

Thermo Scientific
Skant[®] Software for
Varioskan[®] version 2.4.3
User Manual

Rev. 3.1



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Varioskan[®]
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About the User Manual

Intended users

The user manual has been written for the users of the Thermo Scientific Varioskan spectral scanning multimode reader and provides information on Thermo Scientific SkanIt Software for Thermo Scientific Varioskan. The manual contains the operating instructions for Thermo Scientific SkanIt Software version 2.4.3 Research Edition (RE) and Drug Discovery Edition (DDE) that includes features needed for compliance with FDA's 21CFR11.

Read the manual in its entirety before using the software.

For more information

For instrument-related issues, refer to the *Thermo Scientific Varioskan User Manual* (Cat. no. 15018850). The instrument and software user manuals can be found in PDF format on the Thermo Scientific SkanIt Software installation CD and they can also be launched from the same location as the software itself:

Start > Programs > Thermo SkanIt Software > SkanIt RE/DDE for Varioskan User Manual / Varioskan Instrument User Manual.

For the latest information on products and services, visit our worldwide websites on the Internet at:

<http://www.thermo.com>

<http://www.thermo.com/readingroom>

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual to your local Thermo Fisher Scientific representative.

Warning and other markings used in the documentation

The following symbols and markings appear in this User Manual.



Caution Risk of damage to the instrument, other equipment or loss of performance or function in a specific application.



Note Marks a tip, important information that is useful in the optimum operation of the system, or an item of interest.



Tip Gives a helpful hint for getting the most out of the software functionality.

Chapter 1

Introduction

Skant Software

Skant Software is used to control the Varioskan spectral scanning multimode reader.

Skant Software provides features needed to define and run a variety of assays and to make comprehensive calculations and reports. A plate template database covers the most common plate formats. Skant Software allows you to build your own applications and to run or modify ready-made applications. Designing an own application is easy. The protocol steps are simply added to a steplist while their parameters can be viewed and defined on a single window pane. Plate layouts are created with the Fill Wizard.

By using Skant Software the user can:

- Load, create and edit sessions and plate layouts
- Export raw measurement data and perform data reductions
- Execute a session directly from the software with the Varioskan instrument
- Export and import sessions and runs between Skant Software databases in different PCs
- Generate and print measurement and calculation reports

The sessions are made and stored in a database on the PC using Skant Software. Once you have created a session, you can execute it directly from the software.

The PC and the instrument only have to be connected to each other when sessions are run directly from the software. In other words, you can create and edit sessions without a connection to the instrument.



Figure 1-1. Operation principle

Varioskan system

The Thermo Scientific Varioskan (Figure 1-2) is an advanced spectral scanning multimode reader. The Varioskan is used to measure fluorescence intensity, time-resolved fluorescence (TRF), and absorbance in end point, kinetic and spectral measurements in the UV/Vis/NIR range from appropriate microplate formats. In fluorometric measurements appropriate 6 to 1536-well plates can be used, and correspondingly appropriate 6 to 384-well plates in photometric measurements. Incubation can be carried out in a controlled incubation temperature. The instrument also allows shaking and reagent dispensing. The instrument is run with SkanIt Software, which controls all the instrument functions and provides data processing as well as reporting functions.



Figure 1-2. Varioskan spectral scanning multimode reader

Chapter 2

Installing SkanIt Software

This chapter contains information necessary for a successful installation of SkanIt Software.

Before installation

Read these instructions before you attempt to install SkanIt Software version 2.4.3.



Note Failure to follow these instructions may lead to unsuccessful installation of SkanIt Software.

Registering SkanIt Software

Before you can install your SkanIt Software, you have to fill out the registration form on our Web site at:

<http://www.thermo.com/skanit>

During the registration the software serial number is requested. You can find the serial number on the installation CD box. You will receive an installation code as a result of the registration. The installation code is sent to the e-mail address entered on the registration page. This code is required during the installation of SkanIt Software (see “Installing the software” on page 26, Step 2).

Checking the PC requirements

The table below lists the minimum PC requirements for SkanIt Software version 2.4.3.

Table 2-1. Minimum PC requirements

Minimum PC requirements	
Supported operating systems	Microsoft Windows 2000 with Service Pack 4 or Microsoft Windows XP Professional with Service Pack 2
Disk space	5 GB free disk space
Processor	Intel Pentium (or equivalent), 1 GHz or faster
Memory	1 GB RAM
Serial or USB ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	XVGA monitor with 1024 x 768 resolution
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them from the Microsoft web pages at:

<http://www.microsoft.com>

Microsoft Windows settings

The language should be set to *English (United States)* or *English (United Kingdom)* before installation of SkanIt Software. The language settings can be checked and changed in the **Regional Options** window (*Windows 2000*) or in the **Regional and Language Options** window (*Windows XP*):

Windows 2000:

Start > Settings > Control Panel > Regional Options > General > **English**

Windows XP:

Start > Settings > Control Panel > Regional and Language Options > Regional Options > **English**

If for some overriding reason you do not wish to set the language to English, the *Decimal symbol* must, nevertheless, be determined as “.” and the *Digit grouping symbol* preferably as “,” and *not* “.”.

Windows 2000:

Start > Settings > Control Panel > Regional Options > Number > **Decimal symbol & Digit grouping symbol**

Windows XP:

Start > Settings > Control Panel > Regional and Language Options > Customize... > Numbers > **Decimal symbol & Digit grouping symbol**

Make sure the *Turn off hard disk* setting is set to *Never*. In long kinetic sessions the computer is seemingly in an idle state, as the data flow is not monitored. Therefore, if the power option setting is not set to *Never*, the computer may turn off the power in the middle of a measurement. The power options settings can be checked and changed in the **Power Options Properties** window:

Start > Settings > Control Panel > Power Options > Power Schemes > Turn off hard disk > **Never**

Letters permitted with SkanIt Software

You are only allowed to use the letters a to z and A to Z with SkanIt Software. Scandinavian letters (å, ä, ö, æ, ø, and so on) or any other non-standard letters should not be used with SkanIt Software.

Installing

This section will guide you through the software installation procedure.



Note SkanIt Software cannot be installed on a network drive.

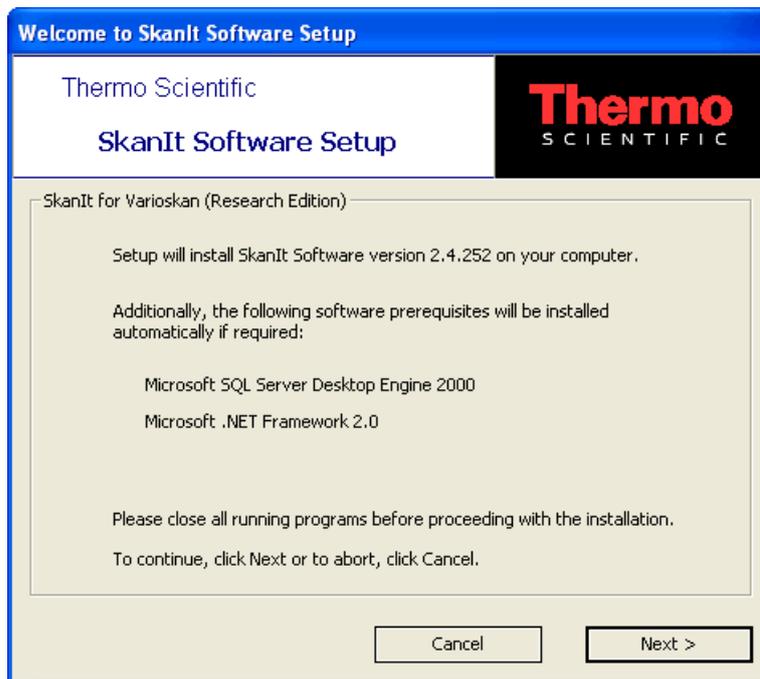


Note You have to be logged on to your computer with administrator privileges to install SkanIt Software.



Note You can stop the installation procedure at any stage by pressing the **Cancel** button. The setup will roll-back your system to the initial state.

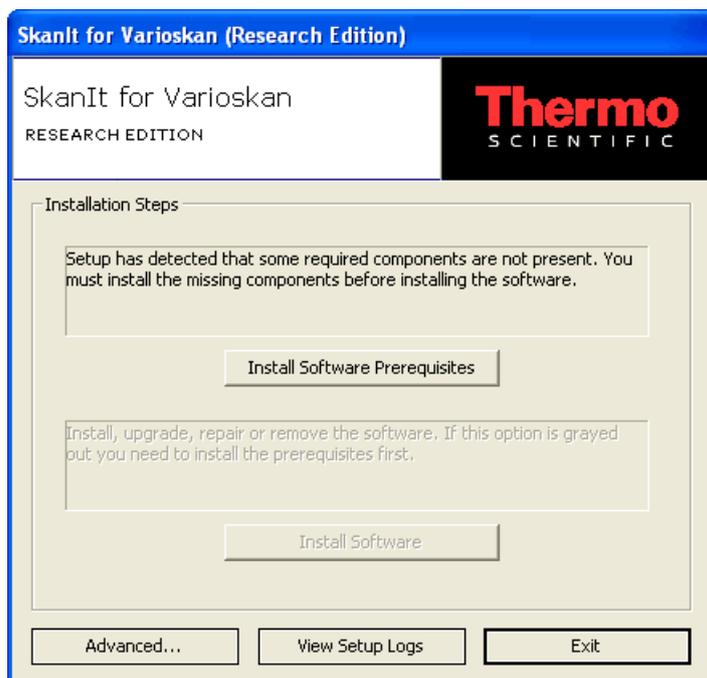
1. Check that SkanIt Software is registered and you have the installation code which is needed in “Installing the software” on page 26, Step 2. For more information about registering SkanIt Software, see “Registering SkanIt Software” on page 19.
2. Check that the PC requirements in Table 2-1 are met.
3. Insert the SkanIt Software installation CD into the CD-ROM drive of your PC.
4. The **SkanIt Software Setup** window appears automatically. If it does not, launch the installation from the CD; double-click the Setup.exe file.



5. The SkanIt Software Setup checks that all the software and system requirements are met and installs the required components:
 - SkanIt Software 2.4.3 always installed
 - Microsoft SQL Server Desktop Engine (MSDE 2000), installed if not found on your PC
 - Microsoft .NET Framework 2.0, installed if not found on your PC
 - SkanIt USB Driver, optionally installed if not found on your PC

Close all other programs from your computer and click **Next**.

6. If the SkanIt Software Setup detects existing MSDE 2000 instances, you are asked if you wish to install a new database engine instance. Select **Yes**, if you do not know the system administration (sa) password of the existing database engine.
7. The installation steps are shown.



If the **Install Software** button is not available, it means that at least one of the prerequisites are not met and you must first install them. Click the **Install Software Prerequisites** button. Proceed with “Installing software prerequisites” on page 23.

If all prerequisites are present on the PC, proceed with “Installing the software” on page 26.

You can also use the advanced installation option to update the instrument's embedded software. Proceed with updating the embedded software as instructed in a separate document *Upgrading Thermo Scientific Varioskan to SkanIt Software 2.4.3*.

Installing software prerequisites

To install software prerequisites, follow these steps:

1. The installation of a new database engine does not affect any other database engines if they are installed. If you want to configure SkanIt Software to use any of the existing MSDE server instances, you must know the system administrator (sa) password for that instance and the password must not be blank. Also, the MSDE 2000 instance must be set to use SQL Server or mixed mode authentication.

If the MSDE2000 database engine is not installed on your computer, it will be installed first. The MSDE System Administrator Password dialog is launched.



- **MSDE Instance Name** - The default name of the newly installed database engine instance. You cannot change the name of the database engine.
- **Enter SA Password** - Enter the sa (database engine's system administrator) password. You cannot leave this field empty. You will need this password in “Installing the software” on page 26, Step 7.

It is not possible to install the MSDE engine with an empty password. You must define a system administrator (SA) password for security reasons. Write down the SA password you define here. It is required for maintaining the database (see “Database” on page 227 and Appendix B: “Database maintenance”).



Caution The SA password is not recoverable. Store the password in such a way that it can be found when necessary. It is needed in installation Step 7 and also required for maintaining the database.

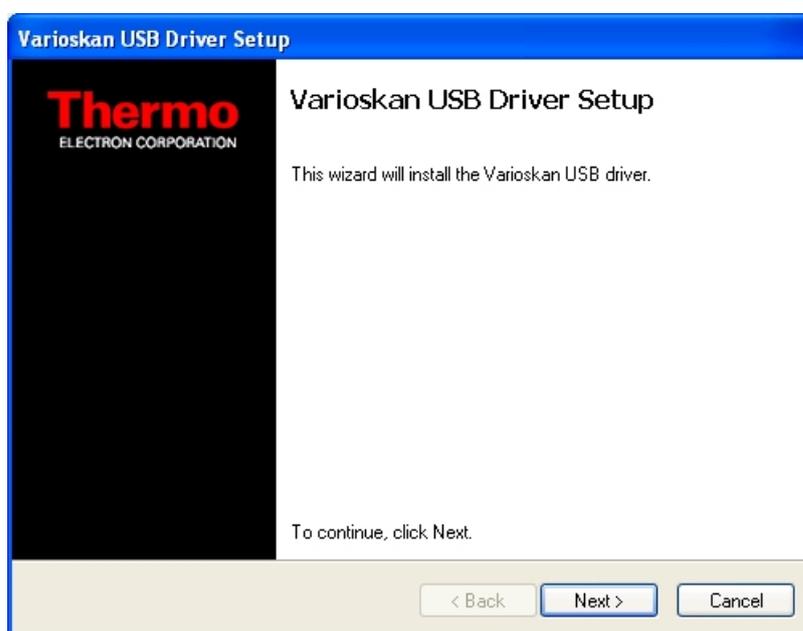
- **Confirm SA Password** - Retype the password to confirm the new password.

Click **OK**. The MSDE is installed.



The MSDE installation may take several minutes depending on your PC.

2. You are asked if you wish to have the USB (Universal Serial Bus) Driver installed. If you are going to connect your instrument to the PC through a USB port, this driver must be installed. Make sure that the instrument is not connected to the PC or that it is turned off before you continue with the USB Driver installation. Click **Yes**. The Varioskan Flash USB Driver Setup is launched.



Click **Next**.

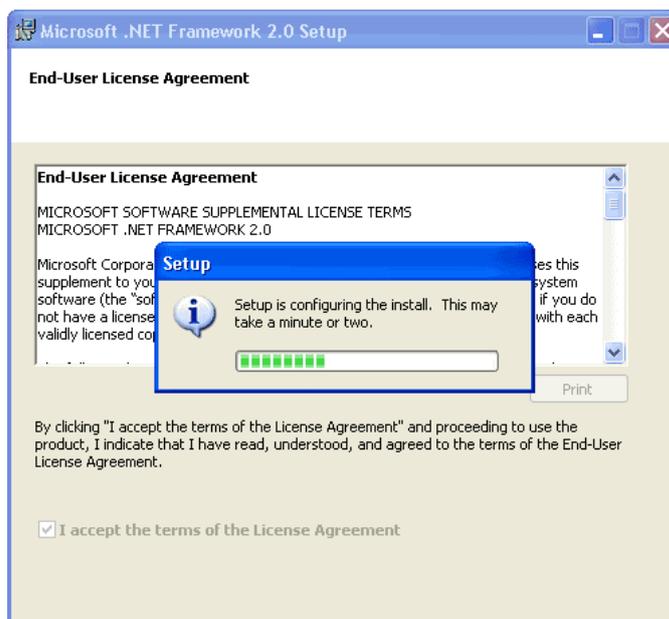
3. You have to first accept the End User License Agreement. Select **I accept this EULA** and click **Next**.
4. The USB Driver is installed. Click **Finish**.
5. If the required version of .NET Framework is not installed on your computer, the Setup will propose to install it.

The Setup checks for the correct version of the .NET Framework. If it is not installed, the setup installs the English version. If you have a correct national version of the .NET Framework already installed, you can cancel the installation of the .NET Framework by clicking **No**.

With Windows 2000, the Windows Installer is required to install the .NET Framework. If you are connected to the network, it is automatically installed. If you are not connected to the network, you

must install it manually. Run the executable in the *Windows Installer* folder on the installation CD.

You have to accept the terms of the End-User License Agreement.

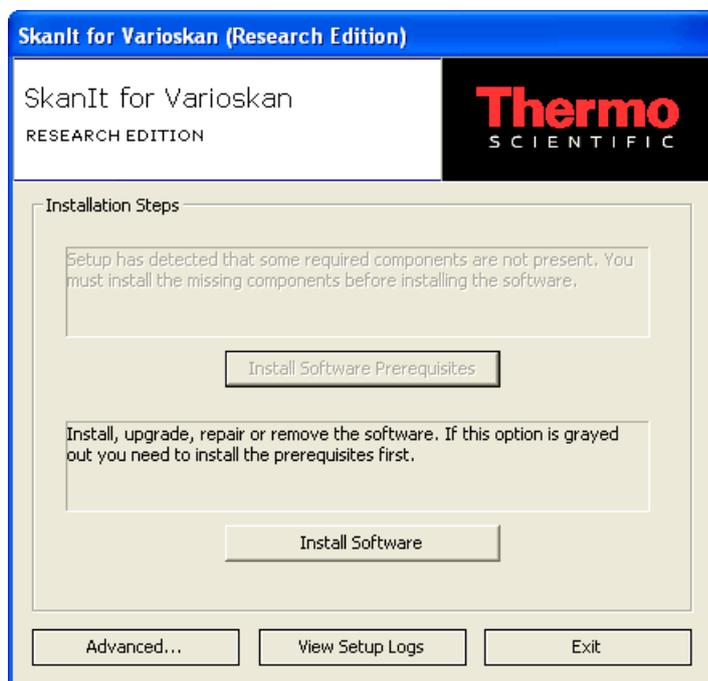


The installation of the .NET Framework takes a couple of minutes. When the installation is complete, click **OK** to continue with the SkanIt Software Setup.

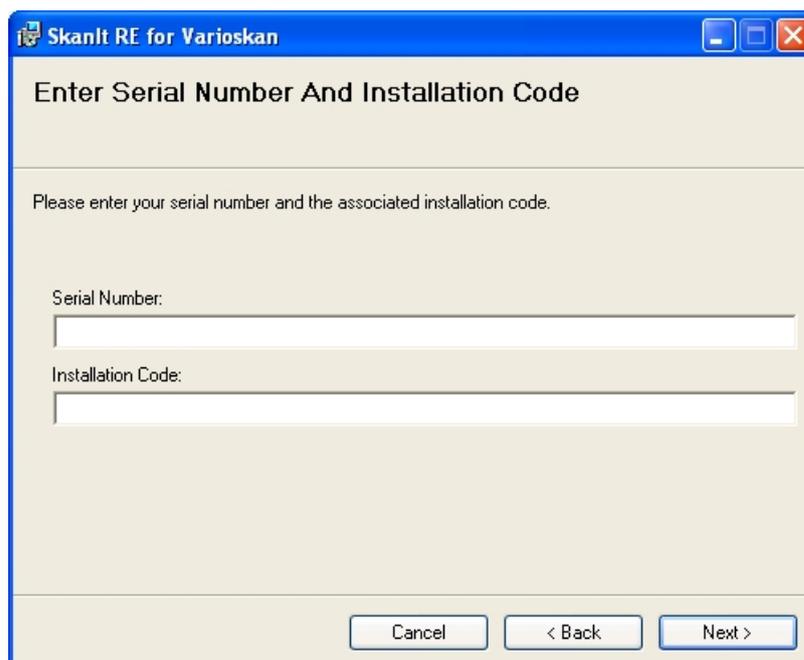
6. You may have to restart your computer after the prerequisites have been installed. However, the setup will inform you about it and the setup will continue after the restart.

Installing the software

When the prerequisites have been installed, the SkanIt for Varioskan Setup will proceed with the actual SkanIt Software installation.

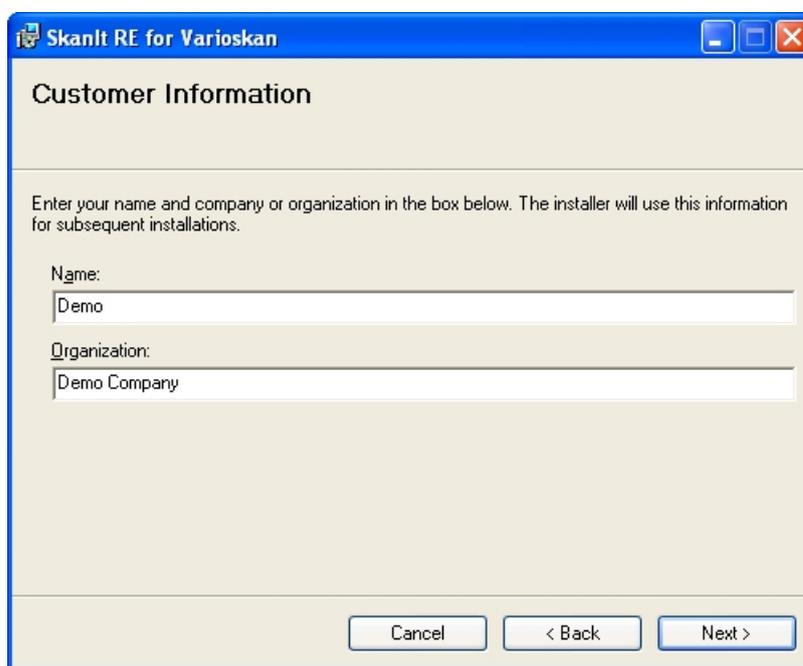


1. Click the **Install Software** button to launch the Setup Wizard. The wizard guides you through the entire installation procedure.
2. The **Enter Serial Number And Installation Code** dialog is shown. You have to first enter SkanIt Software licensing and customer information.



Type the software serial number in the *Serial number* field - the serial number is found on the cover of the SkanIt Software installation CD. Enter the software installation code you have received when registering your copy of SkanIt Software (see “Registering SkanIt Software” on page 19) in the *Installation code* field. Click **Next**.

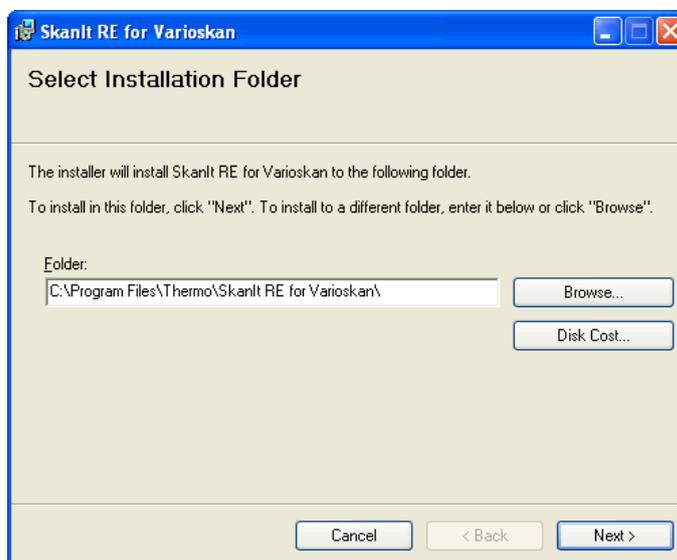
3. Read the License Agreement and agree with it to proceed with the installation. Click **Next**.
4. Fill out your customer information.



The screenshot shows a Windows-style dialog box titled "SkanIt RE for Varioskan" with a blue title bar. The main title is "Customer Information". Below the title, there is a text box containing the instruction: "Enter your name and company or organization in the box below. The installer will use this information for subsequent installations." There are two input fields: "Name:" with the text "Demo" and "Organization:" with the text "Demo Company". At the bottom of the dialog, there are three buttons: "Cancel", "< Back", and "Next >".

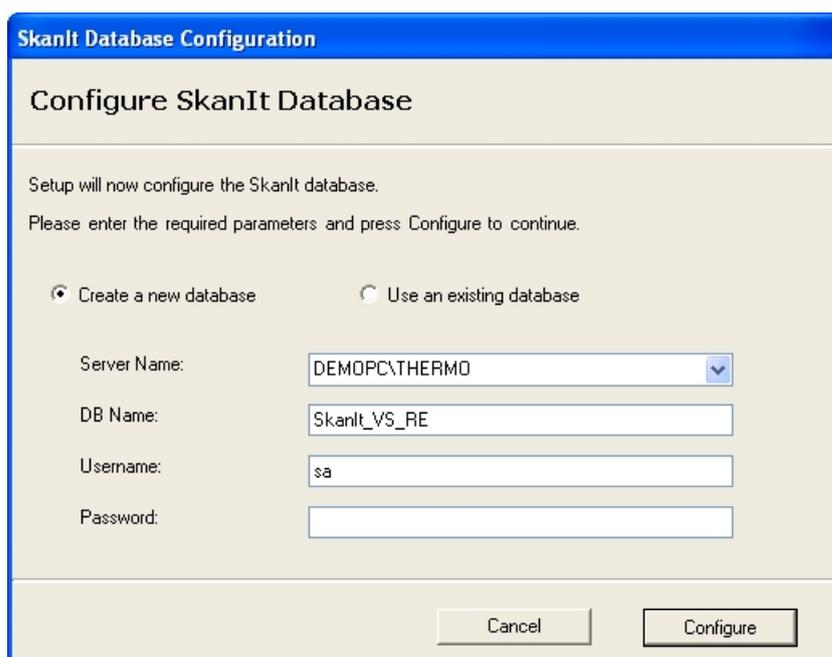
When entering your information in the **Customer Information** dialog, type your name and company in the *Name* and *Organization* fields, if the Setup Wizard has not detected them automatically. Click **Next**.

5. The Setup Wizard automatically suggests file locations for the files to be installed. It is recommended to use these suggestions. Change them only if absolutely necessary. The recommended free disk space is minimum 2.5 GB for the SkanIt Software database.



Click **Browse...** to select another folder or drive. You can click the **Disk Cost...** button to view the available disk space on the available drives. Click **Next**.

6. The Setup Wizard is now ready for the actual installation. Click **Next** to proceed. The installation files are copied to the selected folder.
7. The Database Configuration is performed next.

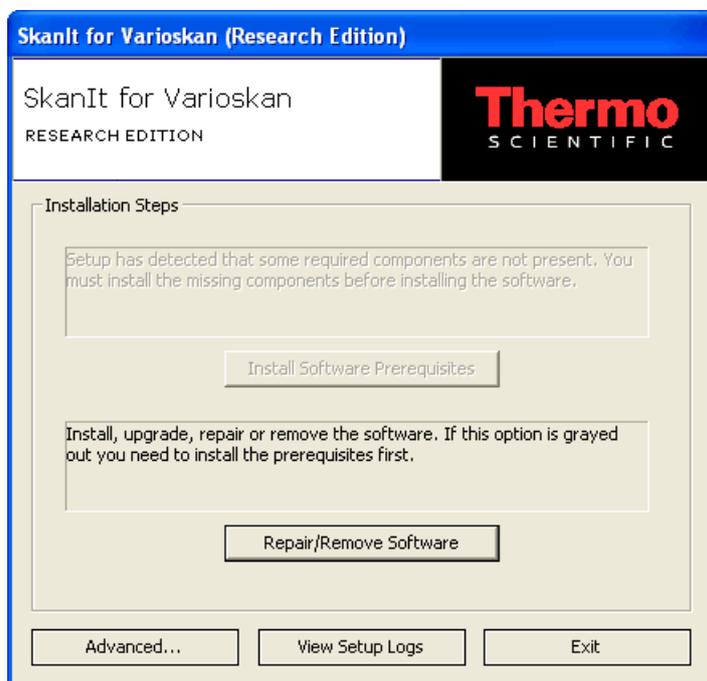


- **Create a new database** - SkanIt Software will be connected to an existing database engine (the one installed in the prerequisites) and a new SkanIt Software database will be created. This is recommended for new installations.
- **Use an existing database** - SkanIt Software will be connected to an existing database engine and an existing SkanIt Software database will be used. If the database you select is not compatible with the current software version, you can upgrade the database (see “Upgrade” on page 290).
- **Server Name** - The setup will automatically detect the computer's name. This is the name of the database engine (Server) where the SkanIt Software database will be created. The database engine has the instance name THERMO, separated by a backslash (\).
- **DB Name** - The name of the SkanIt Software database which you want to create. The default name is SkanIt_RE or SkanIt_DDE. If a database with the defined name already exists, you will obtain an error message. The existing database will not be overwritten.
- **Username** - The administrator login (system administrator) for the database engine (sa or equivalent). “sa” is default, as defined in “Installing software prerequisites” on page 23, Step 1. If you are using a previously installed database engine, it is possible that the username is not “sa”. Make sure that you use the correct username defined for the database engine.
- **Password** - The login password defined for the system administrator (as was entered in Step 1). If you are using a previously installed database engine, make sure that you use the correct password.

Click **Configure**.

The installation proceeds with the database configuration.

8. When the installation is complete, you will receive a notification of it. Click **Close**.

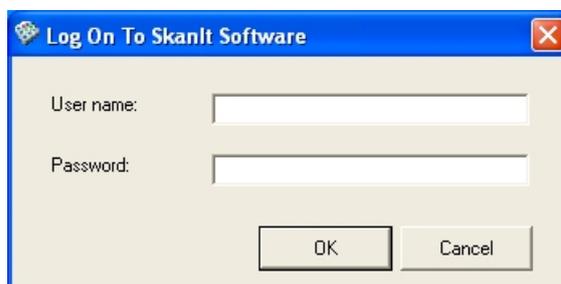


Exit the SkanIt for Varioskan Setup by clicking **Exit**.

9. Now you can launch SkanIt Software from the **Start** menu:

Start > Programs > Thermo SkanIt Software > **SkanIt RE/DDE for Varioskan 2.4.3**.

Or: double-click the SkanIt for Varioskan shortcut icon on your desktop. The **Log On To SkanIt Software** dialog is shown.



10. When logging in for the first time, enter “admin” in the *User name* field and leave the *Password* field empty. Use the **SkanIt Control Panel** to create a new administrator user (see “Adding a new user” on page 275). After creating the new administrator user, you should either remove the old administrator user or change its password in order to

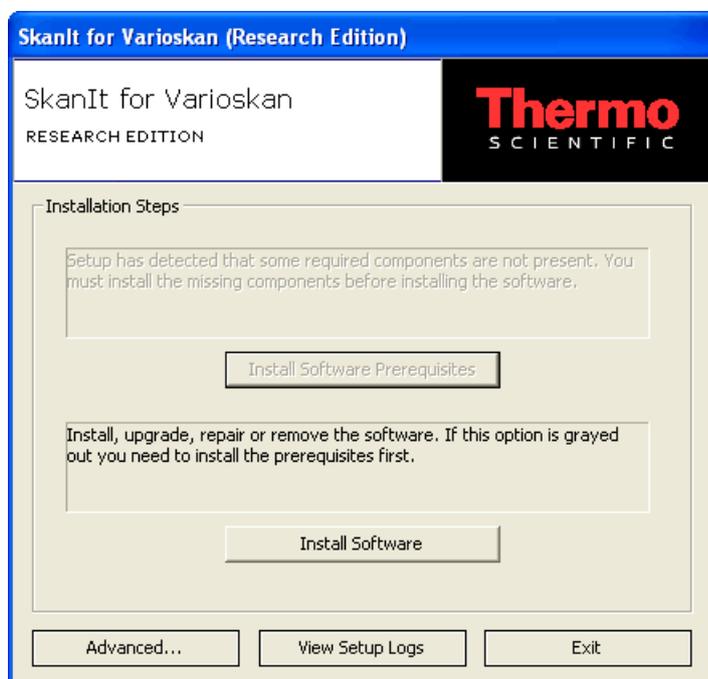
protect the data security. Exit the software and launch it again logging in with the new user name.

Installing DDE and RE versions to one computer

It is possible to install both the Drug Discovery Edition (DDE) and Research Edition (RE) of SkanIt Software to one computer. The software suggests different folders for the different SkanIt Software versions.



Note It is recommended that you make a database backup before a new installation. See “Backup” on page 286.



Click **Install Software**. The installation is performed similarly to the actual installation.

Installing SkanIt Software for different instrument models

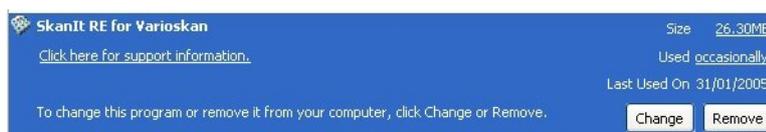
SkanIt Software is published separately for different instrument types. If you want to use the same PC to operate several instruments, such as Varioskan Flash, Multiskan Spectrum and Appliskan, you need to install separate versions of SkanIt Software onto your PC. The installation procedure is similar for all versions.



Note It is recommended that you make a database backup before a new installation. See “Backup” on page 286.

Uninstalling SkanIt Software

You can uninstall SkanIt Software by using the “Add-Remove Programs” applet in the Windows Control Panel.



Click **Remove**. You are asked to confirm the removal of the program. Click **Yes**. The SkanIt Software is uninstalled.

However, your SkanIt database will remain as it is. The database engine and .NET Framework will not be uninstalled, as they may be used by another software application. They must be uninstalled separately. If you are uninstalling version 2.0, the database engine will be uninstalled too.



Caution Do not delete the database engine if any of your other software applications uses a database engine.

The database engine (MSDE) can be uninstalled using the “Add-Remove Programs” applet in the Windows Control Panel. Click **Remove**. You are asked to confirm the removal of the program. Click **Yes**. The database engine is uninstalled.

Backup the database by using standard database engine maintenance tools, if you choose to delete the SkanIt database. The following files will remain in the installation folder after uninstallation: *SkanIt_RE(or _DDE).mdf* and *SkanIt_RE(or _DDE).ldf*.

You can delete these files, make a backup copy of them, or attach them to a database engine (MSDE 2000 or Microsoft SQL Server 2000) using standard maintenance tools. For example, you can use Enterprise Manager for attaching database files to another database engine.

Reinstalling the software

The reinstallation mode is not supported. You have to uninstall SkanIt Software and then install a fresh copy.

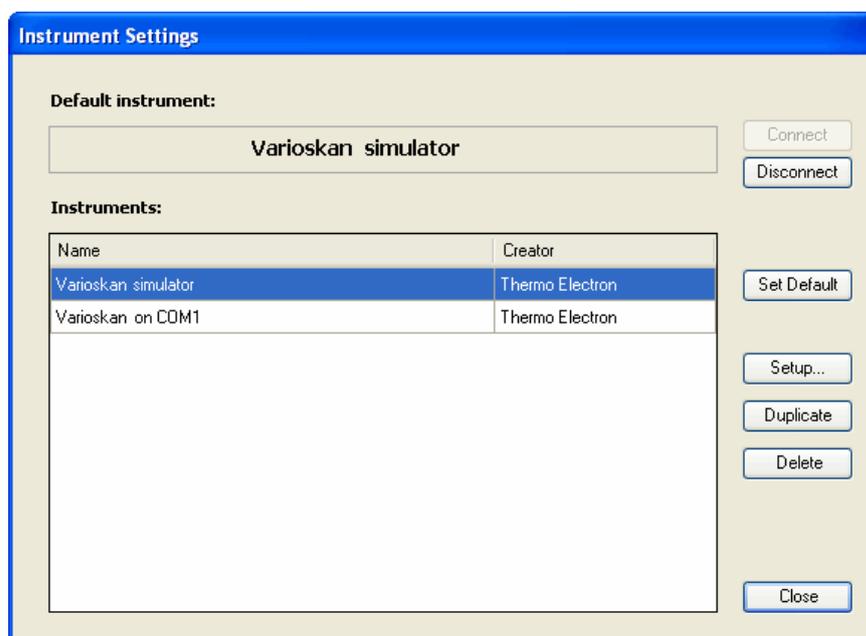
Communicating with instruments

The instrument has to be configured in the SkanIt Software database before a connection can be established between the instrument and the SkanIt Software on the PC.

When the instrument and SkanIt Software are connected, the software checks the serial number of the instrument and compares it to the one in the database. The following sections will describe the instrument and software settings. Also see Chapter 13: “Settings”.

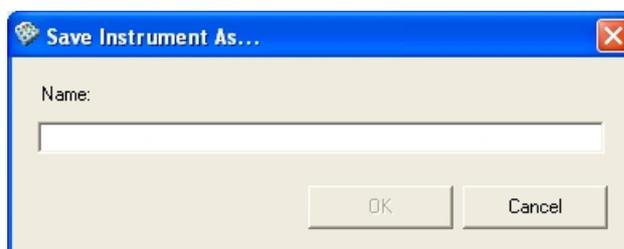
Adding an instrument to the PC database

1. Open the **Instrument Settings** dialog by selecting Settings > **Instrument...**



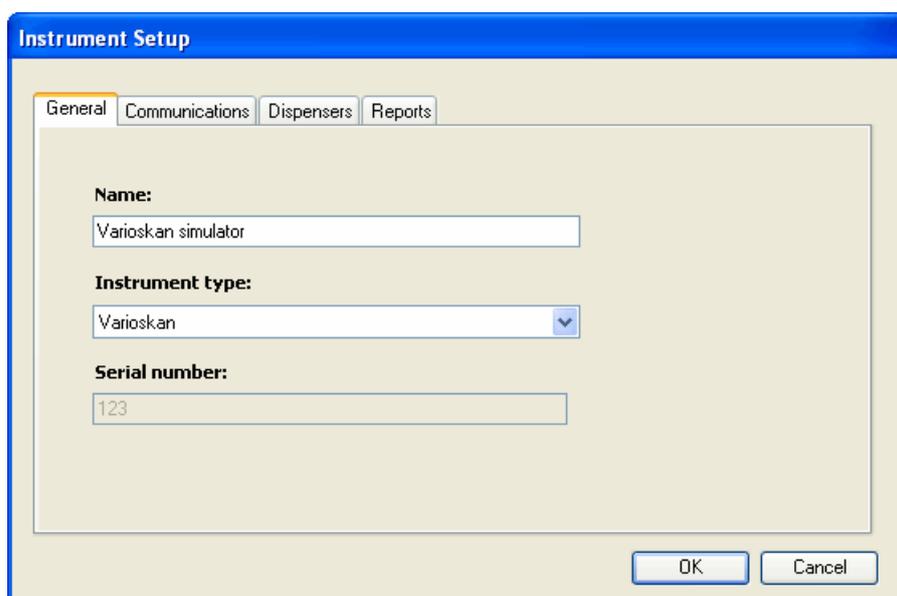
- **Connect** - Opens a connection to the default instrument.
- **Disconnect** - Closes the connection to the default instrument. You must close the connection before setting another instrument as the default.
- **Set Default** - Click the button to set the selected instrument as the default. The software will try to connect to the default instrument when it is launched (see “Launching SkanIt Software” on page 54), if the **Connect to default instrument at startup** setting is selected (see “General” on page 226).
- **Setup...** - You can edit an existing instrument (see below).

- **Duplicate** - If you want to add a new instrument that does not exist in the list, first make a duplicate of an existing instrument and save it with a new name. Then you can modify the setup information (see below).
 - **Delete** - Remove the selected instrument from the database.
 - **Close** - Close the dialog.
2. If your instrument is not included in the list, add the instrument to the database: select *Varioskan on COM1* and click **Duplicate**. The **Save Instrument As...** dialog opens.

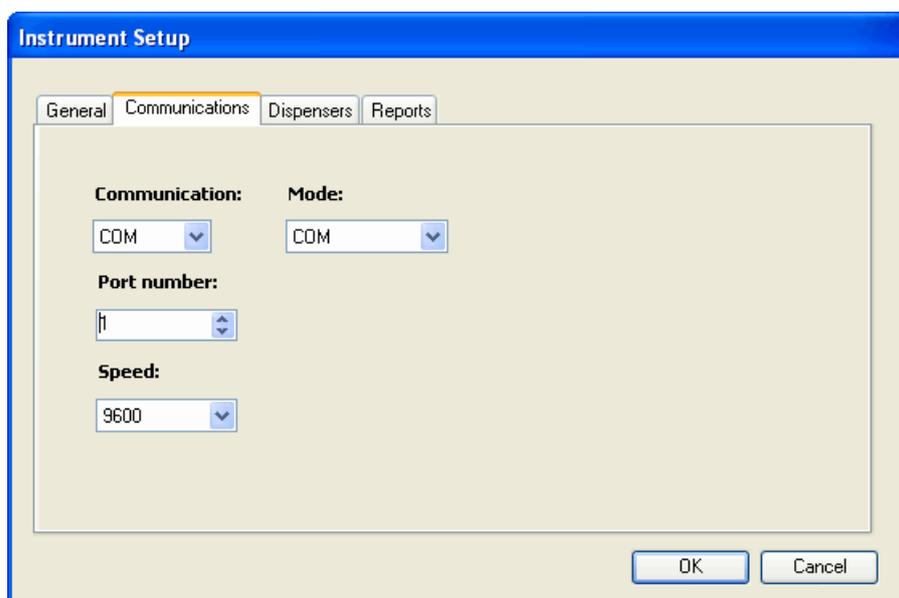


Type the name of your instrument in the field and click **OK**.

3. A duplicate of the selected instrument is added to the database with the new name and you can now modify it. Select the instrument from the Instruments list and click **Setup...**. The **Instrument Setup** dialog opens.



