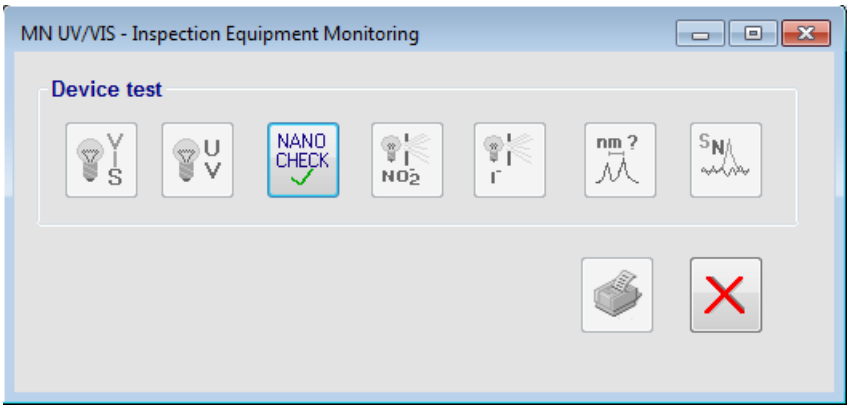


Fig. 116: *Device test* *function area*

7.4.1 Halogen Lamp Test

The  button measures the energy response of the halogen lamp in the photometer in the visible range. The measurement is displayed as a scan in the scan window (see Fig. 117) and finally, a report is printed.

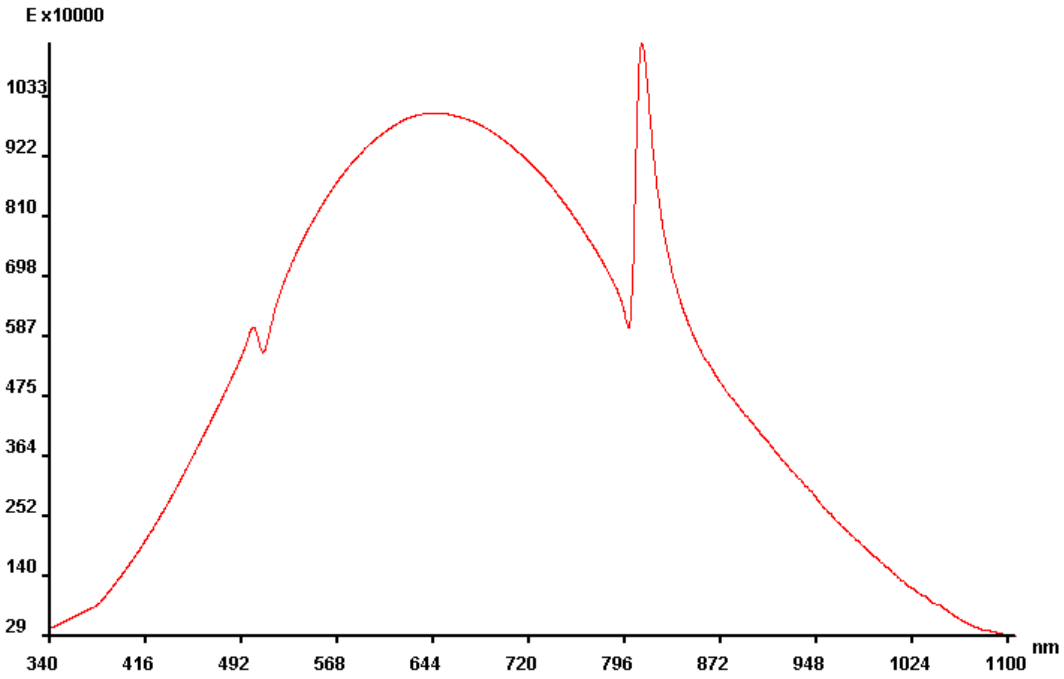


Fig. 117: Example of a lamp characteristic (halogen lamp)

The relative energy values are plotted on the absorbance axis; the values can be used for estimating the power of the lamp and on completion of the measurement, the automatically created log is printed. The energy values of the wavelengths at 340 nm and 1050 nm are measured and compared with the reference values. The lamp should be replaced if the power of the lamp falls below the reference values.

7.4.2 Deuterium Lamp Test

This test is not possible with the photometer ***NANOCOLOR® VIS* photometer.**

In the same manner as described in Chapter 7.4.2, the energy response of the deuterium lamp can be measured with the  button. Fig. 118 shows the graph of the measurement.

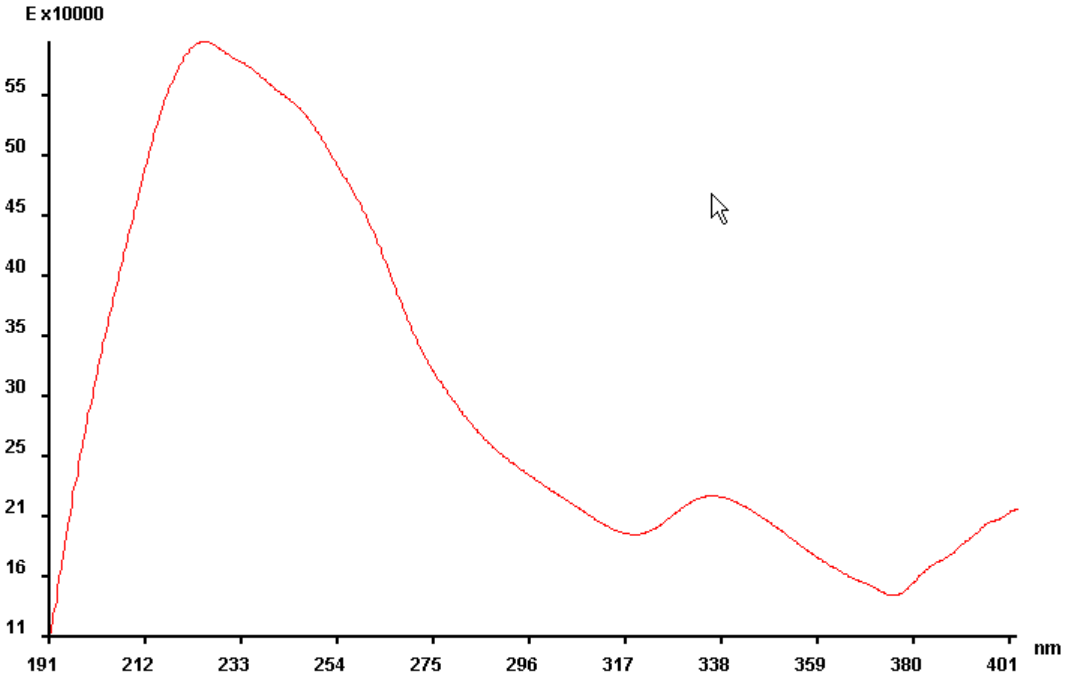


Fig. 118: Example of a lamp characteristic (deuterium lamp)

The relative energy values are plotted on the absorbance axis; the values can be used for estimating the power of the lamp and on completion of the measurement, the automatically created log is printed. The energy values of the wavelengths at 191 nm and 320 nm are measured and compared with the reference values. The lamp should be replaced if the power of the lamp falls below the reference values.

7.4.3 Light Scatter according to DAB, Ph. Eur.

The 

button is used to complete a light scatter test according to the DAB and Ph Eur standards. To perform a light scatter test, a scattered light test kit, e.g. MERCK UV-VIS Standard 2 (Cat. No. 1.08161.0001) and a 10 mm quartz cuvette are required. As an alternative to the test kit, a solution of 50 g sodium nitrite (p.a.) or 61,5 g potassium nitrite (p.a.) in 1 l pure water can be used.

To start the test, click on the button shown above and wait until the zero measurement with respect to air has been completed. Now, insert the 10 mm cuvette with the test solution and after the measurement, print the results.

7.4.4 Light Scatter with Potassium iodide at 220 nm

This test is not possible with the photometer ***NANOCOLOR® VIS* photometer.**

The button  performs an alternative light scattering test at 220 nm.

7.4.5 Wavelength Accuracy

The 

button performs a test of the wavelength accuracy. The photometers ***NANOCOLOR® UV/VIS*** and ***NANOCOLOR® VIS*** are tested as follows:

A) With the ***NANOCOLOR® UV/VIS*** a scan with the deuterium lamp is performed in the range from 450 nm to 700 nm and the Balmer lines of deuterium are analyses. Figure 119 show the relevant part of the spectrum.

   Reference wavelengths are 485.09 nm, 581.1 nm and 656.1 nm,

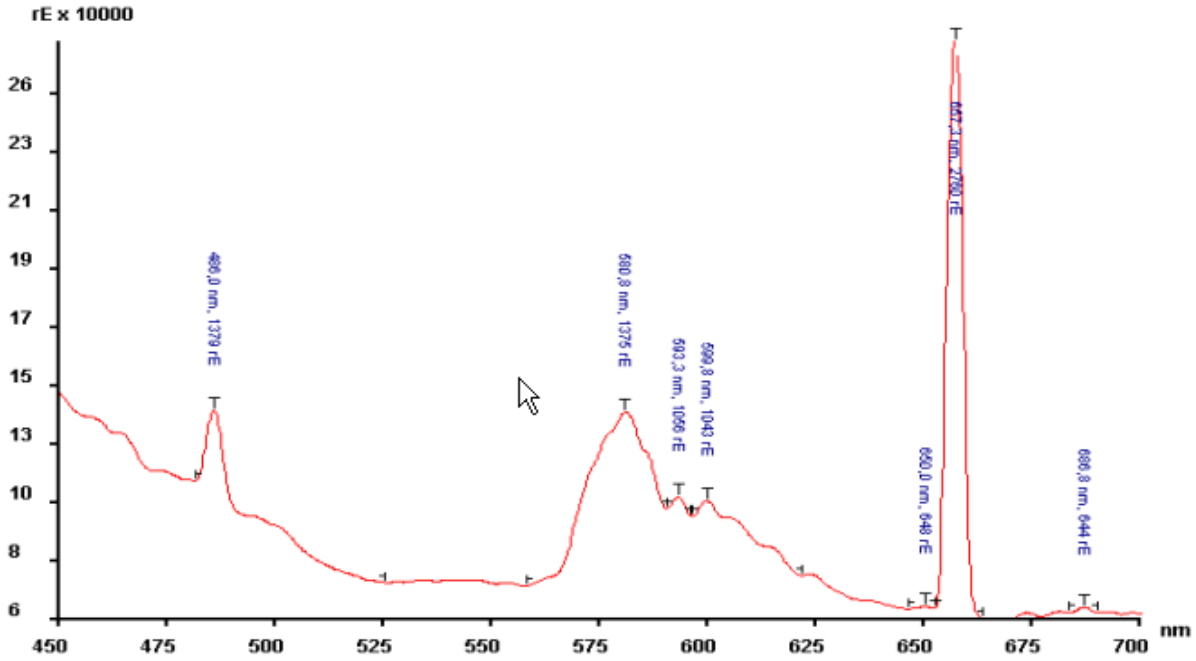


Fig. 119: Test of wavelength accuracy

B) With the ***NANOCOLOR® VIS*** the halogen lamp is used to scan the internal holmium oxide filter in the range from 340 to 700 nm.

Reference wavelengths are 360.9 nm, 453.6 nm, 536.4 nm and 637.7 nm, The deviation measured must not exceed 1 nm per wavelength. After the test is completed, a text log is automatically printed.

7.4.6 Photometric Accuracy with *NANOCHECK*

The 

button starts a test to check the photometric accuracy. To complete this test, a *NANOCHECK* test kit from MACHEREY-NAGEL, Cat. No. 925701 is required.

A click on this button opens the *NANOCHECK* window, see Fig. 120.

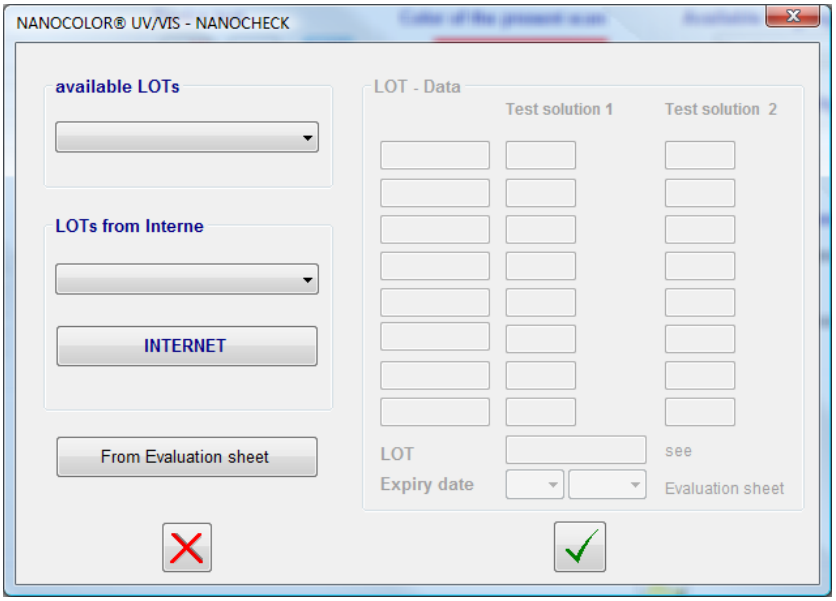


Fig. 120: NANOCHECK window

The *NANOCHECK* test works with batch-specific test values. These must first be entered in the *NANOCHECK* window. This can be achieved in different ways:

A) The easiest method: Click on the *From Evaluation sheet* button. The entry boxes at the right hand side of the *NANOCHECK* window are now enabled. Click on the boxes consecutively with the mouse and enter the absorbance setpoints from the evaluation leaflet of your *NANOCHECK* test, see example in Fig. 121.

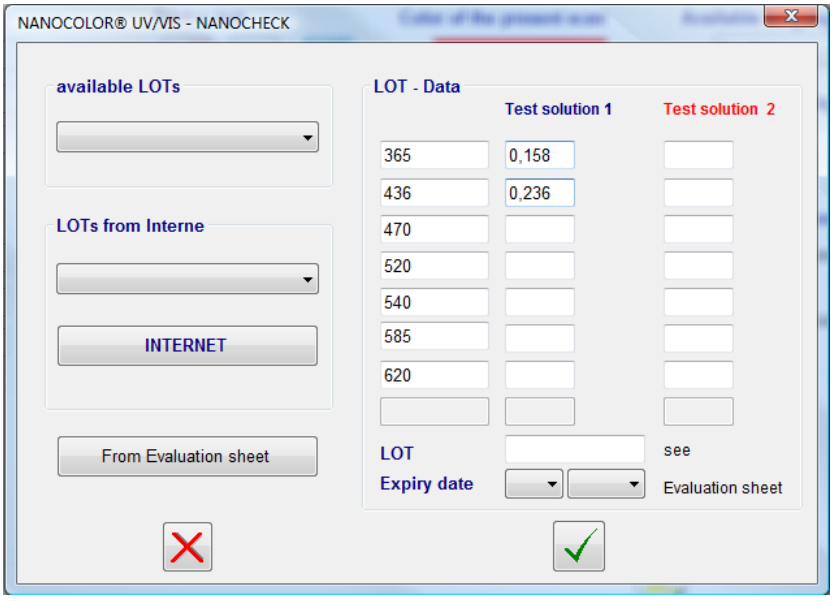


Fig. 121: NANOCHECK window, Entry of test values

Complete all boxes that are in a line with wavelength values. Lines without these values can be left blank.