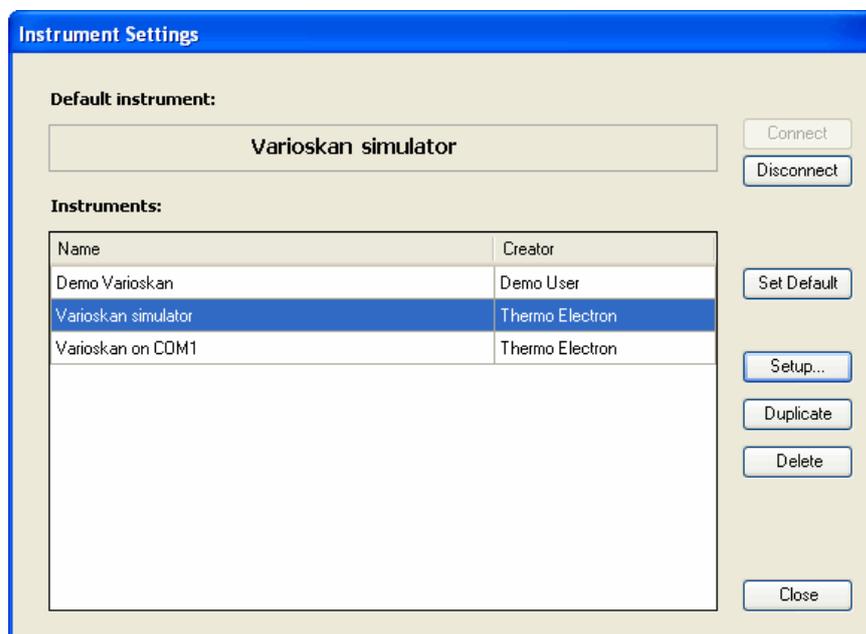
4. Change the instrument information on the **General** tab. See “General” on page 233.
5. Select the **Communications** tab.



6. Enter the data communication information in the boxes. See “Communications” on page 233.
7. Edit the dispenser settings. See “Dispensers” on page 234.
8. Click **OK**.
9. You can now change the default instrument to be the newly added instrument. Select the new instrument from the *Instruments* list and click **Set Default**.



Note You must disconnect from the current default instrument first.



10. Click **Close** to exit the **Instrument Settings** dialog.



Note You can add several new instruments before clicking **Close**.

The new instruments are available immediately in the *Instruments* list and in the list in the **Execution** action panel.

Connecting the instrument to the PC through a COM port

The PC and the Varioskan instrument are connected by using an RS-232 C serial cable.

1. Close SkanIt Software and shut down the PC. Switch OFF the Varioskan instrument from the mains switch on the left side panel of the instrument.
2. Connect the serial cable to a free serial (COM) port on your PC. This port should correspond to the one defined for the instrument in the database (defined in the **Instrument Setup** dialog, under the **Communications** tab).
3. Connect the other end of the cable to the SERIAL RS-232 C connector (Figure 2-1) on the back panel of the instrument.

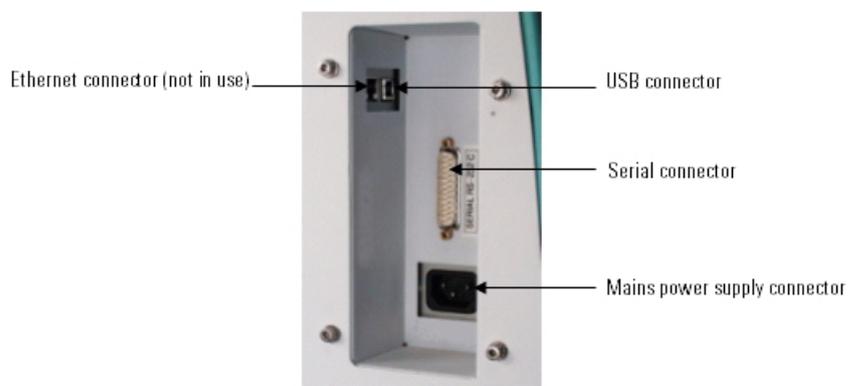


Figure 2-1. Computer and mains power supply connectors in the Varioskan instrument

4. Switch ON the instrument and then the PC.
5. Launch SkanIt Software as described in “Launching SkanIt Software” on page 54.
6. After startup the instrument is ready for operation. Since the instrument calibrates itself, you can start measuring immediately as soon as the instrument has been turned on. However, the stabilization of the incubator can take up to 10 minutes.

It is further recommended to carry out an empty run to verify proper instrument operation.

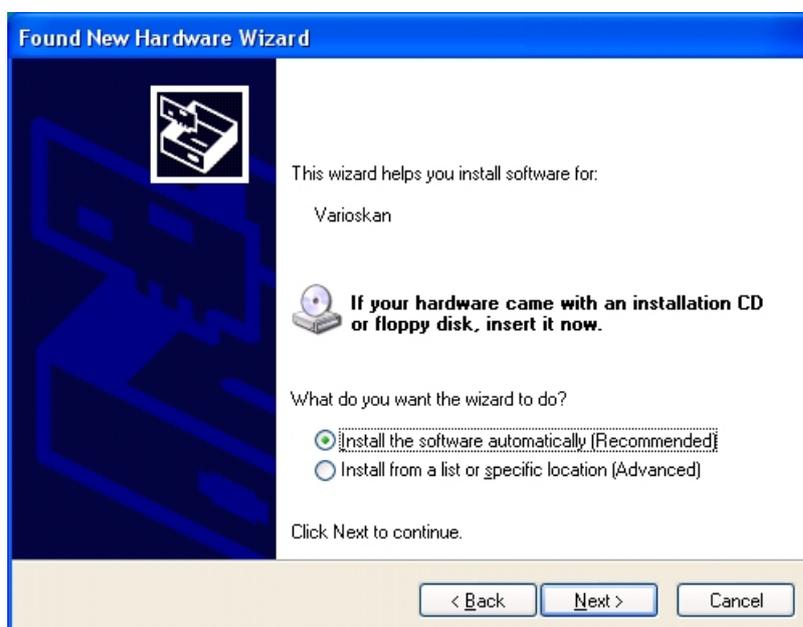
Connecting the instrument to the PC through a USB port

If you want to use your instrument through the USB port, proceed as follows. You have to have the USB Driver installed (see “Installing software prerequisites” on page 23, Step 2). You should also have the SkanIt Software Setup CD-ROM in the CD drive of your computer.

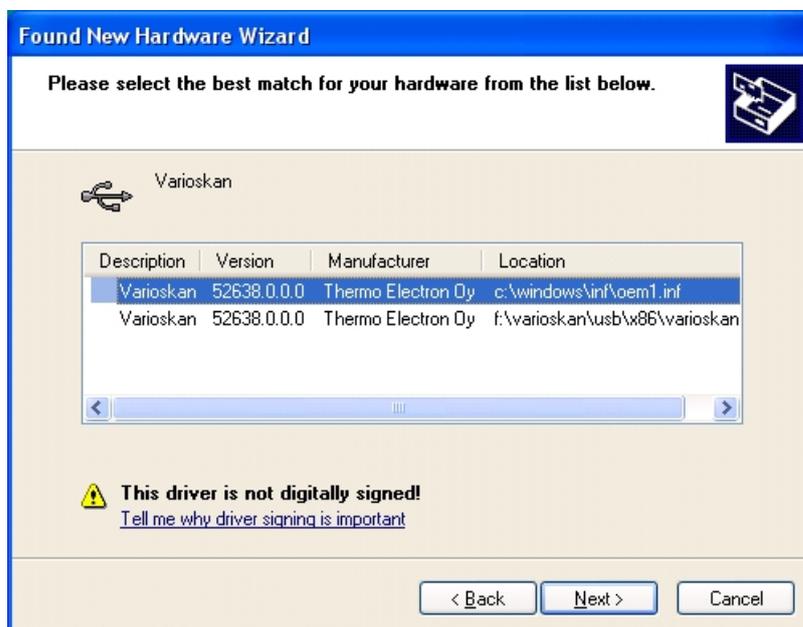
1. Turn off the instrument.
2. Connect the instrument to the computer using a USB cable (see Figure 2-1).
3. Turn on the instrument. The computer will recognize a new hardware.



4. Select *No, not this time* and click **Next**.



5. Make sure the installation CD is in the CD drive. Select *Install the software automatically* and click **Next**.



6. Select the appropriate choice from the list and click **Next**.
7. Click **Finish**. The instrument is now ready for operation.

Chapter 3

User Interface

Navigating in the software

When a session is open, the main window of SkanIt Software for Varioskan is divided into six areas (see Figure 3-1):

- The **Session** action panel on the upper left (1) has buttons to create, open and save sessions and to view the Help. It also has a list to select the part of the session you want to work with: the plate layout, the protocol, the measurement results or the run log. The view on the rest of the window changes according to your selection here.
- The **Steps, Results, Sample Properties, or Run Information** action panel on the middle left (2) has information about the session part selected above. If *Protocol* or *Results* is selected, the panel has buttons and a tree view of the protocol steps, or the measurements and their associated calculations, respectively. When you select any of the items in the tree, the view on the right-hand panel changes accordingly. If *Plate Layout* is selected, the panel allows you to view and edit individual well information. If *Run Log* is selected, the panel shows run information.

Use the buttons on the **Steps** action panel to add control, measurement or action steps to the protocol. Use the buttons on the **Results** action panel to add calculation steps to the measurement. The button selection changes according to what measurements or calculations are allowed for the selected step. When adding a step to the protocol is not allowed, the button is gray. When adding a calculation step to the results is not allowed, the button is not visible at all.

- The **Execution** action panel on the lower left (3) has instrument control functions. You can connect the PC to the instrument or the simulator and close the connection as well. Once you are connected, you can move a plate in or out and execute sessions, for example.
- The view on the largest panel (4) depends on the selection made in both the upper and middle left panels. You can view and edit protocol and calculation parameters as well as the plate layout, and view the measurement and calculation results and the run log.
- The menus on the menu bar (5) vary according to the selection made in the **Session** action panel (see Chapter 14: “Menus and Action Panels”).

- The status bar at the bottom (6) shows to which instrument the software is connected, the logged-on user, the temperature of the instrument and the current date and time. When the software is connected to an instrument, the background color is green, and when not connected, the background color is red.

See “Protocol editing window” on page 42 through “Run Log viewing window” on page 48 for examples of the different views.

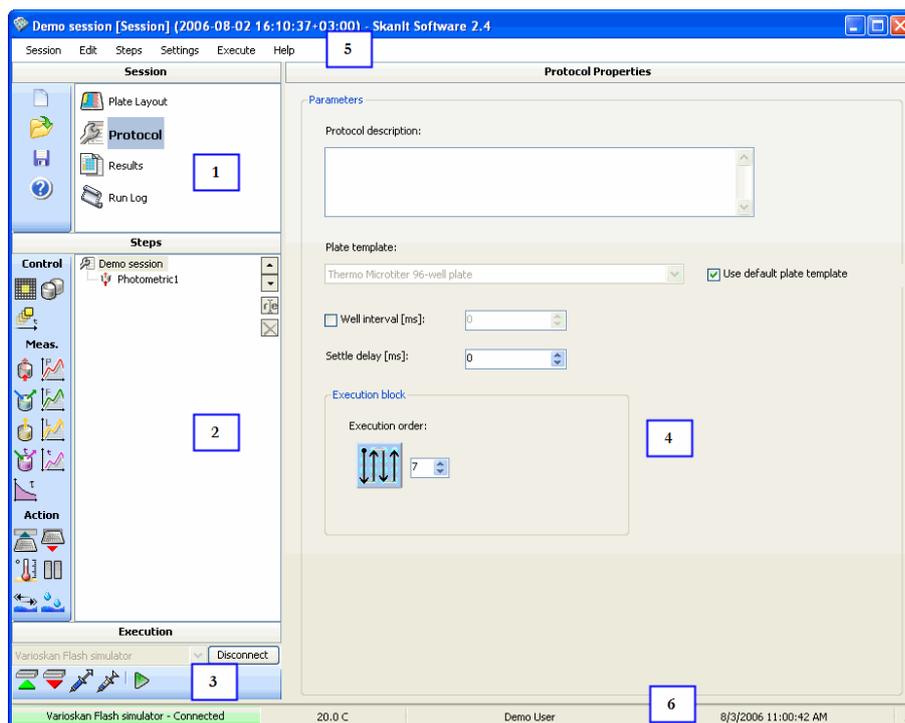


Figure 3-1. Different parts of the main window

Protocol editing window

The following figure shows an example of a protocol that has been opened in the main window. *Protocol* is selected from the session list, and the desired step is added to or selected from the **Steps** tree. The parameters of the selected step can now be viewed or edited in the main panel.

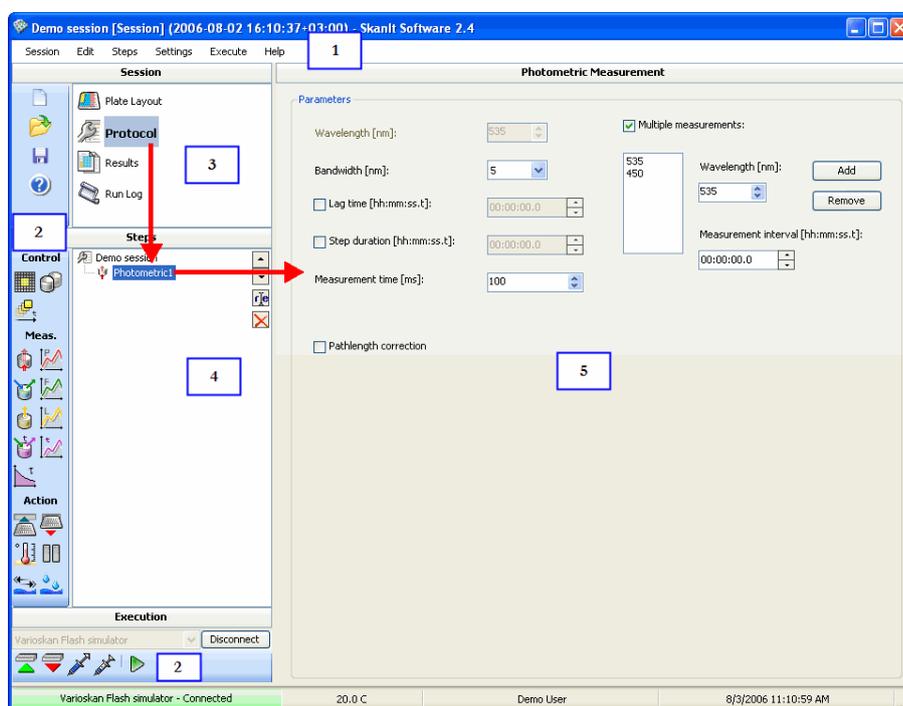


Figure 3-2. Main window for protocol editing

- 1 - Menu bar
- 2 - Action panels
- 3 - Session list
- 4 - Tree view of protocol steps. Use the and buttons to move steps up or down in the protocol, the button to rename steps, and the button to remove steps.
- 5 - Main panel showing the parameters of the protocol itself or the selected protocol step

For more information on editing a protocol, see “Editing a protocol” on page 81 and Chapter 7: “Step Parameters”.

Plate layout editing window

The following figure shows an example of a plate layout that has been opened in the main window. The plate layout is selected from the session list. The plate layout can now be viewed or edited in the main panel. When a well is selected, its properties can be viewed or edited in the **Sample Properties** panel.

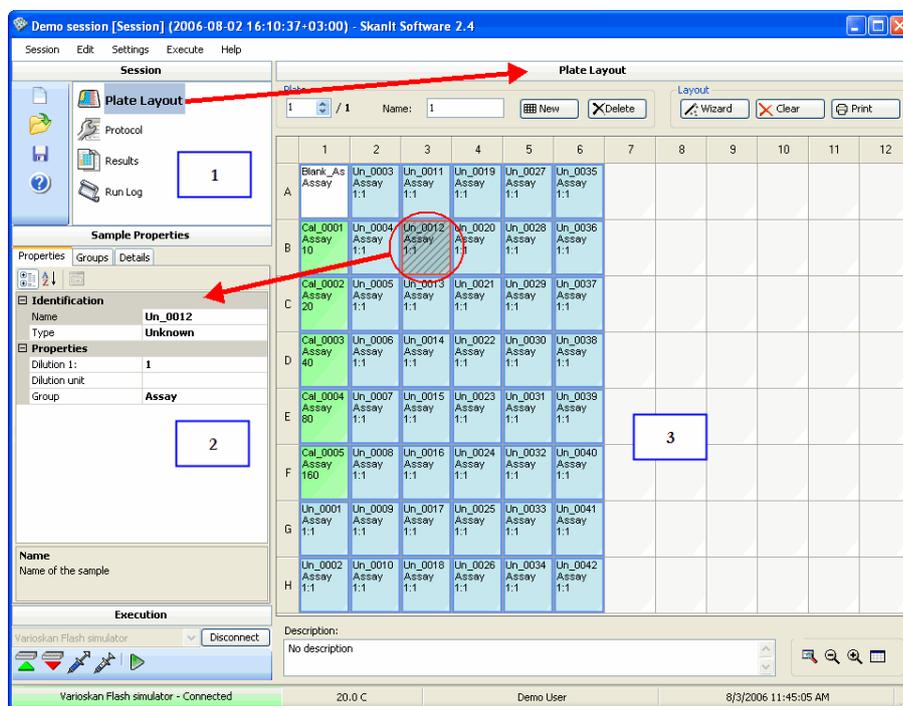


Figure 3-3. Main window for plate layout editing

- 1 - Session list
- 2 - Tab view of sample properties
- 3 - Main panel showing the plate layout and the well definitions

For more information on editing a plate layout, see Chapter 8: “Plate Layouts”.

Results viewing window

The following figures show an example of measurement results that have been opened in the main window.

The view in the main panel depends on the selection made in the **Results** tree:

- When the session is selected (circled in Figure 3-4), the run status report can be viewed.
- When a measurement is selected, the measurement results can be viewed in the table (plate layout) view (Figure 3-5) and the list view.
- When a calculation is selected, the calculation parameters (Figure 3-6) or the calculation results can be viewed in the table and list views (Figure 3-7).

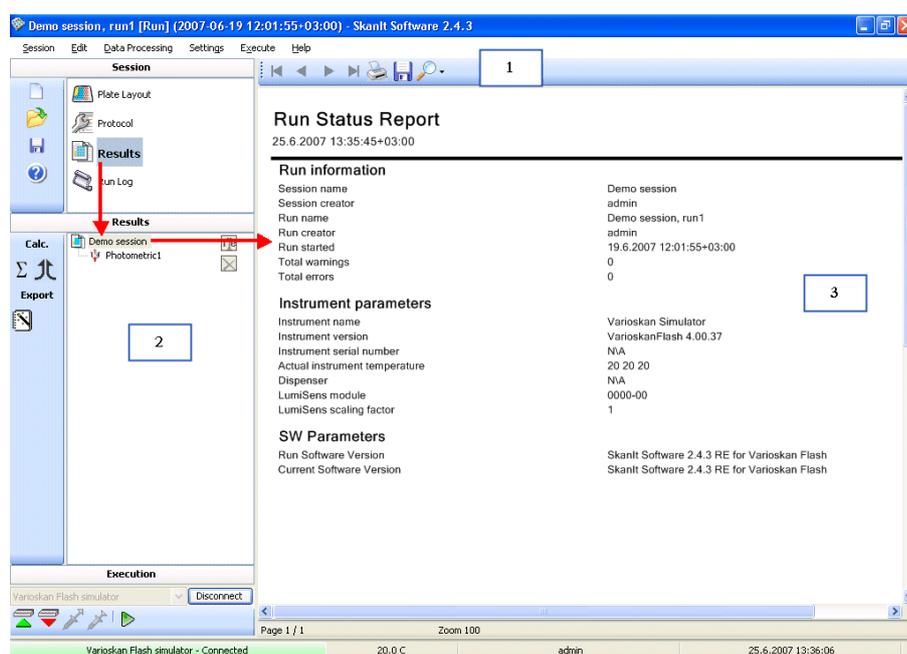


Figure 3-4. Main window for results viewing - status reports

- 1 - Report toolbar
- 2 - Tree view of measurements and their associated calculations
- 3 - Main panel showing the status report of the selected session

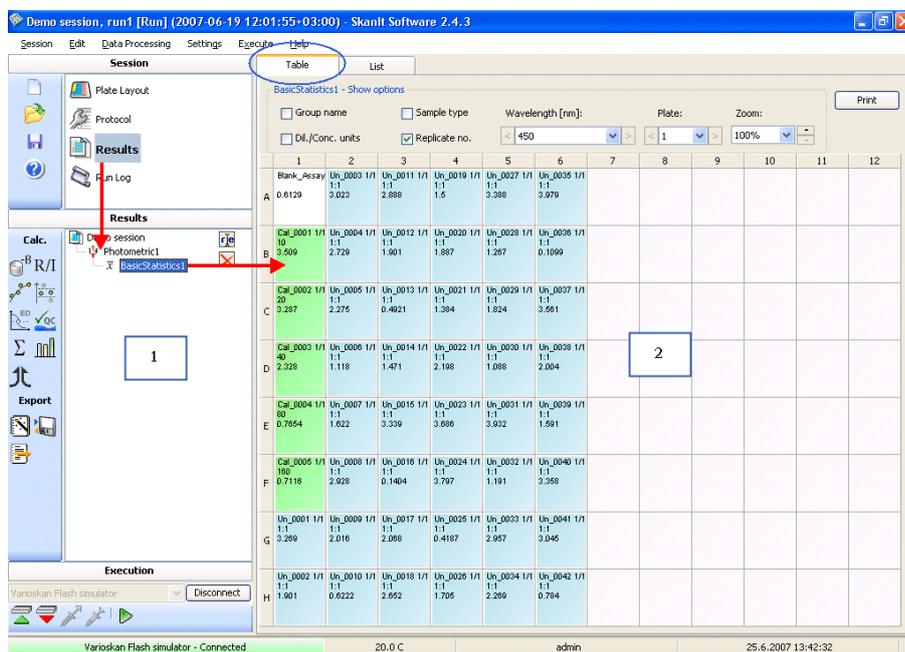


Figure 3-5. Main window for results viewing - measurement results as a table

- 1 - Tree view of the measurements and their associated calculations
- 2 - The main panel showing the results of the selected measurement in the plate layout format

For more information on viewing the measurement results, see “Calculation results” on page 206.

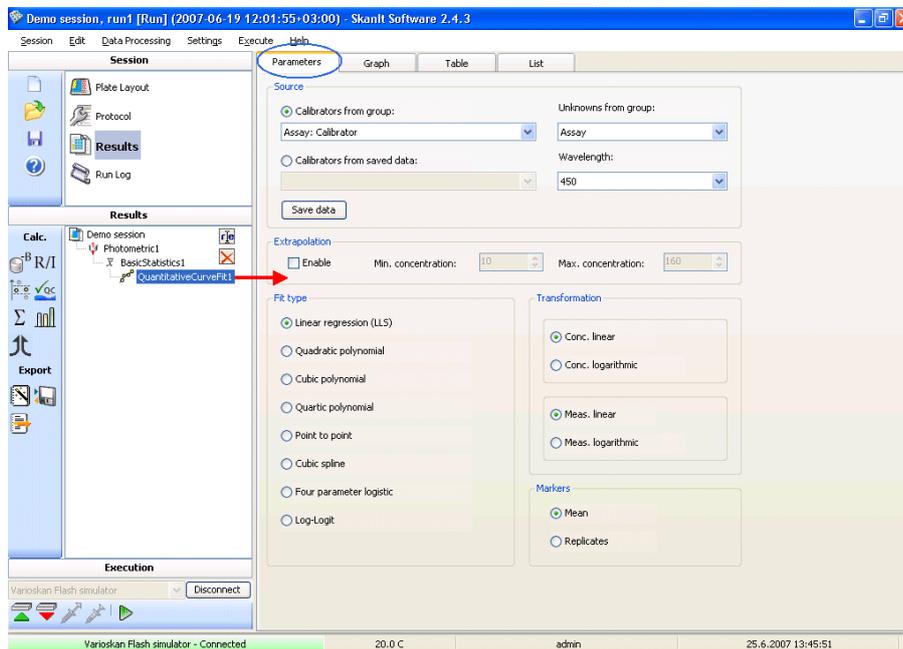


Figure 3-6. Main window for results editing - calculation parameters

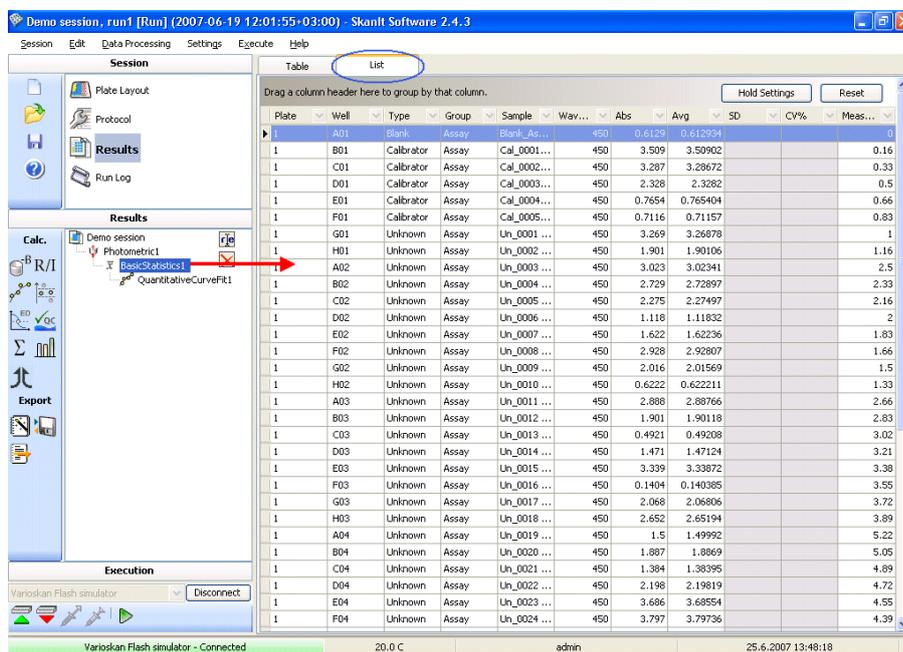


Figure 3-7. Main window for results viewing - calculation results as a list

For more information on measurement results and calculations, see Chapter 11: “Results” and Chapter 10: “Data Processing Parameters”.

Run Log viewing window

The following figure shows an example of a run log that has been opened in the main window.

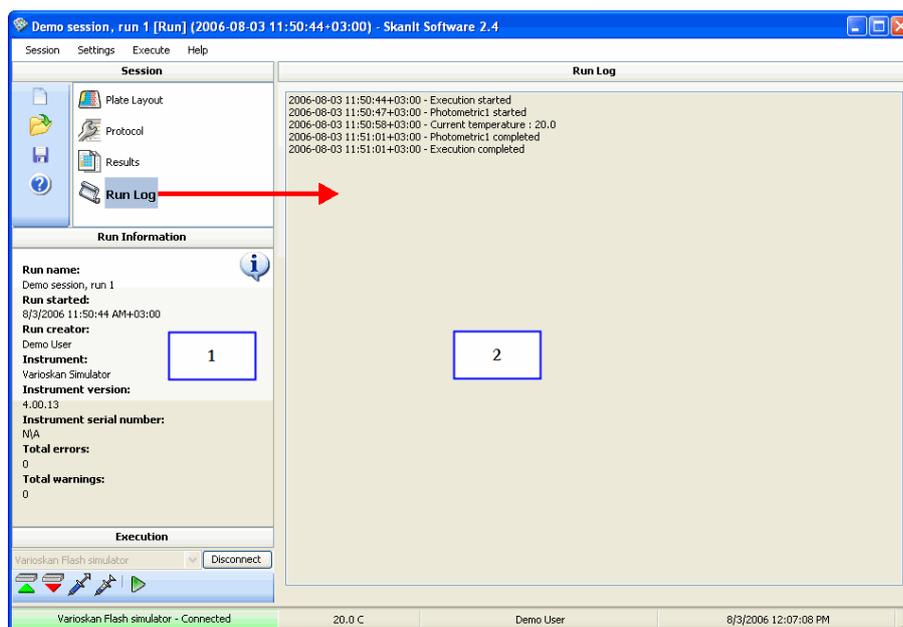


Figure 3-8. Main window for run log viewing

- **1** - Run status information
- **2** - Main panel showing the log of the executed session

Exploiting the software features fully

You can speed up your use of SkanIt Software for Varioskan by using the following features.

Lists

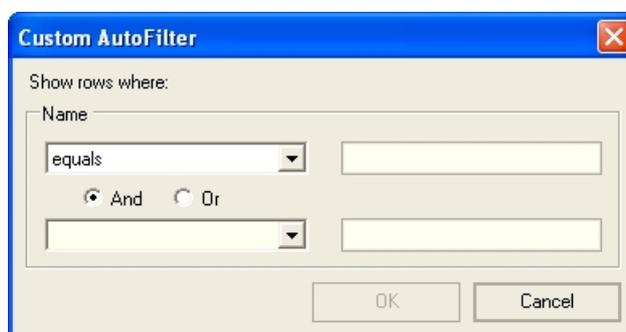
Click the list title bar to sort the entries in alphabetical order. The order can be ascending or descending. The small gray arrow shows the sorting order.

Well	Type	Group	Sample	Wavelength
A01	Blank	Assay	Blank_Assay 1/1	450
B01	Calibrator	Assay	Cal_0001 1/1	450
C01	Calibrator	Assay	Cal_0002 1/1	450

Figure 3-9. Arrow showing the sorting order in lists

Use the  arrow on the list title bar to limit the number of list items displayed according to the different filtering criteria.

If you select **Custom**, the **Custom AutoFilter** dialog opens.



Enter the filtering criteria in the text boxes on the right and select the way they are interpreted from the left.

The results list can be ordered in multiple ways. In addition to the above sorting, you can drag a column header to the darker area above the list. The rows are grouped according to the items in that column. For example, if the header “*Type*” is dragged up, the rows are grouped according to the sample types present in the plate layout.

User Interface

Exploiting the software features fully

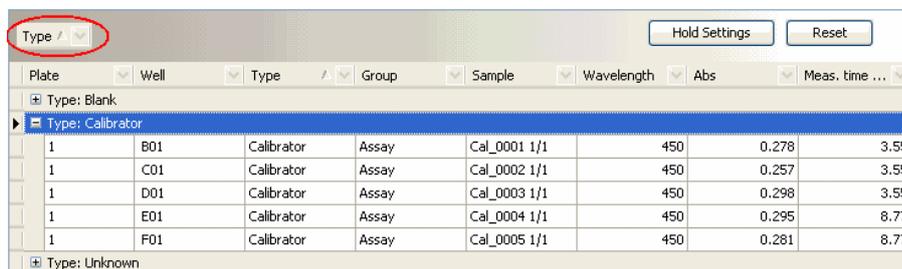


Plate	Well	Type	Group	Sample	Wavelength	Abs	Meas. time ...
Type: Blank							
Type: Calibrator							
1	B01	Calibrator	Assay	Cal_0001 1/1	450	0.278	3.55
1	C01	Calibrator	Assay	Cal_0002 1/1	450	0.257	3.55
1	D01	Calibrator	Assay	Cal_0003 1/1	450	0.298	3.55
1	E01	Calibrator	Assay	Cal_0004 1/1	450	0.295	8.77
1	F01	Calibrator	Assay	Cal_0005 1/1	450	0.281	8.77
Type: Unknown							

Click the  and  buttons on the left to toggle the view of the rows belonging to that group. Use the **Hold Settings** button to keep the grouping in the software memory. Use the **Reset** button to return the list order to the original.

Right-click menus

In many cases you can display an additional menu by right-clicking your mouse. These menus allow quick access to functions relevant to the item you are clicking and even functions that are not available from anywhere else. An example of such a function is the **Disable <item>** menu in the Results list view. You can disable a result row, replicates of the sample, a sample, a whole reading, or a certain wavelength in the raw data of the results, one at a time. The disabled item is not used in calculations. Right-click the row and then select a function from the menu. The disabled rows are marked with a strike-through line. To enable the disabled item, right-click the row and then click **Enable <item>**.

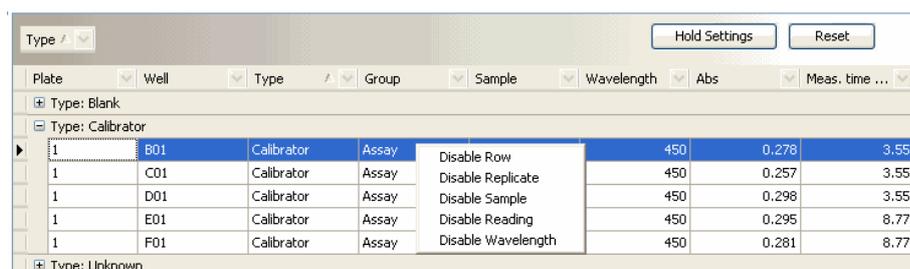


Plate	Well	Type	Group	Sample	Wavelength	Abs	Meas. time ...
Type: Blank							
Type: Calibrator							
1	B01	Calibrator	Assay	Cal_0001 1/1	450	0.278	3.55
1	C01	Calibrator	Assay	Cal_0002 1/1	450	0.257	3.55
1	D01	Calibrator	Assay	Cal_0003 1/1	450	0.298	3.55
1	E01	Calibrator	Assay	Cal_0004 1/1	450	0.295	8.77
1	F01	Calibrator	Assay	Cal_0005 1/1	450	0.281	8.77
Type: Unknown							

- Disable Row
- Disable Replicate
- Disable Sample
- Disable Reading
- Disable Wavelength

You can find right-click menus in all measurement and calculation data lists, in the plate layout view, and in the protocol and results steplists.

Entering information without using the mouse

SkanIt Software requires the use of a mouse, but you can do without one in certain cases. When you are entering information into fields (parameters, text fields, check boxes, and so on), you can use the tabulator on your keyboard instead of the mouse to move from one field to the next. You can use the arrow keys to select radio buttons, move sliders and change the

available values in fields. You can also use the arrow keys to toggle between tabs in a dialog.

Shortcuts You can select menus by pressing **Alt** and the underlined letter; for example, **Session** is selected by pressing **Alt+S**.

Some of the operations have keyboard shortcuts. These are presented in Table 3-1.

Table 3-1. Keyboard shortcuts

Operation	Keyboard shortcut
Open an existing session or run	Ctrl+O
Create a new session	Ctrl+N
Save the session	Ctrl+S
Cut the selected string to the Windows clipboard	Ctrl+X
Copy the selected string to the Windows clipboard	Ctrl+C
Paste string from the Windows clipboard	Ctrl+V
Close an open dialog	Esc
Accept and save changes made through a dialog	Enter

Some of the operations have function key shortcuts. These are presented in Table 3-2.

Table 3-2. Function key shortcuts

Operation	Function key shortcut
Open the help application	F1
Rename a protocol step	F2
Execute a session	F5
Prime the dispenser	F6
Empty the dispenser	F7
Wash the dispenser	F8
Run the plate in	F9
Run the plate out	F10
Connect to the default instrument	F11
Disconnect from the default instrument	F12

Tooltips Tooltips appear when you move the cursor over a button in a toolbar or an action panel.

User Interface

Exploiting the software features fully

When the information icon  appears next to a field or a button in a dialog, it means that a precondition related to the task must be fulfilled first. Move the cursor over the icon to view instructions.

Chapter 4

Sessions

A *session* defines the protocol and plate layout that are required for a successful execution of the session (Figure 4-1). The *protocol* consists of a plate template, step parameters, and data processing parameters. Plate template defines the plate type, for example, a 96-well plate. Step parameters define all the control steps for the instrument, for example, measurement techniques and dispensing. Data processing parameters define the calculations applied to the measurement data. The *plate layout* consists of the samples and their properties. Samples, such as blanks, controls and unknowns, are defined to specific wells on the plate. Samples can further be grouped and their properties, such as concentrations or dilutions, defined.

You can create a new session, save the current session and load an existing one. Only one session can be loaded at a time.

A single execution of a session is called a *run*. A run produces measurement data which is processed with the data processing parameters defined in the session. A session can be executed several times, each of which results in a new run and new measurement data. The runs of a session are saved under the session and they can be opened or exported separately.

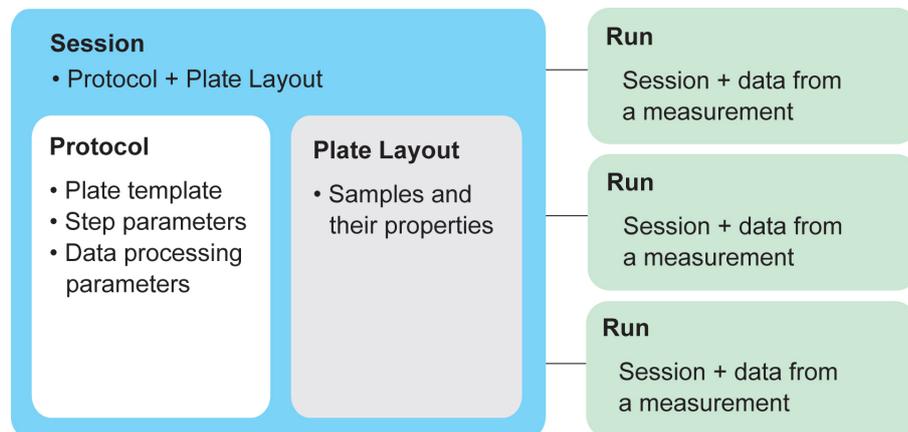


Figure 4-1. Session structure

General operational procedure

The general operational procedure with SkanIt Software is as follows:

- Launch the SkanIt Software application.
- Load an existing session or create a new one.

- Edit the session (protocol, plate layout and calculations) if necessary and save it.
- Execute the session.
- Perform calculations.
- Print and save reports.

Launching SkanIt Software

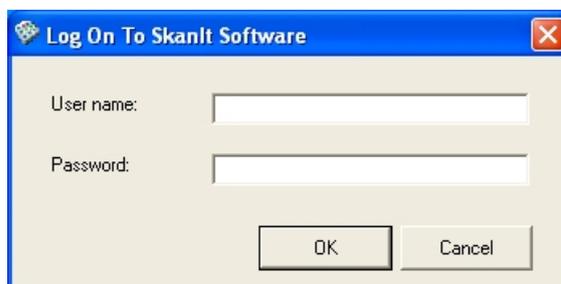
1. Launch the software from the **Start** menu:

Start > Programs > Thermo SkanIt Software > **SkanIt RE/DDE for Varioskan 2.4.3**.

Or: double-click the SkanIt RE/DDE for Varioskan 2.4.3 shortcut icon on your desktop.



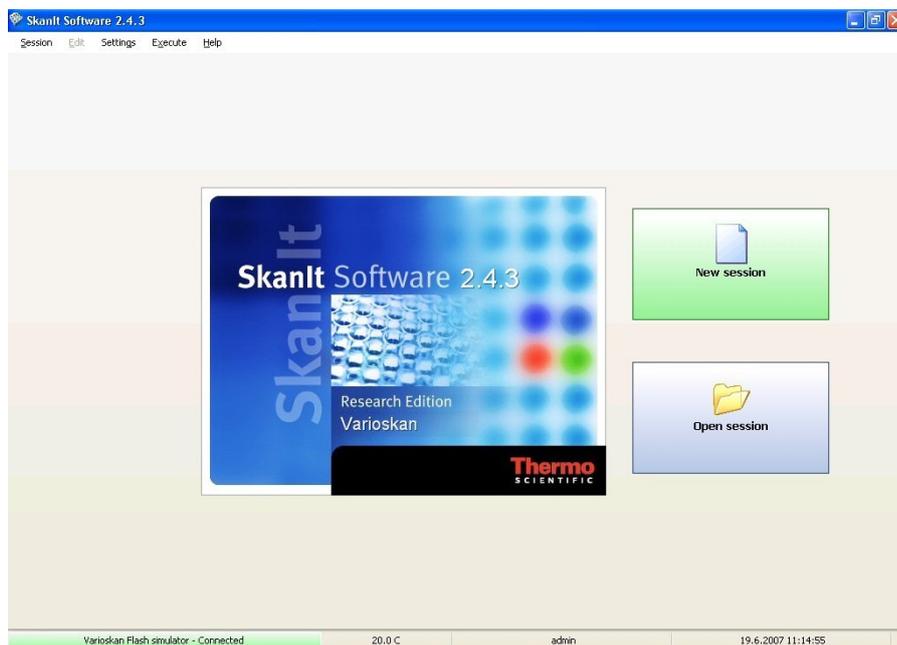
2. The **Log On To SkanIt Software** dialog appears.



3. Enter your user name and password and click **OK**. The software starts to load. The software will try to connect to the default instrument if the **Connect to default instrument at startup** setting is selected from the Settings > **Options...** menu.

When the software is ready for use, the main window opens. You can now create a new session (see “Creating a new session” on page 61) or open an existing session (see “Opening an existing session” on page 59).

If a different user has already launched the software, you have to exit the software and launch it again in order to log in with your own user name.



Managing folders in the tree view

Sessions and their runs are managed using a tree view. Sessions can be organized and stored into folders that you can easily create, move, rename or delete using the buttons available in the dialogs.

The root folder in the tree view is *SkanIt Software* and new folders can be created under it. A new session is stored into the root folder by default. The *Thermo* folder has demonstration protocols created by Thermo Fisher Scientific. It is not possible to remove the root folder or the *Thermo* folder and its demonstration protocols but you can move the protocols to another folder.

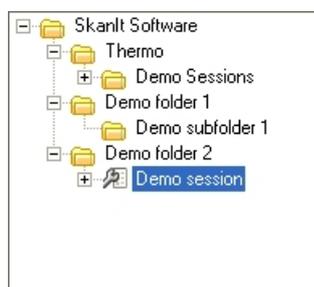


Figure 4-2. Tree view in SkanIt Software

Creating new folders

You can create new folders in the tree view when you:

- create a new session with **Session > New** in the **Definitions done** dialog.

Sessions

Managing folders in the tree view

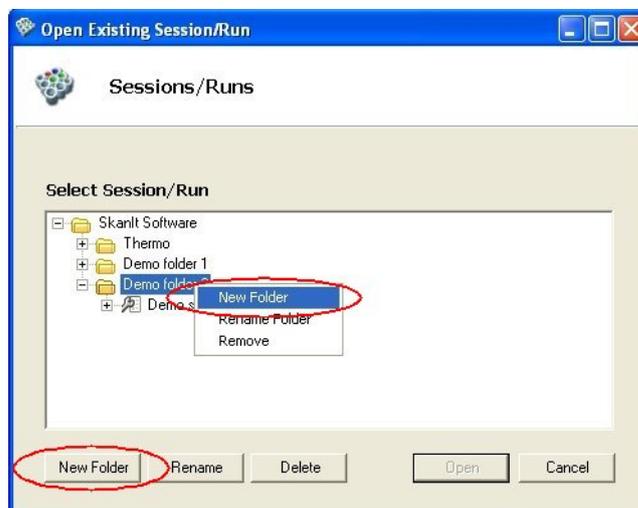
- save a session with Session > **Save As...**
- open a session or a run with Session > **Open** in the **Open Existing Session/Run** dialog.
- import session data with Session > Import/Export > **Import Data...** in the **Definitions done** dialog.

To create a new folder:

1. Select one of the steps above. The dialog where you can create a new folder is not the first appearing dialog in all cases.
2. Select the parent folder under which you want to create the new folder in the tree view.
3. Click **New Folder**.

OR

Right-click on the parent folder and select **New Folder** from the pop-up menu.



A new folder is created.

4. Give a name to the new folder (see also “Renaming folders” on page 57).

5. Now you can close the dialog and the change becomes effective. You can also continue creating, opening, saving, or importing.

Moving folders or sessions

You can move a folder or a session into another folder in the tree view when you:

- create a new session with Session > **New** in the **Definitions done** dialog.
- save a session with Session > **Save As...**
- open a session or a run with Session > **Open** in the **Open Existing Session/Run** dialog.
- import session data with Session > Import/Export > **Import Data...** in the **Definitions done** dialog.

To move a folder or session:

1. Select one of the steps above.
2. Move a subfolder and its contents by dragging and dropping it into the correct folder in the tree view.
3. Move a session and its runs by dragging and dropping the session into the correct folder in the tree view.



Note To move a single run to another folder, move its session.

Renaming folders

You can rename existing folders in the tree view when you:

- create a new session with Session > **New** in the **Definitions done** dialog.
- open a session or a run with Session > **Open** in the **Open Existing Session/Run** dialog.

To rename a folder:

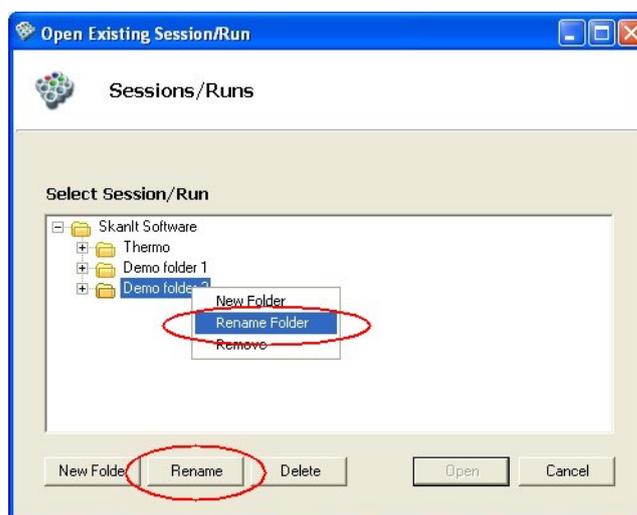
1. Select one of the steps above.
2. Select the folder you want to rename.
3. Click **Rename**.

Sessions

Managing folders in the tree view

OR

Right-click on the folder and select **Rename Folder** from the pop-up menu.



4. Give a new name to the folder.
5. Now you can close the dialog and the change becomes effective. You can also continue creating or opening.

Deleting folders

You can delete folders in the tree view when you:

- create a new session with Session > **New** in the **Definitions done** dialog.
- open a session or a run with Session > **Open** in the **Open Existing Session/Run** dialog.



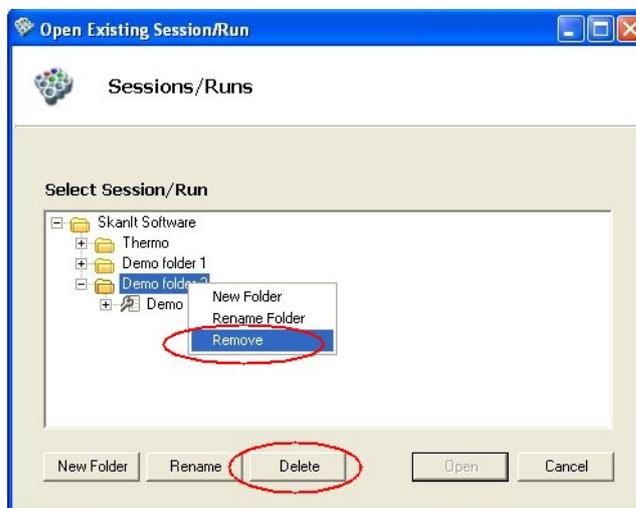
Note Before you can delete a folder you have to move the sessions in it to another folder. If there are more than one session in the folder, you cannot delete it.

To delete a folder:

1. Select one of the steps above.
2. Select a folder you want to delete.
3. Click **Delete**.

OR

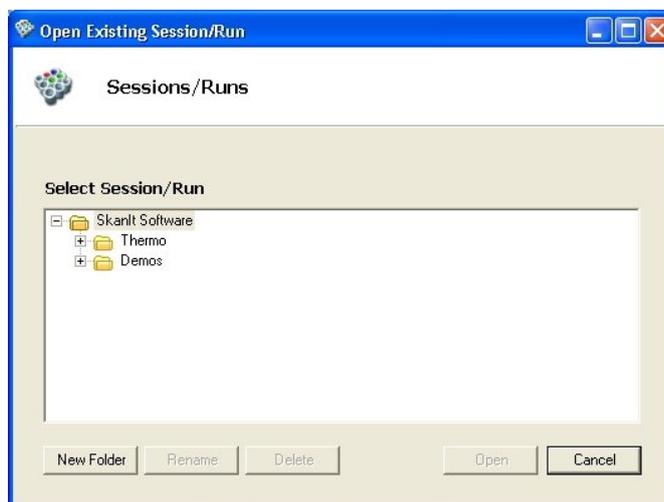
Right-click on the folder and select **Remove** from the pop-up menu.



4. Confirm the deletion by clicking **Yes**. Otherwise click **No**.

Opening an existing session

Select Session > **Open...**, click **Open session** in the main window, click the  button or press **Ctrl+O**. The **Open Existing Session/Run** dialog appears.



The **Open Existing Session/Run** dialog shows all the sessions and runs that are currently in the database in a hierarchical tree view. You can create new folders, rename folders, move sessions between folders and delete folders, sessions or runs.

Sessions

Opening an existing session

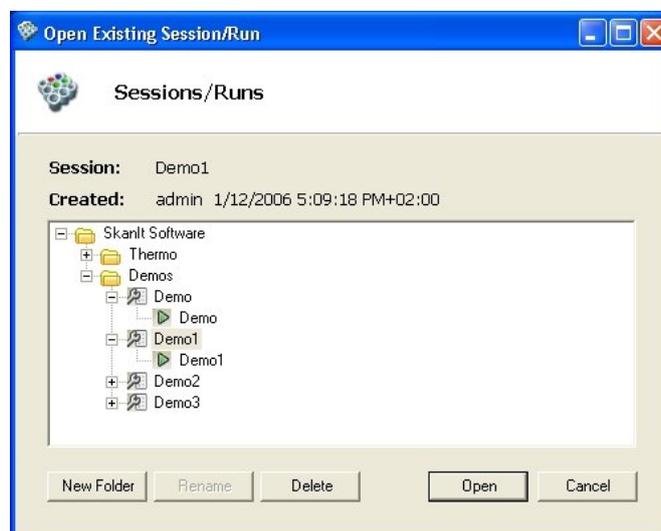
You can open a session with no measurement data or a session with a particular run (executed session) that includes the measurement data from that run.

To open a session:

1. Select the session from an appropriate folder in the tree view by clicking its name.
2. Click **Open** or double-click the session.

The selected session is now opened in the main window.

When you click a session stored in a folder, you will see the executed runs of the session expanding under its name.



To open a run with its measurement data:

1. Open the folder where the session whose run you want to open is stored.
2. Select the run under an appropriate session by clicking its name.
3. Click **Open** or double-click the run.

The selected run is now opened in the main window. You can view the session and measurement data and perform calculations.

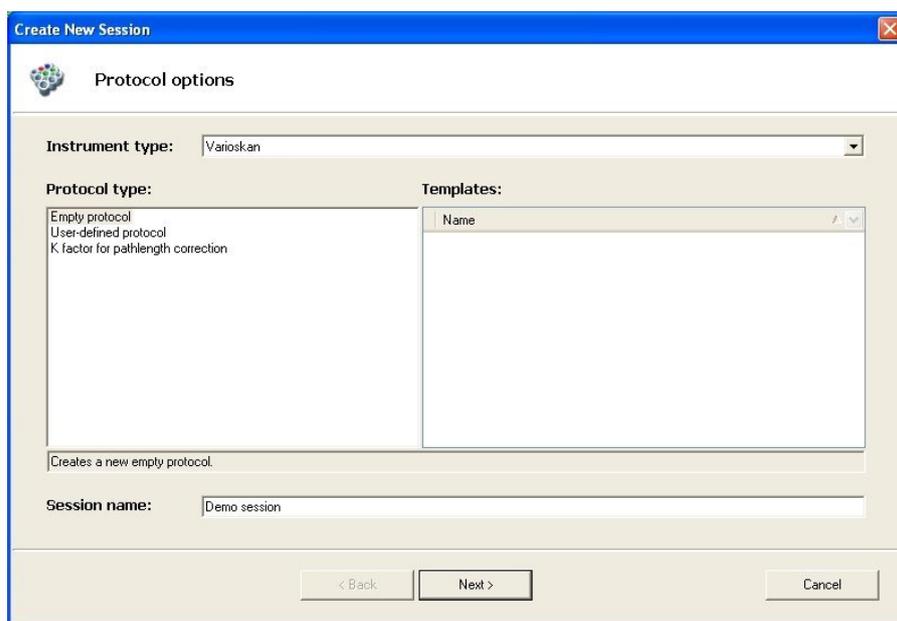
If you only open the session of an executed run, only the session information will be opened and the measurement data will not be available.



Note When a session has been executed, you can no longer edit it. If you want to make any changes to the protocol or the plate layout, save the session with a new name.

Creating a new session

1. Select **Session > New...**, click **New session** in the main window, click the  button or press **Ctrl+N**. The **Create New Session** wizard is launched.

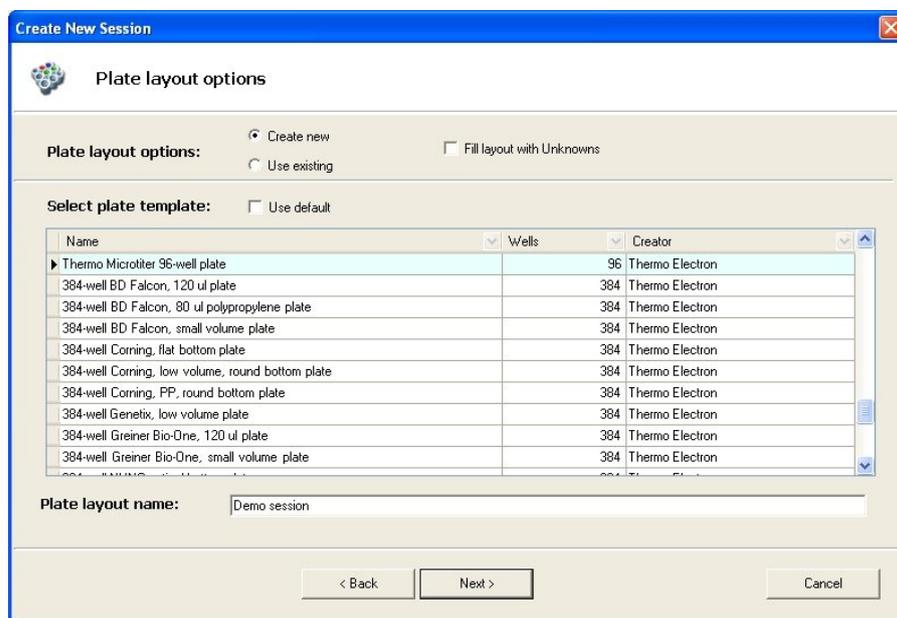


- **Instrument type** - The instrument you are using is shown in the field.

Select from the Protocol type list:

- **Empty protocol** - Start the protocol editing from a blank.
 - **User-defined protocol** - Use one of your own protocol templates as the starting point (see “Protocol template setup” on page 250). Select the template from the list on the right.
 - **K factor for pathlength correction** - Use this protocol to define the pathlength correction K-factor for the type of plate and solvent you are using (see “Editing pathlength correction K-factors” on page 247. Also see “Using pathlength correction in a measurement” on page 97 and “Pathlength correction” on page 72).
2. Give a name to the session in the *Session name* field. The **Next** button becomes available.

3. Click **Next**. The **Plate layout options** is displayed.



Choose whether you want to create a new plate layout or use an existing plate layout. Select from the Plate layout options:

- **Create new** - Create a new plate layout.
 - **Select plate template** - Select the type of plate from the list. The plate template database includes the most commonly used 6 to 1536-well microplates. If the plate is not included in the list, it can be added using the Plate Template Editor (see “Plate template settings” on page 238).
 - **Use default** - Use the default plate type. The default type is Thermo Cliniplate, 96-well plate, but it can be changed in Settings > **Plate Template...** (see “Plate template settings” on page 238).
 - **Fill layout with Unknowns** - The plate layout is automatically filled with unknown samples. Use this option, when you do not need any specific plate layout.
 - **Plate layout name** - Give a name to the new plate layout. The name of the session is automatically suggested.
- **Use existing** - Use a previously created plate layout. The list displays all plate layouts and the user names of the plate layout creators.

Select the plate layout from the list.

4. Click **Next**. The **Definitions done** is displayed showing the session name, plate layout name and plate type name.
5. Select a folder where you want to save the session in the **Select location** tree view. You can also create a new folder by selecting a parent folder in the tree view and clicking **New Folder** or by right-clicking on the parent folder.
6. Click **Finish**. A new session is created and saved to the database. The plate layout editing window opens showing the Plate Layout (see “Editing a protocol” on page 81 and “Plate layout editing window” on page 43).

Editing a session

Here is a list of tasks that have to be carried out when editing new or existing sessions. The tasks below can be performed in any order, as long as they are all checked and saved before you execute the session.

- Edit the Protocol properties (see “Editing a protocol” on page 81).
- Check that the plate layout in the session matches the actual plates used in the measurement. Create a new plate layout or edit the existing one, if necessary (see “Working with plate layouts” on page 125).
- Add, delete or edit protocol steps (see “Adding new protocol steps” on page 83, “Deleting protocol steps” on page 85 and Chapter 7: “Step Parameters”).
- Add, delete or edit calculation steps (see “Adding calculations” on page 143, “Deleting calculations” on page 145 and Chapter 10: “Data Processing Parameters”).



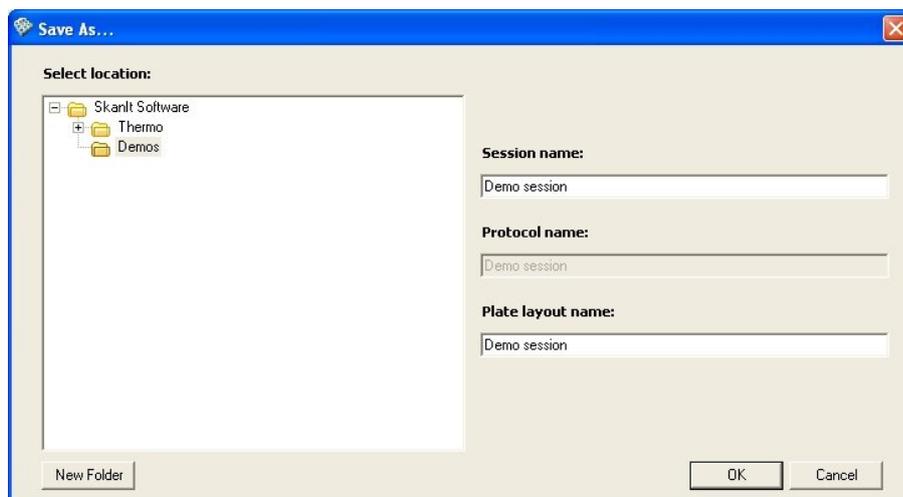
Note When a session has been executed, you can no longer edit it. You can still perform calculations on an executed session. If you want to make any changes to the protocol or the plate layout, save the session with a new name.

Saving a session

Click on the action panel or select Session > **Save (Ctrl+S)** or Session > **Save As...** from the menu. **Save** (or) saves the session with its current name and **Save As...** prompts for a new session name. The protocol and the plate layout connected to the session will automatically be given the same name. You can select an existing folder or create a new one (click **New Folder** or right-click on the parent tree) in the tree view for storing the session.

Sessions

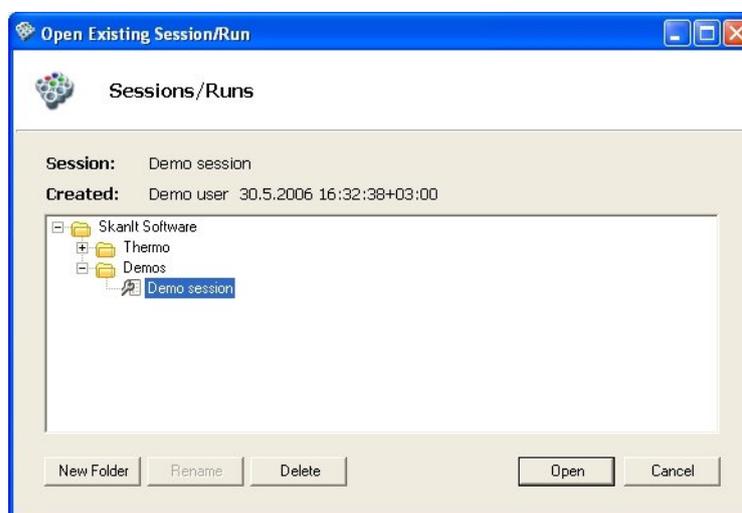
Deleting a session or a run from the database



Deleting a session or a run from the database

To delete a session or a run from the database on the PC:

1. Select Session > **Open...** or click the  button.
2. In the **Open Existing Session/Run** dialog, select the desired session or run in the tree view. If you select a session, and the protocol and plate layout are not used in any other session, they will also be deleted.



3. Click **Delete**, or right-click on the session or run and select **Remove**. If the run or session is currently open, you cannot delete it unless you first close it. Close the item and select it again for deletion.



Note If you delete a session, all of its runs are also deleted.



Note If the folder has more than one session in it, you cannot delete the folder. You have to move the sessions into another folder first or delete them if they are not needed.

Exporting and importing sessions

Sessions and runs can be exported to a separate file that can be imported to another SkanIt Software protocol database. Sessions and runs that have been exported from another instance of SkanIt Software for Varioskan 2.4.1 or 2.4.3 can be imported to the database.

Exporting a session does not export the measurement results. You must export runs to export the measurement results. Exporting a run automatically exports the session used to create the run.

The export/import function is also useful, if you want to take into use sessions or runs that are in archived or backup databases. For more information, see Appendix B: “Database maintenance” and “Database” on page 227.

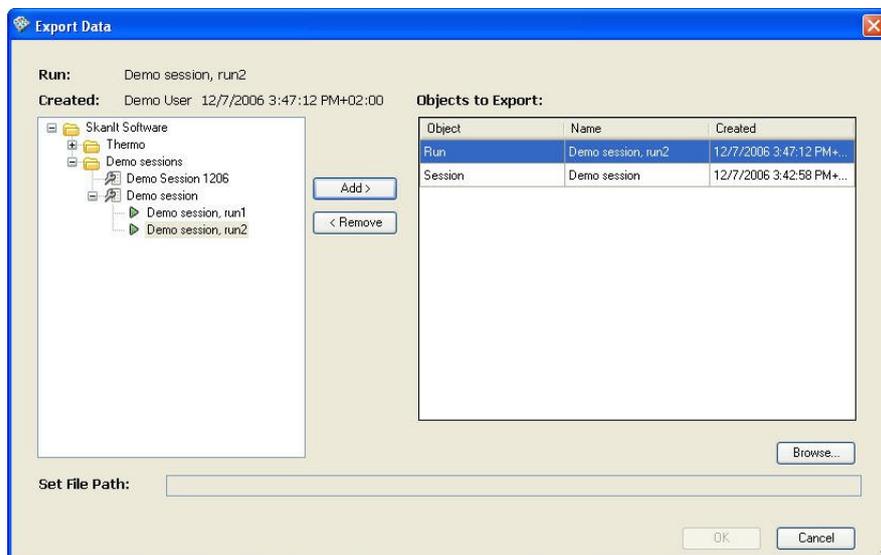
Exporting sessions or runs

1. Select Session > Import/Export > **Export Data...** The **Export Data** wizard is launched.
2. Select the sessions or runs you want to export from the correct folders in the hierarchical folder tree:
 - If you select a session, only the session and its related protocol, plate layout and plate template are exported.
 - If you select a run, the measurement results of the run are exported. The session used to create the run is also automatically exported as well as the saved standard curves.

After each selection, click the **Add>>** button. The run or session is moved to the list of objects to be exported. You can use the **<<Remove** button to remove a selected session or a run from the list.

Sessions

Exporting and importing sessions



3. Enter the file path using the **Browse...** button to open the **Save As** dialog.
4. Select the directory where the SkanIt data file (*.msd) is saved.
5. Enter a name for the file.
6. Click **Save**. The file path appears in the **Set File Path** text box. The filename is automatically appended with “_VS” to distinguish it from data exported from different instruments.
7. Click **OK**.

You will receive a message stating whether the export procedure was successful or not. Click **OK**.

Importing sessions or runs

1. Select Session > Import/Export > **Import data....** The **Import Data** wizard is launched.
2. The **Import options** is displayed.
3. Enter the file path. Use the **Browse...** button to open the **Open** dialog.
4. Select the SkanIt data file (*.msd) by browsing in the **Open** dialog and click **Open**. The file should be indicated by “_VS”. The file path appears in the **File location** text box.



Note Objects imported from SkanIt Software version 2.0 are not indicated by “_VS”

5. Click **Next**. The **Definitions done** is displayed showing the objects included in the selected database. The names of imported objects are prefixed with the text [import]. If there are already objects with the same names in the database, the objects in the database will not be overwritten, but there will be several objects with the same name.
6. Select a folder where you import the selected objects. You can also create a new folder where you can import them.
7. Click **Finish**.

You will receive a message stating whether the database updating procedure was successful or not. Click **OK**.

An imported session is shown in the folder tree with the text [import] in front of it.



If the imported session or run has dispenser information, the information is also shown even if the connected instrument has no dispensers installed.

Sessions

Exporting and importing sessions

Chapter 5

Executing a Session

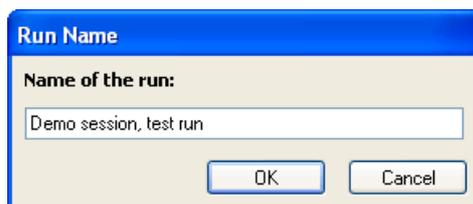
To execute a session:

1. Make sure that the instrument is connected correctly to the PC (see “Connecting the instrument to the PC through a COM port” on page 37).
2. Turn on the instrument (see the *Thermo Scientific Varioskan User Manual*, Cat. no. 15018850).
3. Start up the software (see “Launching SkanIt Software” on page 54).
4. Open the session (see “Opening an existing session” on page 59). If the session is already open, make sure that the session has been saved.
5. Insert the plate into the plate tray (see the *Thermo Scientific Varioskan User Manual*).

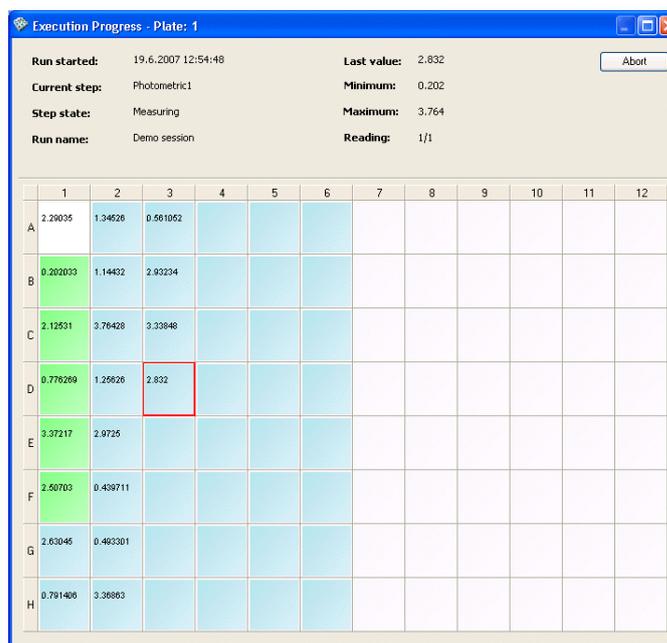


Caution Make sure you do not dispense into the instrument by mistake. Ensure that:

- A correct microplate has been inserted.
 - The microplate or tip priming vessel is not too full.
 - The recommended *Check plate* is ticked (Settings > **Options...**).
6. Prime the tubing if you have a dispensing step in the protocol (see “Priming dispensers” on page 75).
 7. Click  on the Execution action panel or select Execute > **Session**.
 8. Give a name to the run and click **OK**.



The **Execution Progress** dialog is launched and the measurement starts.



The **Execution Progress** dialog shows the plate layout to be measured.

The dialog displays measurement information while measuring. The well that is being measured is framed in red. The measurement values are shown in each well. In multipoint measurements, the last shown value is a mean value of the measured points in a well. In spectrum scanning, kinetic and TRF decay measurements, the measurement curves are shown in each well instead of values.

- **Run started** - Shows when the execution was started (the date and time).
- **Current step** - Shows the step being executed.
- **Step state** - Shows the state of the execution.
- **Run name** - Shows the name of the run you gave.
- **Last value** - Displays the value of the last read measurement.
- **Minimum** - Displays the minimum value that has been measured up to the point.
- **Maximum** - Displays the maximum value that has been measured up to the point.
- **Reading** - Shows the ordinal of the execution loop (kinetic loop or well loop).

- **Abort** - If you want to abort the execution in the middle of the measurement, click **Abort**.

When the measurement is finished, the **Execution Progress** dialog is closed and the software will load the results to the Results view, which shows the measurement results in the plate layout format (*Table*). The measurement value is shown for each well in end-point measurements and the curve in kinetic and spectrum scanning measurements.

The screenshot shows the 'Table' view of the software. The table has 12 columns representing wells. The data is organized into groups (A-H) and rows (1-4). Each cell contains a well ID (e.g., 'Bank_Assay_Uh_0003 1/1') and a numerical value (e.g., '0.613'). The interface includes a menu bar, a toolbar, and a sidebar with options like 'Plate Layout', 'Protocol', 'Results', and 'Run Log'.

Group name	Sample type	Wavelength [nm]	Plate	Zoom
Bank_Assay_Uh_0003 1/1	Uh_0011 1/1	Uh_0019 1/1	Uh_0027 1/1	Uh_0035 1/1
0.613	1.1	1.1	1.1	1.1
2.823	2.888	1.650	2.388	3.979
Ch_0001 1/1	Uh_0004 1/1	Uh_0012 1/1	Uh_0020 1/1	Uh_0028 1/1
3.509	2.729	1.861	1.887	1.207
0.110				
Ch_0002 1/1	Uh_0005 1/1	Uh_0013 1/1	Uh_0021 1/1	Uh_0029 1/1
3.287	2.275	0.482	1.584	1.824
3.981				
Ch_0003 1/1	Uh_0006 1/1	Uh_0014 1/1	Uh_0022 1/1	Uh_0030 1/1
4.233	1.118	1.471	1.1	1.1
1.888	2.004			
Ch_0004 1/1	Uh_0007 1/1	Uh_0015 1/1	Uh_0023 1/1	Uh_0031 1/1
8.785	1.822	2.329	1.685	2.822
1.591				
Ch_0005 1/1	Uh_0008 1/1	Uh_0016 1/1	Uh_0024 1/1	Uh_0032 1/1
3.802	1.1	1.1	1.1	1.1
0.712	2.828	0.140	2.787	1.951
3.288				
Uh_0001 1/1	Uh_0009 1/1	Uh_0017 1/1	Uh_0025 1/1	Uh_0033 1/1
1.1	1.1	1.1	1.1	1.1
2.289	2.216	2.080	0.419	2.957
2.945				
Uh_0002 1/1	Uh_0010 1/1	Uh_0018 1/1	Uh_0026 1/1	Uh_0034 1/1
1.1	1.1	1.1	1.1	1.1
1.481	0.822	2.952	1.795	2.289
0.784				

You can also view the results in a list format by clicking the **List** tab.

The measurement and calculation data is automatically saved to the database each time the session is run.

Click the calculation name in the **Results** steplist on the left to view the calculation results. For more information on the different ways you can view and manipulate measurement and calculation results, see Chapter 11: “Results”.



Note When a session has been executed, you can no longer edit it. You can only make changes to the calculations of the run that is currently open and these calculation results can be saved. However, when the session is run again, the new run will have the original calculation parameters. If you want to change the calculations permanently, save the session with a new name.

9. If you have more than one plate in the plate layout, the software prompts you to insert the subsequent plates into the instrument after measuring the previous plate.

When you have inserted the plate, click **OK**. The measurement starts.

10. If you have a dispensing step in your protocol, you can empty the dispenser tubing after measuring (see “Emptying dispensers” on page 76).

Pathlength correction

When a microplate is used to contain the samples, the beam traverses the well from bottom to top. The pathlength is defined by the volume of liquid that is added to each well. The longer the pathlength is, the greater the absorbance will be. Therefore, the absorbance in a 1 cm cell (cuvette) will be higher than that taken in a microwell. The pathlength correction factor (K-Factor), when applied, accounts for these differences and allows for samples measured in a microplate to be directly comparable to the same samples measured in cuvettes.

The pathlength of a well can be measured using an absorbance measurement of the liquid in each well at a wavelength where the solvent absorbs and the photometric dye does not. The absorbance at such a wavelength is used to correct the actual measured absorbance at the analytical wavelength for the variation in well volume. All results of the correction calculation are calculated for a 1 cm pathlength so that comparisons can be made to cuvette data.

Photometric samples are commonly in a water-based buffer. Therefore, the absorbance of water at a far infrared wavelength can be used for pathlength correction. In SkanIt Software, the measurement is usually done with wavelengths at 975 nm and 900 nm. The 975 nm measurement is used for the calculation and the 900 nm measurement is used as a background value for the 975 nm measurement. See Figure 5-1. This absorption band can be used to calculate the pathlength of the aqueous solution being measured in a microplate and then used to normalize the data.

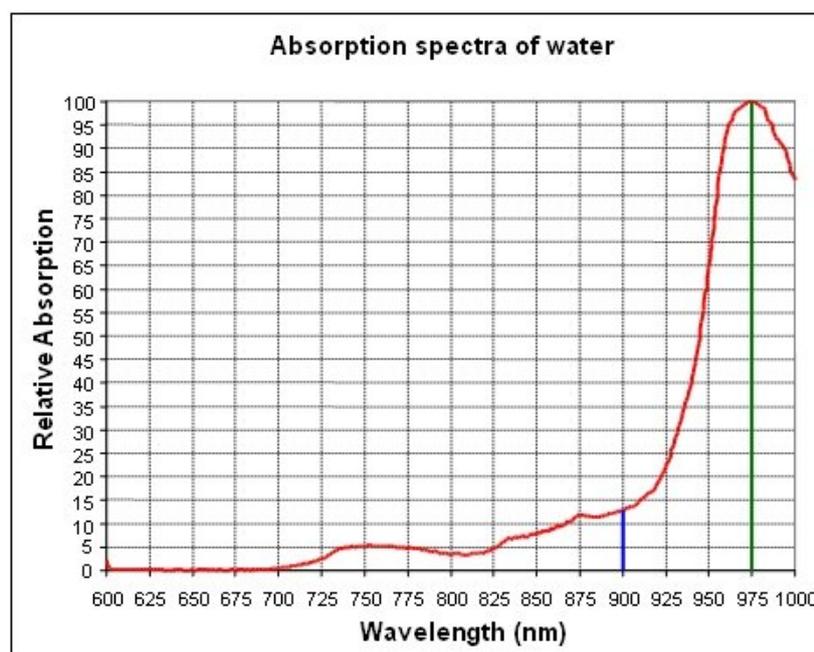


Figure 5-1. Absorption spectra of water

The pathlength correction value (K-Factor) is different for different solvents and should be determined separately for each solvent used.



Note The pathlength correction is dependent on the material used to manufacture the plate, and should be determined separately for each plate type.



Note The pathlength correction factor can only be used with 96- and 384-well plates.

Measuring new pathlength correction K-factors

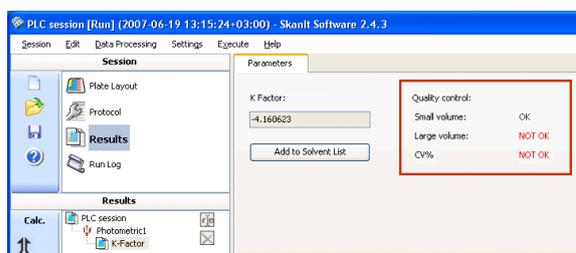
When you use a plate type and/or a solvent for the first time to measure absorbances (photometric measurements) and you are going to perform a pathlength correction, first define the pathlength correction K-factor using a specially defined protocol in the software.



Note The pathlength correction is applicable only to water-based solutions. There must be an aqueous solution in the well when you measure pathlength correction.

1. Create a new session (Session > **New...** or the  button). Select the *K factor for pathlength correction* protocol type and the *Pathlength Correction* template and give a name to the session. Click **Next**.
2. Select the correct plate type from the *Plate layout options* list and click **Next**. Select a folder where the session is saved. Click **Finish**. A ready protocol opens in the main window.

3. Select **Protocol** and follow the instructions given in the *Protocol description* field in **Protocol Properties**. When you select **Plate Layout**, the *Description* field at the bottom of the window provides information on how to fill the wells of the plate with the desired solvent.
4. Make sure the instrument is connected to the software and save the session. Click the  button. Insert the plate into the instrument and click the  button. Give a name to the run in the **Run Name** dialog and click **OK**. The **Execution Progress** window is launched and the measurement starts.
5. After the measurement the result of the K-factor calculation is shown.



Validity checks:

The validity of the measurement data is checked automatically according to the following quality control rules. Select **K-Factor** in the Results tree. If the check passed, you see *OK* on the main window. If the check did not pass, you see *NOT OK*.

- *Small volume*: $\Delta A_{v1} > 0.030$
 - where ΔA_{v1} is the absorbance difference between 975 nm and 900 nm with volume 1
 - *Large volume*: $\Delta A_{v2} = 2 * \Delta A_{v1} \pm 10\%$
 - where ΔA_{v2} is the absorbance difference between 975 nm and 900 nm with volume 2
 - *CV%*: After the session is run, four sets of data are created: vol1 with 900 nm, vol2 with 900 nm, vol1 with 975 nm, and vol2 with 975 nm. The calculated coefficient of variation (CV%) of each set of data must be less than 10%.
6. Click the **Add to Solvent List** button. The **Add New Pathlength Correction Factor** dialog is shown.

If the measurement data is not within the acceptable quality control range, you can still choose to accept and save the K-factor.



Caution Measurements made with such K-factors will cause unreliable PLC measurement results.

7. Give a name to the solvent and add an optional description for the solvent. The K-factor is automatically entered with a 3-decimal accuracy. Click **OK**. The pathlength correction factor is saved and added to the **Pathlength Correction K-Factors** dialog that is launched when the pathlength correction is selected in a photometric measurement (see “Using pathlength correction in a measurement” on page 97).

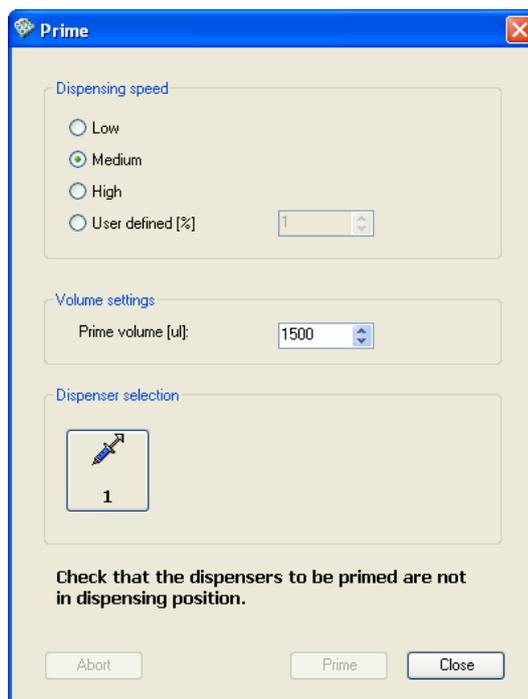
You can perform calculations with the pathlength correction measurement data, if you wish. First add a *User Equation* calculation step and then any other calculation step, such as *Quality Control*.

Priming dispensers



Caution You must always prime into a waste vessel by removing the dispensing head from the dispensing head positioner. Hold on to the dispensing head while priming.

Select Execute > **Prime...** or click the  button.

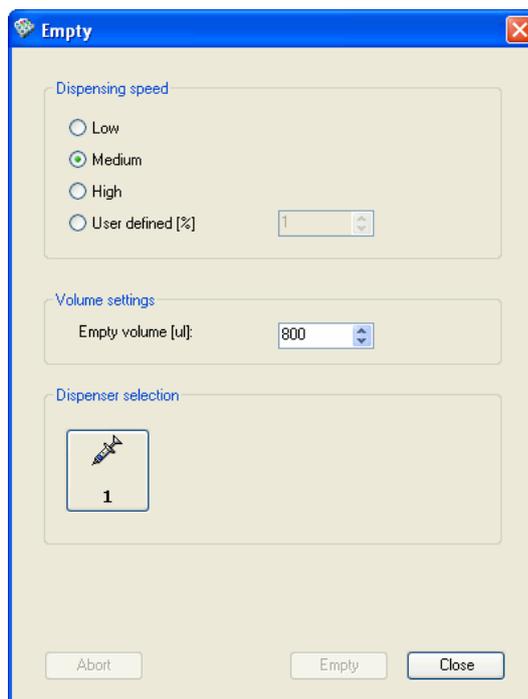


1. Select a dispensing speed to test and find out the optimal dispensing speed.
2. Select the priming volume in increments of 100 μl . The volume range is 100 to 9900 μl . The default is 1500 μl .
3. Select the installed dispenser you want to prime.
4. Click **Prime**. The instrument will prime the dispenser tubing.
5. If you want to stop the priming, click **Abort**.
6. When the priming is finished, click **Close**.

You can also prime the tubing using the Prime/Empty control switch located on top of the dispenser (see the *Thermo Scientific Varioskan User Manual*).

Emptying dispensers

You can empty the dispenser tubing to prevent wasting expensive reagents. Select Execute > **Empty...** or click the  button.

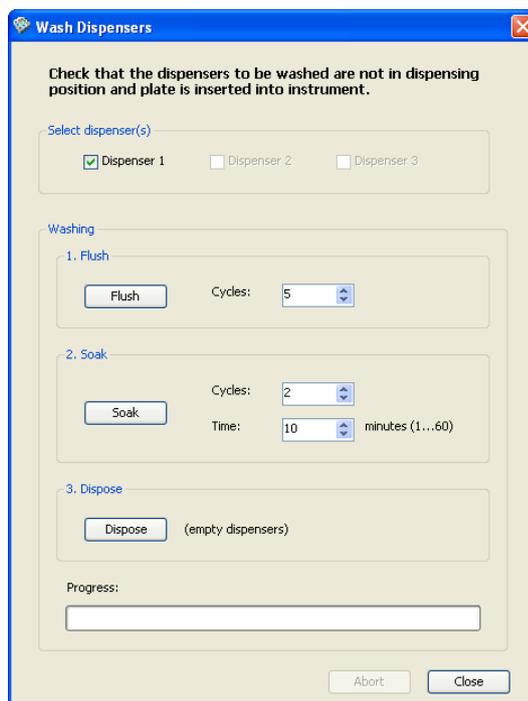


1. Estimate the volume of solution remaining in the tubing and select the volume in μl that you want to empty.
2. Select the dispenser whose tubing to empty.
3. Click **Empty**.

For more information, see the instrument manual.

Washing dispensers

Select Execute > **Wash...** The **Wash Dispenser** dialog opens. For more information, see “Example of dispenser washing” on page 78.



The **Wash Dispensers** dialog has the following functions:

- **Select Dispenser(s)** - The Varioskan is equipped only with one dispenser.
- **Washing** - Select the washing step and its settings. All the selected dispensers perform the step simultaneously.
 1. **Flush** - Select the number of cycles (times) the dispenser tubing is flushed with liquid. Each cycle flushes the full volume of a syringe, that is, 1 or 5 ml of liquid, through the tubing. Click **Flush**.
 2. **Soak** - Select the number of cycles (times) the dispenser tubing is soaked with the washing liquid and the time a cycle lasts. The time range is 1 to 60 minutes with a 1 minute increment. Click **Soak**. An alert sound is played when soaking is finished.
 3. **Dispose** - Empty the dispenser tubing of the washing liquid by clicking **Dispose**.
- **Progress** - Shows the status of the current step.

Example of dispenser washing

This example clarifies the dispenser washing steps when you use the **Wash Dispenser** dialog.

Before you start, check that:

- the dispensing head is not in a dispensing position.
 - the plate is inserted into the instrument.
 - the following liquids are available:
 - washing liquid 1 (0.1 M NaOH solution)
 - distilled water
 - washing liquid 2 (0.1 M HCl)
1. Place the dispensing head into a waste vessel.
 2. Place the aspirate tubing into washing liquid 1.
 3. Flush the dispenser tubing. Select 1 cycle and click **Flush**. After the flushing, the whole syringe is filled with the washing liquid.
 4. Soak the syringe for 1 to 60 minutes. Select the soaking time (default is 10 minutes), the cycles and click **Soak**.

The syringe is filled with the washing liquid for the defined time.

5. Place the aspirate tubing into distilled water.
6. Flush the dispenser syringe and tubing between 1 to 20 times. Select the required 1 to 20 cycles and click **Flush**.

The syringe and tubing are flushed with distilled water into the waste vessel.