B) The second possibility is to call up a *NANOCHECK* test that has been saved previously. Each **completed** *NANOCHECK* test is automatically saved on the hard disk in your PC, so that the test values are available for further tests.
Open the *available LOTs* list box, where all *NANOCHECK* LOTs are shown that are on your hard disk. After selecting a LOT, the entry boxes are automatically completed. **If you have not yet run any *NANOCHECK* tests or data has not been downloaded from the Internet, this list is empty.**

C) If your computer has an Internet connection, you can also click on the *INTERNET* button below *LOTs from Internet*. The software searches through the MACHEREY-NAGEL Internet server for updated *NANOCHECK* information. All LOT numbers found on the Internet are shown in the list box above the *INTERNET* button. When a LOT number is selected from this list, the relevant LOT information is downloaded to your hard disk. This LOT can now be selected from the *available LOTs* list box.

When all text boxes are completed, click on the *Run* button. The program now requests that a blank cuvette be inserted. Wait until the photometer has measured all wavelengths. Then, when requested by the program, insert test solution 1. Wait until all wavelengths have been measured, insert test solution 2 and again measure all wavelengths.

The test results are printed in log format.

7.4.7 Determination of signal/noise ratio

The button  performs a signal-to-noise test. This test can be performed in the visual range at 345 nm, 436 nm, 540 nm, 585 nm and 700 nm or in the UV-range (only ***NANOCOLOR*® *UV/VIS*** photometer) at 220 nm, 260 nm, 280 nm, 334 nm and 366 nm. In addition it can be performed with or without BLANK cuvette. To select the kind of test required use the **[CTRL]** and **[ALT]** button on your keyboard:

visual reange without BLANK 

UV range without BLANK **[CTRL]** and 

visual range with BLANK **[ALT]** and 

UV range with Blank **[CTRL]** & **[ALT]** and 

The test performs 50 single measurements, reading detector values. **Warning: This test takes over 10 minutes to complete!** Figure 122 shows a part of the measurement protocol.

Inspection Report

NANOCOLOR® UV / VIS

Signal / Noise

Photometer S/N : NUV0027
Company : Support
Operator : WieczorrekC
Measurement against calibration cuvette
N = 50 , α = 95%

345 nm : I0 = 8606 , s = 3,6 = 0,04 % , Max-Min = 20
Delta 2 mA (0.5 A), 5 mA (1.0 A), 58 mA (2.0 A)

436 nm : I0 = 75859 , s = 25,3 = 0,03 % , Max-Min = 132
Delta 1 mA (0.5 A), 4 mA (1.0 A), 46 mA (2.0 A)

540 nm : I0 = 191940 , s = 13,4 = 0,01 % , Max-Min = 56
Delta < 1 mA (0.5 A), 1 mA (1.0 A), 9 mA (2.0 A)

585 nm : I0 = 248412 , s = 19,9 = 0,01 % , Max-Min = 86
Delta < 1 mA (0.5 A), 1 mA (1.0 A), 11 mA (2.0 A)

700 nm : I0 = 246049 , s = 40,9 = 0,02 % , Max-Min = 215
Delta 1 mA (0.5 A), 2 mA (1.0 A), 22 mA (2.0 A)

Fig. 122: Signal-to-noise measurement protocol

For each wavelength the mean detector value *I0*, the standard deviation *s* is calculated.

Furthermore, the anticipated mean error *Delta* of a single measurement is shown in milli absorbances (mA) for the anticipated measures 0.5 A, 1 A and 2 A.

7.5 Show LOG Files

The *Show LOG file* menu function opens a window that allows the user to read all LOG files that have been created, see Fig. 123.

The *ERROR*, *SYSTEM* and *PC* buttons open the corresponding LOG files for display in the text window. The button  copies the log file or selected text into the windows clipboard.

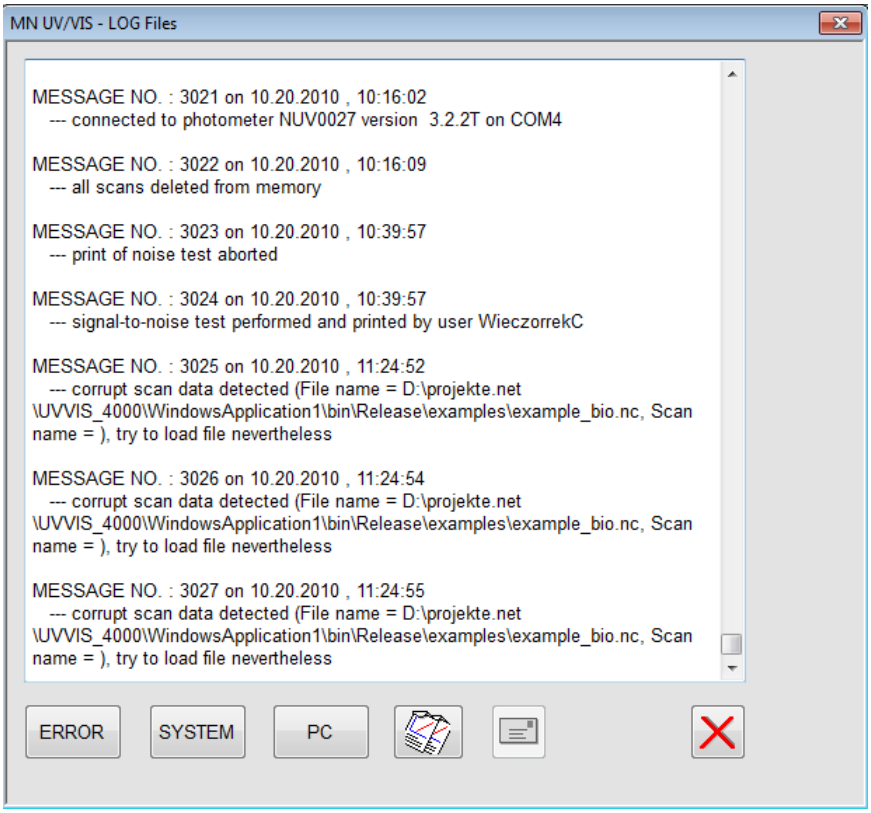


Fig. 123 : Show LOG files

If the option *Allow error report* in the general settings window is enabled, the button  is enabled (depending on an existing internet connection).

When the  button is clicked, the software asks whether an error report is to be sent to MACHEREY-NAGEL. A click on *Yes* opens a window with text boxes for entering your name, telephone number and e-mail address, together with a text box for entering a short description of the fault. Now click on the *OK* button. The time taken for transmitting the information depends on the quality of the internet connection. The relevant window is shown in Fig. 124.

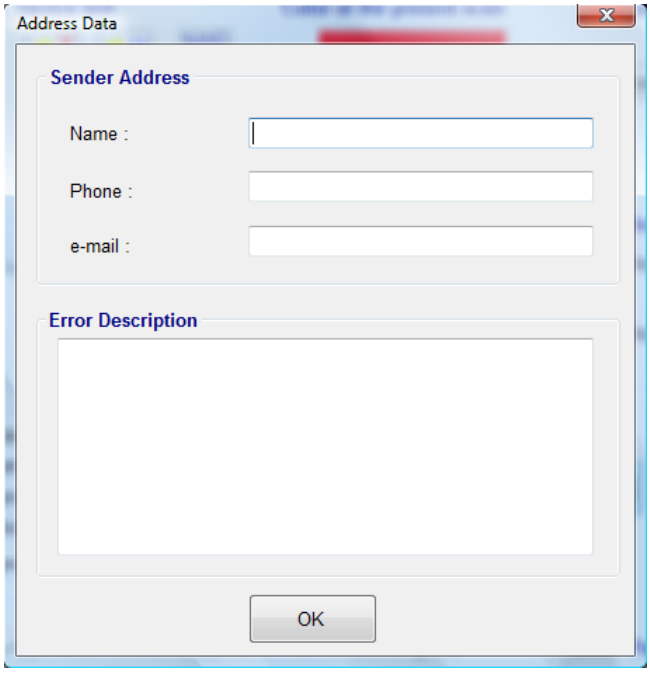


Fig. 124: Address window of the error report

This function uses port 25. This port on your local PC may be inhibited by a virus scanner or firewall. In this case, consult your system administrator. If there is no active Internet connection, this function is disabled.