7. Place the aspirate tubing into washing liquid 2.
8. Flush the dispenser tubing. Select 1 cycle and click **Flush**. After the flushing, the whole syringe is filled with the washing liquid.
9. Soak the syringe for 1 to 60 minutes. Select the soaking time (default is 10 minutes), the cycles and click **Soak**.

The syringe is filled with the washing liquid for the defined time.

10. Place the aspirate tubing into distilled water.
11. Flush the dispenser syringe and tubing between 1 to 30 times. Select the required 1 to 30 cycles and click **Flush**.

Executing a Session

Washing dispensers

12. Empty the dispenser syringe and tubing of distilled water. Click **Dispose**.

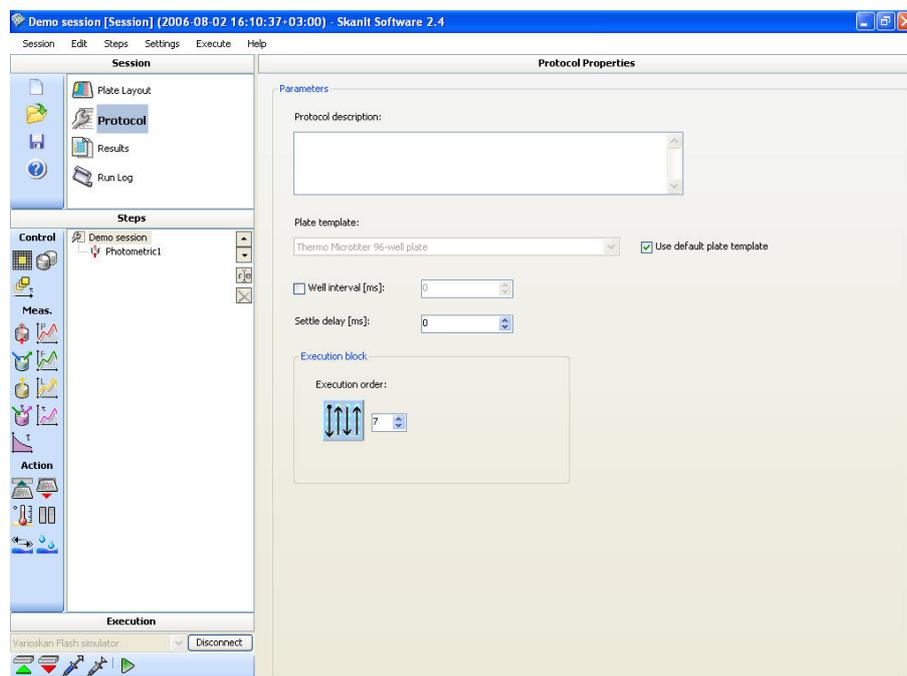
Chapter 6

Protocols

A protocol contains all the information required by the instrument to perform the desired functions. This information includes the plate type and the measurement order, so that the instrument can determine the actual movements of the plate and the reader. The protocol steps tell the instrument which functions are required (for example, the measurements, heating, dispensing or shaking steps) and in which order they should be performed.

Editing a protocol

When you have created a new session, a new blank protocol appears showing the *Plate Layout*. The plate layout can be defined before or after defining the protocol properties (see Chapter 8: “Plate Layouts”). Then select **Protocol** from the *Session* action panel. A new blank protocol appears showing the *Protocol Properties*. These properties should be defined before proceeding to add more protocol steps.



The *Protocol Properties* includes the following parameters:

- **Protocol description** - Text description of the protocol (optional).
- **Plate template** - Select the plate template used. The plate type selected in the **Create New Session** wizard (see “Creating a new session” on page 61) is suggested. You can only change the plate type to another

plate of the same size; for example, a 96-well plate can only be changed into another 96-well plate.



Note If the protocol has multipoint measurements, changing the plate template clears all the multipoint selections. You must confirm the change first.

- **Use default plate type** - Tick the check box to use the default plate type. The default is set in Settings > **Plate Template** (see “Plate template settings” on page 238).
- **Well interval** - Define the time that elapses between the starting times of two consecutive wells. If selected, the value must be at least 10 ms greater than the *Settle delay* value.
- **Settle delay [ms]** - Define the time delay after the plate has moved to a new reading position before the instrument performs the following reading. The range is 1 to 10,000 ms (10 seconds) and the default value 0 ms.

When liquid in the wells is exposed to acceleration or deceleration, surface resonance waves occur in the wells. As the plate moves fast from one well and stops at the next well prior to a measurement, the surface waves start propagating in the liquid. The propagation continues for a certain time depending on the liquid and the well size.

The surface waves may affect the results and thus it is necessary to ensure that certain actions are taken to optimize the measurement. The surface wave effect can be seen as noise in the signal in certain cases. There are two methods to minimize surface wave effects when they occur:

1. Use detergent in the well, if possible.
 2. Increase the Settle delay time and wait until the surface waves have been sufficiently reduced. For example, in water-based samples the surface waves may keep propagating up to:
 - 5 seconds in a 6-well plate.
 - 300 ms in a 96-well plate.
 - < 20 ms in a 384-well plate.
- **Execution order** - Select the direction in which the steps are executed on the plate. It also sets the direction in which the points inside a well are measured in a multipoint measurement. The black dot designates the starting point and the arrows show the execution order (Figure 6-1). Use the up and down arrows to switch between the different options.

Use an execution order that is best aligned to the defined measurement area(s).

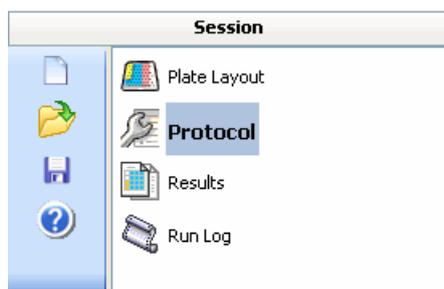


Figure 6-1. Available execution order types

Adding new protocol steps

Once a session has been created (see “Creating a new session” on page 61) or opened (see “Opening an existing session” on page 59), new steps can be added to the protocol while it has not been run. Once the session has been run, if you need to edit it further, you have to first save it with a new name.

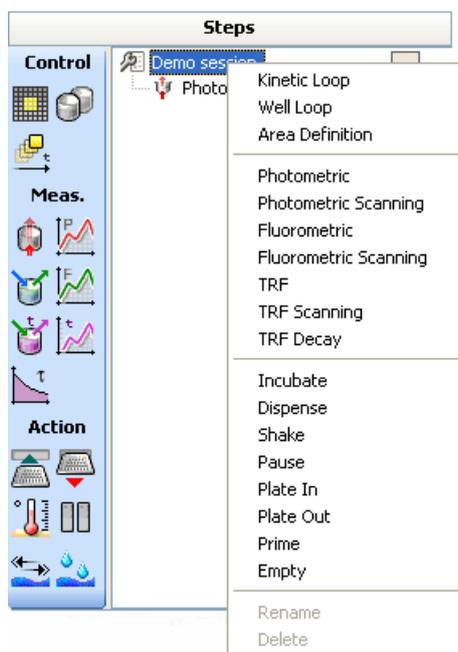
Select **Protocol** from the **Session** action panel.



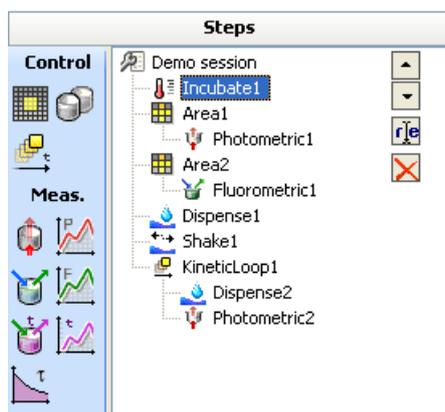
Add steps to the **Steps** tree in the order in which you want them to be executed. To add a step, go to the **Steps** menu and select the type of step you wish to add, click the desired step button on the action panel (see “Steps action panel” on page 264) or right-click in the **Steps** tree and select the step from the menu.

Protocols

Editing a protocol



New steps appear after the step selected by clicking or, if no step has been selected by clicking, after the last step in the step tree. You can move steps up and down in the tree structure using the arrows in the top-right corner of the action panel. You can rename steps using the  button and delete them using the  button.



In the example above, the *Incubate1* step is performed first, then the *Photometric1* measurement is performed only for the measurement area defined by the area definition step *Area1* followed by the *Fluorometric1* measurement performed for *Area2*. The *Dispense1* and *Shake1* steps are applied next for the entire layout, followed by *KineticLoop1*, which includes the *Dispense2* and *Photometric2* steps.

For more information on protocol steps, see Chapter 7: “Step Parameters”.

Deleting protocol steps

1. Select (click) the protocol step you wish to delete.

If you delete a step that has child steps, such as the *Area1* step in the example above, the child steps (*Photometric1* in the example) are deleted too.

2. Select Edit > **Delete**, or right-click and select **Delete**, or click the  button.



Caution Before selecting Edit > **Delete**, always check that you have selected the item you wish to delete.

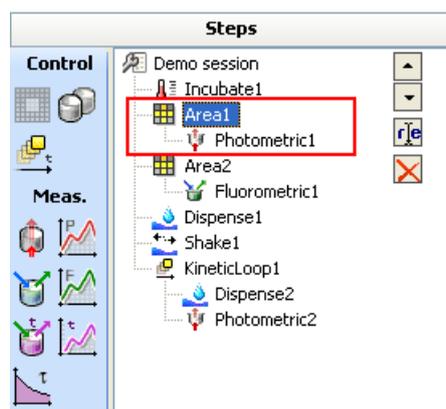
3. Confirm the step deletion by clicking **OK**.



Note If the session has been executed, you can no longer edit it. If you want to delete steps from the protocol, save the session with a new name.

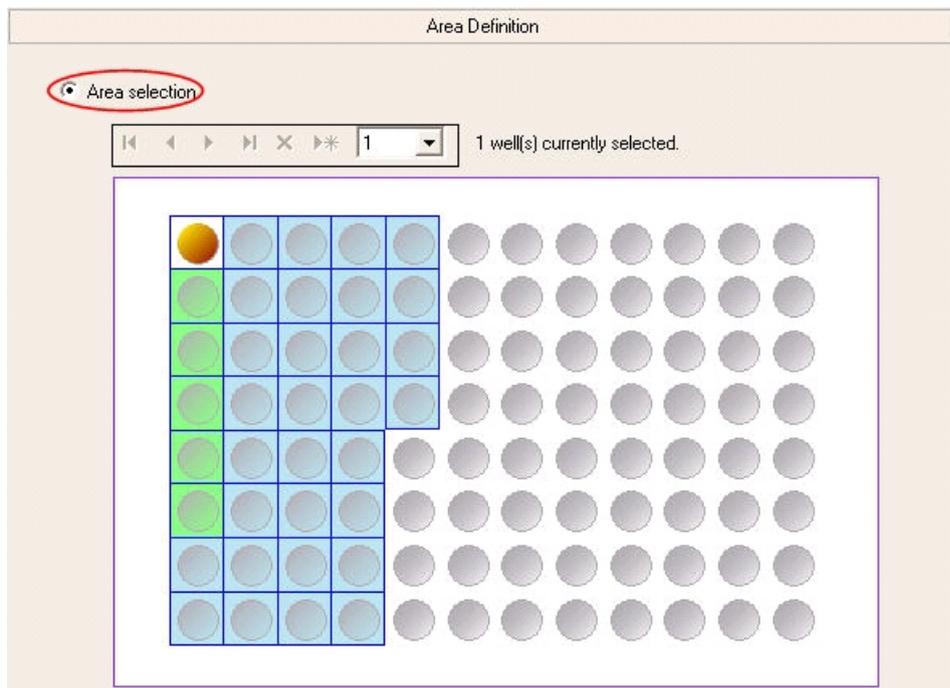
Area definition

The *Area Definition* step is used to define the operation area for the steps below it in the **Steps** step tree.

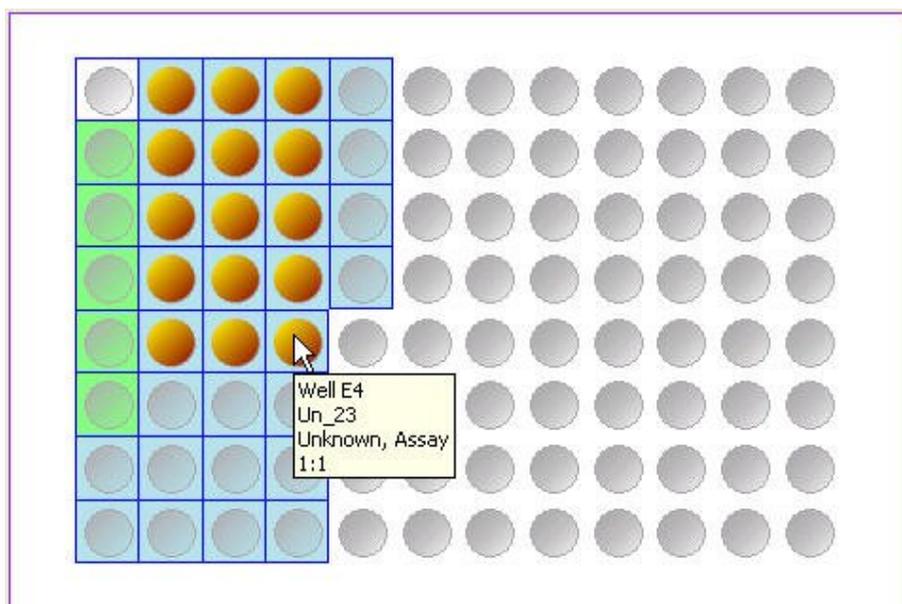


If there is no explicit area definition, steps apply by default to all the samples in the layout. For steps that are not inside any area definition, the operation area consists of all the wells that contain blanks, unknowns, controls and/or calibrators.

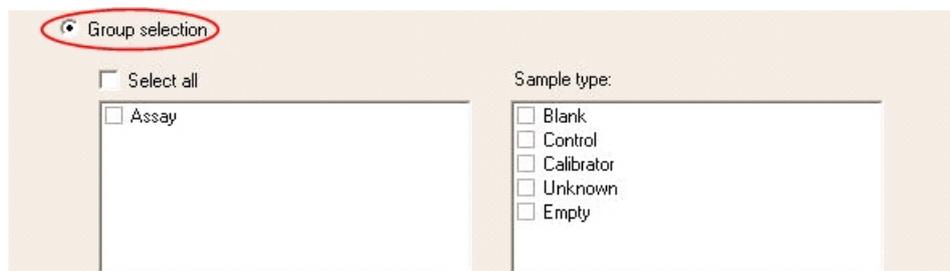
You can select according to an area or a group.



In **Area selection**, select the operation area by painting it. The big orange point shows the selected well(s). Paint a rectangular area by keeping the mouse button down while moving the mouse. You can add several rectangular areas by holding down the **Ctrl** key while dragging. The selected areas should be continuous and the execution order of the step should be aligned to the areas.



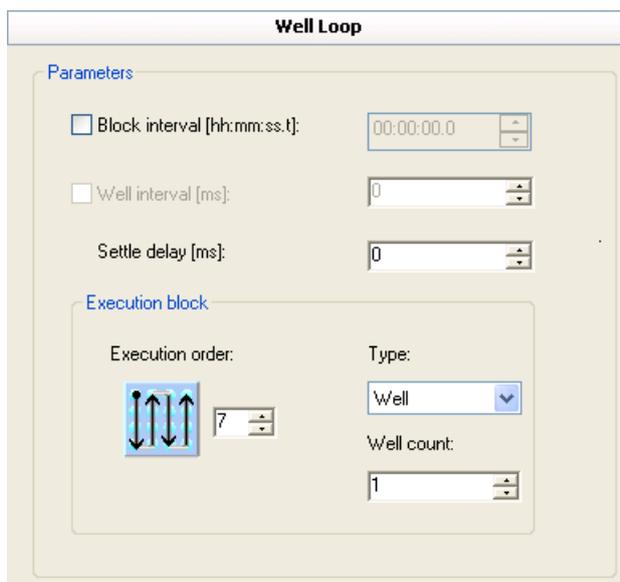
In **Group selection**, select the group and/or the sample type(s) by ticking the appropriate check box(es).



Well loops

You can use a well loop to join together a selection of protocol steps and to redefine the protocol parameters for them. The steps are run in the order specified in the **Steps** step tree in a loop until all the wells are measured. You can specify the number of wells in the loop or you can select the whole plate.

In addition, you can control the execution order of kinetic measurements, kinetic dispense and measurement steps, and dispense and measurement steps.



- **Block interval [hh:mm:ss]** - Enter the duration of the well loop execution. If no block interval is defined, the instrument performs consequent loops with no delay in between.
- **Well interval [ms]** - Enter the time that elapses between the starting times of two consecutive wells. For example, to keep the time between the measurement after dispensing and the next measurement constant in a kinetic loop. If selected, the value must be at least 10 ms greater than the *Settle delay* value. This option is not available when the execution block type is set to *Well* and the well count is 1 (see below).

- **Settle delay [ms]** - Define the time delay before the instrument performs the following reading after the plate has moved to a new reading position. The range is 0 to 10,000 ms; the default value is 0 ms. For more information, see “Editing a protocol” on page 81.
- **Execution block** - Define the general order in which the steps are applied to the individual wells on the plate. See the example below.

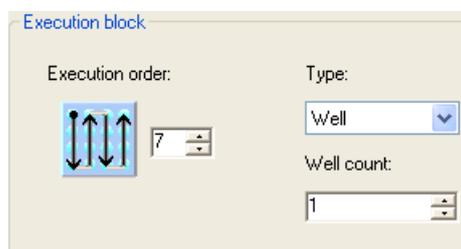


Note Spectral scanning and kinetic loop with dispense and measurement steps are always performed for one well at a time, regardless of the selections in the **Execution block**.

- **Type** - Select the group for which each step is executed before moving to the next group. The available options are *Plate* and *Well*. The default selection is *Plate*.

When *Plate* is selected, the first step is performed for the whole plate, then the second step for the whole plate and so on.

When *Well* is selected, all steps under the well loop are performed for the number of wells defined. Use the **Well count** parameter to define how many wells you want to measure at a time.



- **Execution order** - Select the direction in which the steps are executed on the plate. It also sets the direction in which the points inside a well are measured in a multipoint measurement. The black dot designates the starting point and the arrows show the execution order (Figure 6-1). Use the up and down arrows to switch between the different options. Use an execution order that is aligned to the defined measurement area(s).

Example of a well loop in a kinetic dispense and fluorometric measurement

The following 24-well plate will be measured with a kinetic dispense and fluorometric measurement with dispense in reading 1:

A1	A2	A3	A4	A5	A6
B1	B2	B3	B4	B5	B6
C1	C2	C3	C4	C5	C6
D1	D2	D3	D4	D5	D6

The following settings are selected:

- **Type** - Well
- **Well count** - 1
-

Execution order - 

The instrument performs the execution as follows:

1. The instrument dispenses into well A1 and measures A1 with the fluorometric parameters as many times as defined in the kinetic loop.
2. The instrument drives the plate to position B1.
3. The instrument dispenses into well B1. Well B1 is measured with the fluorometric parameters as many times as defined in the kinetic loop.
4. The instrument drives the plate to position C1.
5. The well loop is thus executed until all the wells are measured.

When the setting **Type** is changed from *Well* to *Plate*, the execution order is as above. However, if only dispense and measurement steps are used in the well loop without the kinetic loop, the execution order is as follows:

1. All the wells are dispensed (in the order A1, B1, C1, D1, D2, C2, B2, ..., B6, A6).
2. The instrument returns the plate to the original position.
3. Well A1 is measured with the fluorometric parameters.
4. The instrument drives the plate to position B1 and measures B1.
5. The well loop continues until all the wells are measured once.
6. The instrument returns to position A1 and measures A1 again, then well B1, and so on.
7. The wells are thus measured as many times as defined in the kinetic loop.

Measurement with dispensing

In fast kinetic measurements, it is often necessary to start the measurement of the sample immediately when the dispensing is started, not when the dispensing has been completed. Figure 6-2 shows a measurement with dispensing. The delay between the start of dispensing and the start of measurement is so small that no liquid has yet reached the well when the

measurement starts. The delay is caused when the plate is moved from dispensing position to the measurement position.

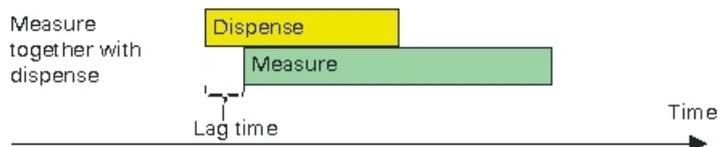
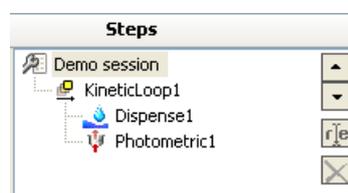
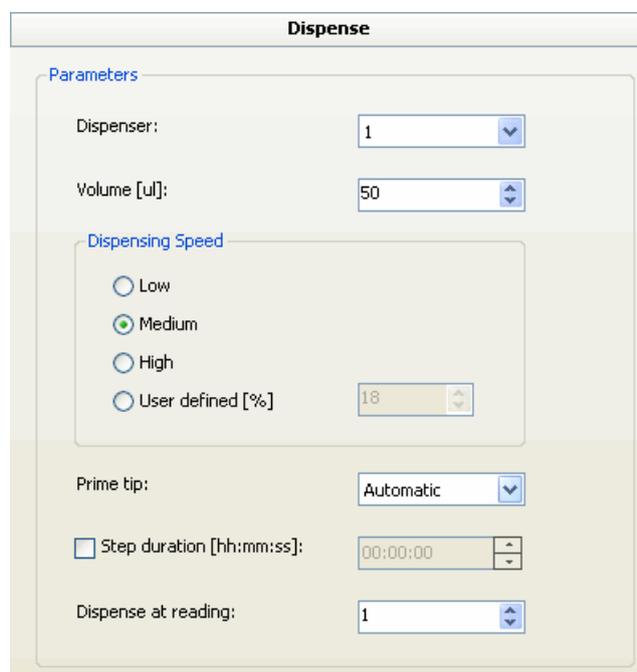


Figure 6-2. Measurement after dispense and measurement together with dispense

Select Steps > **Kinetic Loop** and below the kinetic loop, select Steps > **Dispense** and then Steps > **[measurement]**.



You can modify the dispensing parameters (see “Dispense” on page 120) by selecting the *Dispense* step from the **Steps** step tree.



Use the *Dispense at reading* parameter if you want to specify that dispensing is started only after a number of readings. Select at which reading the dispensing will be performed. The possible values are from 1 to the number of readings defined for the kinetic loop, the default being 1.



Note The readings are numbered in the measurement results so that the reading where the dispensing is performed is reading no. 0 and the readings before it are given negative numbers. For example, if the *Dispense at reading* value is set to 5, the readings are numbered -4, -3, -2, -1, 0, 1, 2,....

The *Lag time* and *Step duration* parameters are not available in the measurement step.

Kinetic loops

The kinetic loop step is used for executing the child steps multiple times before continuing with the protocol, that is, for kinetic measurements. The kinetic loop can be used with photometric, fluorometric and TRF measurements. It is also possible to add a dispense step to the kinetic loop before any of the allowed measurement steps. In this case a well is first dispensed and then measured immediately before dispensing and measuring the next well.

You can set the number of readings as well as the interval time. These values will define the delay between the steps in the loop as well as the total time of the kinetic loop (Figure 6-3).

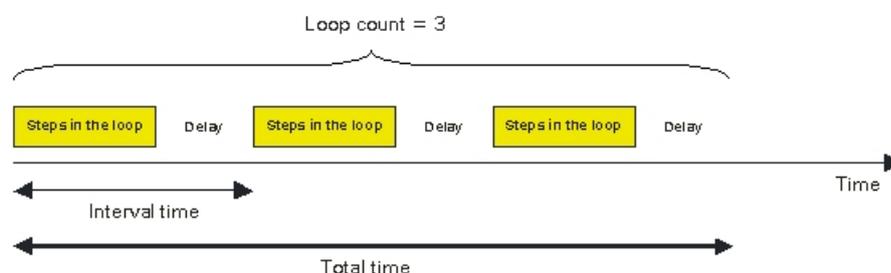
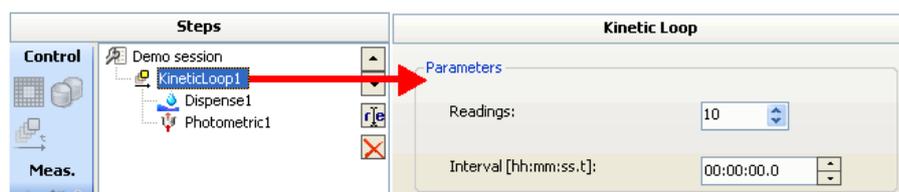


Figure 6-3. Interval time, total time and readings in a kinetic loop

When you add a measurement to the kinetic loop, the editing pane shows the selected measurement step parameters. The kinetic loop parameters can be edited by selecting the kinetic loop in the **Steps** step tree.



- **Readings** - Enter the number of times the kinetic loop is executed. The default value is 10.
- **Interval [hh:mm:ss:t]** - Enter the time that elapses between the starting times of two consecutive loops.

The *Lag time* and *Step duration* parameters are not available in the measurement step.

Chapter 7

Step Parameters

Step parameters are described in the following sections:

Area definition

Select Steps > **Area Definition** or click the  button on the action panel.

For more information, see “Area definition” on page 85.

Well loop

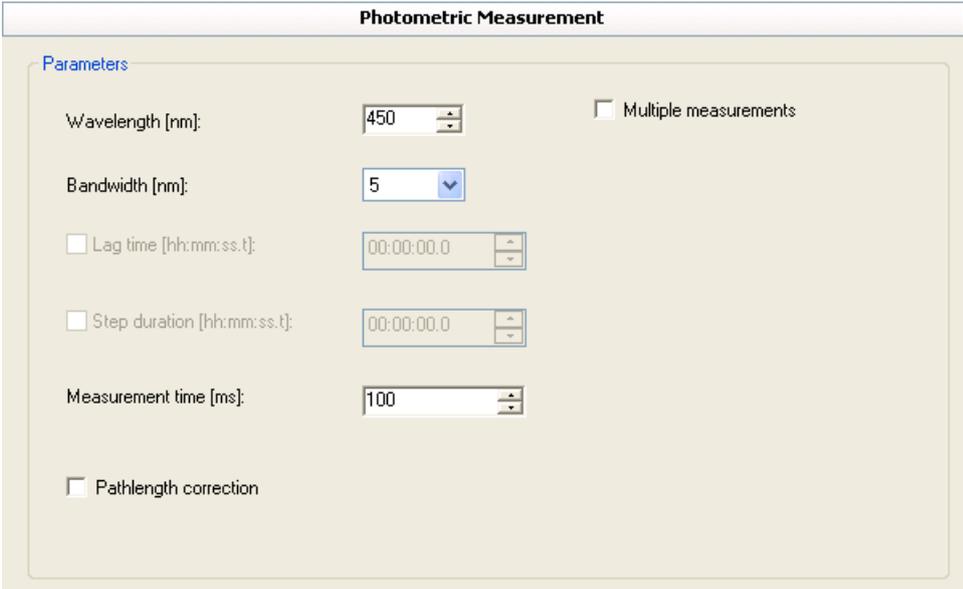
Select Steps > **Well Loop** or click the  button on the action panel.

For more information, see “Well loops” on page 87.

Photometric measurement

The *Photometric Measurement* step is used to measure absorbance (Abs). In the photometric calibration procedure the instrument reads the air blank level. In long measurement procedures calibration is performed in a suitable phase without disturbing the measurement timing. The calibration is valid for 1 hour.

Select Steps > **Photometric** or click the  button on the action panel.



The screenshot shows a software window titled "Photometric Measurement" with a "Parameters" section. The parameters are as follows:

Parameter	Value	Control Type
Wavelength [nm]	450	Spin box
Bandwidth [nm]	5	Dropdown menu
Lag time [hh:mm:ss.t]	00:00:00.0	Spin box (disabled)
Step duration [hh:mm:ss.t]	00:00:00.0	Spin box (disabled)
Measurement time [ms]	100	Spin box
Multiple measurements	<input type="checkbox"/>	Checkbox
Pathlength correction	<input type="checkbox"/>	Checkbox

The photometric measurement step includes the following parameters:

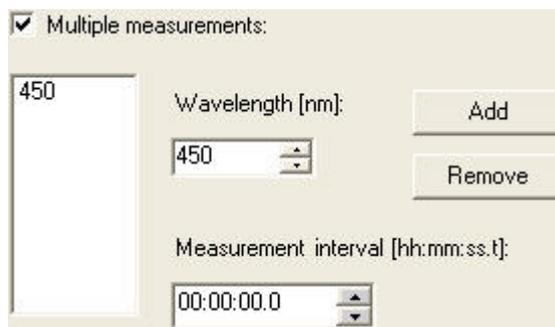
- **Wavelength [nm]** - Select the wavelength to be used in the measurement. The wavelength range is 200 to 1,000 nm; the default value is 450 nm.
- **Bandwidth [nm]** - The bandwidth value in a photometric measurement is 5 nm.
- **Lag time [hh:mm:ss.t]** - Define the time before a measurement is executed. Lag time is not available for kinetic loops.
- **Step duration [hh:mm:ss.t]** - Define the total time for the step. The software waits after the instrument has performed the action until the time is up before proceeding with the following step. Step duration is not available for kinetic loops.
- **Measurement time [ms]** - The measurement time defines the light collection time used in the measurement. The wells are measured with a selected measurement time. For more information, see “Measurement times” on page 113.
- **Pathlength correction** - Tick the check box to launch the **Pathlength Correction K-Factors** dialog. Select the pathlength correction factor for the solvent you are using (see “Using pathlength correction in a measurement” on page 97).
- **Multipoint** - Tick the check box to select which points in a well are measured. If the multipoint measurement is not selected, only the center of the well is measured. Multipoint is only available for 6 to 48-well plates. For more information, see “Using multipoint in a measurement” on page 114.
- **Multiple measurements** - Tick the check box to select multiple wavelengths for measurement. All wavelengths are measured one after the other from the same well before moving to the next well. For more information, see the section called “Defining multiple photometric measurements”.



Note In the photometric calibration procedure the instrument reads the air blank level. In long measurement procedures calibration is performed in a suitable phase without disturbing the measurement timing. The calibration is valid for 1 hour.

Defining multiple photometric measurements

When you select the **Multiple Measurements** check box while defining the photometric measurement parameters, the following dialog opens:



- **Add** - Enter the new wavelength value into the *Wavelength [nm]* box and then click **Add**. The new value is added to the value list on the left.
- **Remove** - Select the value you want to remove from the list on the left and click **Remove**.
- **Measurement interval [hh:mm:ss]** - Define the time between measurements with different wavelengths.

Photometric spectrum scanning

The *Photometric Spectrum Scanning* is a measurement carried out over a defined spectral range.

The bandwidth of 5 nm by means of a slit is used in the measurement.

Select Steps > **Photometric Scanning** or click the  button on the action panel.

Step Parameters

Photometric spectrum scanning

The screenshot shows a software dialog box titled "Photometric Spectrum Scanning". It contains several input fields and checkboxes for configuring the scanning process. The "Scanning wavelengths" section includes "Start [nm]" (400), "End [nm]" (600), and "Step size [nm]" (5). Other parameters include "Bandwidth [nm]" (5), "Lag time [hh:mm:ss.t]" (00:00:00.0), "Step duration [hh:mm:ss]" (00:00:00), "Measurement time [ms]" (100), and a "Pathlength correction" checkbox.

Parameter	Value
Start [nm]	400
End [nm]	600
Step size [nm]	5
Bandwidth [nm]	5
Lag time [hh:mm:ss.t]	00:00:00.0
Step duration [hh:mm:ss]	00:00:00
Measurement time [ms]	100
Pathlength correction	<input type="checkbox"/>

The photometric spectrum scanning step includes the following parameters:

- **Scanning wavelengths** - Select the starting and ending wavelengths and the step size (scanning resolution) for the scanning measurement. A step size is automatically suggested. The wavelength range is 200 to 1,000 nm.
- **Lag time [hh:mm:ss.t]** - Define the time before the measurement is executed.
- **Step duration [hh:mm:ss]** - Define the total time for the step. The software waits after the instrument has performed the action until the time is up before proceeding with the following step.
- **Measurement time [ms]** - The measurement or integration time defines the light collection time used in the measurement. The wells are measured with a selected measurement time. For more information, see "Measurement times" on page 113.

- **Pathlength correction** - Tick the check box to launch the **Pathlength Correction K-Factors** dialog. For more information, see “Using pathlength correction in a measurement” on page 97.

Using pathlength correction in a measurement

Pathlength correction calculates the measurement value from each well in the microplate so that it corresponds to a measurement in a 10 mm cuvette. Note that these factors are dependent on the solvent and the polymer used for the plastic plate. If a new solvent or a new type of plate is used, then you must generate a new K-factor.



Note If you have added a pathlength correction to a photometric measurement and you want to remove the photometric measurement, you must untick the *Pathlength correction* check box first.



Note The pathlength correction is applicable only to water based solutions. There must be an aqueous solution in the well when you measure pathlength correction.



Note The pathlength correction is dependent on the material used to manufacture the microplate, and should be determined each time the plate type is changed (see “Measuring new pathlength correction K-factors” on page 73).



Note The pathlength correction factor can only be used with either a 96-well or a 384-well plate.

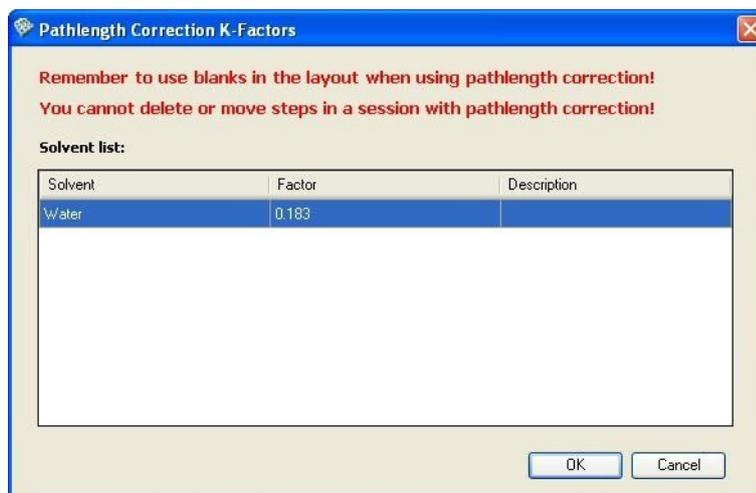


Note A background subtraction step is automatically added to the pathlength correction calculation. Therefore, blanks are needed in the plate layout.

Tick the *Pathlength correction* check box. The **Pathlength Correction K-Factors** dialog is launched.

Step Parameters

Using pathlength correction in a measurement

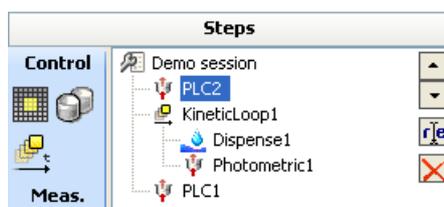


Select the appropriate value and click **OK**. If the solvent is not on the list, you can add a known K-factor to the database (see “Editing pathlength correction K-factors” on page 247) or measure the pathlength K-factor (see “Measuring new pathlength correction K-factors” on page 73).

An additional photometric measurement step (PLC) is automatically added after the measurement step for determining the pathlength correction of the selected solvent. You cannot delete this measurement step unless you remove the pathlength correction. In a kinetic measurement with dispensing, two PLC steps are added, one before dispensing and another after dispensing.



Note Readings numbered with 0 or negative numbers are calculated using the PLC performed before the dispensing. Readings numbered with positive numbers are calculated using the PLC performed after the dispensing.



The chosen pathlength correction K-factor will be shown for the measurement.



Add the pathlength correction for each measurement step separately.



Note If you use a pathlength correction factor which did not pass the quality control check, the measurement results will be unreliable

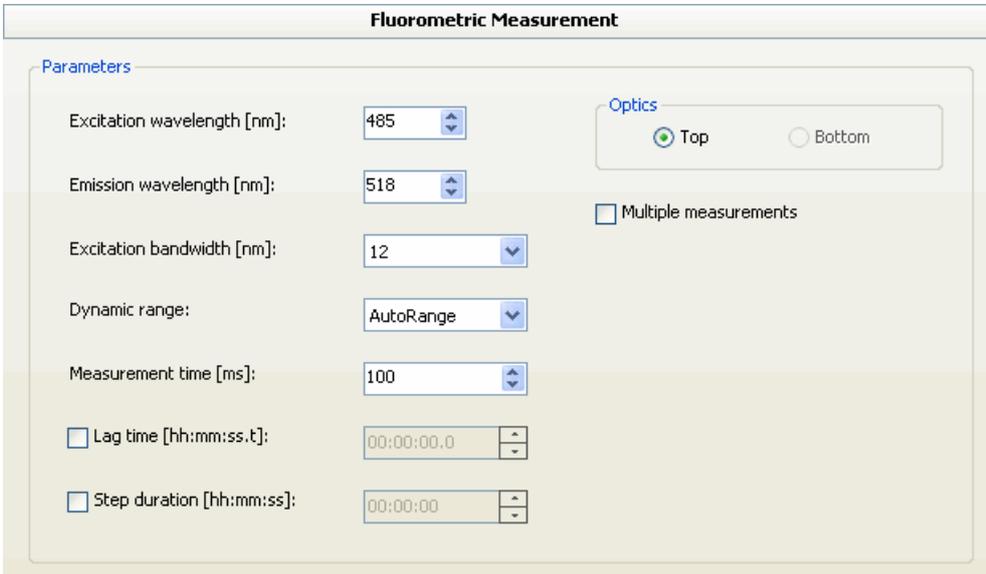
(see “Measuring new pathlength correction K-factors” on page 73).

For more information on pathlength correction and pathlength correction factors, see “Editing pathlength correction K-factors” on page 247 and “Pathlength correction” on page 72.

Fluorometric measurement

The *Fluorometric Measurement* step is used for measuring fluorescence intensity (FI, RFU) at selected excitation and emission wavelengths.

Select Steps > **Fluorometric** or click the  button on the action panel.



The fluorometric measurement step includes the following parameters:

- **Excitation wavelength [nm]** - Select the excitation wavelength to be used in the measurement. The wavelength range is 200 to 1,000 nm; the default value is 485 nm.
- **Emission wavelength [nm]** - Select the emission wavelength to be used in the measurement. The wavelength range is 270 to 840 nm; the default value is 518 nm.
- **Excitation bandwidth [nm]** - Select the bandwidth of the excitation wavelength to be used in the measurement. Bandwidth is selected using an excitation slit that has 5 nm and 12 nm options. The default value is 12 nm. The emission bandwidth is permanently set to 12 nm and cannot be changed.



Note To prevent any stray light from the excitation light from interfering with the emission light, the difference between the

excitation and emission wavelengths should be at least the sum of the bandwidths used. If the 5 nm excitation and 12 nm emission (permanent) bandwidths are used, the difference between excitation and emission wavelengths should be at least 17 nm. With the 12 nm excitation and emission bandwidths the difference should be at least 24 nm.

- **Dynamic range** - Select the dynamic range to be used in the measurement: AutoRange, Low, Medium Low, Medium High, or High. The default selection is AutoRange. For more information, see “Dynamic range selections” on page 112.
- **Measurement time [ms]** - The measurement or integration time defines the light collection time used in the measurement. The wells are measured with a selected measurement time. For more information, see “Measurement times” on page 113.
- **Lag time [hh:mm:ss.t]** - Define the time before the measurement is executed. Lag time is not available for kinetic loops.
- **Step duration [hh:mm:ss]** - Define the total time for the step. The software waits after the instrument has performed the action until the time is up before proceeding with the following step. Step duration is not available for kinetic loops.
- **Multipoint** - Tick the check box to select which points in a well are measured. If the multipoint measurement is not selected, only the center of the well is measured. Multipoint is only available for 6 to 48-well plates. For more information, see “Using multipoint in a measurement” on page 114.



Note If there are not any points selected in the multipoint measurement, the protocol cannot be run.

- **Optics** - Fluorescence can be measured from one position:
 - **Top** - Fluorescence is measured at the measurement position above the plate.
 - **Bottom** - This option is available with the Varioskan Flash only.
- **Multiple measurements** - Tick the check box to enter multiple wavelength pair values. All wavelengths are measured one after the other from the same well before moving to the next well. For more

information, see “Defining multiple fluorometric measurements” on page 101.

Defining multiple fluorometric measurements

When you select the **Multiple Measurements** check box while defining the fluorometric measurement parameters, the following dialog opens:

Ex	Em
490	520

Ex [nm]: 490 Add

Em [nm]: 520 Remove

Measurement interval [hh:mm:ss.t]: 00:00:00.0

- **Add** - Enter the new excitation and emission wavelength values in the *Ex [nm]* and *Em [nm]* boxes and then click **Add**. The new value pair is added to the list on the left.
- **Remove** - Select the value pair you want to remove from the list on the left and click **Remove**.
- **Measurement interval [hh:mm:ss]** - Define the time between measurements with different wavelengths.

Fluorometric spectrum scanning

In the *Fluorometric Spectrum Scanning* step, either the emission or the excitation wavelength is fixed and the other wavelength is scanned.

Select Steps > **Fluorometric Scanning** or click the  button on the action panel.

Step Parameters

Fluorometric spectrum scanning

The screenshot shows the 'Fluorometric Spectrum Scanning' window. It is divided into several sections:

- Parameters:**
 - Fixed wavelength:** Radio buttons for 'Excitation' (selected) and 'Emission'. A 'Wavelength [nm]' field is set to 450.
 - Optics:** Radio buttons for 'Top' (selected) and 'Bottom'.
- Scanning wavelengths:** Fields for 'Start [nm]' (490), 'End [nm]' (600), and 'Step size [nm]' (2).
- Excitation bandwidth [nm]:** Set to 5.
- Dynamic range:** Set to AutoRange.
- Measurement time [ms]:** Set to 100.
- Lag time [hh:mm:ss.t]:** Set to 00:00:00.0.
- Step duration [hh:mm:ss]:** Set to 00:00:00.

The fluorometric spectrum scanning step includes the following parameters:

- **Fixed wavelength** - Select either excitation or emission wavelength as the fixed wavelength and enter its wavelength. The wavelength range is 200 to 1,000 nm for the excitation and 270 to 840 nm for the emission wavelength.
- **Scanning wavelengths** - Select the starting and ending wavelengths and the step size (scanning resolution) for the scanning measurement. The wavelength range is 200 to 1,000 nm for excitation and 270 to 840 nm for emission.
- **Excitation bandwidth [nm]** - Select the bandwidth of the excitation wavelength to be used in the measurement. Bandwidth is selected using an excitation slit that has 5 nm and 12 nm options. The default value is 12 nm. The emission bandwidth is permanently set to 12 nm and cannot be changed.



Note To prevent any stray light from the excitation light from interfering with the emission light, the difference between the excitation and emission wavelengths should be at least the sum of the bandwidths used. If the 5 nm excitation and 12 nm emission (permanent) bandwidths are used, the difference between excitation and emission wavelengths should be at least 17 nm. With the 12 nm

excitation and emission bandwidths the difference should be at least 24 nm.

- **Dynamic range** - Select the dynamic range to be used in the measurement: AutoRange, Low, Medium Low, Medium High, or High. For more information, see “Dynamic range selections” on page 112.
- **Measurement time [ms]** - The measurement time defines the light collection time used in the measurement. The wells are measured with a selected measurement time. For more information, see “Measurement times” on page 113.
- **Lag time [hh:mm:ss.t]** - Define the time before the measurement is executed.
- **Step duration [hh:mm:ss]** - Define the total time for the step. The software waits after the instrument has performed the action until the time is up before proceeding with the following step.
- **Optics** - Fluorescence can be measured from one position:
 - **Top** - Fluorescence is measured at the measurement position above the plate.
 - **Bottom** - This option is available with the Varioskan Flash only.

TRF measurement

The *TRF Measurement* step is used for measuring time-resolved fluorescence using special TRF labels at selected excitation and emission wavelengths.

Select Steps > **TRF** or click the  button on the action panel.

The TRF measurement step includes the following parameters:

- **Excitation wavelength [nm]** - Select the excitation wavelength to be used in the measurement. The wavelength range is 200 to 840 nm; the default value is 340 nm.
- **Emission wavelength [nm]** - Select the emission wavelength to be used in the measurement. The wavelength range is 270 to 840 nm; the default value is 615 nm.
- **Excitation bandwidth [nm]** - Select the bandwidth of the excitation wavelength to be used in the measurement. Bandwidth is selected using an excitation slit that has 5 nm and 12 nm options. The default value is 12 nm. The emission bandwidth is permanently set to 12 nm and cannot be changed.



Note To prevent any stray light from the excitation light from interfering with the emission light, the difference between the excitation and emission wavelengths should be at least the sum of the bandwidths used. If the 5 nm excitation and 12 nm emission (permanent) bandwidths are used, the difference between excitation and emission wavelengths should be at least 17 nm. With the 12 nm excitation and emission bandwidths the difference should be at least 24 nm.

- **Dynamic range** - Select the dynamic range to be used in the measurement: AutoRange, Low, Medium Low, Medium High, or High. For more information, see “Dynamic range selections” on page 112.
- **Measurement time [ms]** - The measurement or integration time defines the light collection time used in the measurement. The wells are measured with a selected measurement time. For more information, see “Measurement times” on page 113.
- **Lag time [hh:mm:ss.t]** - Define the time before the measurement is executed. Lag time is not available for kinetic loops.
- **Step duration [hh:mm:ss]** - Define the total time for the step. The software waits after the instrument has performed the action until the time is up before proceeding with the following step. Step duration is not available for kinetic loops.
- **Multipoint** - Tick the check box to select which points in a well are measured. If the multipoint measurement is not selected, only the center of the well is measured. Multipoint is only available for 6 to 48-well plates. For more information, see “Using multipoint in a measurement” on page 114.



Note If there are not any points selected in the multipoint measurement, the protocol cannot be run.

- **Optics** - Fluorescence can be measured from one position:
 - **Top** - Fluorescence is measured at the measurement position above the plate.
 - **Bottom** - This option is available with the Varioskan Flash only.
- **Multiple measurements** - Tick the check box to enter a multiple wavelength pair and TRF parameter values. For more information, see “Defining multiple TRF measurements” on page 106.
- **TRF delay time [μs]** - Define the waiting period (TRF delay) between the excitation flash and the beginning of the emission light measurement (see Figure 7-1).
- **TRF integration time [μs]** - Define the length of the measurement window, that is, the time the emitted signal is collected for each measurement cycle (see Figure 7-1).

The sum of TRF delay time and TRF integration time can be maximum 2400 μs.

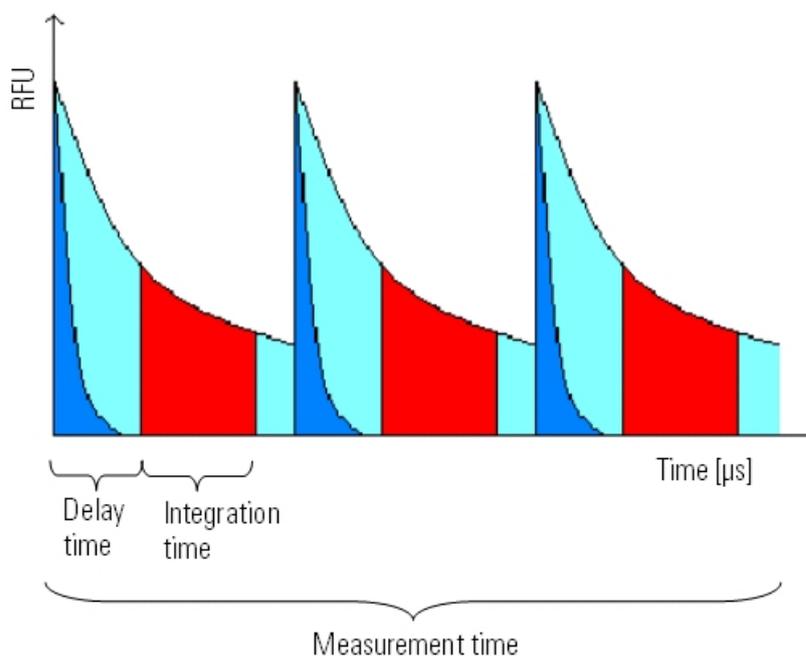
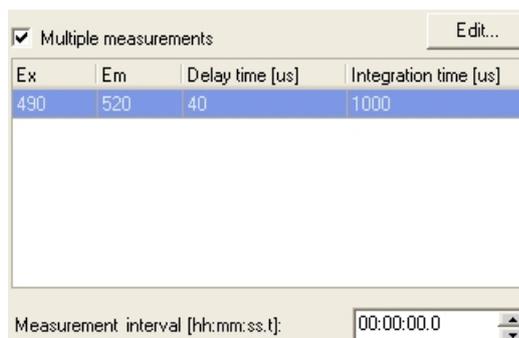


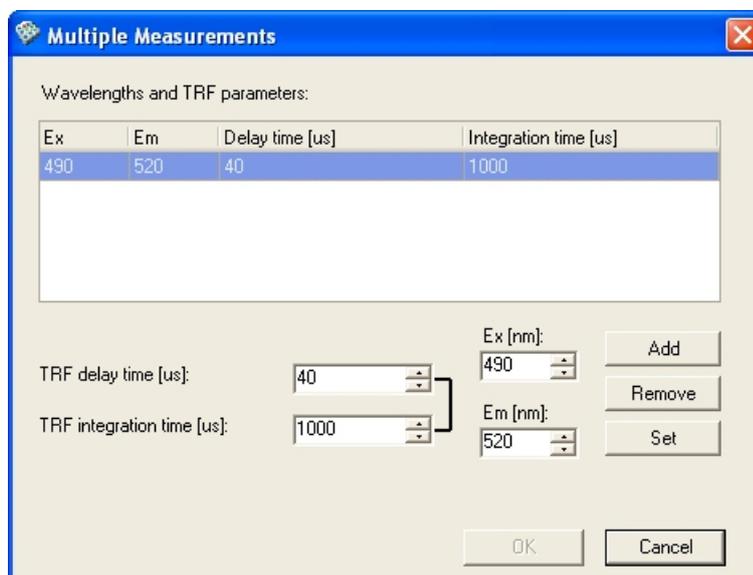
Figure 7-1. TRF principle

Defining multiple TRF measurements

When you select the **Multiple Measurements** check box while defining TRF measurement parameters, the following dialog opens:



The list shows the current measurement parameters, that is, the excitation (Ex) and emission (Em) wavelengths and the delay time and integration time values. To add new measurements and edit the current values, click **Edit...**



- **Add** - Enter the new measurement parameters in the *Ex [nm]*, *Em [nm]*, *Delay time [μs]* and *Integration time [μs]* boxes and then click **Add**. The new value pair is added to the list on the left.
- **Remove** - Select the measurement parameters you want to remove from the list above and click **Remove**.
- **Set** - The parameters of the currently selected measurement are substituted with the values defined on the left.

Click **OK** to close the dialog and add the new measurements to the step parameters.

- **Measurement interval [hh:mm:ss.t]** - Define the time between measurements with different wavelengths.

TRF scanning

In the *TRF Scanning* step, either the emission or the excitation wavelength is fixed and the other wavelength is scanned.

Select Steps > **TRF Scanning** or click the  button on the action panel.

The screenshot shows the 'TRF Scanning' window with the following parameters:

- Fixed wavelength:** Excitation (selected), Wavelength [nm]: 340
- Optics:** Top (selected), Bottom
- Scanning wavelengths:** Start [nm]: 550, End [nm]: 660, Step size [nm]: 2
- Excitation bandwidth [nm]:** 12
- Dynamic range:** AutoRange
- Measurement time [ms]:** 100
- Lag time [hh:mm:ss.t]:** 00:00:00.0
- Step duration [hh:mm:ss]:** 00:00:00
- TRF delay time [us]:** 200
- TRF integration time [us]:** 1000

The TRF scanning step includes the following parameters:

- **Fixed wavelength** - Select either excitation or emission wavelength as the fixed wavelength and enter its wavelength. The wavelength range is 200 to 840 nm for the excitation and 270 to 840 nm for the emission wavelength.
- **Scanning wavelengths** - Select the starting and ending wavelengths and the step size (scanning resolution) for the scanning measurement. The wavelength range is 200 to 840 nm for excitation and 270 to 840 nm for emission.
- **Excitation bandwidth [nm]** - Select the bandwidth of the excitation wavelength to be used in the measurement. Bandwidth is selected using an excitation slit that has 5 nm and 12 nm options. The default value is 12 nm. The emission bandwidth is permanently set to 12 nm and cannot be changed.



Note To prevent any stray light from the excitation light from interfering with the emission light, the difference between the excitation and emission wavelengths should be at least the sum of the bandwidths used. If the 5 nm excitation and 12 nm emission (permanent) bandwidths are used, the difference between excitation and emission wavelengths should be at least 17 nm. With the 12 nm excitation and emission bandwidths the difference should be at least 24 nm.

- **Dynamic range** - Select the dynamic range to be used in the measurement: AutoRange, Low, Medium Low, Medium High, or High. For more information, see “Dynamic range selections” on page 112.
- **Measurement time [ms]** - The measurement time defines the light collection time used in the measurement. The wells are measured with a selected measurement time. For more information, see “Measurement times” on page 113.
- **Lag time [hh:mm:ss.t]** - Define the time before the measurement is executed.
- **Step duration [hh:mm:ss]** - Define the total time for the step. The software waits after the instrument has performed the action until the time is up before proceeding with the following step.
- **Optics** - Fluorescence can be measured from one position:
 - **Top** - Fluorescence is measured at the measurement position above the plate.
 - **Bottom** - This option is available with the Varioskan Flash only.
- **TRF delay time [μs]** - Define the waiting period (TRF delay) between the excitation flash and the beginning of the emission light measurement (see Figure 7-1).
- **TRF integration time [μs]** - Define the length of the measurement window, that is, the time the emitted signal is collected for each measurement cycle (see Figure 7-1).

The sum of TRF delay time and TRF integration time can be maximum 2400 μs.

TRF decay

The *TRF Decay* step is used to determine the life time of the TRF signal.

Select Steps > **TRF Decay** or click the  button on the action panel.

Step Parameters

TRF decay

The screenshot shows the 'TRF Decay' software window. It is divided into two main sections: 'Parameters' and 'Time settings'.
In the 'Parameters' section, there are five rows of controls:

- 'Excitation wavelength [nm]': A dropdown menu set to 340.
- 'Emission wavelength [nm]': A dropdown menu set to 615.
- 'Excitation bandwidth [nm]': A dropdown menu set to 12.
- 'Dynamic range:': A dropdown menu set to 'AutoRange'.
- 'Measurement time [ms]': A dropdown menu set to 100.

To the right of these parameters is an 'Optics' section with two radio buttons: 'Top' (which is selected) and 'Bottom'.
The 'Time settings' section is located below the parameters and contains four rows of input fields:

- 'Integration time [us]': A text box containing '100' and an 'Edit...' button to its right.
- 'Start time [us]': A text box containing '50'.
- 'Stop time [us]': A text box containing '2350'.
- 'Step [us]': A text box containing '10'.

The TRF decay step includes the following parameters:

- **Excitation wavelength [nm]** - Select the excitation wavelength to be used in the measurement. The wavelength range is 200 to 840 nm; the default value is 340 nm.
- **Emission wavelength [nm]** - Select the emission wavelength to be used in the measurement. The wavelength range is 270 to 840 nm; the default value is 615 nm.
- **Excitation bandwidth [nm]** - Select the bandwidth of the excitation wavelength to be used in the measurement. Bandwidth is selected using an excitation slit that has 5 nm and 12 nm options. The default value is 12 nm. The emission bandwidth is permanently set to 12 nm and cannot be changed.



Note To prevent any stray light from the excitation light from interfering with the emission light, the difference between the excitation and emission wavelengths should be at least the sum of the bandwidths used. If the 5 nm excitation and 12 nm emission (permanent) bandwidths are used, the difference between excitation and emission wavelengths should be at least 17 nm. With the 12 nm excitation and emission bandwidths the difference should be at least 24 nm.

- **Dynamic range** - Select the dynamic range to be used in the measurement: AutoRange, Low, Medium Low, Medium High, or High. For more information, see “Dynamic range selections” on page 112.
- **Measurement time [ms]** - The measurement time defines the light collection time used in the measurement. The wells are measured with a selected measurement time. For more information, see “Measurement times” on page 113.
- **Time Setting** - Edit the TRF Decay time settings by clicking **Edit...**. You are asked to enter the values one by one. When you have given all four values, the software may change the values, if they are not compatible. You can use the **Back** and **Next** buttons to modify the values. Click **Finish** to confirm the values.



- **Integration time [μs]** - Define the length of the measurement window, that is, the time the emitted signal is collected for each measurement cycle (see Figure 7-2).
- **Start time [μs]** - Define the time from the flash to the middle point of the first measurement (see Figure 7-2).
- **Stop time [μs]** - Define the time from the flash to the middle point of the last measurement (see Figure 7-2).
- **Step [μs]** - Define the time difference between two sequential middle points of the measurement (see Figure 7-2).

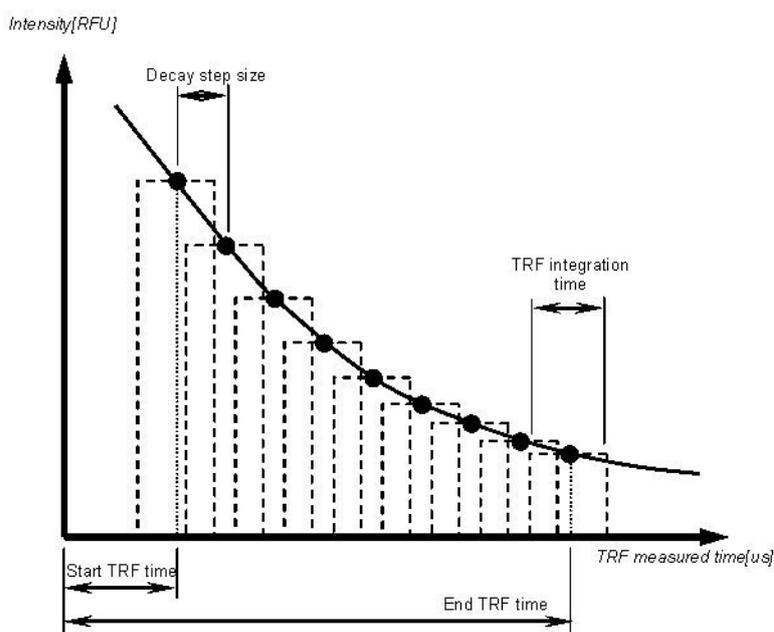


Figure 7-2. TRF decay definitions

- **Optics** - Fluorescence can be measured from one position:
 - **Top** - Fluorescence is measured at the measurement position above the plate.
 - **Bottom** - This option is available with the Varioskan Flash only.

Dynamic range selections

AutoRange (default) selects automatically that the optimal reading range is used. It is based upon signal intensity in the well and uses the lowest possible reading range to obtain best sensitivity.

However, if you need a faster reading speed or your kinetic reaction causes overrange with the *AutoRange* setting, use the manual dynamic range selection of Low, Medium Low, Medium High, or High range.

- *High range* is intended for highest-concentration samples. It covers a wide dynamic range with somewhat lower sensitivity than with other dynamic ranges.
- *Low range* produces on the other hand the highest sensitivity with a limited dynamic range.
- *Medium Low range* and *Medium High range* offer sensitivity and dynamics in between the Low and High ranges.

When selecting a fixed gain, the principle for achieving the best sensitivity is to select the lowest possible range, without receiving overrange results in the measurement.

The measured values are comparable regardless of the dynamic range selection.

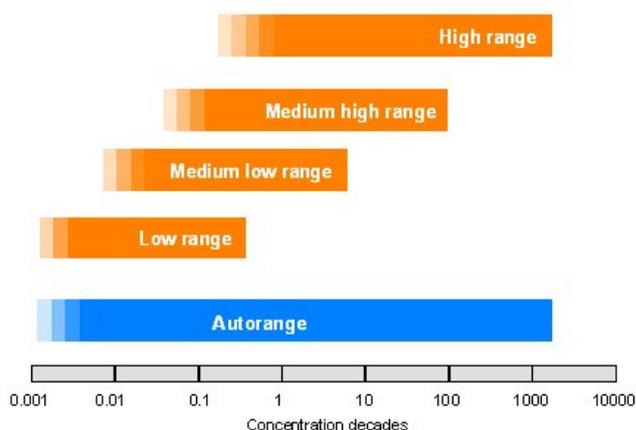


Figure 7-3. Dynamic range selections in the FI and TRF measurements

Measurement times

There is one xenon lamp flash for each 10 ms period of measurement time. The amount of flashes can be set to 1 to 100 flashes per measurement (10 – 1,000 ms) for fluorescence intensity, photometric and TRF decay measurements and 1 to 1,000 flashes per measurement (10 – 10,000 ms) for TRF measurements. The amount of xenon lamp flashes affects the quality of the measurement result. Thus, the more flashes, the better the quality of the result.

Use the default measurement times which normally produce good results (see Table 7-1). If there is a necessity to improve the quality of the results, the flash amount should be increased. In photometric measurements, longer than 100 ms measurement times are recommended to reduce noise if the measured absorbance is too high.

The result is the mean value of individual 10 ms readings during the total measurement time.

Table 7-1. Measurement times for each measurement technique and mode

Measurement technique	Mode	Measurement time (ms)		
		Default (Recommended)	min.	max.
Absorbance (photometric)	Fixed wavelength	100	10	1,000
	Scanning wavelength	100	10	1,000
Fluorometric intensity (FI)	Fixed wavelength	100	10	1,000
	Scanning wavelength	100	10	1,000
Time-resolved fluorescence (TRF)	Fixed wavelength	1000	10	10,000
	Scanning wavelength	1000	10	10,000
TRFdecay		100	10	1,000

Using multipoint in a measurement

In a multipoint well measurement, different parts of a well can be measured separately. Multipoint measurement is only available for 6 to 48-well plates.

If the multipoint measurement is not selected, only the middle point of the well is measured.

You can define which points are measured in the wells. The multipoint pattern is only defined for the current step and is applied to all the wells that are measured with that step. Points inside the well are measured in the same direction as defined with the **Execution order** setting.

The measurement points in a well are equidistant from each other. The location and distance of a single measurement point is measured from the origin. The origin (0:0) is in the center of the well. The location is shown as a coordinate value on the X and Y axes. A negative or positive value indicates the direction from the origin. The distance is shown in units of 0.01 mm. The coordinates of measurement points are shown in the list format of the measurement results.

In Figure 7-4, the measured points are orange. Their coordinates are shown as values on the X and Y axes. For example, $-190 : 0$ means 1.90 mm left in the X direction from the origin.

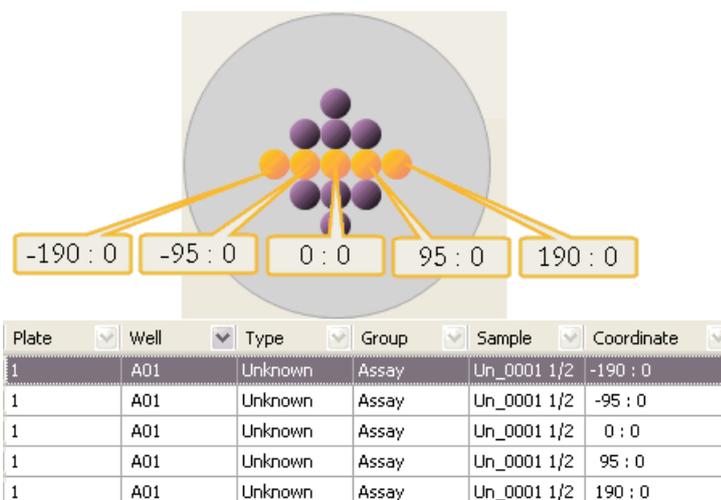


Figure 7-4. Multipoint example: measurement points in a well and their coordinates in the results

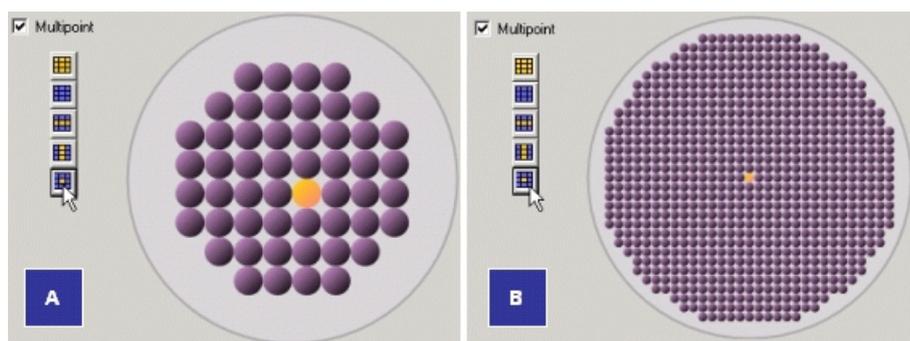


Figure 7-5. Multipoint selection: center point of a well on a 48-well plate (A) and on a 6-well plate (B)

Purple points in the well are not selected; orange points are selected for measurement. You can select a rectangular area by dragging with the mouse. You can select several areas by holding the **Ctrl** key down while you drag. You can also select several single points in a well by holding the **Ctrl** key down while you select.



Note If there are not any points selected in the multipoint measurement, the protocol cannot be run.

-  **Select all** - Selects the entire well.
-  **Clear all** - Clears all selections from the well.
-  **Horizontal line across well** - Selects a horizontal line in the middle of the well.
-  **Vertical line across well** - Selects a vertical line in the middle of the well.

 **Center point of well** - Selects the middle point of the well.

Kinetic measurements

Select Steps > **Kinetic Loop** or click the  button on the action panel. Then select the measurement step you want to add to the kinetic loop with the action panel button or from the **Steps** menu.

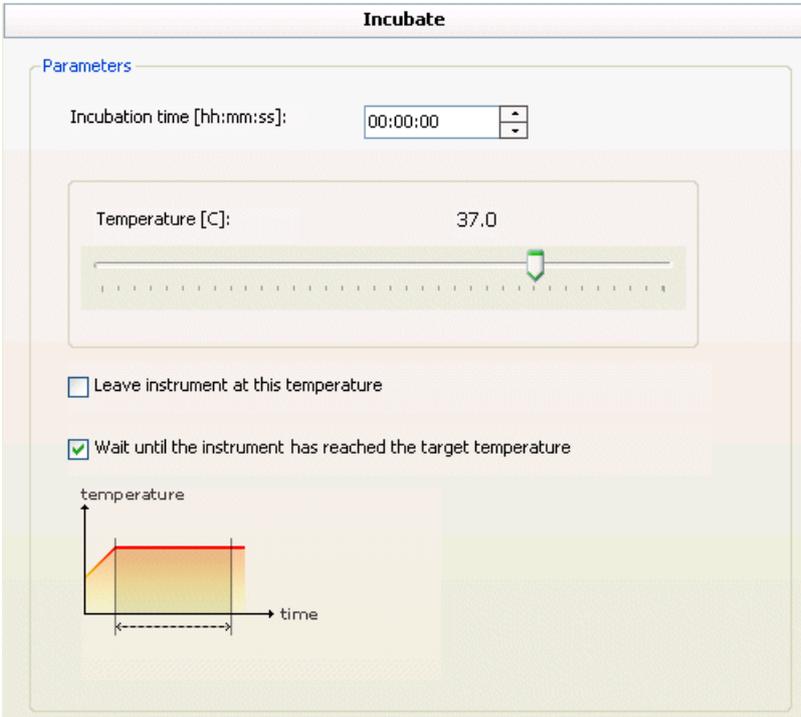
The kinetic loop can be used with photometric, fluorometric, and TRF measurements. It is also possible to add a dispense step to the kinetic loop before any of the allowed measurement steps. In this case a well is first dispensed and then measured immediately before dispensing and measuring the next well.

For more information, see “Kinetic loops” on page 91.

Incubate

The *Incubate* step is used to control the temperature of the incubator (if present) of the instrument.

Select Steps > **Incubate** or click the  button on the action panel. Incubation can be carried out with 24 to 1536-well plates.



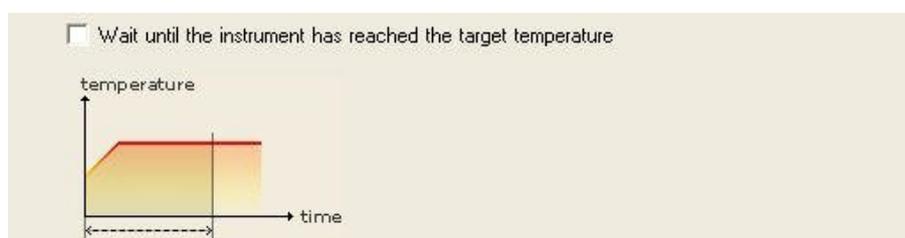
The incubation step includes the following parameters:

- **Incubation time** - The duration of the incubation (hh:mm:ss).

- **Temperature [°C]** - The target instrument temperature. The available temperature range is from 10°C to 45°C. Temperatures below ambient + 4°C cannot be reached. The default value is 37°C.
- **Leave instrument at this temperature** - When selected, the incubator is left on at the defined temperature after the step ends. The temperature is kept for the duration of the entire session or until the next incubation step in the session. If there is another incubation step, the incubator is heated up to the temperature defined in the step or it cools down passively to the defined temperature. After the session ends, the incubator reverts to the defined plate startup temperature or is switched off if no startup temperature is defined (see “Temperature” on page 229).

When not selected, the incubator is reverted to the previous set temperature. For example, if the protocol has one *Incubate* step only and the option is not selected, the incubator reverts to the plate startup temperature. If the protocol has two *Incubate* steps and the first step has the option selected but the second one does not, the incubator reverts to the temperature set in the first step.

- **Wait until instrument has reached the target temperature** - When selected, the incubation time is only counted from the time that the instrument has reached the target temperature. If not, also the time it takes for the instrument to reach the target temperature is included.



Note It is possible to set the instrument temperature to be kept at a defined temperature at all times. Select Settings > Options > **Temperature** (see “Temperature” on page 229).

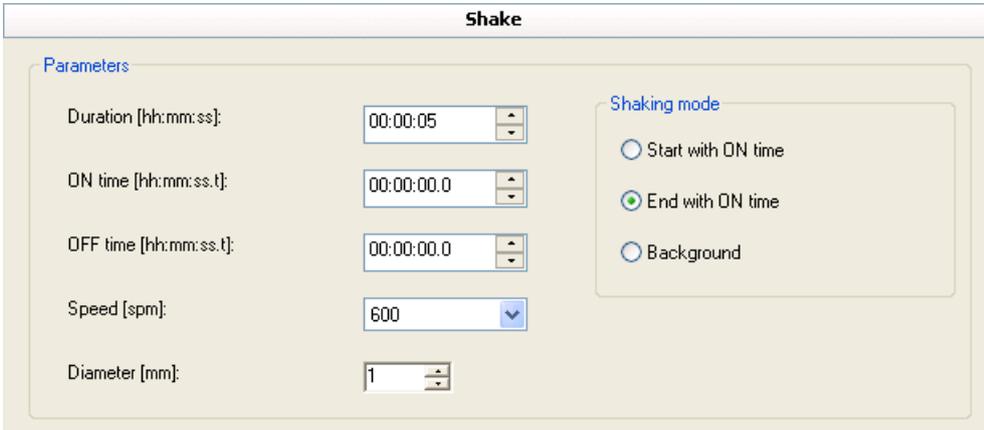


Note The samples in the microplate usually reach the target temperature much later than the instrument. For example, for a covered 96-well plate with 200 µl water/well, the liquid warm-up time from 25°C to 37°C takes approximately 1 h. The warm-up time also depends on the liquid that is used.

Shake

The *Shake* step is used for shaking the microplate (orbital shaking) in order to mix the samples.

Select Steps > **Shake** or click the  button on the action panel.



Shake

Parameters

Duration [hh:mm:ss]: 00:00:05

ON time [hh:mm:ss.t]: 00:00:00.0

OFF time [hh:mm:ss.t]: 00:00:00.0

Speed [spm]: 600

Diameter [mm]: 1

Shaking mode

Start with ON time

End with ON time

Background

The shaking step includes the following parameters:

- **Duration [hh:mm:ss]** - Enter the total shaking time (step duration).
- **ON time [hh:mm:ss.t]** - If you want the shaking to be intermittent, enter here how long each shaking period lasts. If you want to have a constant shake, then the ON time equals the duration or total time of the shake.
- **OFF time [hh:mm:ss.t]** - Enter how long the pause is between shaking periods.
- **Speed [spm]** - Enter the shaking speed in shakes per minute (spm). The available shaking speed range is 60 to 1,200 in 60 spm increments.
- **Diameter [mm]** - Enter the orbital shaking diameter. The available shaking diameter is 1 to 50 mm in 1 mm steps.
- **Shaking mode** - Select whether the shaking sequence starts or ends with a shaking ON time. If you select *Background*, the instrument shakes during idle periods. With background shaking the shaking always ends with an ON time. **Duration** is not available in the background mode.

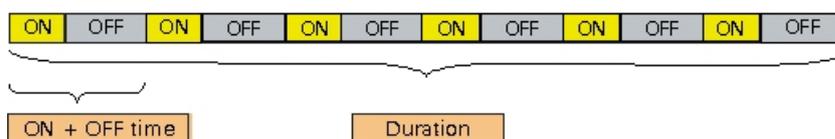


Figure 7-6. Shaking sequence: ON and OFF time and total shaking time

Shaking speed and diameter combinations

Only certain combinations of speed and diameter are allowed by the software. The software automatically prevents all those combinations that would cause too strong mechanical stress to the instrument and would definitely cause spilling over of the liquid from the wells. This feature is based on the limitation that angular acceleration of the performed shaking may not be over 1.0 G.



Caution Some combinations of speed and diameter may cause too high centrifugal forces inside the well area resulting in spills inside the measurement chamber. Centrifugal forces greater than 1.0 G are automatically prevented.



Caution Certain speed and diameter combinations that are allowed by the software can cause spilling from the wells with some plate types. With large wells (6-, 12- and 24-well plates), it is recommended to use lower diameter and speed combinations than are accepted by the software. See Figure 7-7 for recommended maximum speed and diameter combinations. The usable combinations are dependent on the volume used in the wells and on the viscosity of the liquid inside the wells.

The recommendations shown in Figure 7-7 are based on the situation where the wells are filled with 50% of the total volume and pure water is used as a liquid. The allowed combinations are those that are accepted by the software but may cause spilling. Only the recommended (dark gray) speed and diameter combinations should be used.

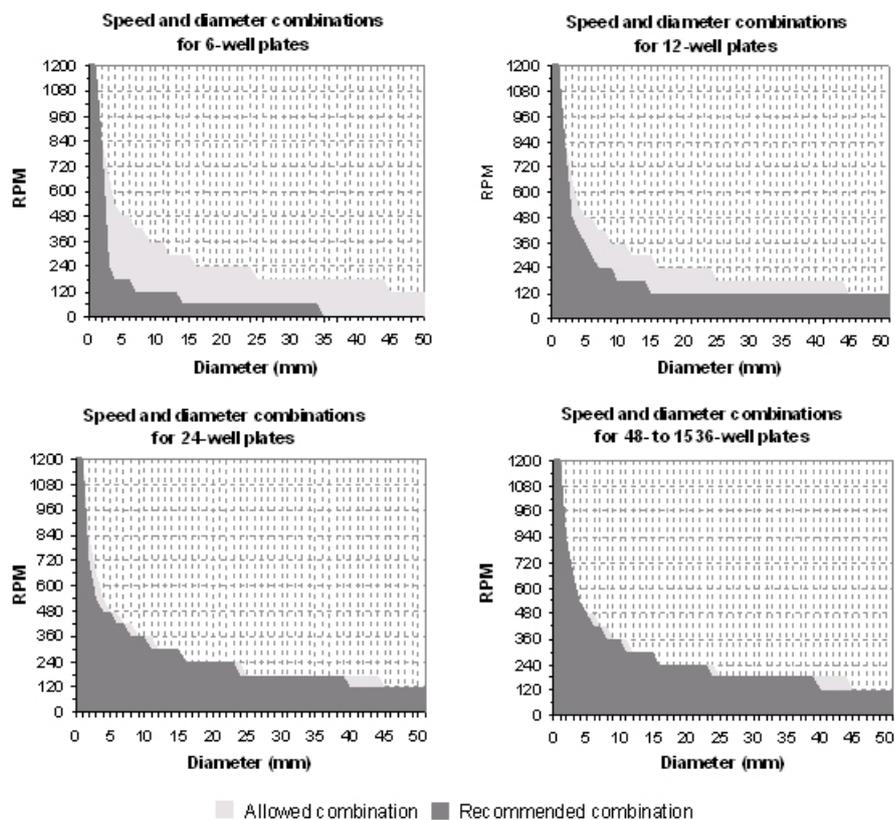


Figure 7-7. Recommended shaking speed and diameter combinations

Dispense

The *Dispense* step is used for instructing the instrument to use the installed optional dispenser to dispense a given amount of reagent into selected wells. The dispensing and measurement operations can be synchronized with an exact time interval on a well-by-well basis. You can use a dispensing step with all microplates suitable for the instrument. For more information about dispensing and dispenser rinsing, see the instrument manual.



Caution Make sure that there is a plate in the plate carrier before dispensing to avoid dispensing into the instrument.

Select Steps > **Dispense** or click the  button on the action panel.

The dispensing step includes the following parameters:

- **Dispenser** - Select which dispenser will be used. Only one dispenser is available with the Varioskan instrument. Also see “Dispensers” on page 234.
- **Volume [µl]** - Enter the volume to be dispensed into all the wells in the defined area. The range is 1 to 1,000 µl in 1 µl increments. The size of the well can affect the available upper limit. The default volume is 50 µl.

During the execution of a session, the total dispensing volume of each well is calculated by summing up all the dispensed volumes into a well. The execution of a session is prevented if the total dispensed volume exceeds 80% of the well volume in any well.

- **Dispensing Speed** - Select the speed at which the instrument dispenses the reagent into the wells.

The optimal speed depends on the viscosity of the dispensed solution. Too low a speed prevents adequate mixing of solutions in the well, or leaves the dispensed liquid as a droplet on the dispenser tip. Too high a speed can cause the solution to splash outside the well. However, too high a speed can also speed up the mixing of the dispensed liquid and the solution in the well.

- *Low* - The instrument dispenses at a speed that is 10% of the maximum speed.

- *Medium* - The instrument dispenses at a speed that is 18% of the maximum speed.
- *High* - The instrument dispenses at a speed that is 40% of the maximum speed.
- *User defined [%]* - You can define the exact speed as a percentage of the maximum speed. The speed range is 1 to 100 where 100 is the maximum speed and equal to the high dispensing speed.
- **Prime tip** - Select whether tip priming should be performed when necessary or not at all. Tip priming parameters are defined in the instrument setup, see “Dispensers” on page 234.
 - *Automatic* - Tip priming is performed if **Volume** < 50 µl or if over 1 min has elapsed from the previous dispensing (default). You will be informed if priming will be carried out.
 - *No* - Never perform tip priming.



Note Tip priming is a different procedure from manual priming that must be performed when a reagent bottle is installed into the dispenser and the dispenser tubes are completely empty.

- **Step duration [hh:mm:ss]** - Define the total time for the step. The software waits after the instrument has performed the action until the time is up before proceeding with the following step. Step duration is not available for kinetic loops.
- **Dispense at reading** - This selection is only available in Kinetic Loops. Use this if you want to specify that dispensing is started only after a certain number of readings. Select at which reading the dispensing will be performed. The possible values are from 1 to the number of readings defined for the kinetic loop, the default being 1.



Note The readings are numbered in the measurement results so that the reading where the dispensing is performed is reading no. 0 and the readings before it are given negative numbers. For example, if the *Dispense at reading* value is set to 5, the readings are numbered -4, -3, -2, -1, 0, 1, 2,...

Pause

The *Pause* step is used for stopping the execution of a protocol for a moment. A message is displayed during the pause if a message has been entered in the step parameters. The instrument continues to execute the session after the condition set in **Continuation options** is met.

Select Steps > **Pause** or click the  button on the action panel.

The pause step includes the following parameters:

- **Waiting time [hh:mm:ss]** - Enter the time.
- **Continuation options** - Select the condition for continuing the session execution. When *User action* is selected, no *Waiting time* can be set. When *Time expired* or *User action* is selected, the session execution continues after either of the conditions has been met.
- **Message** - Type in the message that appears on the screen during the execution of the *Pause step* (max. 50 characters).

Plate In

The *Plate In* step is used for running the plate into the instrument.

Select Steps > **Plate In** or click the  button on the action panel.

The step has no parameters.

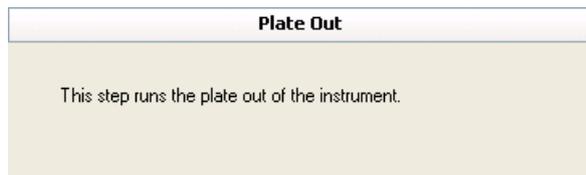
Plate Out

The *Plate out* step is used for running the plate out of the instrument.

Select Steps > **Plate Out** or click the  button on the action panel.

Step Parameters

Plate Out



The step has no parameters.

Chapter 8

Plate Layouts

A plate layout is used for telling the software how to handle the measurement results in the calculations according to the different sample types: unknowns, blanks, calibrators or standards, controls, and empty wells.

- *Unknown* samples are the samples under investigation.
- *Blanks* are used for blank subtraction.
- *Calibrators* are samples with known concentrations used for quantitative curve fit (see “Quantitative curve fit” on page 153).
- *Controls* are used for quality control (see “Quality control (QC)” on page 167) or for qualitative classification (see “Qualitative classification” on page 160).
- *Empty* wells are kept empty and unavailable for the filling process.

You must define a plate layout before you can execute a measurement. If you do not need a layout (if you only need to obtain raw measurement data), tick the *Fill layout with Unknowns* check box when creating a new session. Alternatively, you can also right-click on the plate layout and select **Fill Plate With Unknowns** from the menu (see “General operational procedure” on page 53).

Samples of the same type have the same functionality in the assay, but their concentration/dilution, name and other parameters may differ. Samples can consist of individual replicates, each occupying one well on a microplate.

A plate layout can consist of several plates.

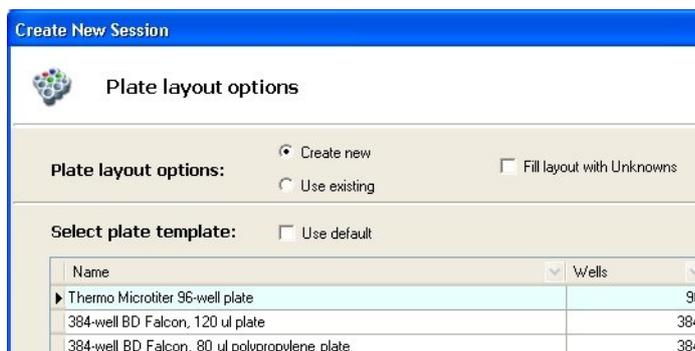
Working with plate layouts

When you have created a new session as described in “General operational procedure” on page 53, you can start creating a new plate layout. Click **Plate Layout** on the **Session** view to display the plate layout (Figure 8-1).

The plate layout is based on the selection made in the Create New Session wizard.

Plate Layouts

Working with plate layouts



- If the **Use existing** plate layout option was selected, you only need to ensure that the plate layout matches the actual plates. If it does not, you will have to edit it.
- If the **Create new** plate layout option was selected, the empty plate layout is based on the selected plate type. You can start editing it.
- If the **Fill layout with Unknowns** was selected, all wells in the plate are filled with unknown samples. You can edit the layout, if you want.