7.6 Show Hash Code

To meet all the requirements of GLP, the protocols that are output incorporate special codes, the so-called hash codes that contain a coding of the user name. This feature is included to render falsification of the measurement logs more difficult. When it becomes necessary to check a protocol in real time, click on the *Show hash code* menu command. The hash code window is opened as shown in Fig. 125.

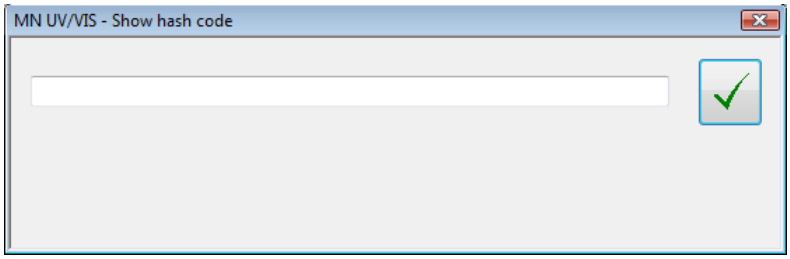


Fig. 125 : Show hash code

For example: Enter the user name given in the log and click on the button with the green check-mark. The user hash code is calculated and displayed below the text box. The code displayed and the code on the measurement log must be identical.

7.7 GUID's Management

A Globally Unique Identifier (GUID) is a globally unique number of 128 bit (that is 16 bytes) and that comes into play in distributed computing systems. GUID's enable you to implement the Universally Unique Identifier Standards (UUID) whereby documents etc. may be uniquely identified across the world.

For additional security, you have the option to identify the printout of the document with such a GUID. This function is enabled via the settings menu with the function *Manage GUID's* (see Chapter 6.1). When this function is used, all GUIDs created by the software are saved in a database. The *Manage GUID's* menu function opens this database and displays the GUIDs that have been created. By comparing the entries, it is possible to ascertain that a GUID associated with a document, has actually been created by this software.

Fig. 126 shows the GUID database.

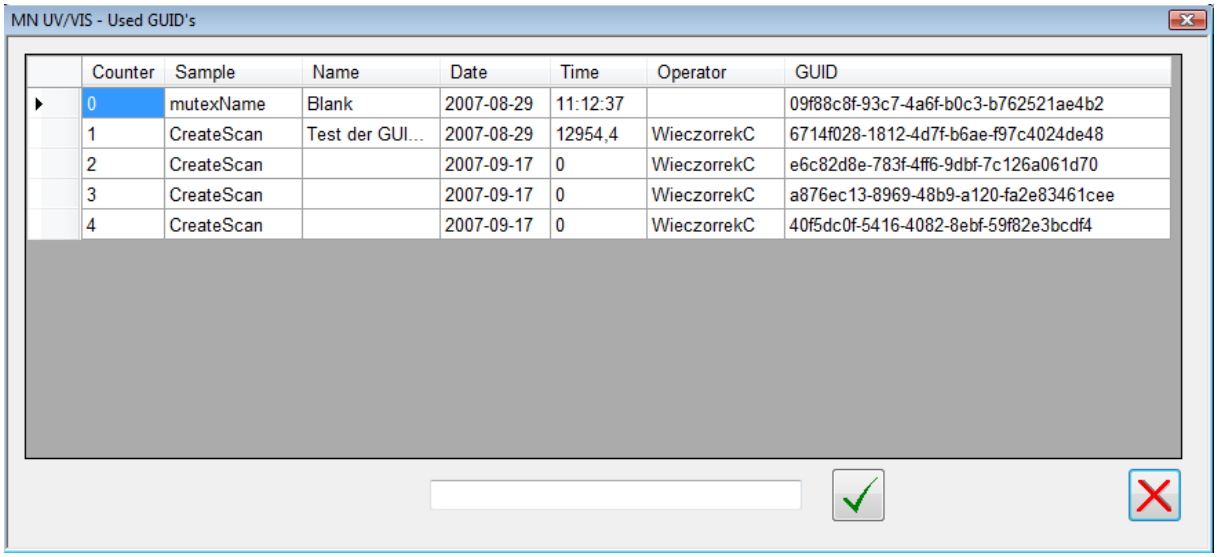


Fig. 126 : GUID database

Fig. 127 shows a section of a printed wavelength scan. The GUID that has been used is shown in the last line. This GUID is identical to the second line in the database.

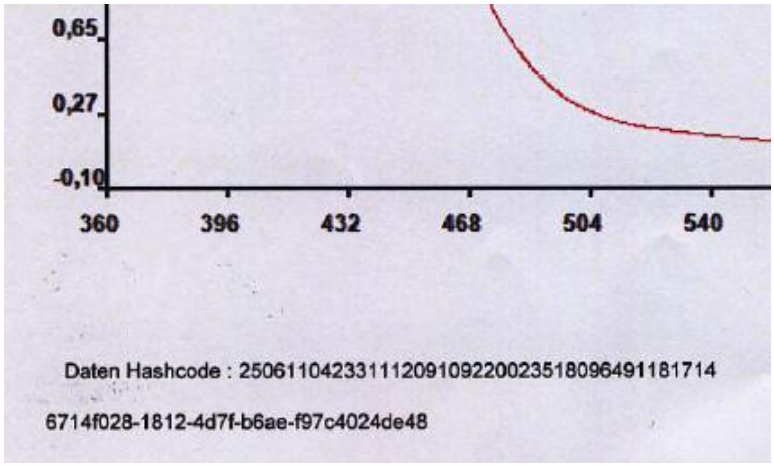


Fig. 127 : GUID on a scan printout

8 Appendix

8.1 Export of the Photometer Memory in ASCII Format

This function exports the contents of the RAM in a semicolon-formatted text file. Each data field is separated by a semicolon, each data record is in one line terminated with CR+LF. The first line of the file contains the column headings. Printout 1 shows a short example file.

```
Method_Number;Method_Name;Sample_place;Date;Time;Counter;Value;Unit;Remark;
Dilution;Operator;Serial_number;Decimals;Send;ST_CONC;ST_CONC_DE;LOT_TEST;L
OT_ST;IQC_M_Counter;IQC_D_Counter;A;deleted
903;EXTINKTION;Place;26.07.2010;10:49;1;0.434;E;;0;WIECZORREK;NUV0027;3;Tru
e;0;0;;;;;0.434
0042;AMMONIUM10;Place;21.06.2010;15:45;11;3;mg/l
NH4;;0;WIECZORREK;NUV0027;1;True;0;0;;;;;0.412
0042;AMMONIUM10;Place;21.06.2010;15:45;10;3;mg/l
NH4;;0;WIECZORREK;NUV0027;1;True;6;0.2;289632;75369;;;0.412
0042;AMMONIUM10;Dueren;21.06.2010;15:44;9;3;mg/l
NH4;;0;WIECZORREK;NUV0027;1;True;3;0.2;289632;75369;;;0.412
0042;AMMONIUM10;Place;21.06.2010;15:44;8;3;mg/l
NH4;;0;WIECZORREK;NUV0027;1;True;0;0;;;;;0.412
```