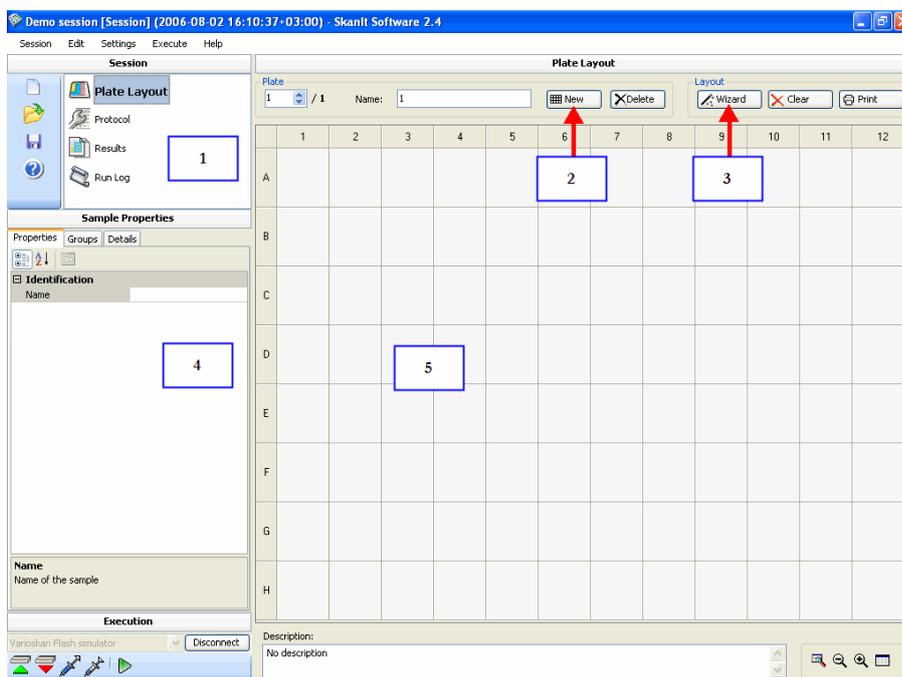


Figure 8-1. Plate layout editing window

- **1** - Plate layout is selected
- **2** - Several plates can be defined
- **3** - Samples are added with Fill Wizard

- 4 - Tabbed view of sample properties
- 5 - Editing panel showing the plate layout and well definitions

The Plate Layout panel includes the following functions:

- *Plate:*
 - **No.** - The plate number. Browse between different plates with the arrows.
 - **Name** - The name of the plate. By default, the number of the current plate is given as the name. It will be displayed as Plate 1, Plate 2, and so on.
 - **New** - Add a new empty plate to the plate layout.
 - **Delete** - Delete the selected plate from the plate layout. The plate may not contain any samples.
- *Layout*
 - **Wizard** - Launch the Fill Wizard for entering well information. See “Editing a plate layout” on page 127.
 - **Clear** - Delete the contents of the selected well(s).
 - **Print** - Print the current plate layout(s). See “Printing the plate layout” on page 140.
- **Description** - Enter a description of the plate layout (optional).

You can modify the way the plate is displayed using the plate view buttons:

 **Whole Plate** - View the entire plate.

 **Zoom Out** - The plate will appear smaller.

 **Zoom In** - Enlarge the wells.

 **Optimized View** - The well height and width are set to default, "best fit".

Editing a plate layout

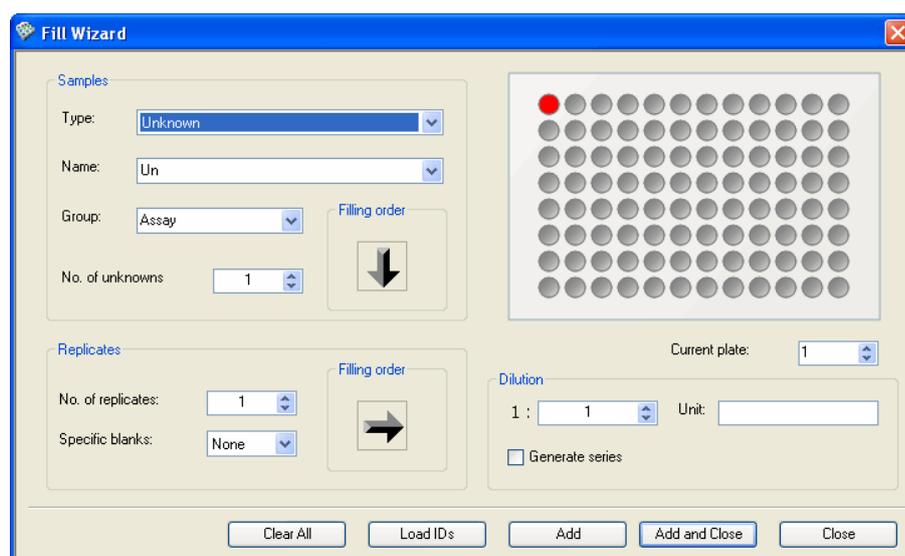
You can add samples to the plate layout in several ways. The best way is to use the **Fill Wizard** (see “Adding samples to the plate layout using Fill Wizard” on page 128 below), but you can also add samples using the Sample

Properties panel of the Plate Layout view (see “Editing sample properties and adding samples with Sample Properties” on page 134).

Adding samples to the plate layout using Fill Wizard

The **Fill Wizard** is used to provide each sample with a unique name and to assign them with a type (blank, calibrator, control, empty or unknown) so that they can be used in calculations. Click the **Fill Wizard** button.

The **Fill Wizard** dialog opens.

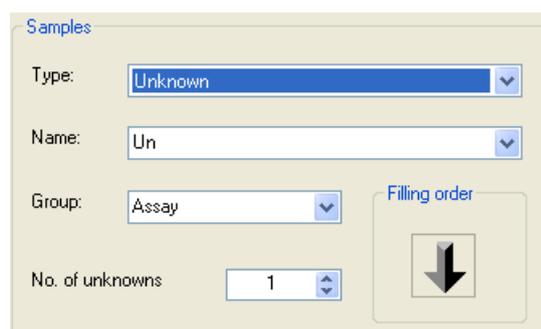


The dialog has the following selections:

Plate view:

The plate indicated by **Plate** is shown on the right and the wells filled will be shown in color. The well from which the filling will start is indicated by a bright red dot. You can change the well from which you start filling by clicking it. If you start filling from the right side of the plate, remember to change the **Filling order** arrows accordingly (see below).

Samples:



- **Type** - Select the type of sample from the drop-down list. The available types are *Blank*, *Calibrator*, *Control*, *Empty* and *Unknown*.



Note If you want to create several calibrators with the same concentration, it is recommended to create one calibrator and then create an appropriate number of replicates of the calibrator by using the **No. of replicates** setting. Otherwise the cubic spline as a fit type in the quantitative curve fit calculation will not process correctly.

- **Name** - The name is automatically suggested. The name is used in the calculations. If the value of No. of <samples> is more than one, the names are given running numbers, for example, Un_0001, Un_0002, and so on. You can change the name. You can also start the numbering from a number of your own choice by writing the name and an underscore (_) followed by the desired starting number, for example, Un_0100. The samples will be named Un_0100, Un_0101, etc.
- **Group** - The name of the sample group. The default group is *Assay*, but other groups may be defined by entering a new group name in the *Group* field. You can enter several groups for a sample in the field by separating the groups with commas.
- **No. of <samples>** - Enter the number of samples of the selected type. Not available for Blanks.
- **Filling order** - Select in which order the samples are added to the plate layout.

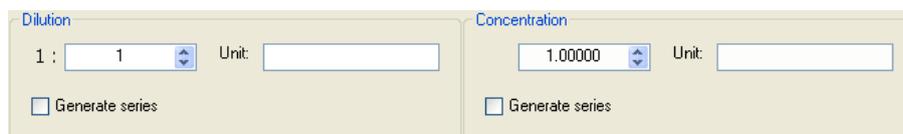
Replicates:

The screenshot shows a software interface with two main sections: 'Replicates' and 'Filling order'. Under 'Replicates', there are two dropdown menus: 'No. of replicates' is set to '1' and 'Specific blanks' is set to 'None'. To the right, under 'Filling order', there is a button with a right-pointing arrow.

- **No. of replicates** - Enter the number of replicates for each sample of the selected type. The number of replicates can vary from one (1) to the number of wells on the plate (i.e., 96 on a 96-well plate, and so on).
- **Filling order** - Select in which direction the replicates are added to the plate layout.
- **Specific blanks** - Select the number of specific blanks (individual sample blanks) in use: none, one or two. If you select one or two specific blanks,

a blank group with the same number of samples as in the target group is constructed. Each sample has its own corresponding blank sample from the blank group. The average of the blank sample replicates is calculated and then subtracted from the unknown samples on a sample-by-sample basis when blank subtraction is performed. Not available for Blanks or Empty wells.

Dilution/Concentration:



The screenshot shows two side-by-side input panels. The left panel is titled 'Dilution' and contains a numeric input field with the value '1', a 'Unit:' label followed by an empty text box, and a 'Generate series' checkbox. The right panel is titled 'Concentration' and contains a numeric input field with the value '1.00000', a 'Unit:' label followed by an empty text box, and a 'Generate series' checkbox.

- **Dilution/Concentration** - Enter the reagent volume of the first sample in the dilution or concentration series. *Dilution* is available for Unknowns, and *Concentration* for Controls and Calibrators.

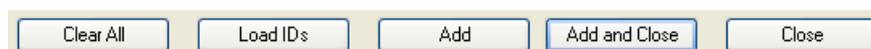
- **Unit** - Enter the unit for the dilution or concentration volume.



Tip In some keyboards, you can enter the character "µ" by pressing **AltGr+M**.

- **Generate series** - Tick the check box to generate concentration and dilution series. The **Concentrations** or **Dilutions** dialog opens (see “Adding a concentration or dilution series” on page 131). Not available for Blanks or Empty wells.

Buttons:



The screenshot shows a horizontal bar with five buttons: 'Clear All', 'Load IDs', 'Add', 'Add and Close', and 'Close'.

- **Clear All** - Clicking this button will clear all wells.
- **Load IDs** - You can load a text file (.txt) containing the IDs of the unknown samples. The unknowns will be named according to the IDs in the file. The file must only contain sample names (no spaces allowed) separated by line breaks (see Example 8-1, “Text file for defining names for unknown samples” below). The **Load IDs** button is only available for Unknowns.

Example 8-1 Text file for defining names for unknown samples

Each line in the text file represents one sample. No spaces are allowed and the sample names are separated by line breaks. The sample names must not begin with a number. For example, “A_100” is an acceptable sample name, whereas “100_A” is not.

```
DemoSample_A_151204
```

```
DemoSample_B_151204
```

```
DemoSample_C_151204
```

- **Add** - Adds the sample information to the wells.
- **Add and Close** - Adds the sample information to the wells and closes the Fill Wizard.
- **Close** - Closes the Fill Wizard without adding the sample information.

When you have entered all sample information to the plate, click **Close** to return to the main window. If all the samples did not fit into one plate, the software automatically adds new plates.

If you need several plates, click **New** in the **Plates** section and use **Fill Wizard** again.

If you have to delete a sample from the plate layout, select the sample and click **Clear**.

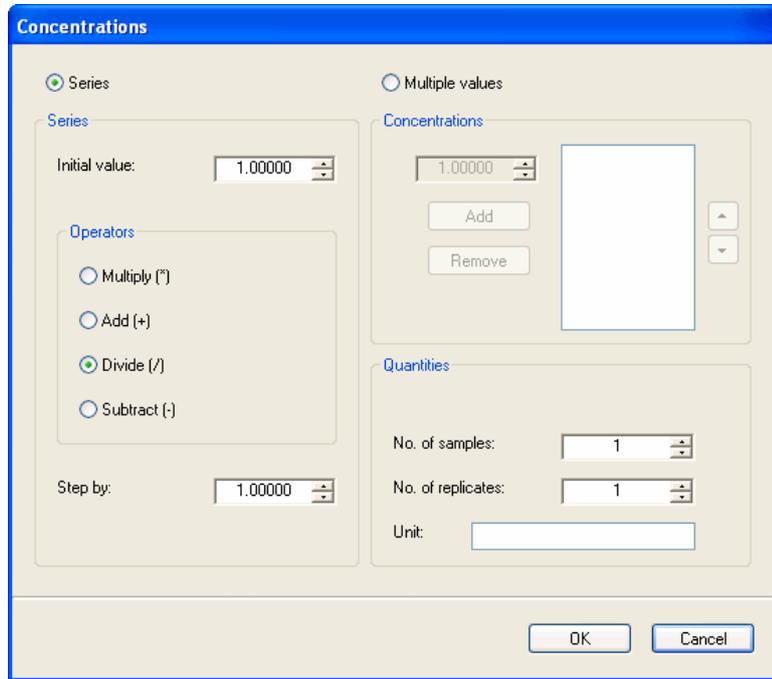


Note If all the samples and replicates do not fit in one plate, additional plates are automatically added.

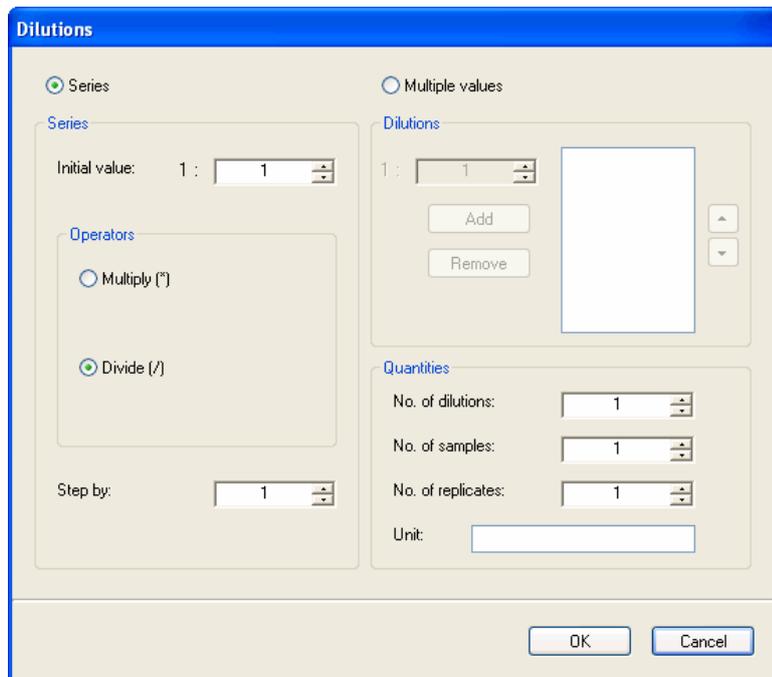
Adding a concentration or dilution series

You can add a concentration series for calibrators and controls and a dilution series for unknowns.

When you select *Generate series* in the **Fill Wizard** dialog and click **Concentrations** or **Dilutions**, the **Concentrations** or **Dilutions** dialog is launched.



See below for an explanation of the fields. Example 8-2, “Adding a concentration series” shows an example of adding automatically a concentration series.



Quantities

- **No. of dilutions** - Enter the number of dilutions (not with calibrators or controls).
- **No. of samples** - Enter the number of samples.
- **No. of replicates** - Enter the number of replicates.
- **Unit** - Enter the unit for concentration or dilution volume.

You can let the software calculate the dilution/concentration values by selecting *Series*, or enter individual values manually by selecting *Multiple values*. See Example 8-2, “Adding a concentration series” below.

If you select *Series*, the following parameters are available:

- **Initial value** - Enter the initial concentration or dilution value.
- **Operators** - Select the way in which the amount of reagent changes in the series: the factor given in the *Step By* box is applied to the consecutive concentration or dilution values according to the selection.
- **Step By** - Enter the factor for the concentration/dilution series.

The screenshot shows the 'Dilutions' dialog box. It has a title bar 'Dilutions' and two radio buttons: 'Series' (unselected) and 'Multiple values' (selected). Under 'Series', there is an 'Initial value' field with a spinner set to 1, an 'Operators' section with radio buttons for 'Multiply (*)' and 'Divide (/)' (selected), and a 'Step by' field with a spinner set to 1. Under 'Multiple values', there is a 'Dilutions' section with a spinner set to 1, 'Add' and 'Remove' buttons, and a 'Quantities' section with spinners for 'No. of dilutions' (0), 'No. of samples' (1), and 'No. of replicates' (1), plus a 'Unit' text field. 'OK' and 'Cancel' buttons are at the bottom.

If you select *Multiple Values*, the following parameters are available:

- **Dilutions/Concentrations** Enter each concentration or dilution value into the small box and click **Add**. The value is added to the larger box.

You can change the order of the values in the larger box with the arrow buttons on the right and remove values using the **Remove** button.

When you have entered the concentration or dilution series information, click **OK**. The software offers to rename the samples automatically.

The **Dilutions/Concentrations** dialog is closed and the information is added to the **Fill Wizard** dialog.

If the dilution or concentration information was entered incorrectly, the software prompts you to correct the data. You have to return to **Fill Wizard** and select **Generate series** again.

Example 8-2 Adding a concentration series

To enter the concentration series "1, 10, 100, 1000, 10000" with two replicates for each concentration, make the following selections in the **Concentrations** dialog:

- Select **Series**.
- **Initial value:** 1.00000
- **Operators:** Multiply (*)
- **Step By:** 10
- **No. of samples:** 5
- **No. of replicates:** 2

Click **OK**. Five calibrators with two replicates each are added to the **Fill Wizard** dialog.

Editing sample properties and adding samples with Sample Properties

You can edit the properties of the samples or define new samples using the **Sample Properties** panel.

Sample Properties	
Properties	Groups Details
<div style="border: 1px solid gray; padding: 2px;"> 🔍 📄 ⬇️ </div>	
Identification	
Name	Un_0002
Type	Unknown
Properties	
Dilution 1:	1
Dilution unit	
Group	Assay
<div style="border: 1px solid gray; padding: 2px;"> Name Name of the sample </div>	

The following parameters are found under the **Properties** tab:

Identification:

- **Name** - The name of the selected sample.
- **Type** - The type of the selected sample.

Properties:

- **Dilution/Concentration 1:** - The dilution or concentration of the current sample as a ratio of 1 to the value shown.
- **Dilution/Concentration unit** - Enter the unit for dilution or concentration values.



Tip On some keyboards, you can enter the character "μ" by pressing **AltGr+M**.

- **Group** - The group to which the selected sample belongs. You can set multiple groups for one sample by separating the group names with commas. You can also edit the group name. This lets you use, for example, one control or calibrator series in several assay groups.

Adding new samples to the plate layout

To add new samples to the plate layout:

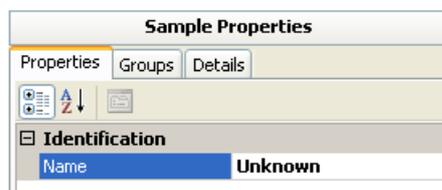
1. Select the well or wells you want to fill in the **Plate Layout** panel. You can paint several wells while keeping the right mouse key pressed down. Use the Ctrl key to select several areas or to select wells one by one.

Plate Layouts

Editing a plate layout

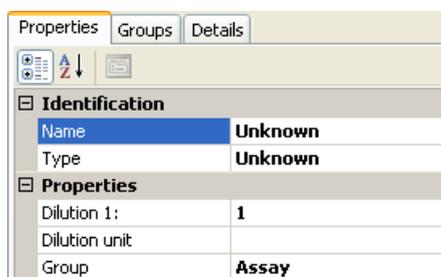
	1	2	3	4
A	Well D1			
B	Well D1			
C	Well D1			
D	Well D1			
E				

2. Give a name to the sample in the **Sample Properties** panel and press the Enter key or double-click the **Name** field.



The samples in all the selected wells are given the same name. When you execute the measurement, the sample names are appended with a fractional number. For example, if you have four unknown samples, they are named as *Unknown 1/4*, *Unknown 2/4*, *Unknown 3/4*, and *Unknown 4/4*. If you want to give samples unique names so that they can be used in calculations, it is recommended to use **Fill Wizard** (see “Adding samples to the plate layout using Fill Wizard” on page 128).

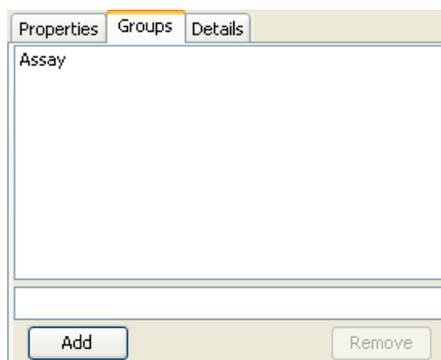
3. Enter the rest of the sample information in the fields. The same information is given to all the samples in the group.



Creating new groups for the samples

To define which groups the sample belongs to:

1. Select the well(s) with the sample in the **Plate Layout** panel. You only have to select one of the wells because all the replicates are given the same information.
2. Select the **Groups** tab in the **Sample Properties** panel.
3. Give a name to the new group in the lower field and click **Add**. The sample is added to the group. The Assay group name is the default.

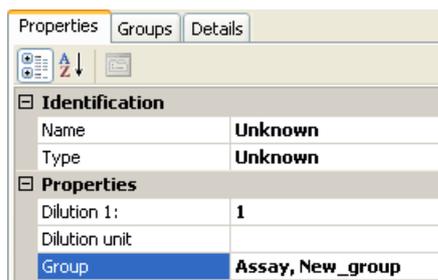


You can remove a group from the list by selecting it and clicking **Remove**.

Alternatively, to add new groups:

1. Select the well(s) with the sample in the **Plate Layout** panel. You only have to select one of the wells because all the replicates are given the same information.
2. Select the **Properties** tab in the **Sample Properties** panel.

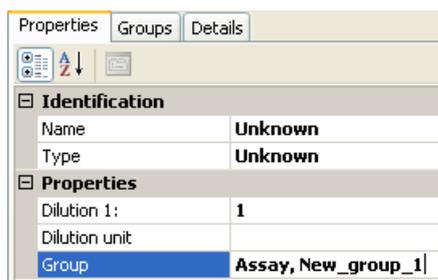
3. Enter the name of the new group in the **Group** field and press the Enter key. Separate the groups with commas. The name can only contain the following characters: a – z, 0 – 9, and an underscore.



Modifying group names

To modify the group name of the sample:

1. Select the well(s) with the sample in the **Plate Layout** panel. You only have to select one of the wells because all the replicates are given the same information.
2. Select the **Properties** tab in the **Sample Properties** panel.
3. Click on **Group** field and modify the group name. Press the Enter key.

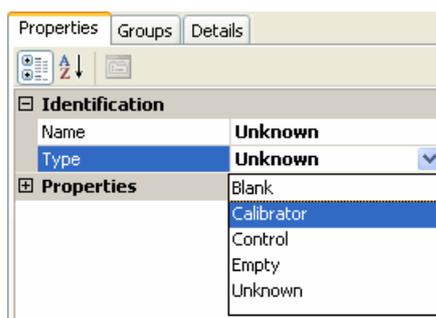


Changing sample types

To change the type of the sample:

1. Select the well(s) with the sample in the **Plate Layout** panel. You only have to select one of the wells because all the replicates are given the same information.
2. Select the **Properties** tab in the **Sample Properties** panel.

- Click on the **Type** field and select a new type for the sample from the drop-down menu. For example, to change an unknown sample to a calibrator, select *Calibrator*.



Adding additional information to replicates

To add additional information to replicates:

- Select the replicate(s) of the sample to which you want to add details in the **Plate Layout** panel. You can use the Ctrl key to select several replicates.
- Select the **Details** tab in the **Sample Properties** panel.
- Click **Add**. The **Add details to <n> replicate(s)** is opened.

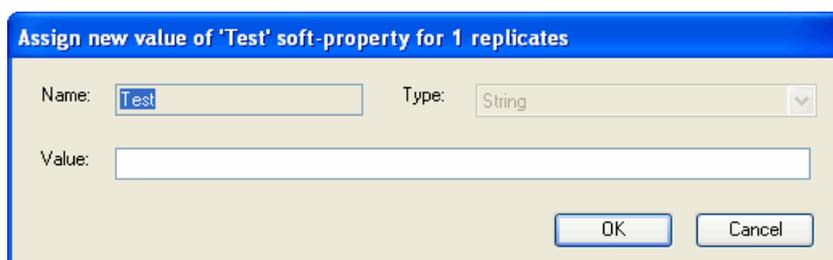


- Give a name to the property.
- Choose the type of value: *String*, *Integer*, or *Decimal*.
- Enter the value and click **OK**. The property is added to the list of sample details.

To edit the value of a property:

- Select the value of the property from the list of sample details.

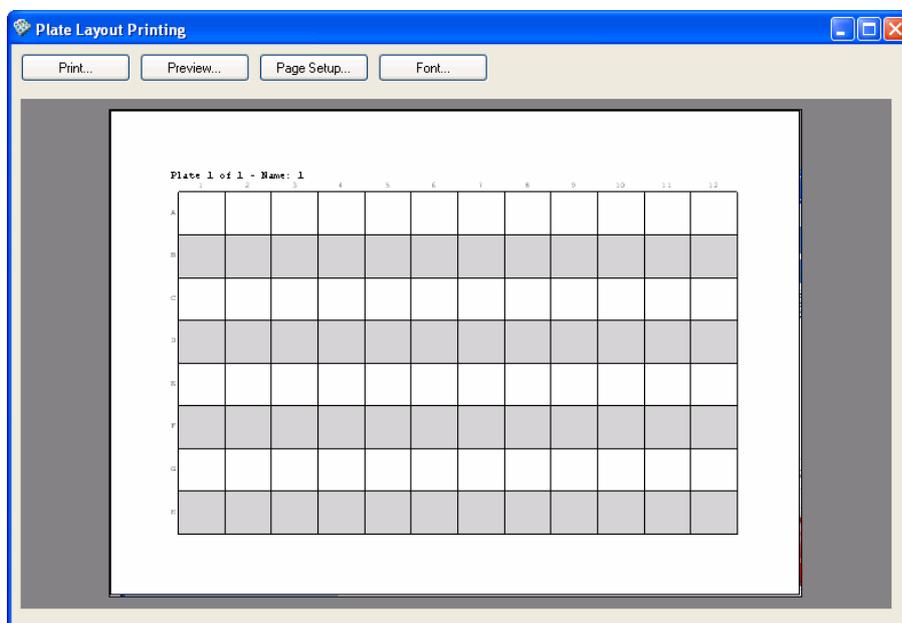
2. Click **Edit**. The **Assign new value of '<PropertyName>' soft-property for <n> replicates** dialog opens.



3. You can only edit the value. Click **OK**.

Printing the plate layout

You can print the plate layout of the currently open session by clicking **Print** in the main window. The **Plate Layout Printing** window is launched.



The Plate Layout Printing window has the following functions:

- **Print...** - The **Print** dialog is opened and you can print the page.
- **Preview...** - Select how many pages you want to view at a time. This selection will not affect the printing.
- **Page Setup...** - You can define paper size, paper source, orientation and margins for printing.

- **Font...** - Select the font and font size used in the plate layout.

Plate Layouts

Printing the plate layout

Chapter 9

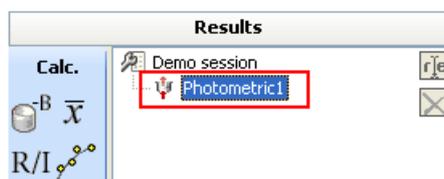
Calculations

Adding calculations

You can define calculations for each measurement result. Select **Results** from the **Session** action panel.



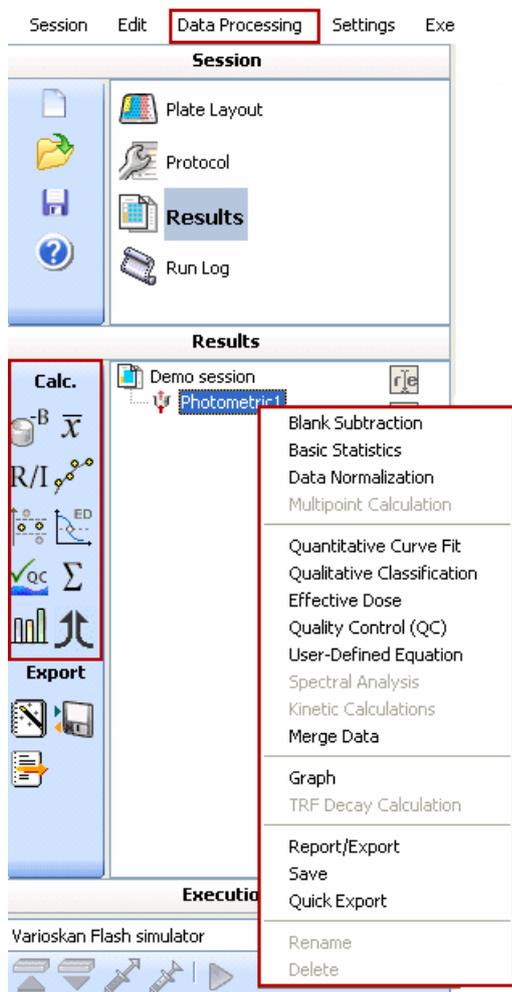
In the **Results** tree you will see the measurements that were defined in the **Steps** tree (see “Adding new protocol steps” on page 83).



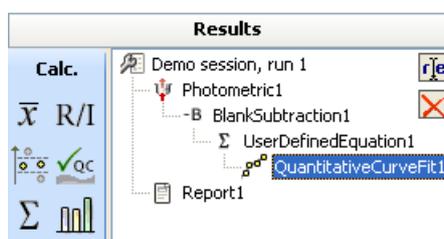
Select the measurement for which you want to define calculations. Select the calculation from the **Data Processing** menu, click the button of that calculation on the action panel or right-click the measurement in the **Results** tree.

Calculations

Adding calculations



The calculation appears in the **Results** tree below the measurement.



Each added calculation appears on a branch below the selected item. You can also right-click one of the items in the tree to display a menu of all calculations that can be added below that item. You can also select the calculation by clicking the available buttons or by using the **Data Processing** menu. Select the one you want to add.

The software allows you to add only certain calculation steps for different measurement types. Table 9-1 shows the required data reductions for different measurement types.

The software checks the validity of the calculation and the validity of the previous calculation step. If the calculation cannot be performed with the parameters set, the calculation step will be displayed in red. For example, in kinetic measurements, you must include a kinetic calculation step before you can add a qualitative classification calculation. If you have first defined the type as “Sum” in the kinetic calculation and then change the type to “Ignore reading”, the qualitative classification calculation will turn red because an additional kinetic calculation step is now required.

1. Select the measurement.
2. Add the data reduction step, when necessary.
3. Add the calculation. The calculation will appear as a branch below the data reduction step.

Table 9-1. Required data reductions for different measurement types

Measurement type	Required data reductions	Possible calculations without data reduction
Multipoint measurement	“Multipoint calculation” on page 152	“Basic statistics” on page 150, “Blank subtraction” on page 147
Spectral scanning measurement	“Spectral analysis” on page 174	“Blank subtraction” on page 147, “Graph” on page 198, “Basic statistics” on page 150
Kinetic measurement	“Kinetic calculations” on page 183	“Blank subtraction” on page 147, “User-defined equation” on page 169, “Graph” on page 198, “Basic statistics” on page 150
Measurement with multiple wavelengths	“User-defined equation” on page 169	“Basic statistics” on page 150, “Blank subtraction” on page 147

If you want to perform several independent calculations for the same measurement, select the measurement from the **Results** tree each time prior to adding the calculation.

Deleting calculations

You can remove calculations in the following ways:



Note If you delete a calculation that has other calculations added as a branch, those calculations will also be deleted.

- Select a calculation from the **Results** tree and right-click it. Select **Delete** from the menu that opens next to the calculation.
- Press the **Delete** button on your keyboard.
- Select Edit > **Delete** or click the button.

Calculations

Deleting calculations

Chapter 10

Data Processing Parameters

This chapter contains information describing all data processing (calculation) parameters.

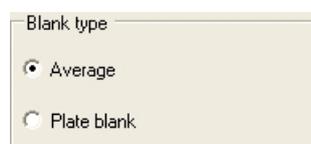
Blank subtraction

Blank subtraction is used for subtracting the mean blank value from all the wells in the layout. Specific blanks are subtracted from their own samples and no normal blank average is subtracted from samples with specific blanks.



Note When calculating the blank subtraction for dilutions series, the mean value of all the specific blanks of the series is calculated. The mean blank value is then subtracted from all the samples, replicates, and blanks in the series.

Select Data Processing > **Blank Subtraction**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.



- **Average** - The average of all non-specific blanks in an assay is calculated and subtracted from all the samples in the same assay (blanks, calibrators, controls and unknowns). The blanks can be on the same plate or on different plates, but they are all in the same assay and they are subtracted from all the samples belonging to the same assay. See Example 10-1, “Average blank subtraction”. Specific blanks are subtracted from their own samples.
- **Plate blank** - The average of all non-specific blanks available on each plate in each assay is calculated separately. The average of the blanks from each plate in each assay is then subtracted from the samples on each specific plate in the same assay only. See Example 10-2, “Plate blank subtraction”. Specific blanks are subtracted from their own samples.

Examples of blank subtraction

Blanks are positioned in the first well on both plates:

Plate 1

0.01					
0.3					
0.4					
0.5					

Plate 2

0.02					
0.35					
0.45					
0.55					

Example 10-1 Average blank subtraction

The blank average is: $(0.01 + 0.02)/2 = 0.015$

The blank subtracted data would then be (with an accuracy of three decimals):

Plate 1

-0.005					
0.285					
0.385					
0.485					

Plate 2

0.005					
0.335					
0.435					
0.535					

Example 10-2 Plate blank subtraction

The blank subtracted results calculated from the same raw data would be (with an accuracy of two decimals):

Plate 1

0.00					
0.29					
0.39					
0.49					

Plate 2

0.00					
0.33					
0.43					
0.53					

Blank subtraction in kinetic measurements

You can use all modes of blanking in kinetic measurements as well. Normally this means that the kinetic data is first reduced to rates, peak maximums, integrals, and so on by using kinetic calculations (see “Kinetic calculations” on page 183), and then the blank subtraction is performed to the reduced data.

Consider, for example, a kinetic measurement with a plate containing one blank, the rest being other samples, and an average rate calculation with linear regression. The rate is calculated separately for all samples. The reduced rate is then calculated by subtracting the rate of the blank sample from the rates of other samples.

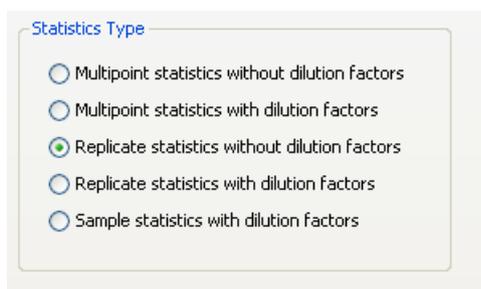
Alternatively, you may specify point-wise kinetic blank reduction directly from measurement data. In this mode, the raw blank signal at each time point is subtracted from raw sample signals at that specific time. This produces reduced curves for all samples. Only then is the kinetic processor applied to the reduced curves to determine the reduced rate, and so on.



Note If a certain rate cannot be determined, for example, increasing maximum rate if the whole reaction is decreasing, the software returns NaN (not a number).

Basic statistics

Select Data processing > **Basic Statistics**, click the \bar{x} button on the **Results** action panel, or right-click the measurement in the **Results** tree.



- **Multipoint statistics without dilution factors** – Calculates the statistics per well without combining data from different replicates or dilutions. It can be used to calculate the statistics within each well in case of multipoint measurements.

For example, a multipoint measurement with input data of eight dilutions and two replicates for each dilution results in eight dilutions with two replicates for a dilution. The average of the multipoint data is calculated and the SD and CV% of the values are calculated.

- **Multipoint statistics with dilution factors** – The same as **Multipoint statistics without dilution factors** except that the values are multiplied by dilutions before calculating the average
- **Replicate statistics without dilution factors** – Calculates the statistics per dilution without combining data from different dilutions. It combines data from different replicates of the dilution. It can be used to calculate the statistics within each dilution in case of dilution series.

For example, a multipoint measurement with input data of eight dilutions and two replicates for each dilution results in eight dilutions with one replicate for each dilution. The average of the multipoint and replicate data is calculated and the SD and CV% of the replicate values are calculated.

- **Replicate statistics with dilution factors** – The same as **Replicate statistics without dilution factors** except that the values are multiplied by dilutions before calculating the average.
- **Sample statistics with dilution factors** – Calculates the statistics per sample while combining data from different dilutions and replicates

into a single value. It can be used to calculate the statistics within each sample in case of dilution series.

For example, a multipoint measurement with input data of eight dilutions and two replicates for each dilution results in one value. The average of the multipoint, replicate and dilution data is calculated and the SD and CV% of the dilution values are calculated. The values are multiplied by dilutions before calculating the average.



Note If the dilution factors are already calculated, they are not calculated in subsequent basic statistics calculations.

The basic statistics calculates the average (Avg), the standard deviation (SD) and the coefficient of variation (CV%) of the replicates.

The SD is calculated using the following formula of variance:

$$s = \sqrt{\frac{\sum (x - m)^2}{n - 1}}$$

In the formula, x is the measured value from a single measurement point, m is the mean of the measured replicate values, and n is the number of replicates.

The average is displayed in both the table and list views and the SD and CV% only in the list view.

To compare how the basic statistics and the user-defined equation calculate the average, see the section called “Comparison of the calculation of average in User-Defined Equation and Basic Statistics”.

Data normalization

Select Data Processing > **Data Normalization**, click the **R/I** button on the **Results** action panel, or right-click the measurement in the **Results** tree.

- **Calculation** - Select either **Ratio** or **Inhibition**.

$$\text{Ratio} = \frac{B}{B_0} \times 100\%$$

$$\text{Inhibition} = 100\% - \frac{B}{B_0} \times 100\%$$

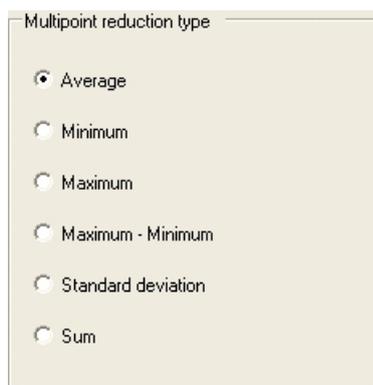
- **Source** - Select which sample is used as the B_0 and the group that contains that sample. The mean value of the selected sample (if replicates do exist) will be used in the calculation.

The B_0 can be any sample type (calibrator, control, blank or unknown), and you can define which specific sample should be used as B_0 , for example, Cal_5 or Unknown_56. The ratio or inhibition value is calculated for all the samples belonging to a certain group.

Multipoint calculation

Multipoint calculation is used to reduce multiple measurement points in each well to just one point per well (see “Using multipoint in a measurement” on page 114). Multipoint calculation must be performed before any other data reduction, except Basic Statistics or Blank Subtraction, is attempted. Multipoint calculation is only available for 6- to 48-well plates.

Select Data Processing > **Multipoint Calculation**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.

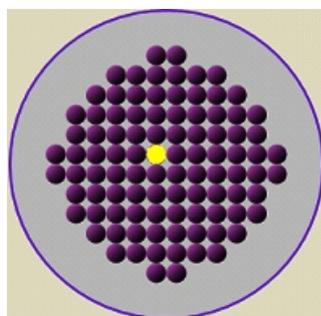


- **Average** - The average value of all the measurement points in each well.
- **Minimum** - The minimum value of all the measurement points in each well.
- **Maximum** - The maximum value of all the measurement points in each well.
- **Maximum - Minimum** - The minimum value of all the measurement points in each well is subtracted from the maximum value of all measurement points in the same well.
- **Standard deviation** - The standard deviation of all the measurement points in each well.
- **Sum** - The sum of all the measurement points in each well.

By default, the **Average** value is always shown in the raw data *Table* view if a multipoint measurement has been run. The separate measurement points for each well are shown in the data list. If another multipoint reduction calculation type is desired, this can be obtained by adding a *Multipoint* calculation step.

For a multipoint, kinetic measurement, the curve shown in the *Table* view is based on the middle point of each well. The middle point is calculated as the coordinate (number of points in the x direction/2, number of points in the y direction/2). The number is always rounded to the nearest integer (for example, $3.5 \approx 4$), and it is added from the upper-left corner.

See the example below:



(number of points in the x direction/2, number of points in the y direction/2) = (12/2, 12/2) = (6, 6)

Quantitative curve fit

Quantitative curve fit is used for quantitative analysis. It calculates the concentration of the samples based on a standard curve made from a series of calibrators with known concentrations.

Any dilutions defined for unknowns in the layout are taken into account in the calculations.



Note Curve fit requires that calibrators have been defined in the plate layout. The number of calibrators required for different fit types are given in Table 10-1.

Quantitative curve fit definitions

Select Data Processing > **Quantitative Curve Fit**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.

The screenshot shows the 'Source' and 'Extrapolation' sections of a software interface. The 'Source' section has two radio buttons: 'Calibrators from group:' (selected) and 'Calibrators from saved data:'. Below the first radio button are two dropdown menus: 'Assay: Calibrator' and 'Unknowns from group: Assay'. Below the second radio button is a dropdown menu for 'Wavelength: 450' and a 'Save data' button. The 'Extrapolation' section has an 'Enable' checkbox (unchecked), 'Min. concentration: 10', and 'Max. concentration: 160'. Below this are two panels: 'Fit type' with radio buttons for 'Linear regression (LLS)' (selected), 'Quadratic polynomial', 'Cubic polynomial', 'Quartic polynomial', 'Point to point', 'Cubic spline', 'Four parameter logistic', and 'Log-Logit'; and 'Transformation' with radio buttons for 'Conc. linear' (selected), 'Conc. logarithmic', 'Meas. linear', and 'Meas. logarithmic'. A 'Markers' section has radio buttons for 'Mean' (selected) and 'Replicates'.

- **Source** - Select the groups (assays) for both the calibrators and the unknowns used. The calibrators can either be calibrators or controls, depending on the plate layout. You can also select the calibrators from previously saved data (standard curve). Select the wavelength whose information is used.

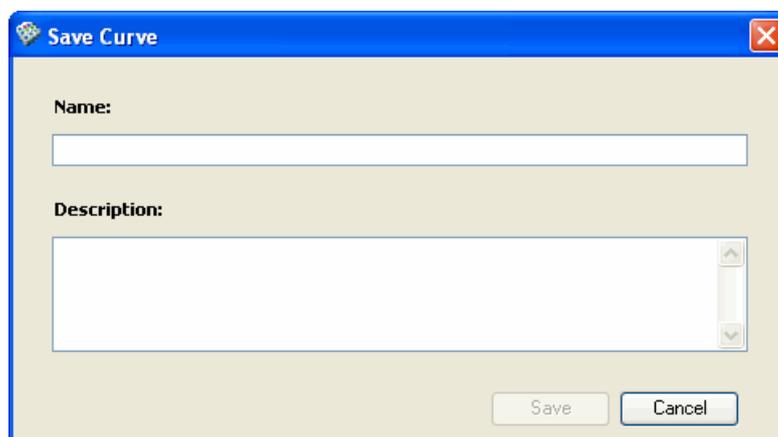


Note When you select a calibrator from the saved data, you can only select a standard curve saved from data of the same type of measurement technique. That is, if you performed a photometric measurement, you can select a standard curve saved from another photometric measurement.

To save the data as a new standard curve for use in other sessions:

1. Select the groups for calibrators and unknowns.
2. Select a correct wavelength. Only information related to the selected wavelength is saved.

3. Click **Save data**. Enter a name and description for the curve in the **Save Curve** dialog. For example, enter the wavelength as a description so that it is available for later use.



Click **Save**. For more information, see “Saved curves” on page 249.

- **Extrapolation** - Tick the check box if you want to enable extrapolation. Enter the minimum and maximum concentrations for the extrapolation. Extrapolation is not available for point-to-point or cubic spline fit types. Extrapolation is absolutely reliable only for the linear regression fit type, due to mathematical reasons.
- **Fit type** - Select the quantitative curve fit type from the list. The curve fit types are explained in “Polynomial fit types” on page 156 through “Four parameter logistic and Log-Logit” on page 158. The number of calibrators required for each curve fit type is shown in Table 10-1.

Table 10-1. Number of calibrators required in different curve fit types

Curve fit type	Minimum number of calibrators
Linear regression (LLS)	1 (3 is recommended - see the Note in “Polynomial fit types” on page 156)
Quadratic polynomial	3
Cubic polynomial	4
Quartic polynomial	5
Point to point	2
Cubic spline	3
Four parameter logistic	4
Log-Logit	4

- **Transformation** - Select the type of data transformation trailing. Transformation of statistical data means in brief a change of scale used

to improve the validity of statistical analyses. Logarithmic transformation is employed when data dynamics is considerable. This facilitates mathematical adaptations, that is, curve fitting.

The Conc. logarithmic transformation cannot be selected for the four-parameter logistic or Log-Logit fit types.



Note If the concentration range of the calibrators is more than four decades, logarithmic transformation should be used to obtain more precise curve fitting.

- **Markers** - Select whether the means of the measured calibrator replicates or individual measurements (replicates) are displayed in the curve. The replicates cannot be selected for point-to-point or cubic spline fit types.

Polynomial fit types

The *polynomial fit* function is determined as:

$$y = a + bx + cx^2 \dots mx^n, \text{ where}$$

- $n = 1, 2, 3$ or 4 ,
- $a, b, \dots, m =$ coefficients,
- $x =$ the concentration value, and
- $y =$ the measured signal.

Derived from the above equation:

- $y = a + bx$ for the Linear regression (LLS) fit type. The minimum number of calibrators is two.



Note If only one calibrator is used, then the second point is assumed to cross the origo (0,0).

It is recommended to use at least three (3) calibrators in order to raise the quality of the result.

- $y = a + bx + cx^2$ for the Quadratic polynomial fit type. The minimum number of calibrators is three.
- $y = a + bx + cx^2 + dx^3$ for the Cubic polynomial fit type. The minimum number of calibrators is four.
- $y = a + bx + cx^2 + dx^3 + ex^4$ for the Quartic polynomial fit type. The minimum number of calibrators is five.

Coefficients of the functions are solved with matrix calculations using Gauss-Jordan elimination with backsubstitution algorithm, except for the linear regression, where the linear least squares (LLS) method is used.

Roots of the polynomial, that is, “the results”, are calculated with an iterative Laquerre’s method. If more than one root is found in the calibration area, the software produces a warning.

Point to point

The point-to-point method connects the adjacent response-concentrations coordinates, that is, the calibration points, together using a straight line (see the equation below), which is different for each of the intervals. The results are calculated by first searching for the correct interval and then using the corresponding equation. The minimum number of calibrators for this fit type is two.

$$y_i = a_i + b_i x_i$$

Extrapolation is not available for the point-to-point fit type. Only the means of the measured calibrator replicates are used in the calculations. The replicates are visible in the graph but they are not used in calculations.

Cubic spline

This method is a smoothed point-to-point method where the adjacent calibration points are connected together using cubic polynomials (see the equation below) and optimizing the connecting points as smoothly as possible to avoid sharp angles. The results are calculated by first searching for the correct interval and then using a bisectional method to find the answer from the corresponding equation. The minimum number of calibrators for this fit type is three.



Note If you want to create several calibrators with the same concentration, it is recommended to create one calibrator and then create an appropriate number of replicates of the calibrator by using the **Fill Wizard** and the **No. of replicates** setting. Otherwise the cubic spline as a fit type in the quantitative curve fit calculation will not process correctly.

$$y_i = a_i + b_i x_i + c_i x_i^2 + d_i x_i^3$$

Extrapolation is not available for the cubic spline fit type. Only the means of the measured calibrator replicates can be used, not the individual replicate values.

Four parameter logistic and Log-Logit

The four-parameter logistic and Log-Logit logistic methods use the same fit model:

$$y = d + \frac{a - d}{1 + (x/c)^b}$$

In the equation above, y is the signal, x the concentration, a the minimum signal (asymptote below), d the maximum signal (asymptote above), c the concentration at the inflection point, and b a slope-related term at the inflection point.

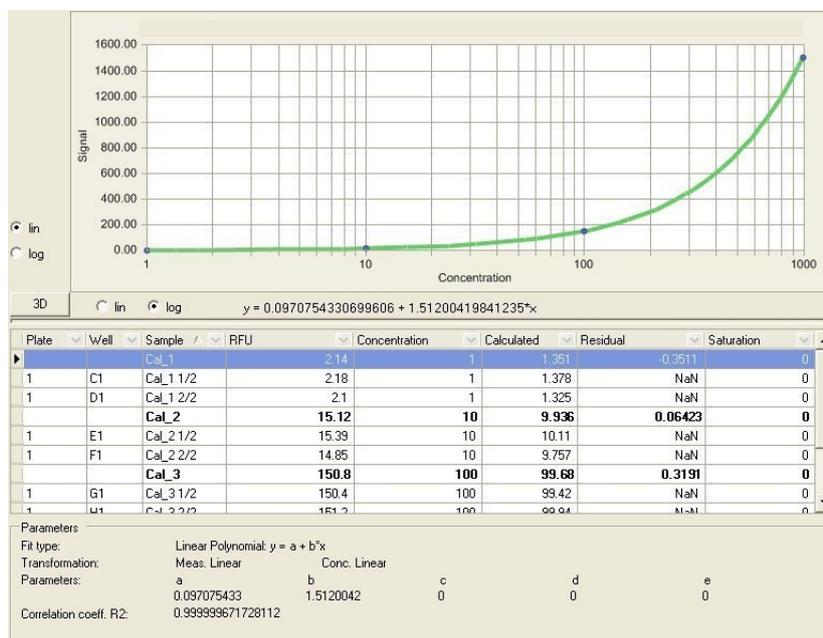
The minimum number of calibrators for these fit types is four.

- **Four-parameter logistic** fit type. The coefficients of the function are solved using the Levenberg-Marguardt method.
- **Log-Logit** fit type. The a and d coefficients are treated as known, otherwise it is the same as the four-parameter logistic (4PL) fit type.

The Conc. logarithmic transformation is not available for these fit types.

Quantitative Curve Fit results

The graph view of the calculation results displays the calculated fit function and its parameters as well as the correlation coefficient R^2 . The list below the graph shows the calibrators used for defining the curve fit. The column *Residual* shows the difference between the calculated and actual values.



The bolded results on the list are average values of all the replicates.

You can select whether to display the data on linear or logarithmic X- and Y-axes by ticking the corresponding *lin* and *log* radio buttons.

You can change the scale of the Y-axis. Right-click within the graph and select **Properties...** Select the *Y axis* tab and modify the *Minimum* and *Maximum* values.



Note If you want to remove a calibrator point or a replicate, you must disable it in the raw data. See the end of “Raw measurement data” on page 203.

Qualitative classification

In a qualitative classification analysis, the raw data is divided into separate categories. You can define limits as boundaries for the different categories (see Figure 10-1). There can be up to five limits in SkanIt Software, thereby dividing the data into a maximum of six different categories. The limits (boundaries) can be set as a number, a formula, a specified sample, or a formula containing both samples and numbers. The formulas can contain the operations +, -, / and *. The limits are defined as “less than” (<) at the lower limit of the category and “less or equal than” (<=) at the higher limit.

For example, if there are two limits (Limit for cat. 2 and Limit for cat. 3) and therefore three categories (Category 1, Category 2 and Category 3), the limits are as follows:

Category 1 < Limit for cat. 2 = Category 2

Category 2 < Limit for cat. 3 = Category 3

Any dilutions defined for samples in the layout are taken into account in the calculations. You can also select to ignore the dilutions in the calculations.

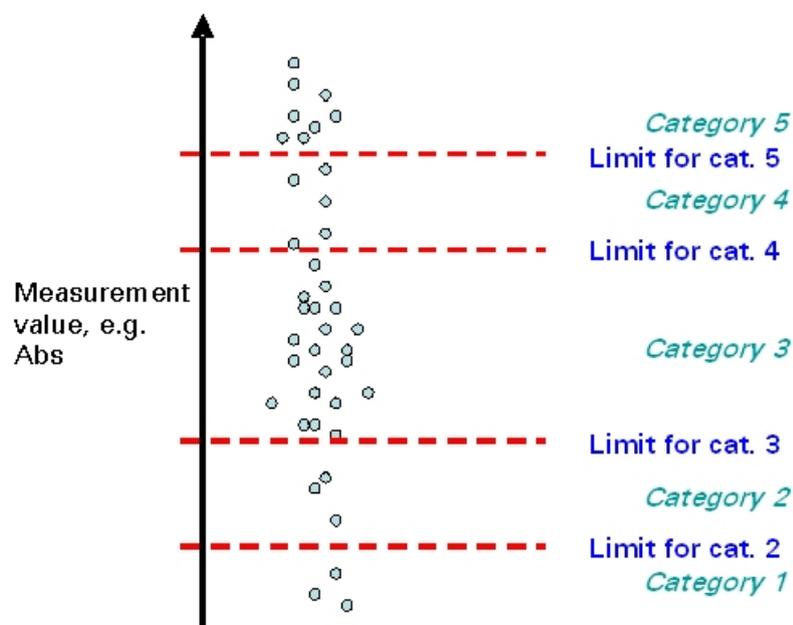


Figure 10-1. Dividing measurement results into categories using cut-off limits

Select Data Processing > **Qualitative Classification**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.

- **Source** - Select which group is used as the source data. If you select **Ignore dilutions**, the dilutions are not taken into account in the calculation.
- **Limit to category** - Select the number of categories that you want to have by ticking the *Limit to category* check box for each desired category starting from the bottom (see Figure 10-1). Those categories become available and **Qualitative Classification Limit Wizard** is launched.



- **Sample** - Select a sample or replicate to be used in the formula. The software allows you to select samples or replicates existing in the layout.
- **Values** - Enter the formula by using the number and operator buttons and the *Sample* box. The  button is the backspace button for deleting one character at a time, and the  button for clearing the entire formula.
- **Formula** - The formula is visible here in the way it will be shown in the **Limit to category** field.

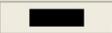
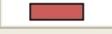
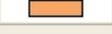
Click **OK** to accept the formula. The value or formula entered using the Limit Wizard is shown in the text box.

- **Limit Wizard** - The Limit Wizard button is available for the selected categories. Click the button to launch **Qualitative Classification Limit Wizard** to edit the Limit to category formula.



Note To obtain correct limit values, it is important to update formulas if the controls used in the respective formulas are removed, deleted or all replicates disabled.

- **Interpretation** - Enter a text string to describe each category. The text string, for example, “+” or “HighPos”, will be displayed for the Cut-Off result categories in the Table and List views.
- **Color** - Select a color for displaying each category value in the list view. Clicking the color field displays a small dialog for selecting the color.

Category 4	
<input type="checkbox"/> Limit to category:	Interpretation: Category4
<input type="text"/>	Color: 
<input type="button" value="Limit Wizard"/>	
Category 3	
<input checked="" type="checkbox"/> Limit to category:	Interpretation: Category3
<input type="text" value="AVG(Ca_0001)*2"/>	Color: 
<input type="button" value="Limit Wizard"/>	
Category 2	
<input checked="" type="checkbox"/> Limit to category:	Interpretation: Category2
<input type="text" value="AVG(Ur_0001)/2"/>	Color: 
<input type="button" value="Limit Wizard"/>	
Category 1	Source
Interpretation: Category1	Group: Assay
Color: 	<input type="checkbox"/> Ignore dilutions

The result from the qualitative classification calculation is shown as the category text strings in the results list and table views.

Effective dose

The ED₅₀ value is a concentration value where 50% of the followed activity of the sample has been lost. Typically, the followed activity is cell death or cell survival or certain enzymatic activity. This value is used to demonstrate the efficiency (such as toxicity) of the sample. In addition to ED₅₀, other ED values are also counted, typically ED₂₀ and ED₈₀. The ED₂₀ value is a concentration where 20% of the activity is lost, and similarly, ED₈₀ is the value where 80% has been lost. An example of the ED determination is shown in Figure 10-2.

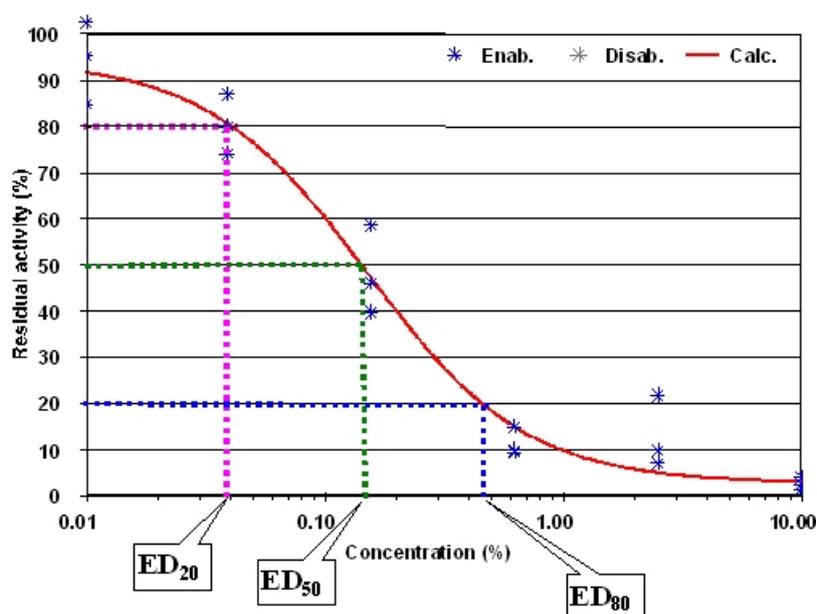


Figure 10-2. Effective dose (ED) determination

The curve values are defined according to the following equations:

$$ED_{100} = \frac{\text{Sample}}{X} \times 100\%$$

$$ED_0 = 100\% - \frac{\text{Sample}}{X} \times 100\%$$

where X is the sample selected as the ED₁₀₀ (or the sample giving the maximum signal).

When the ED value determination has been performed, the software also automatically generates the graphics presenting the results. All results (curves and ED values) are collected into one graph from the selected assay. The calculated ED values are also shown in a list.

Select Data Processing > **Effective Dose**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree. Select the Parameters tab to edit the calculation parameters.

The screenshot shows a software interface for setting data processing parameters. It is divided into several sections:

- ED:** Radio buttons for 'ED0' (selected) and 'ED100'.
- Use series:** Radio buttons for 'Dilution' (selected) and 'Concentration'.
- Reference value:** A checked box for 'Use maximum value', a 'Wavelength' dropdown set to '470', a 'Group' dropdown set to 'Assay', and a 'Sample' dropdown set to 'Un_0001 A1 (1)'.
- ED values:** Three checked boxes with numerical values: '1: 20', '2: 50', and '3: 80'.
- Fit type:** Radio buttons for 'Linear regression (LLS)', 'Quadratic polynomial', 'Cubic polynomial', 'Quartic polynomial', 'Point to point', 'Cubic spline' (selected), 'Four parameter logistic', and 'Log-Logit'.
- Transformation:** Two groups of radio buttons. The first group has 'Dil./Conc. linear' (selected) and 'Dil./Conc. logarithmic'. The second group has 'Meas. linear' (selected) and 'Meas. logarithmic'.
- Markers:** Radio buttons for 'Mean' (selected) and 'Replicates'.

- **ED** - Select whether you want an inhibition curve (ED0) or an activity curve (ED100).
- **Use series** - Select whether you want to base the effective dose calculation on Dilution series or on Concentration series.

If you select the **Dilution** series, the *Reference value* selection can be made from Controls and Unknowns. If you select the **Concentration** series, the *Reference value* selection can be made from Controls and Calibrators.

- **Reference value** - Select a sample or a replicate, which acts as the ED₁₀₀ or ED₀ value.
- **Wavelength** - Select a desired wavelength for the calculation if multiple wavelengths were measured.
- **Group** - Select the groups (assays) for both the controls and the unknowns used. The calculation is done for all unknown series found in the selected assay.
- **Sample** - Select the individual sample.

When **Dilution** is selected, you can select a Control or an Unknown sample.

If you select a *Control*, the same graph will display all the dilution series. If you select an *Unknown*, the graph will only display the dilution series of that particular sample.

When **Concentration** is selected, you can select a Control or a Calibrator.

If you select a *Control*, the same graph will display all the concentration series. If you select a *Calibrator*, the graph will only display the concentration series of the series where that particular calibrator belongs.

- **Use maximum value** - You can also select a maximum value to be used as the ED₁₀₀ value, which means that the software selects the replicate with the highest measured value from the selected assay to be the ED₁₀₀ or ED₀ value.
- **ED values** - Up to three different ED values can be calculated within one calculation. The default values are ED₂₀, ED₅₀ and ED₈₀, but you can set any three ED values between 0 and 100.



Note All other selections are equal to quantitative curve fit selections. In contrast to the Quantitative curve fit function, the ED determination does not allow extrapolation of the data. All calculated ED values are between the lowest and highest dilution given in the source data.

- **Fit type** - ED values are calculated based on the function generated by the quantitative curve fit. With this function, the concentration/dilution values corresponding to the required ED values are solved and listed as results. Select the curve fit type from the list. The default fit type is Cubic spline. The curve fit types are explained in “Quantitative curve fit” on page 153.
- **Transformation** - Select the type of data transformation trailing. Transformation of statistical data means in brief a change of scale used to improve the validity of statistical analyses. Logarithmic transformation is employed when data dynamics is considerable. This facilitates mathematical adaptations, that is, curve fitting.



Note If the concentration range of the calibrators is more than four decades, logarithmic transformation should be used to obtain more precise curve fitting.

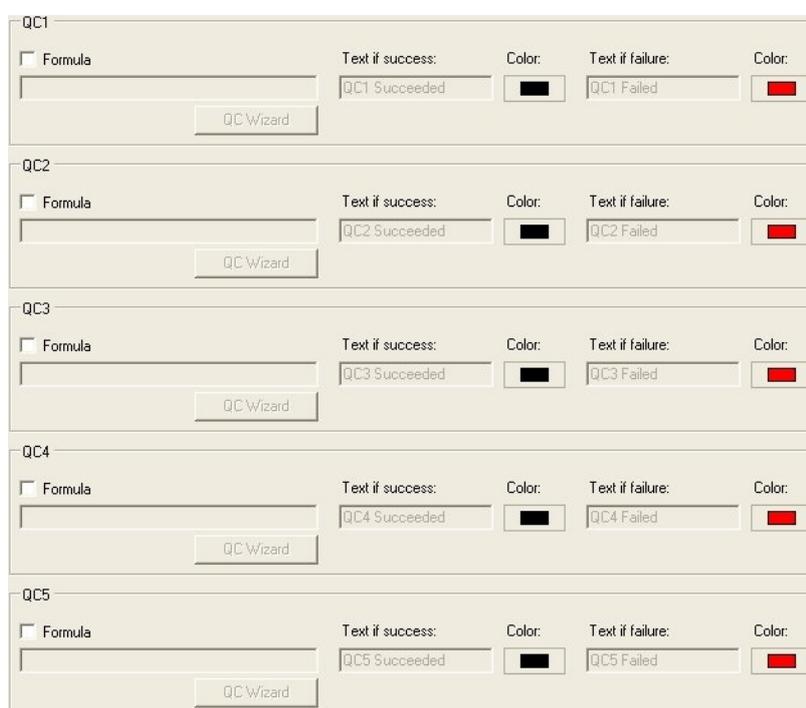
- **Markers** - Select whether the means of the measured calibrator replicates or individual measurements (replicates) are displayed in the curve.

Quality control (QC)

You can select up to five (5) quality control checks with one (1) quality control calculation step. You can name succeeded and failed quality control checks and define the color in which they are displayed.

Any dilutions defined for unknowns in the layout are taken into account in the calculation.

Select Data Processing > **Quality Control (QC)**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.



The screenshot displays a configuration window for five quality control checks, labeled QC1 through QC5. Each check configuration includes:

- A checkbox labeled 'Formula'.
- A text input field for the formula, with a 'QC Wizard' button below it.
- A 'Text if success:' field with a color selection button (black).
- A 'Text if failure:' field with a color selection button (red).

For QC1, the success text is 'QC1 Succeeded' and the failure text is 'QC1 Failed'. Similar configurations are shown for QC2, QC3, QC4, and QC5.

- **QC[number]** - Select the number of quality control checks that you want to have by ticking the check box for each desired check. The selected QC check becomes available and the **QC Wizard** is launched (see “Creating formulas for Quality Control (QC)” on page 168).
- **Formula** - The value or formula entered using the **QC Wizard** is shown in this text box.
- **QC Wizard** - Click the button to launch the QC Wizard and edit the QC formula.
- **Text if success** - Enter a text string for each passed quality control check. The text string, for example, QC1 Succeeded, will be displayed for the quality control check value in the List view.

- **Text if failure** - Enter a text string for each failed quality control check. The text string, for example, QC1 Failed, will be displayed for the quality control check value in the List view.
- **Color** - Select a color for displaying each quality control check result.

The screenshot shows three instances of the QC Wizard dialog box, each for a different quality control check (QC3, QC4, and QC5). Each instance has a 'Formula' checkbox checked and a 'QC Wizard' button. The fields are as follows:

QC	Formula	Text if success	Color	Text if failure	Color
QC3	AVG(Blank_Assay)<=0.2	QC3 Succeeded	Black	QC3 Failed	Red
QC4	(AVG(Cal_0002)/AVG(Cal_0003))>2	QC4 Succeeded	Black	QC4 Failed	Red
QC5	AVG(Cal_0001)>=0.8	QC5 Succeeded	Black	QC5 Failed	Red

The result from the quality control check is shown as the QC text strings in the results list.

Creating formulas for Quality Control (QC)

You can create formulas for quality control checks using the **QC Wizard**. The wizard is launched when you select a quality control check for the *Quality Control* calculation (see “Quality control (QC)” on page 167).

The screenshot shows the QC Wizard dialog box with the 'Parameters' and 'Values' sections. The 'Parameters' section has 'Groups' and 'Samples' dropdown menus. The 'Values' section has a numeric keypad with buttons for digits 0-9, operators (+, -, *, /), and comparison operators (<, >, >=, <=). There are also buttons for parentheses, a red 'C' button, and a 'Lambda' symbol. A 'Formula' text box is located below the keypad. 'OK' and 'Cancel' buttons are at the bottom.

- **Groups** - Select which assay is used as the source data. The list includes all the available assays in the plate layout.

- **Samples** - Select a sample to be used in the formula. The list includes all the available sample names and replicate names with replicate IDs within the selected group.
- **Values** - Enter the formula by using the number and operator buttons and the *Groups* and *Samples* boxes. The  button is the backspace button for deleting one character at a time, and the  button is for clearing the entire formula.
- **Formula** - The formula is visible here in the way it will be shown in the *Formula* field.

Click **OK** to accept the formula. The software attempts to verify that the created formula is valid.



Note The software only checks the validity of the formula, not the validity of the samples. Since it is possible to measure different areas from the layout with different wavelengths, different number of points in each well and different number of loops in kinetic loops, the actual result from the calculation may be invalid.

User-defined equation

The user-defined equation allows you to define basic calculations to calculate data from different measurements, calculations or the same measurement with a different wavelength. It is used, for example, to reduce data from dual measurements or to calculate basic calculations, such as the purity of DNA, using for instance $50 * (\text{the absorbance at } 260 \text{ nm})$.

In a kinetic measurement, the calculation is made for each reading.



Note The user-defined equation is always saved with the protocol. It is not possible to make a user-defined equation in one protocol and use the same user-defined equation in another protocol.

If you want to be able to use all the measurement steps and data processing steps in the user-defined equation, place the *User-Defined Equation* step on the root level (that is, select the session name from the **Results** tree and add the *User-Defined Equation* step). If you want to use only the data from one measurement or data processing step, select that step from the **Results** tree.

To add a user-defined equation step, select Data Processing > **User-Defined Equation**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.

Data Processing Parameters

User-defined equation

The screenshot shows a software interface for defining a user equation. It is divided into several sections:

- Data Source:** A vertical stack of five dropdown menus labeled 'Step:', 'Wavelength:', 'Group:', 'Sample:', and 'Column:'.
- Values:** A calculator-style keypad with buttons for digits 0-9, a decimal point, a negative sign, and various mathematical functions: ABS, SQRT, SUM, MAX, MIN, LOG, LN, EXP, AVG, SD, and CV. A red 'C' button is at the top.
- Formula:** A checkbox labeled 'With dilutions' followed by a large, empty text area for entering the formula.
- Notes:** A text area for additional notes.

- **Step** - Select the measurement raw data or data processing step that contains the data you want to include. The list includes all the available measurements and data processing steps.
- **Wavelength** - Select the wavelength to be used in the formula. If only one wavelength has been used in the measurement, you must select that wavelength.
- **Group** - Select the group name.
- **Sample** - Select the sample or replicate. If this selection is left empty, all the samples from the selected step will be used.
- **Column** - Select the data type. This selection is step-dependent.
- **Values** - Add numbers and calculation types to the formula. For more information about the available functions, see the section called “Functions in user-defined equation”.

When adding a function, click first the appropriate function button, then select the parameter(s) and close the parenthesis with the  button.



Note *User-Defined Equation* calculates the SD, CV and AVG functions differently from *Basic Statistics*. The user-defined equation calculates the average, SD or CV of all replicates and dilutions of the sample. The basic statistics separates the dilutions and only calculates the averages, SD or CV values of their respective replicates. See the example at the end of this section.

The  button is the backspace button for deleting one character at a time, and the  button is for clearing the entire formula.

- **With dilutions** - Select if dilutions for unknowns should be taken into account in the data processing. If dilutions have already been used (for example, in a previous curve fit calculation), they will not be calculated again.
- **Formula** - The entered formula is visible here.
- **Notes** - You can add an optional description.

The software verifies that the created formula is valid.



Note The different variables are assumed to have an equal amount of measurements. If there is not an equal amount of measurements in different variables, the data processing result is invalid.



Note The software only checks the validity of the formula, not the validity of the measurements. Since it is possible to measure different areas from the layout with different measurement steps, different number of points in each well and different number of loops in kinetic loops, the actual result from the data processing may be invalid.

Functions in user-defined equation

This section describes the functions you can use in the formula of a user-defined equation. After selecting a function, define the data source parameters: step, wavelength, group, sample, and column. The effect of group and sample combinations are described for each function.

- **ABS** - Calculates the absolute values of negative values in the results. To remove every negative value from the data, select *[all groups in session]* from the **Group** menu and *[all samples in group]* from the **Sample** menu. To remove negative values of a specific group, select the group from the **Group** menu and *[all samples in group]* from the **Sample** menu.
- **SQRT** - Calculates the square root of the selected results. To calculate square root from the data, select *[all groups in session]* from the **Group** menu and *[all samples in group]* from the **Sample** menu. To calculate

square root of a specific group, select the group from the **Group** menu and *[all samples in group]* from the **Sample** menu.

- **SUM** - Calculates the sum of all the samples of the selected group. Select a specific group or *[all groups in session]* from the **Group** menu and *[all samples in group]* from the **Sample** menu. The calculation returns the sum of all the sample values of the selected group(s). If you select a specific sample from the **Sample** menu, the sample value or the replicate value is returned.
- **MAX** - Searches the samples of the selected group(s) for their maximum value. Select *[all groups in session]* from the **Group** menu to search all the samples in the groups. Alternatively, select a specific group from the **Group** menu to search all the samples in the group. Then select *[all samples in group]* from the **Sample** menu. If you select a specific sample from the **Sample** menu, the sample value or the maximum replicate value is returned.
- **MIN** - Searches the samples of the selected group(s) for their minimum value. Select *[all groups in session]* from the **Group** menu to search all the samples in the groups. Alternatively, select a specific group from the **Group** menu to search all the samples in the group. Then select *[all samples in group]* from the **Sample** menu. If you select a specific sample from the **Sample** menu, the sample value or the minimum replicate value is returned.
- **LOG** - Calculates the 10-based logarithm of the selected sample. Select a specific group from the **Group** menu and a single sample from the **Sample** menu. The calculation returns the 10-based logarithm of the sample. If you select *[all samples in group]* from the **Sample** menu, the calculation returns the value of the sample in each well separately.
- **LN** - Calculates the natural algorithm of the selected sample. Select a specific group from the **Group** menu and a single sample from the **Sample** menu. The calculation returns the natural logarithm of the sample. If you select *[all samples in group]* from the **Sample** menu, the calculation returns the value of the sample in each well separately.
- **EXP** - Raises e to the selected power. Select a specific group from the **Group** menu and a single sample from the **Sample** menu. The calculation returns the value of the sample raised to the selected power. If you select *[all samples in group]* from the **Sample** menu, the calculation returns the value of the sample in each well separately.
- **AVG** - Calculates the average of all the samples in the selected group(s). Select either *[all groups in session]* or a specific group from the **Group** menu and *[all samples in group]* from the **Sample** menu. The calculation returns the average of all the samples either in the session or in the

selected group. If you select a specific sample from the **Sample** menu, the sample value or the replicate value is returned.

- **SD** - Calculates the standard deviation of the samples in the selected group(s). Select either *[all groups in session]* or a specific group from the **Group** menu and *[all samples in group]* from the **Sample** menu. The calculation returns the standard deviation. If you select a specific sample from the **Sample** menu, the sample value or the replicate value is returned.

The formula of variance is used in calculating the SD, refer to “Basic statistics” on page 150.

- **CV** - Calculates the coefficient of variation of the samples in the selected group(s). Select either *[all groups in session]* or a specific group from the **Group** menu and *[all samples in group]* from the **Sample** menu. The calculation returns the coefficient of deviation as a percentage. If you select a specific sample from the **Sample** menu, the sample value or the replicate value is returned.

Comparison of the calculation of average in User-Defined Equation and Basic Statistics

User-Defined Equation calculates the SD, CV and AVG functions differently from *Basic Statistics*. The user-defined equation calculates the average, SD or CV of all replicates and dilutions of the sample. The basic statistics separates the dilutions and only calculates the averages, SD or CV values of their respective replicates.

This example demonstrates the difference when calculating the average (AVG). The sample has two dilutions with three replicates each.

1:1	Un_0001_01 1/3 1.432	1:2	Un_0001_02 1/3 0.723
1:1	Un_0001_01 2/3 1.234	1:2	Un_0001_02 2/3 0.711
1:1	Un_0001_01 3/3 1.322	1:2	Un_0001_02 3/3 0.692

The basic statistics separates the dilutions and only calculates the averages of their respective replicates when the **Combine dilutions** option is not selected. The dilutions are not taken into account.

1:1	Un_0001_01 1/3 1.329	1:2	Un_0001_02 1/3 0.709
-----	-------------------------	-----	-------------------------

Both the user-defined equation and the basic statistics with the **Combine dilutions** option selected calculate the average of all replicates and dilutions of the sample. The dilutions are taken into account. All the dilutions of a sample are multiplied by the dilution factor and the average is calculated.

Un_0001_01 1/3
1.019

Spectral analysis

The spectral analysis calculation is used for handling spectral scanning measurement data. The spectral analysis calculation assumes that there is only one measurement point for each wavelength in a well. In multipoint measurements, the data must be reduced to only one measurement value per wavelength before spectral analysis can be performed.

The wavelength range in spectral reduction is from 200 to 1,000 nm.

Select Data Processing > **Spectral Analysis**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.

Calculation type	Parameters
<input checked="" type="radio"/> Spectral peak search	Wavelength 1: <input type="text" value="200"/>
<input type="radio"/> Spectral maximum	Wavelength 2: <input type="text" value="1000"/>
<input type="radio"/> Spectral minimum	Threshold: <input type="text" value="0.000"/>
<input type="radio"/> HBW calculation	Window (1..3): <input type="text" value="1"/>
<input type="radio"/> Ratio within spectrum	
<input type="radio"/> Ratio between spectra	
<input type="radio"/> Select wavelengths	

Spectral peak search

The spectral peak search seeks all peak wavelengths with an intensity higher than the given threshold value. You can also define a wavelength area for which this calculation is performed (Figure 10-3).

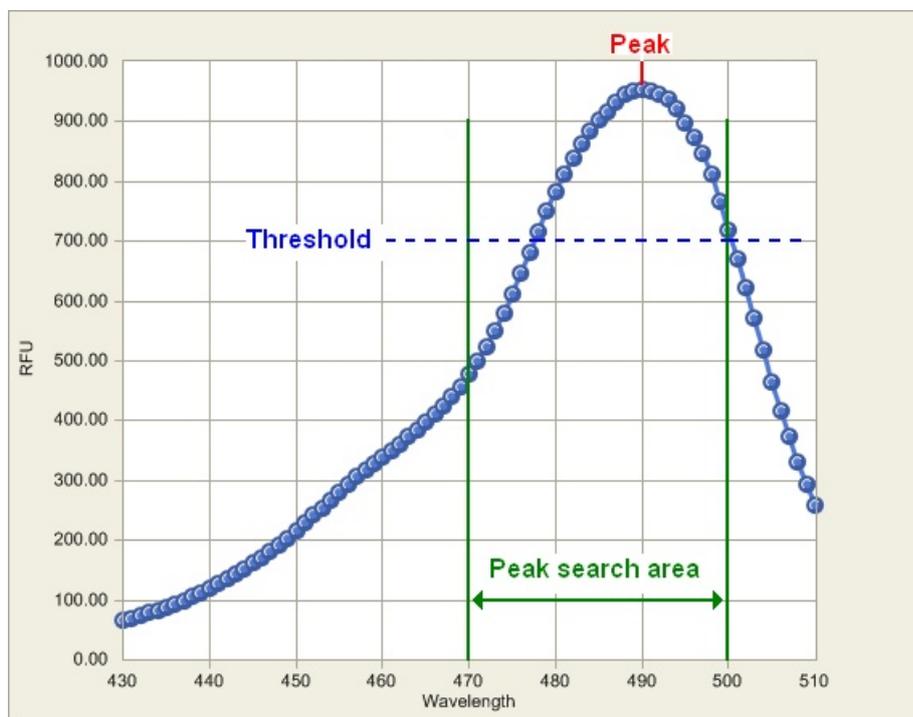


Figure 10-3. Spectral peak search: threshold and wavelength area

A peak is defined by comparing the window count of adjacent measurement values to a possible peak value. If the window count of values before and after a possible peak value is smaller than the possible peak value, then the possible peak value is a true peak value (Figure 10-4 and Figure 10-5).

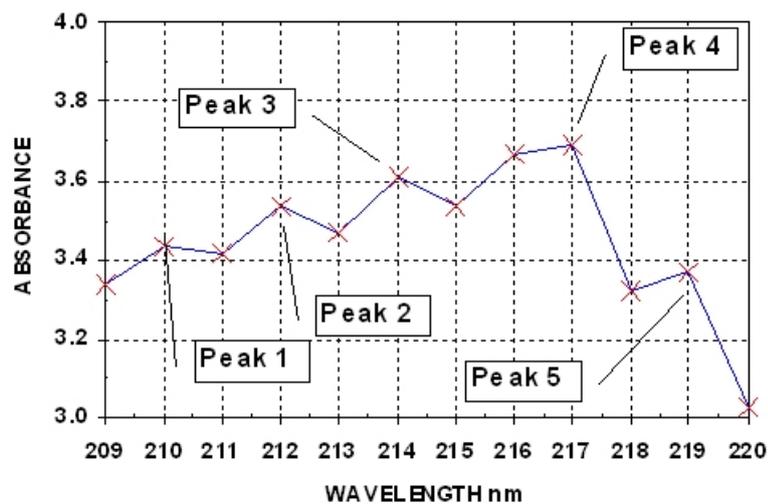


Figure 10-4. Spectral peak search with window count 1

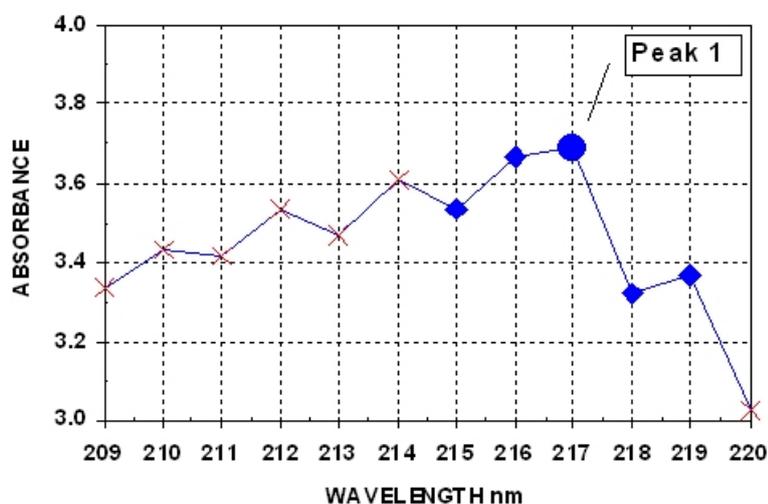


Figure 10-5. Spectral peak search with window count 2

Wavelength 1:	<input type="text" value="200"/>
Wavelength 2:	<input type="text" value="1000"/>
Threshold:	<input type="text" value="0.000"/>
Window (1..3):	<input type="text" value="1"/>

- **Wavelength 1** - Select the starting wavelength for the peak search area.
- **Wavelength 2** - Select the end wavelength for the peak search area.



Note User-selectable wavelength values are not checked against real measured values. Therefore, you may select wavelength values that are not even measured.

- **Threshold** - Enter the intensity threshold that the peak is required to exceed. The minimum value is 0.000 units and the maximum is 10,000,000.
- **Window (1...3)** - See Figure 10-4 and Figure 10-5.

Spectral maximum

The spectral maximum seeks the maximum measurement value [Abs] in each well. You can define a wavelength area for which this calculation is performed. It is also possible to receive the maximum without defining the wavelength area or the threshold.

Wavelength 1:	<input type="text" value="200"/>
Wavelength 2:	<input type="text" value="1000"/>
Threshold:	<input type="text" value="0.000"/>

- **Wavelength 1** - Select the starting wavelength for the spectral maximum calculation.
- **Wavelength 2** - Select the end wavelength for the spectral maximum calculation.



Note User-selectable wavelength values are not checked against real measured values. Therefore, you may select wavelength values that are not even measured.

- **Threshold** - Enter the intensity threshold that the peak is required to exceed. The minimum value is $-10,000,000$ units and the maximum is $10,000,000$.

Spectral minimum

The spectral minimum seeks the minimum measurement value in each well. You can define a wavelength area for which this calculation is performed. It is also possible to receive the minimum without defining the wavelength area or the threshold.

Wavelength 1:	<input type="text" value="200"/>
Wavelength 2:	<input type="text" value="1000"/>
Threshold:	<input type="text" value="0.000"/>

- **Wavelength 1** - Select the starting wavelength for the scanning.
- **Wavelength 2** - Select the end wavelength for the scanning.



Note User-selectable wavelength values are not checked against real measured values. Therefore, you may select wavelength values that are not even measured.

- **Threshold** - Enter the intensity threshold that the peak is required to exceed. The minimum value is $-10,000,000$ units and the maximum is $10,000,000$.

HBW calculation

In HBW calculations, a certain peak is identified and its intensity is measured. The calculation analyses the shape of the curve between the 100% (maximum) and the 50% values. The wavelengths on both sides of the peak corresponding to 50% of the peak intensity are identified and the difference between these two wavelengths is calculated. This is the HBW value. The result of this calculation will be the HBW value and the peak wavelength (Figure 10-6).

The HBW can only be calculated for one well at a time.

The peak value should first be identified using either spectral peak search (see “Spectral peak search” on page 174) or spectral maximum (see “Spectral maximum” on page 176).