Printout 1 : ASCII export file of the photometer data

8.2 Export of the Photometer Memory in XML Format

The XML format provides simple data transfer to database programs. The XML representation is shown in Printout 2.

```
<xs:schema                    id="NewDataSet"                    xmlns=""
xmlns:xs="http://www.w3.org/2001/XMLSchema"      xmlns:msdata="urn:schemas-
microsoft-com:xml-msdata">
    <xs:element          name="NewDataSet"          msdata:IsDataSet="true"
msdata:UseCurrentLocale="true">
      <xs:complexType>
        <xs:choice minOccurs="0" maxOccurs="unbounded">
          <xs:element name="Table1">
            <xs:complexType>
              <xs:sequence>
                <xs:element      name="Method_Number"      type="xs:string"
minOccurs="0" />
                <xs:element       name="Method_Name"       type="xs:string"
minOccurs="0" />
                <xs:element       name="Sample_place"      type="xs:string"
minOccurs="0" />
                <xs:element name="Date" type="xs:string" minOccurs="0" />
                <xs:element name="Time" type="xs:string" minOccurs="0" />
                <xs:element name="Counter" type="xs:int" minOccurs="0" />
                <xs:element name="Value" type="xs:double" minOccurs="0" />
                <xs:element name="Unit" type="xs:string" minOccurs="0" />
                <xs:element   name="Remark"   type="xs:string"   default=""
minOccurs="0" />
                <xs:element  name="Dilution"  type="xs:string"  minOccurs="0"
/>
                <xs:element  name="Operator"  type="xs:string"  minOccurs="0"
/>
                <xs:element      name="Serial_number"      type="xs:string"
minOccurs="0" />
                <xs:element name="Decimals" type="xs:int" minOccurs="0" />
                <xs:element  name="Send"  type="xs:boolean"  default="true"
minOccurs="0" />
                <xs:element   name="ST_CONC"   type="xs:double"   default="0"
minOccurs="0" />
                <xs:element  name="ST_CONC_DE"  type="xs:double"  default="0"
minOccurs="0" />
                <xs:element   name="LOT_TEST"   type="xs:string"   default=""
minOccurs="0" />
                <xs:element   name="LOT_ST"   type="xs:string"   default=""
minOccurs="0" />
                <xs:element        name="IQC_M_Counter"        type="xs:int"
minOccurs="0" />
                <xs:element        name="IQC_D_Counter"        type="xs:int"
minOccurs="0" />
                <xs:element name="A" type="xs:double" minOccurs="0" />
                <xs:element         name="deleted"         type="xs:boolean"
default="false" minOccurs="0" />
              </xs:sequence>
            </xs:complexType>
          </xs:element>
        </xs:choice>
      </xs:complexType>
```

```
    </xs:element>
  </xs:schema>
```

Printout 2 : XML format of the photometer data

An example data record is shown in Printout 3. The data for sample place, dilution and user is output only when it has been specified in the photometer and with the measurement. If the measured value is outside of the measurement range used in the test, identified by the characters for greater than (>) or less than (<), the value is output in the remarks field.

```
<Table1>
    <Method_Number>903</Method_Number>
    <Method_Name>EXTINKTION</Method_Name>
    <Sample_place>Probeort</Sample_place>
    <Date>26.07.2010</Date>
    <Time>10:49</Time>
    <Counter>1</Counter>
    <Value>0.434</Value>
    <Unit>E </Unit>
    <Remark />
    <Dilution>0</Dilution>
    <Operator>WIECZORREK</Operator>
    <Serial_number>NUV0027</Serial_number>
    <Decimals>3</Decimals>
    <Send>true</Send>
    <ST_CONC>0</ST_CONC>
    <ST_CONC_DE>0</ST_CONC_DE>
    <LOT_TEST />
    <LOT_ST />
    <A>0.434</A>
    <deleted>false</deleted>
  </Table1>
```

Printout 3 : XML data record

8.3 Export of a Scan in ASCII Format

When a scan is exported in ASCII format, each line is terminated with CR+LF. All fields are separated by a semicolon. The file contains 3 to 4 data sections.
Section 1 gives the supplementary information of the scan, in the form:

Description – semicolon - Value

Section 2 gives the data table of the scan, in the form:

No. – semicolon – Nanometre – semicolon – Abs.– semicolon – smoothed Abs.

In the third section, data is output in transmission:

No. – semicolon – Nanometre – semicolon – Trans% – semicolon – smoothed Trans%

If a zero line was calculated for this scan, it is output in the fourth section:

Number – semicolon – Nanometre – semicolon – Abs.

Printout 4 following, shows a shortened example file.

```
Titel : ;not_set
Description;not_set
Cuvette;14 mm Tube
Sample ID :;
Operator :;WieczorrekC
Date;03.03.2008;Time : ;14:49
Photometer : ;NANOCOLOR® UV/VIS
Serial number;NUV0027
File name :;D:\data\test_3.txt


TimeStamp = 2709,7

Document ID : 76bb3f8d-fab4-44ab-85f2-cc67c03c181a

No.;nm;Ext;Ext2
37;259,2;0;-0,003
38;259,5;0;-0,01
39;259,8;0;-0,025
40;260;-0,05;-0,045
41;260,2;-0,1;-0,054


No.;nm;T[%];T2[%]
37;259,2;100;100,7
38;259,5;100;102,3
39;259,8;100;105,9
40;260;112,2;110,9
41;260,2;125,9;113,2


No.;nm;Ext
37;259,2;0,232
38;259,5;0,232
39;259,8;0,232
40;260;0,232
41;260,2;0,232
```

Printout 4 : ASCII export of a scan

8.4 Export of a Scan in netCDF / ANDI Format

The ANDI protocol (Analytical Data Interchange) is an American standard for program and platform independent storage of chromatographic and spectroscopic data. The aim is to provide the possibility of comparing all data with one another,

regardless of the instrument and computer platform that were used for the measurements. At the same time, it is possible to achieve high rates of compression for large volumes of data to save on storage space.

The standard is defined in **ASTM E 1947 -98 (2004)** and **ASTM E 1948 -98 (2004)**. Further information can be found under www.astm.org.

The technical platform of the ANDI protocol is the netCDF (network Common Data Form) interface. netCDF is a set of several interfaces for array-oriented data access. The netCDF libraries support a platform independent format for representing scientific data. Further information on netCDF can be found under www.unidata.ucar.edu

Since the saved ANDI / netCDF files are binary coded, they cannot be read without appropriate software. If you wish to see the information stored in a netCDF file, the command box in Windows® must be opened as follows:
Click on *Start/Run*. In the *Open:* text box, enter **cmd** and click on *OK*. The Windows® command line is opened, as in Fig. 128.

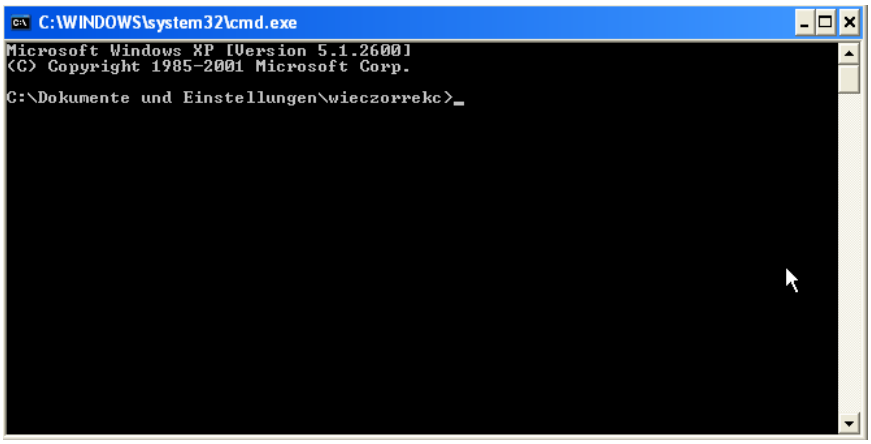


Fig. 128 : Windows® command line

Type in **cd** and press the **[ENTER]** key. Then, change to the installation directory of your ***NANOCOLOR*® *UV/VIS* software** and there, to the folder *ncdump* (see Fig. 129).

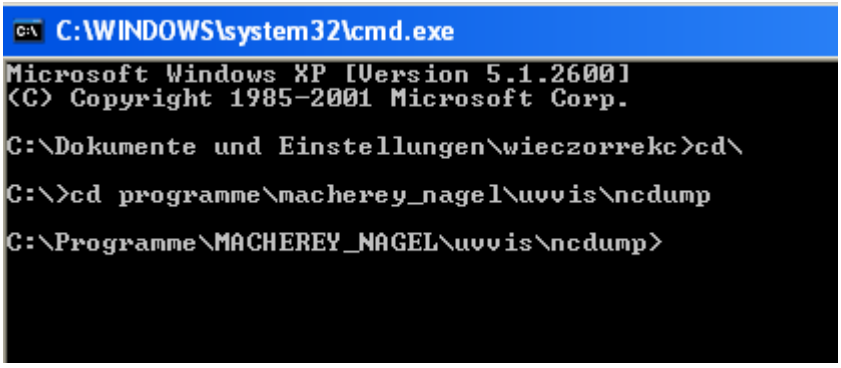


Fig. 129 : Change to the installation directory

Now enter the following program call (the path and file names are only an example):

ncdump c:\export\uvvis.cdf > c:\export\uvvis.txt

The program *NCDUMP* reads all data of the binary-coded file *c:\export\uvvis.cdf* and writes the data as readable text in the file *c:\export\uvvis.txt*.
The file *c:\export\uvvis.txt* can now be opened. This file **cannot** be read with the ***NANOCOLOR*®** PC Software for Spectrophotometer or any other spectroscopic software.

8.5 Literature References for Bio Tests

Concentration Determination of ss-DNA, ds-DNA and ss-RNA

Frederick M. Ausubel et. al.
Short Protocols in Molecular Biology, 4th Ed
A3-11 (1999)
John Whiley.

Concentration Determination of Proteins, A280 nm – A260 nm Method

C. K. Woodward, B. D. Hilton
Annu. Rev. Biophys. Bioeng. *8*
99-127 (1979).

Concentration Determination of Proteins, A215 nm – A225 nm Method

W. Jencks
Catalysis in Chemistry and Enzymology
417-436 (1969)
McGraw-Hill, New York.

Concentration Determination of Proteins, A235 nm – A280 nm Method

S. W. Englander, N. R. Kallenbach
Q. Rev. Biophys. *16*
521-655 (1984).

ZERO-value Determination at Reference Wavelength 320 nm

Warburg, O.; Christian, W.
Biochem. Z. *310*

384-421 (1942).

8.6 Details of the Smoothing Function

The smoothing factor used determines the smoothed value measured at a point **$F(x_i)$** from the weighted averaging of 27 measurement points f_i ° x_i with the specified measurement point as the centre. The weighting factors f_i are taken from a standard Gaussian function (see formula 1). The magnitude of smoothing is determined by the standard deviation from the Gaussian function. The adjustable smoothing factor in this software is represented by σ.

$$F(x_i) = \sum_{i-13}^{i+13} f_i \cdot x_i$$

$$f_i = \frac{1}{\sqrt{2\pi}\cdot\sigma} \cdot e - \frac{i^2}{2\sigma^2}$$

Formula 1 : Smoothing function

8.7 Language Selection in the Software

Version 4.0 of the ***NANOCOLOR***® PC Software for Spectrophotometer is issued in German, English, French, Polish and Spanish versions. MACHEREY-NAGEL is endeavouring to include other languages. If you wish to program a language yourself, follow the procedure given here:

1. First, make a backup copy of the file *mn_uvis_language.xml* from the *language* folder in the installation directory of this software.

2. Start the program LANGUAGE_TOOL from the *language* folder in the installation directory of this software.

3. Click on *Language File / Open Language File*

4. In the file selection window, select the file *mn_uvis_language.xml*.

5. Click on *Modify Language File / New Language*

6. Enter the name of the language without special characters (i.e. “Francais” and **not** “Français”)

7. Translate all texts and complete the new language column.

8. Click on *Language File / Save Language File*

The new language is now available in the ***NANOCOLOR*®** PC Software for Spectrophotometer.

8.8 Selection of Units

At various locations in this software, physical units can be selected from list boxes. The lists contain numerous units commonly used in photometry. However, if you require a particular unit that is not included in the list, there are two possibilities available: a) simply click in the text box of the list and enter the unit required from the keyboard or, b) open the Windows® Editor and enter the unit in the form

Unit 1 [ENTER] Unit 2 [ENTER] Unit 3

in the Editor and save the text under the file name *units.ini* in the *ini* subdirectory of your ***NANOCOLOR*®** PC Software for Spectrophotometer. The next time the software is started; the units are read from the *units.ini* file and added to the units list upon start-up.

8.9 Outlier elimination and multiple measurements

During a calibration outliers may appear by measurement errors or weighing/dilution errors of the calibration solutions. This chapter shows you how to handle outliers and gives you measures to improve the accuracy of your calibration.

8.9.1 Outlier

In mathematical statistics, several tests exist to determine, within a given probability, if a measured value is an outlier or not. One of these tests, the Grubbs outlier test, is integrated in the ***NANOCOLOR*®** PC Software for Spectrophotometer . The software detects automatically if a measured value should be declared as an outlier or if its deviation is within the confidence interval. Figure 130 shows the result of a linear calibration.

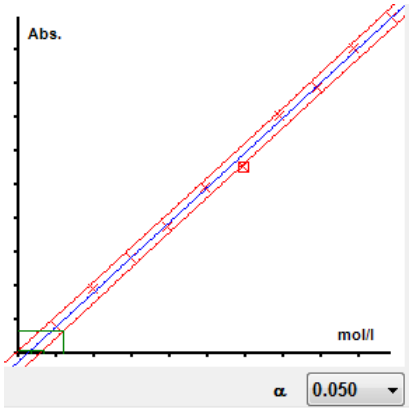


Fig. 130 : Calibration curve with outlier

The software draws a red square around all detected outliers. In the figure above, the value no. 6 is obviously not correct. The result is a quite wide confidence interval. Figure 131 gives the corresponding values.

	No.	Abs.	Conc.	Y/N
	1	0,057	10	☑
	2	0,135	20	☑
	3	0,202	30	☑
	4	0,265	40	☑
	5	0,347	50	☑
▸	6	0,392	60	☑
	7	0,499	70	☑
	8	0,557	80	☑
	9	0,641	90	☑
	10	0,705	100	☑

Fig. 131 : Calibration data with outlier at position 6

There are now two possibilities to handle the outlier: The first and simplest way is to deactivate the option button in the column *Y/N* ; see figure 132.

	No.	Abs.	Conc.	Y/N
	1	0,057	10	☑
	2	0,135	20	☑
	3	0,202	30	☑
	4	0,265	40	☑
	5	0,347	50	☑
▸	6	0,392	60	☐
	7	0,499	70	☑
	8	0,557	80	☑
	9	0,641	90	☑
	10	0,705	100	☑

Figure 132 : Disabling the outlier

The calibration curve is created new automatically as shown in figure 133.

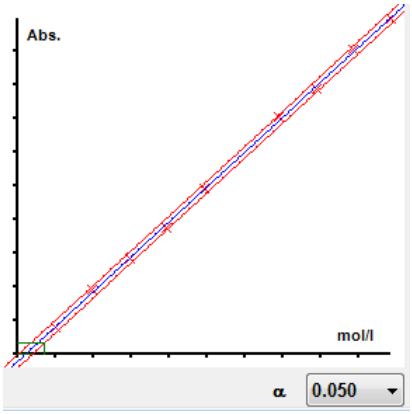


Figure 133 : Calibration curve without outlier

The confidence interval is now significantly narrower. In this way, you can either exclude or include any given measured value. The second possibility for outlier correction is to prepare the incorrect solution again and re-measure it. To do this double-click with the left mouse button on the data field with the incorrect value which in the example is field “0.454”. The software will then prompt you to insert the new solution into the photometer as shown in figure 134.

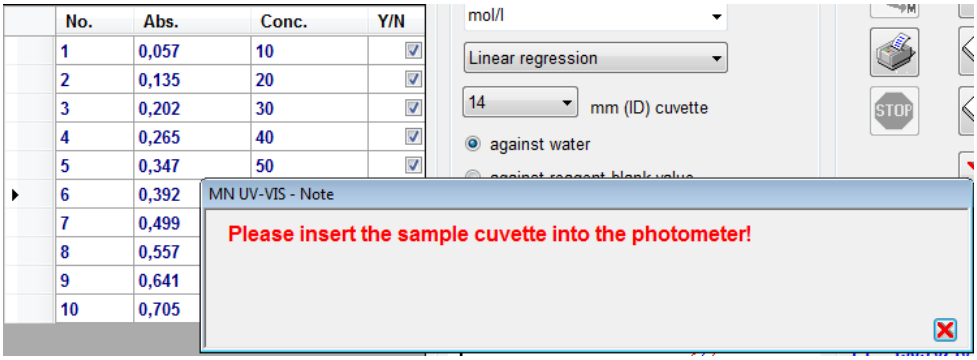


Figure 134 : Re-measuring a sample

As shown in figure 135, the new measured value is immediately incorporated into the spreadsheet. When the cuvette is removed, the graphic will also be updated.

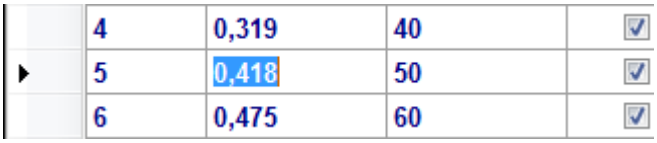


Figure 135 : Displaying the new measured value

8.9.2 Multiple measurements

The example above shows a calibration series with 10 test solutions of different concentrations. There is a statistical trick how to improve the accuracy of your

measurement and reduce your workload at the same time. The method is called multiple measurement. Instead of preparing 10 different solutions with concentrations ranging from 10 to 100, you prepare only 4 different solutions with concentrations let's say for example 20, 40, 60 and 80. However, the solutions with concentrations of 20 and 80 are used to carry out 3 to 4 single experiments, and the other ones with concentrations of 40 and 60 are used to carry out say 2 single experiments. Figure 136 shows this method for the same calibration as described in chapter 8.9.1.

	No.	Abs.	Conc.	Y/N
	1		20	☑
	2		20	☑
	3		20	☑
	4		40	☑
	5		40	☑
	6		60	☑
	7		60	☑
	8		80	☑
	9		80	☑
✎	10		80	☑

Figure 136 : Creating multiple measurements

If you are carrying out three experiments each with the solutions that have a concentration of 20 and 80, and two experiments each for the solutions that have a concentration of 40 and 60, then altogether this amounts to 10 photometric measurements. In the spreadsheet in figure 126, you can see that the solutions 1 to 3 all have the required concentration "20", then twice "40", twice "60" and again three times "80". Figure 137 shows the respective measurement data.

	No.	Abs.	Conc.	Y/N
	1	0,134	20	☑
	2	0,132	20	☑
	3	0,133	20	☑
	4	0,265	40	☑
	5	0,263	40	☑
	6	0,392	60	☑
	7	0,399	60	☑
	8	0,558	80	☑
	9	0,562	80	☑
▶	10	0,561	80	☑

Figure 137 : Measurement data of multiple measurement

As you have performed different experiments with those individual solutions, you will not get the exact same results. However, using the principle of multiple measurement increases the statistical accuracy as is shown in figure 138.

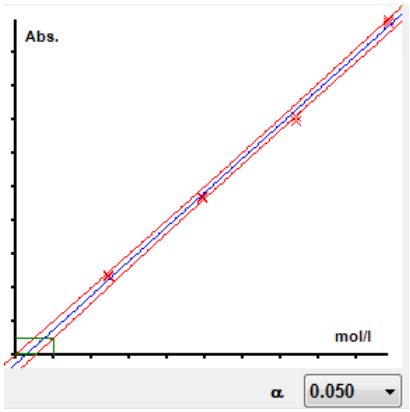


Figure 138 : Narrow confidence interval using multiple measurements

What does “different experiments mean? It does not mean measuring one cuvette three times! You have to prepare three tests with three different cuvettes, but for this you only need one test solution. This method covers all cuvette errors, measuring errors, and test errors, but reduces the influence of weighing/dilution errors and reduces your workload.

This method only works with linear calibrations! Always enter concentrations in increasing order as shown in the example!

For non-linear functions, always use the method described in chapter 8.9.1!

8.9.3 Level of significance α

For the calculation of the Grubbs outlier test and the confidence intervals as well as the detection limit, decision limit and determination limit, it is necessary to provide the accuracy with which the results are to be calculated. This probability or level of significance α can be selected from the list box below the graphics area as shown in figure 116 or 119. For the Grubbs test, the levels $\alpha = 0.1$, $\alpha = 0.05$ and $\alpha = 0.01$ can be used. For the confidence intervals and the detection limit, decision limit and determination limit, the α values 0.005 and 0.001 are also possible. If you select these small values, the Grubbs test is calculated with $\alpha = 0.05$.

A value of $\alpha = 0.05$ means that the probability of the value that was identified as an outlier actually being an outlier is 95%. Or to put it another way: There is a probability of 5% that the value may not be an outlier after all.

8.10 Bioanalysis Scan

The photometric determination of the purity of proteins, DNA and RNA requires measurements at several wavelength, usually 260 nm, 280 nm and 320 nm. As every wavelength requires its own BLANK measurement, the effort is quite high, Much easier and more convenient is to measure a single scan per sample and to evaluate all wavelengths at the same time.

Because the absorption of these samples are very high – and usually only several µl sample are present – it is not possible to use standard cells for this kind of measurement. Applicable cells are, e. g., LableGuard™ MicroCell (Implen). These cells offer a path length of 0.2/1.0 mm and a sample volume of 0.7 to 5 µl.

To evaluate a bioanalysis scan, create a scan in the range from 200 nm to 340 nm. All bioanalysis scans must use this wavelength range. Alternativly, open the file *example_bio.nc* from the scan example folder. Figure 139 shows the scans from file *example_bio.nc* in the scan window:

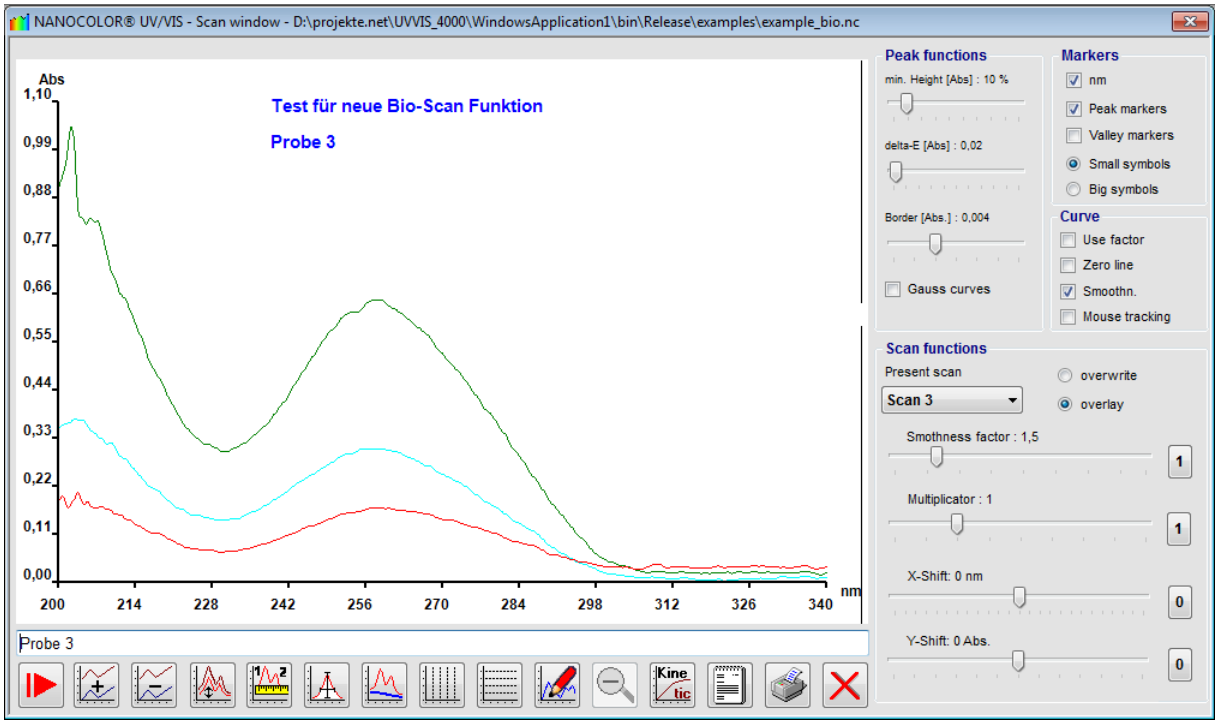


Fig. 139 : Bioanalysis scan

The scan windows shows the curves of three samples in different concentrations. **Important for the following evaluation is to use a scan range from 200 nm to 34 nm!**

Now select the menu command Scan/Bioanalysis Scan . The software opens the evaluation window, see Figure 140.

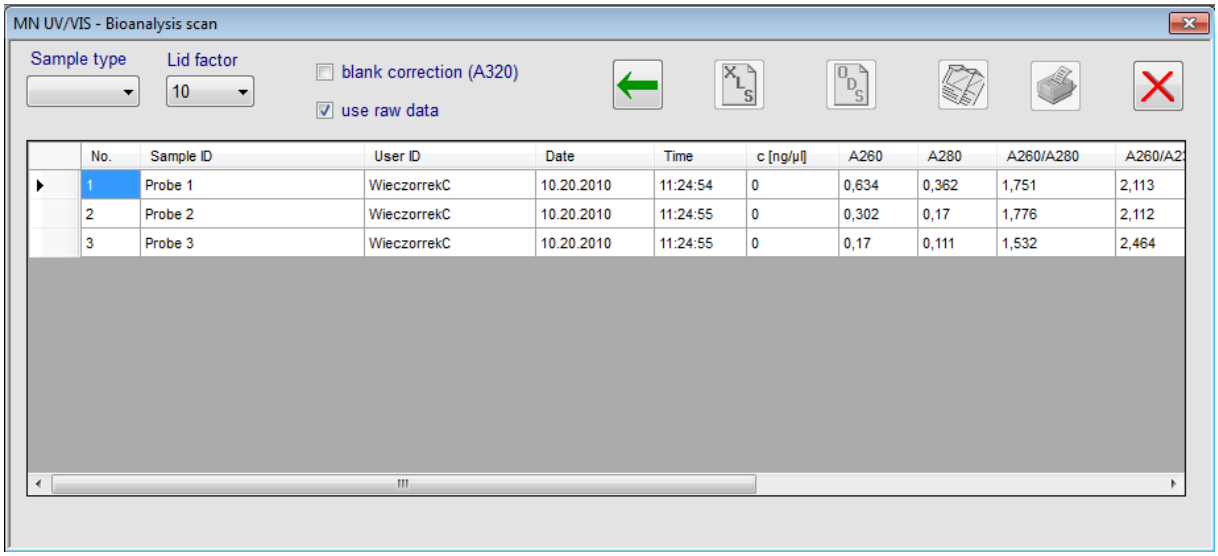


Fig. 140 : Bioanalysis Scan evaluation window

Beside several GLP relevant data the table shows the absorption values at 230 nm, 260 nm and 320 nm, the quotients A_{260}/A_{280} and A_{260}/A_{230}. Select now from the list *Sample type* the type of your samples, see figure 141:

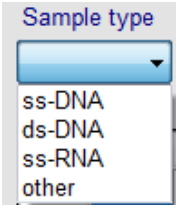


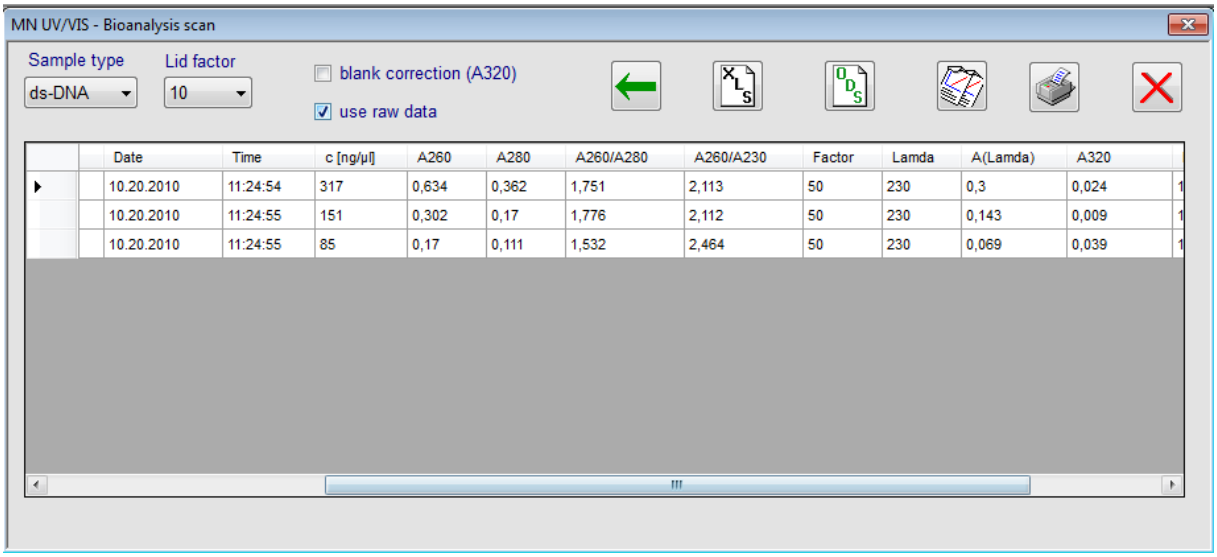
Fig. 141 : Available sample types

If you select “other” you must define the necessary factor in the column *Factor* manually. Finally you must select the correct lid correction factor from the list Lid factor . The lid correction factor depend on the used micro cell or on the cell / lid combination. The lid factor is calculated as

Lid F. = 10 mm / path length of the used micro cell / lid combination in mm

If you select “other” you must define the necessary factor in the column *Lid. F.* manually. The option *blank correction (A320)* determines if a blank correction with the absorbance at 320 nm is applied or not.
The second option *use raw data* determines if the smooth factor of the scan window is applied to the calculation or not.

Figure 142 shows a complete evaluation.



	Date	Time	c [ng/µl]	A260	A280	A260/A280	A260/A230	Factor	Lamda	A(Lamda)	A320
▶	10.20.2010	11:24:54	317	0,634	0,362	1,751	2,113	50	230	0,3	0,024
	10.20.2010	11:24:55	151	0,302	0,17	1,776	2,112	50	230	0,143	0,009
	10.20.2010	11:24:55	85	0,17	0,111	1,532	2,464	50	230	0,069	0,039

Fig. 142 : Evaluation of a bioanalysis scan

The results can be printed or exported to EXCEL or OPENOFFICE.

8.11 Software Errors

Despite all checks and test runs, errors in software cannot be completely ruled out. Please help to improve our software products by reporting to us any errors or faults that may have occurred.
Send a short e-mail to: sales@mn-net.com, Ref.: “UV/VIS software errors” with a brief description of the fault (what happened or did not happen after clicking on a particular button or menu item) and include an attachment with the following three files:

1. *error.log* from the folder *errorlog*

2. *sys.log* from the folder *syslog*

3. environment.log from the folder *syslog*

Both folders can be found in the installation directory of your ***NANOCOLOR***® PC Software for Spectrophotometer.

If possible, use the software function “Allow error report” to send an error report to MACHEREY-NAGEL (see Chapter 7.1).