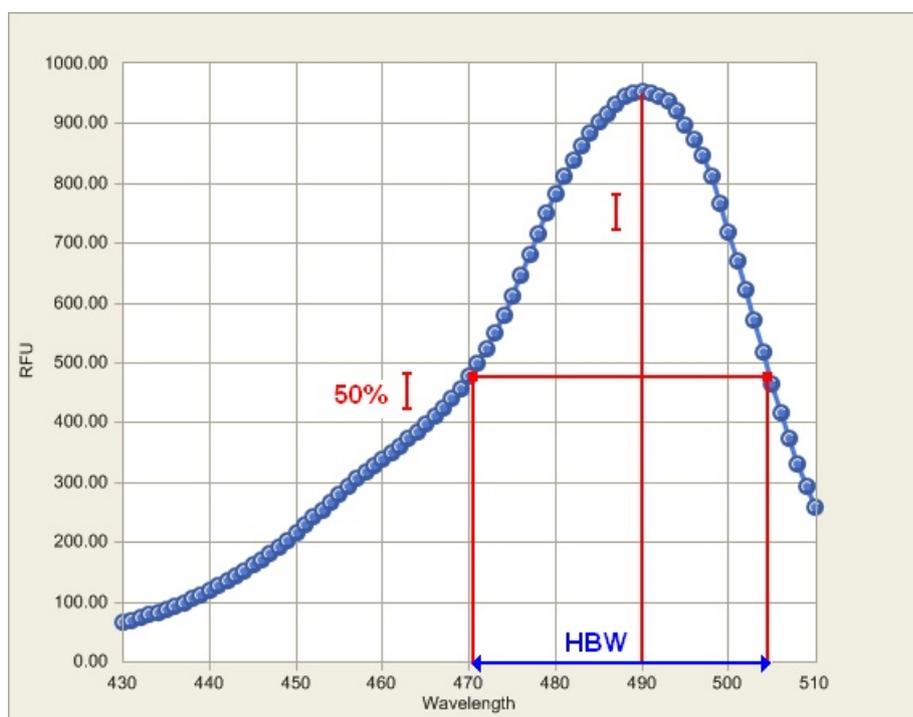


Figure 10-6. Definition of the HBW value

If the 50% intensity value is not found from either lower or higher wavelengths, the calculation cannot be performed. See the following examples:

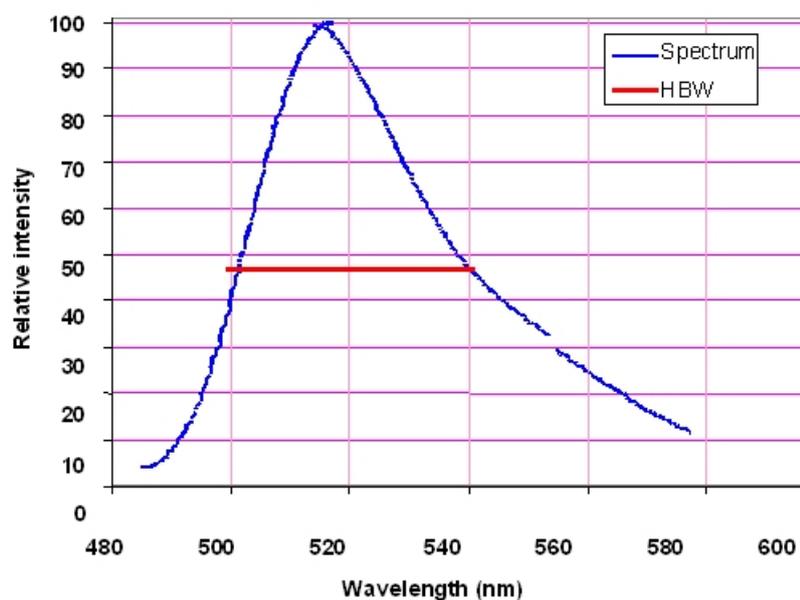


Figure 10-7. HBW calculation in a simple case

In the case shown in Figure 10-7 a reliable HBW is calculated because the spectrum has a good shape.

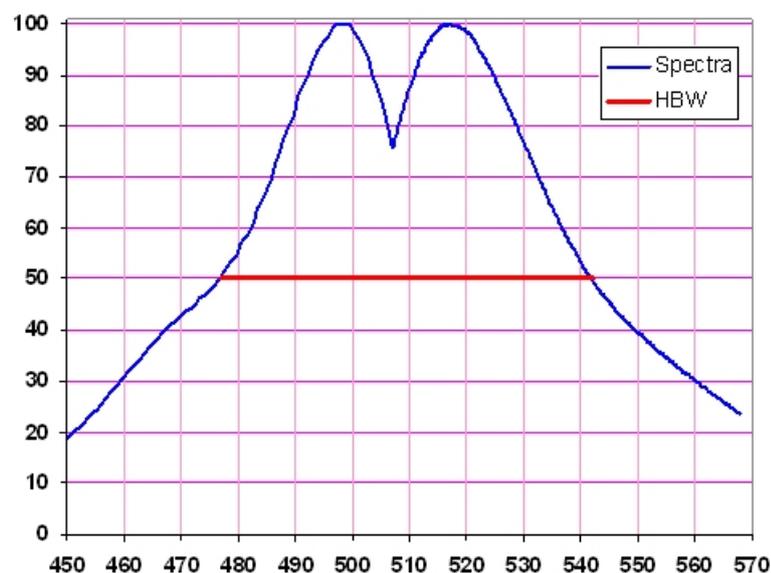


Figure 10-8. HBW calculation in a spectrum with two peaks

In the case shown in Figure 10-8 the HBW calculation gives the same result for both peaks.

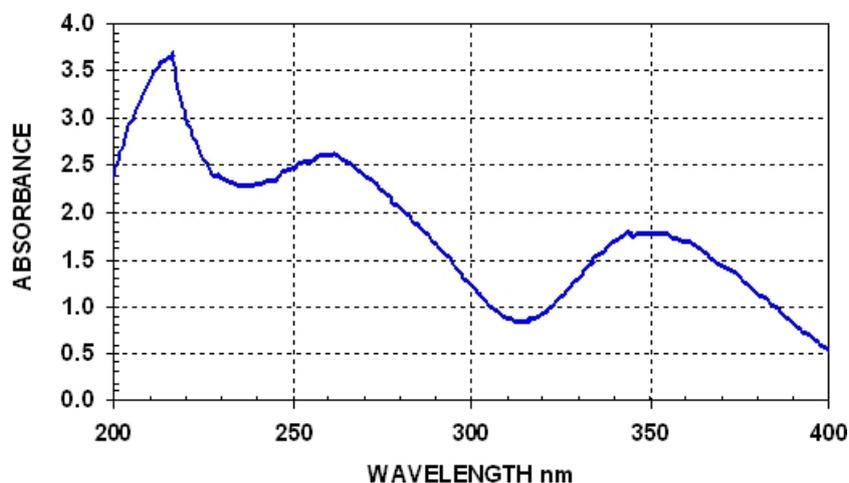


Figure 10-9. Case where an HBW calculation is not possible

In the case shown in Figure 10-9 there is no possibility to calculate HBW values for any of the peaks. There are no lower wavelength values available for any of the peaks.

Wavelength 1:

Group:

Sample:

- **Wavelength 1** - Select the wavelength for the peak. Use spectral peak search (see “Spectral peak search” on page 174) or spectral maximum (see “Spectral maximum” on page 176) to identify the wavelength of the peak.



Note User-selectable wavelength values are not checked against real measured values. Therefore, you may select wavelength values that are not even measured.

- **Group** - The calculation is performed for one replicate. Select the group to be used in the calculation.

- **Control** - Select the sample/replicate for which the calculation is performed.

Ratio within spectrum

Ratio within spectrum calculates the ratio of intensities for two different wavelengths within the same spectrum.

$$x_1 = \frac{I_A}{I_B}$$

Two wavelengths, n_A and n_B , corresponding to the intensities I_A and I_B , are given. For each spectrum, the ratio of the intensities for the given wavelengths is calculated according to the formula given below. The result is displayed in a matrix.

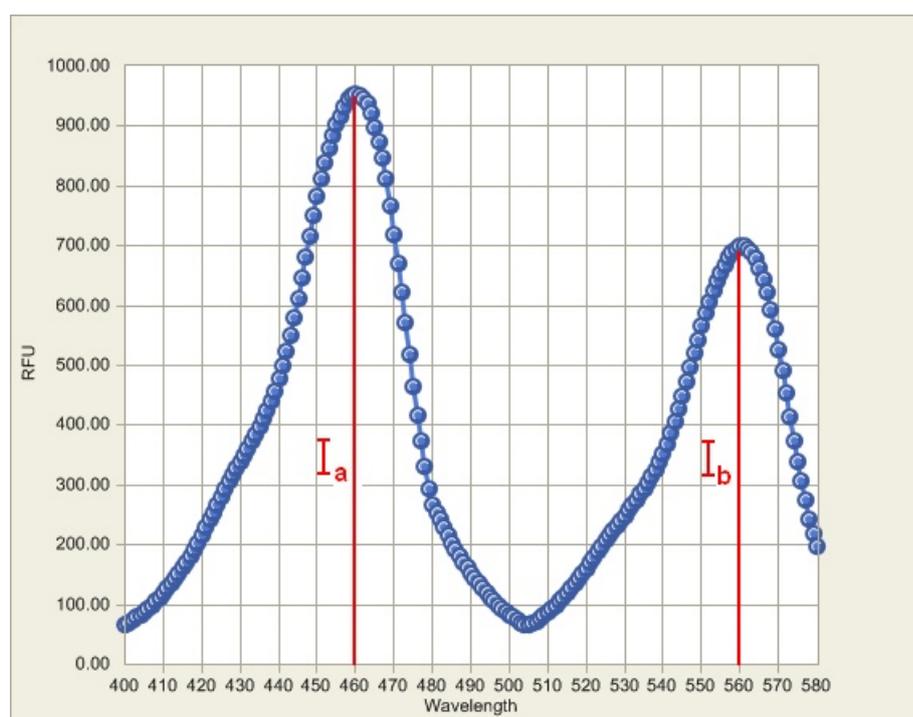


Figure 10-10. Intensities I_A and I_B in ratio within spectrum

Wavelength 1:	<input type="text" value="200"/>
Wavelength 2:	<input type="text" value="1000"/>

- **Wavelength 1** - Select the wavelength of the first peak, I_A .
- **Wavelength 2** - Select the wavelength of the second peak, I_B .

You can use spectral peak search (see “Spectral peak search” on page 174) to identify the wavelengths.



Note User-selectable wavelength values are not checked against real measured values. Therefore, you may select wavelength values that are not even measured.

Ratio between spectra

Ratio between spectra calculates the ratio curve over a range of two wavelengths. All the sample intensities of a certain wavelength n are divided by the control intensity corresponding to the same wavelength n . The ratio between spectra can be used to calculate the signal to blank spectra.

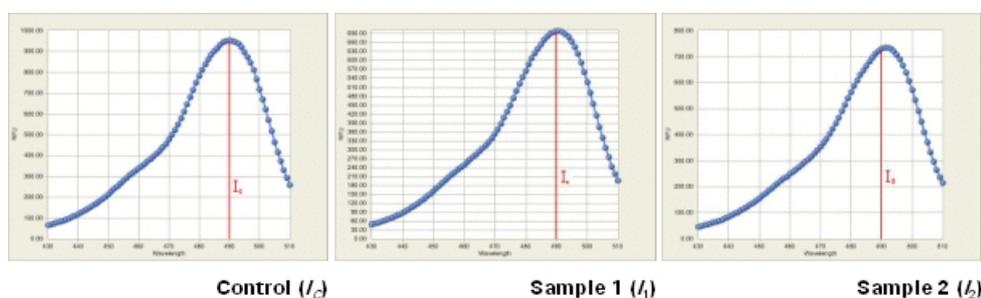


Figure 10-11. Control and sample intensities in ratio between spectra

The formulas for the calculation are as follows:

$$I_{[Sample1]} = \frac{I_1}{I_C}$$

$$I_{[Sample2]} = \frac{I_2}{I_C}$$

Wavelength 1:

Wavelength 2:

Group:

Sample:

- **Wavelength 1** - Select the starting wavelength to be used in the Ratio between spectra calculation.
- **Wavelength 2** - Select the end wavelength to be used in the Ratio between spectra calculation.



Note If you want the calculation to be made only with one wavelength, make sure you enter the same wavelength for both **Wavelength 1** and **Wavelength 2**.



Note User-selectable wavelength values are not checked against real measured values. Therefore, you may select wavelength values that are not even measured.

- **Group** - Select the source/assay name from the plate layout.
- **Sample** - Select the sample/replicate to be used as a base of the ratio calculation. The spectra of all samples are divided with calued from this sample.

Select wavelengths

Wavelength 1: 200
Wavelength 2: 1000

- **Wavelength 1** - Select the starting wavelength to be used in the scanning.
- **Wavelength 2** - Select the end wavelength to be used in the scanning.



Tip If you want to select only one wavelength for further calculations, select that wavelength as both **Wavelength 1** and **Wavelength 2**.



Note User-selectable wavelength values are not checked against real measured values. Therefore, you may select wavelength values that are not even measured.

Kinetic calculations

Kinetic calculations are used to reduce data from kinetic measurements.

Select Data Processing > **Kinetic calculations**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.

Calculation type

- Average rate
- Maximum rate
- Time to maximum rate
- Time to maximum rate / 2
- Time to change
- Maximum of well (Peak)
- Maximum - Minimum (Change)
- Time to maximum (Peak)
- Time to maximum (Peak) / 2
- Select reading
- Ignore readings
- Integral
- Sum
- Average value
- Baseline subtraction

Parameters

Kinetic rate

/s /min

Ignore

First (n) readings:

Last (n) readings:

Average rate

Also known as normal rate. The average kinetic rate (slope of the absorbance (Abs) vs. time curve) will be calculated by linear regression (linear least squares method or LLS) using all the measurement readings within the selected raw data and time range.

You can define the following settings:

Kinetic rate

/s /min

Ignore

First (n) readings:

Last (n) readings:

- **Kinetic rate** - Select to view the results in either seconds (s) or minutes (min). The Kinetic rate is always calculated per second but if needed the time is converted to minutes after the calculations.
- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.

- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Maximum rate

If the maximum rate is selected, the software searches the data for the maximum rate found in each well. To obtain the maximum rate, a series of linear curve fits will be performed for different segments of the measurement value, absorbance or fluorescence intensity vs. time curve (Figure 10-12). The first segment starts at the first data point within the selected time and measurement range, the second segment starts at the second data point, and so on, until all the data points have been analyzed. All the rate calculations are evaluated to determine the maximum rate. In other words, the LLS fit of m span points are sequentially fitted through each of the n data points. There will be $n - m + 1$ curves produced from this. You can specify the number of data points in a segment with the **Window (2...100)** setting.

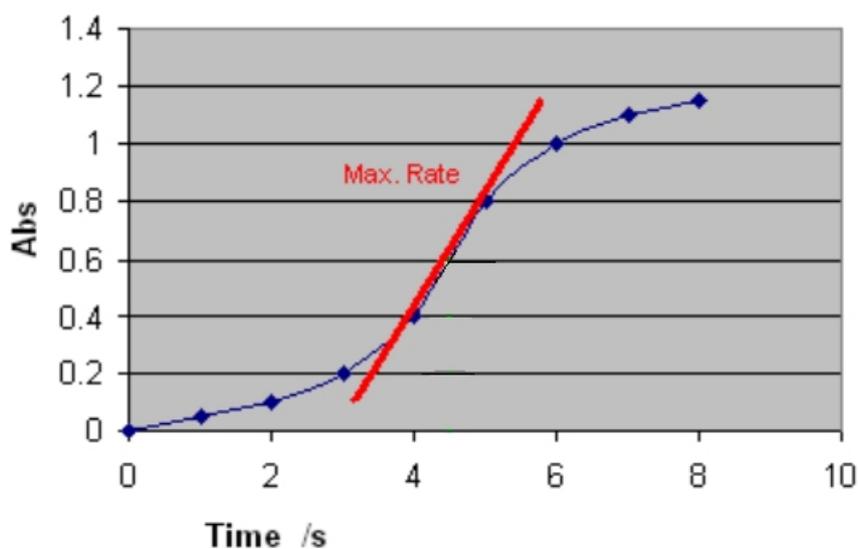


Figure 10-12. Determining the maximum rate with window value 2

You can define the following settings:

Reaction	
<input checked="" type="radio"/> Undefined	
<input type="radio"/> Increasing	
<input type="radio"/> Decreasing	
Kinetic rate	
<input checked="" type="radio"/> /s	<input type="radio"/> /min
Ignore	
First (n) readings:	<input type="text" value="0"/>
Last (n) readings:	<input type="text" value="0"/>
Window (2...100)	
	<input type="text" value="2"/>

- **Reaction** - Define whether the reaction is supposed to produce increasing or decreasing signal levels. There are three options for this setting:
 - **Undefined** (default) - The reaction can be increasing, decreasing or both (for example, first increase and then decrease, that is, peaking). The software does not produce any warnings in either case. It searches for the absolute maximum rate. If the highest absolute rate value is increasing, it is shown as a positive number. If the maximum rate is decreasing, it will be presented with the prefix "-".
 - **Increasing** - The software searches for the maximum increasing rate. The rate is shown as a positive number. If no increasing rate is found, the result is "NaN" (not a number).
 - **Decreasing** - The software searches for the maximum decreasing rate. The rate is shown as a positive number. If no decreasing rate is found, the result is "NaN" (not a number).
- **Kinetic rate** - Select to view the results in either seconds (s) or minutes (min). The kinetic rate is always calculated per second but if needed, the time is converted to minutes after the calculations.
- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

- **Window (2...100)** - Select the number of consecutive readings to use for evaluation. The highest reaction rate for each well is calculated using a sliding window. A window defines how many measurement points are included in the measurement calculations. The size of this window is given in the Window parameter box. For example, if the number of measurements is ten and the Window parameter is three, the system will calculate the first rate using the measurements 1 to 3, the second rate using the measurements 2 to 4, and so on up to measurements 8 to 10. The maximum rate will be the maximum value among these calculated rates.

Time to maximum rate

The time to maximum rate is calculated similarly to the maximum rate, but the result is reported as the time in seconds from the first reading to the middle point of the sliding window where the maximum rate occurs instead of the rate itself. See Figure 10-13.

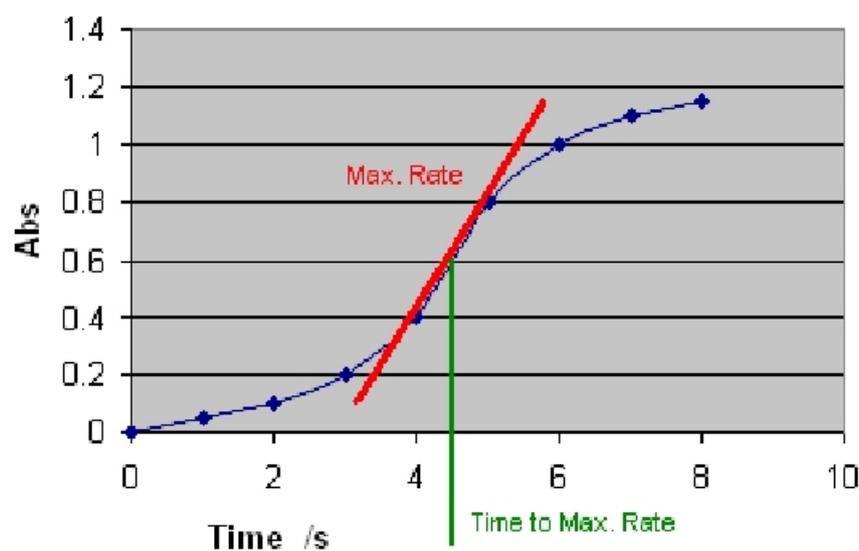


Figure 10-13. Determining the time to maximum rate

You can define the following settings:

Reaction	
<input checked="" type="radio"/> Undefined	
<input type="radio"/> Increasing	
<input type="radio"/> Decreasing	
Ignore	
First (n) readings:	<input type="text" value="0"/>
Last (n) readings:	<input type="text" value="0"/>
Window (2...100)	
	<input type="text" value="2"/>

- **Reaction** - Define whether the reaction is supposed to produce increasing or decreasing signal levels. There are three options for this setting (see “Maximum rate” on page 185):
 - **Undefined** - Searches for the absolute maximum rate and returns the corresponding time as a positive number.
 - **Increasing** - Searches for the maximum increasing rate and returns the corresponding time as a positive number. If no increasing rate is found, the result is “NaN” (not a number).
 - **Decreasing** - Searches for the maximum decreasing rate and returns the corresponding time as a positive number. If no decreasing rate is found, the result is “NaN” (not a number).
- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.
- **Window (2...100)** - The number of consecutive readings to use for evaluation.

Time to maximum rate / 2

The time to maximum rate / 2 is the time taken to reach half of the maximum rate. The maximum rate is determined as shown in “Time to maximum rate” on page 187. This rate is halved and the data is scanned to determine the first rate, which is equal or exceeds this rate. The result is the time when this found rate occurs.

You can define the following settings:

Reaction	
<input checked="" type="radio"/>	Undefined
<input type="radio"/>	Increasing
<input type="radio"/>	Decreasing
Ignore	
First (n) readings:	<input type="text" value="0"/>
Last (n) readings:	<input type="text" value="0"/>
Window (2...100)	
	<input type="text" value="2"/>

- **Reaction** - Define whether the reaction is supposed to produce increasing or decreasing signal levels. There are three options for this setting (see “Maximum rate” on page 185):
 - **Undefined** - Searches for the absolute maximum rate, calculates the half of the maximum rate, and returns the corresponding time as a positive number.
 - **Increasing** - Searches for the maximum increasing rate, calculates the half of the maximum rate, and returns the corresponding time as a positive number. If no increasing rate is found, the result is “NaN” (not a number).
 - **Decreasing** - Searches for the maximum decreasing rate, calculates the half of the maximum rate, and returns the corresponding time as a positive number. If no decreasing rate is found, the result is “NaN” (not a number).
- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

- **Window (2...100)** - The number of consecutive readings to use for evaluation.

Time to change

The time to change is used for calculating the time required to reach a defined change in the signal (in each well). The time is given in seconds from the first reading to the endpoint of the sliding window in seconds.

First define the base value by entering the number of readings from the start in baseline. The base value is the average of results of the given baseline count from the beginning or end of results. Then select whether the change from the base value is evaluated as a relative (%) or an absolute value and define that percentage or value. This change is then added to the base value to create a required change value. The measurements are then compared to the base value using a sliding window of measurement values. The result is the exact interpolated time where the given change occurs.

You can define the following settings:

Reaction

Undefined
 Increasing
 Decreasing

Ignore

First (n) readings: 0
Last (n) readings: 0

Window (1...100) 1

Baseline

Readings (0...100): 0
 first last

Change

Absolute 0.000
 Relative [%]

- **Reaction** - Define whether the reaction is supposed to produce increasing or decreasing signal levels. There are three options for this setting:

- **Undefined** - Searches for the defined change in signal, regardless of the rate polarity (positive or negative), and returns the corresponding time as a positive number.
- **Increasing** - Searches for the increasing defined change in signal, and returns the corresponding time as a positive number. If no increasing rate is found, the result is “NaN” (not a number).
- **Decreasing** - Searches for the decreasing defined change in signal, and returns the corresponding time as a positive number. If no decreasing rate is found, the result is “NaN” (not a number).
- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.
- **Window (1...100)** - The number of consecutive readings to use for evaluation.
- **Baseline (0...100)** - The Baseline parameter is the number of initial readings, calculated from the beginning (*first*) or end (*last*) of the measurement, used for the baseline calculation. The baseline can also be zero, which means that the comparison will be made against zero. In the Baseline the software chooses as many measurement points as exist in the baseline and calculates the mean thereof. The mean equals the baseline value. If *last* is selected, the change is extrapolated starting from the end of the measurement.
- **Change (Relative [%] or Absolute)** - The relative change from the baseline in percent or the absolute change. The change in signal is specified in the *Change* parameter and is compared to the baseline. The *Window* parameter here means the number of consecutive measurements which should reach the change before the result is accepted.

Maximum of well (Peak)

The maximum of well is used to search for the maximum measurement value in each well.

You can define the following settings:

- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Maximum - Minimum (Change)

The minimum measurement value of each well is subtracted from the maximum measurement value of each well.

You can define the following settings:



Ignore
First (n) readings: 0
Last (n) readings: 0

- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Time to maximum (Peak)

The time to maximum is used to calculate the time elapsed before the maximum measurement signal in each well (peak) is reached.

You can define the following settings:



Ignore
First (n) readings: 0
Last (n) readings: 0

- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Time to maximum (Peak) / 2

The time to maximum / 2 equals the time to reach half of the maximum value.

You can define the following settings:



- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Select reading

Select reading is used to select a specific kinetic measurement point (reading).

You can define the following settings:



- **Reading (-1000...1000)** - The number of the kinetic measurement reading. You can select a reading that you want to use in further calculations.

Ignore readings

Ignore readings is used to dismiss a selected number of readings from the beginning and/or the end of the measurement.

You can define the following settings:



- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Integral

The integral calculation estimates the area under the measurement curve (Figure 10-14). The range of readings can be limited with the *Ignore readings* selection.

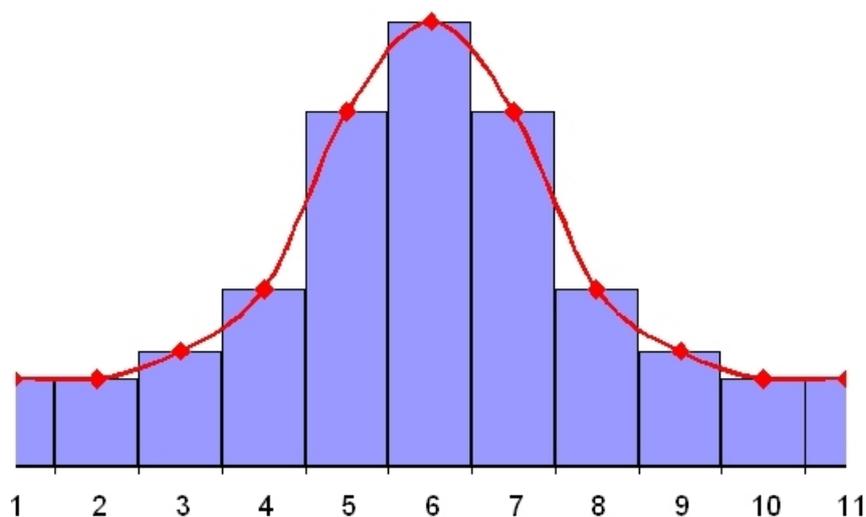


Figure 10-14. Integral calculation

You can define the following settings:

Ignore	
First (n) readings:	<input type="text" value="0"/>
Last (n) readings:	<input type="text" value="0"/>

- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Sum Sum calculates the sum of the selected range of readings. The range of readings can be limited with the *Ignore readings* selection.

In the example below (Figure 10-15), the kinetic processor sum gives the result

$$(0.00 + 0.30 + 0.50 + 0.60 + 0.65 + 0.67) \text{ Abs} = 2.72 \text{ Abs.}$$

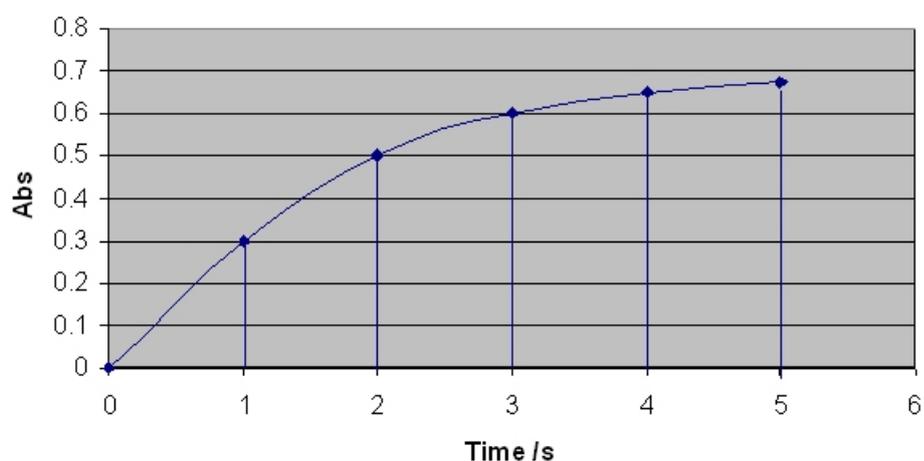


Figure 10-15. Example of an absorbance curve

You can define the following settings:

Ignore	
First (n) readings:	<input type="text" value="0"/>
Last (n) readings:	<input type="text" value="0"/>

- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Average value Average value is used to calculate the average value of all kinetic readings.

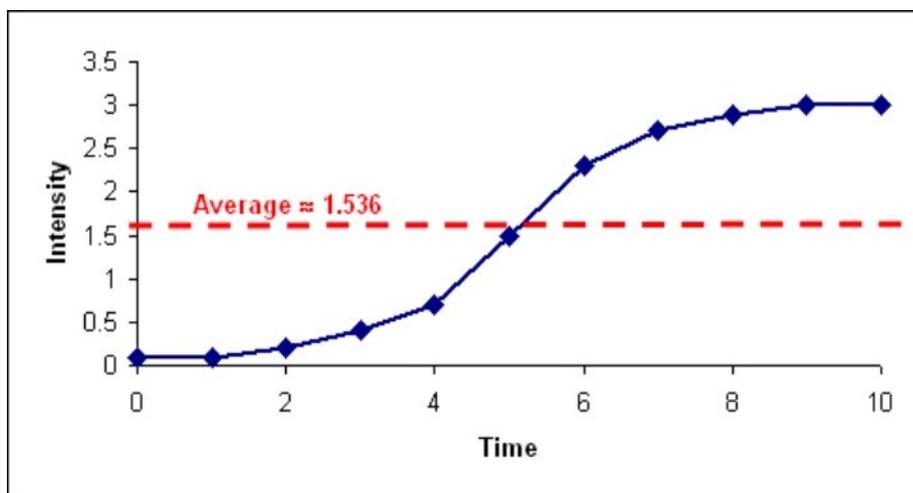


Figure 10-16. Determining the average value of a kinetic measurement

You can define the following settings:

Ignore	
First (n) readings:	<input type="text" value="0"/>
Last (n) readings:	<input type="text" value="0"/>

- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.

Baseline subtraction

Baseline subtraction is used to transform the measured values to start from 0 by removing the measured baseline before the actual reaction. It subtracts the average of a defined number of readings from the beginning or the end of all other readings. This is useful when used with a kinetic loop with dispensing and measurement. For example, you can set the dispensing to occur in reading 5 to obtain 4 measurements from the non-reactive state and then do a baseline subtraction to ensure that the reaction in all wells starts from 0.

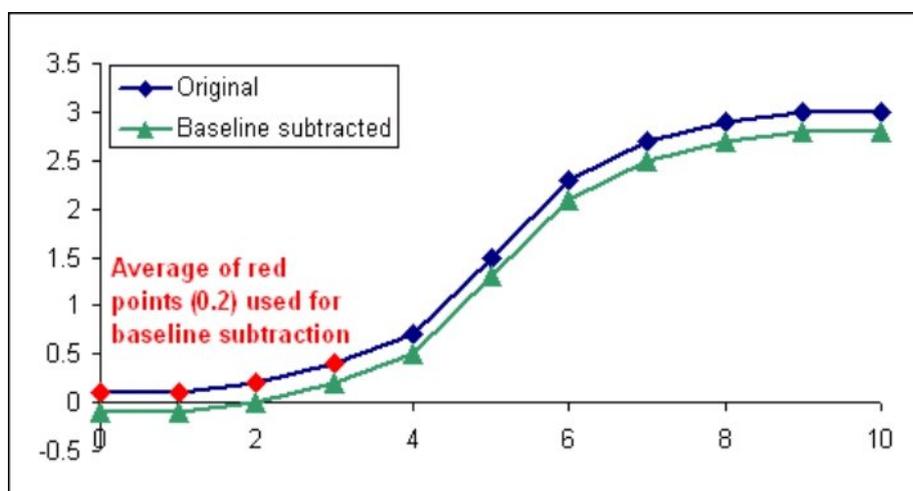


Figure 10-17. Determining the baseline subtracted kinetic curve

You can define the following settings:

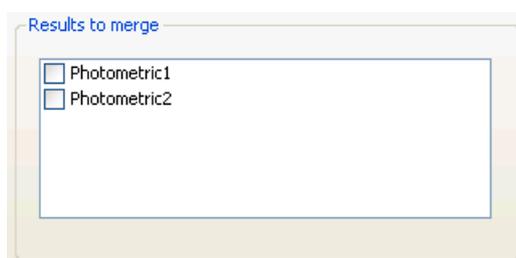
Ignore	
First (n) readings:	0
Last (n) readings:	0
Baseline	
Readings (0...100):	0
<input checked="" type="radio"/> first	<input type="radio"/> last

- **Ignore first (n) readings** - Ignores a defined number of points (readings), counted from the first reading.
- **Ignore last (n) readings** - Ignores a defined number of points (readings), counted from the last reading.
- **Baseline** - Select the number of points [**Readings (0...100)**], counted from the first or last reading, to be used as the baseline. If the number of readings is 0, no subtraction is made.

Merge data

Merge data calculation is used to combine measurement data and measurement times from separate measurement steps into one data set. You can only combine raw measurement data from measurements with the same measurement technology. It is possible to combine data from both endpoint and kinetic measurements. Data from spectral scanning measurements cannot be combined.

Select Data Processing > **Merge Data**, click the  button on the Results action panel, or right-click the measurement in the **Results** steplist.



Select the measurements whose data to combine. The combined data is shown as a kinetic curve for each well in the table view and as a kinetic data list in the list view. You can also perform calculations on the combined data using calculations available for kinetic measurements.

Graph

You can create graphs from the measurement results. Unlike most other calculations, the graph should only be created after the session is executed.

Select Data Processing > **Graph**, click the  button on the **Results** action panel, or right-click the measurement in the **Results** tree.

Graph title:

X axis title:

Y axis title:

X axis:

Well/sample label
 Well Sample name

Wells: Readings: Ex [nm]: Em [nm]:

<input checked="" type="checkbox"/> Blank_Assay 1/1 (1: A01)	<input checked="" type="checkbox"/> 1	<input checked="" type="checkbox"/> 450	<input checked="" type="checkbox"/> 0
<input checked="" type="checkbox"/> Cal_0001 1/1 (1: B01)			
<input checked="" type="checkbox"/> Cal_0002 1/1 (1: C01)			
<input checked="" type="checkbox"/> Cal_0003 1/1 (1: D01)			
<input checked="" type="checkbox"/> Cal_0004 1/1 (1: E01)			
<input checked="" type="checkbox"/> Cal_0005 1/1 (1: F01)			
<input checked="" type="checkbox"/> Un_0001 1/1 (1: A02)			
<input checked="" type="checkbox"/> Un_0002 1/1 (1: B02)			
<input checked="" type="checkbox"/> Un_0003 1/1 (1: C02)			
<input checked="" type="checkbox"/> Un_0004 1/1 (1: D02)			
<input checked="" type="checkbox"/> Un_0005 1/1 (1: E02)			
<input checked="" type="checkbox"/> Un_0006 1/1 (1: F02)			
<input checked="" type="checkbox"/> Un_0007 1/1 (1: A03)			
<input checked="" type="checkbox"/> Un_0008 1/1 (1: B03)			

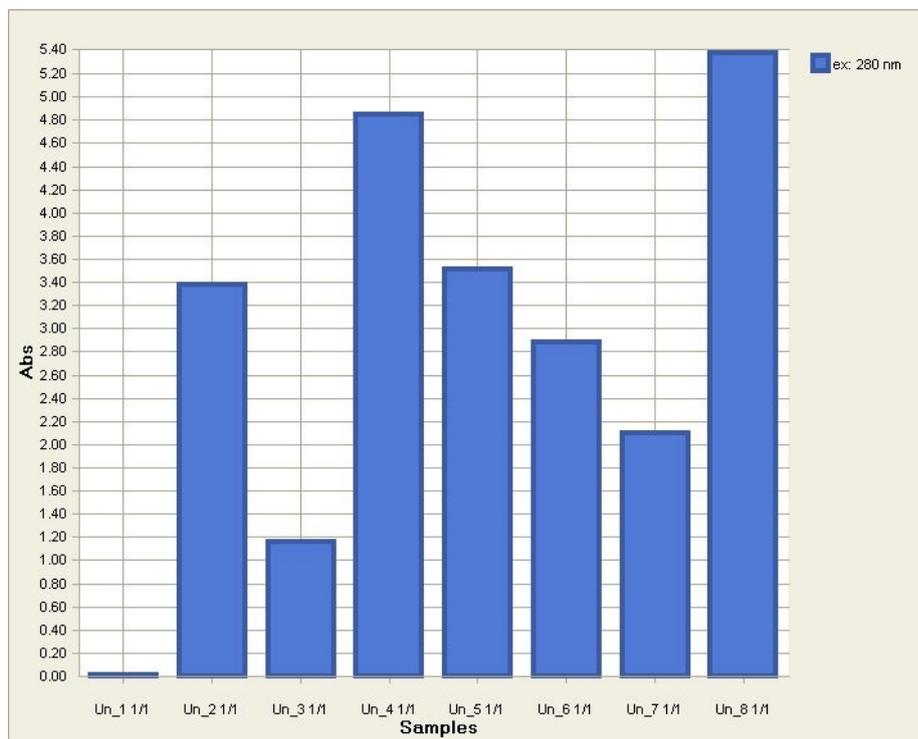
+ - + - + - + -

- **Graph title, X axis title and Y axis title** - Enter titles for the graph and the X- and Y-axes.
- **X axis** - Select which data you want to present on the X-axis: the samples, the readings (in kinetic measurements), the measurement time, the excitation wavelength or the emission wavelength.
- **Well/sample label** - You can display the names of the samples according to the sample names or the well position. Not available for readings, excitation or emission.
- **Wells, Readings, Ex [nm] or Em [nm]** - Select here which samples, readings and excitation and emission wavelengths you want to display on the graph by ticking the check boxes. Use the button to select all and the button to clear all selections.

The graph can be viewed when *Graph* is selected in the **Results** tree and the *Graph* tab is selected on the main window area. An example of an absorbance bar chart is shown below.

Data Processing Parameters

Graph



For more information on how the graph is displayed, see “Viewing results in graph format” on page 208.

TRF decay calculation

The *TRF Decay Calculation* step is used to calculate the τ (tau) and a (alpha) values based on the data from a TRF (time-resolved fluorescence) decay measurement. The tau value is the half-life of the TRF label signal. The alpha value is the peak of the measured signal. The correlation coefficient R^2 shows the validity of the signal; it should be as close to 1 as possible.

The tau and alpha values are calculated using the following formula as a starting point:

$$I(t) = \frac{-t}{e^{-\tau}}$$

The τ (tau) value is then rounded to the nearest μs .

The Recommended integration time (RIT [μs]) is calculated using the formula below:

$$RIT[\mu\text{s}] = 1.256 \times \tau$$

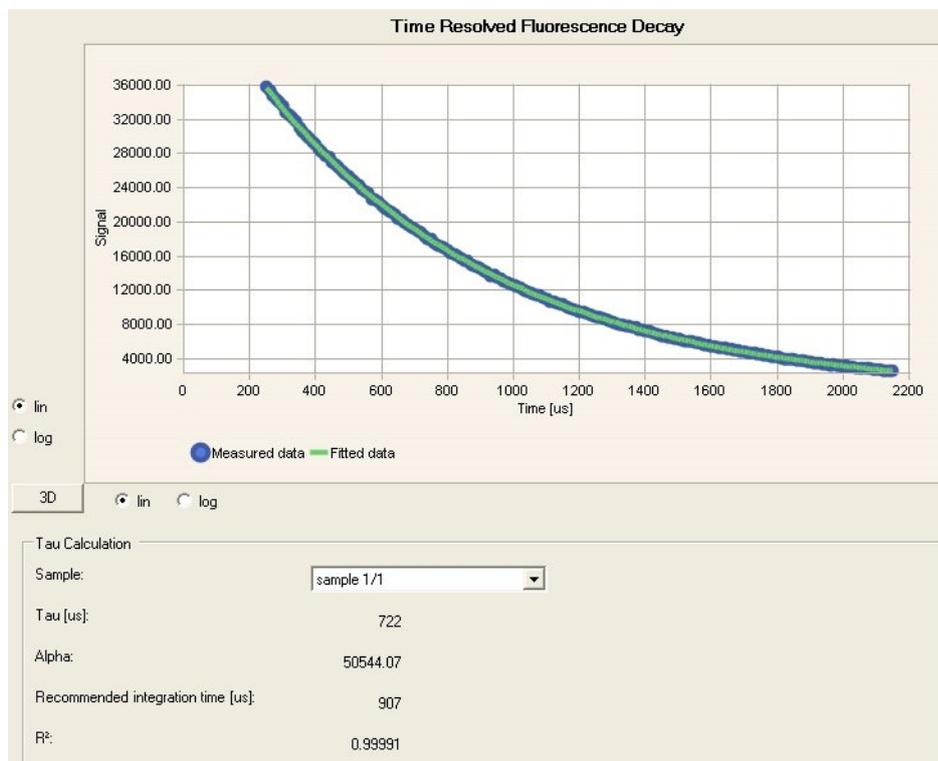
The RIT value is then rounded to the nearest μs .

The TRF Decay measurement can produce a high peak in the first few measurements if the background fluorometric decay is slow and the start time for TRF Decay is too low. You can disable the first few measurements from the raw data if the peak seems to be too high. This will ensure that you get the best possible fit for the data. For information on how to disable measurements, see “Right-click menus” on page 50.

Select Data Processing > **TRF Decay Calculation**, click the τ button on the **Results** action panel, or right-click the measurement in the **Results** tree.

Data Processing Parameters

TRF decay calculation



- **Sample** - Select the sample for the Tau (τ) and Alpha (α) calculation from the list.

Chapter 11

Results

After you have created the session (the protocol and the plate layout), you can execute it (see Chapter 5: “Executing a Session”). The instrument performs the measurements as defined in the protocol and sends the measurement data to the software.

Calculations and other data handling procedures are made to the raw data as defined by the user (see “Adding calculations” on page 143).

Raw measurement data

When the measurement is finished, the Execution Progress dialog is closed and the *Results* view shows the measurement results in plate layout format (*Table*).

The screenshot shows the SkanIt Software 2.4.3 interface. The 'Results' tree on the left is expanded to show 'Demo session' and 'Photometric'. The 'Table' tab is selected in the 'Results' view. The data table below shows the following information:

Well	Sample Name	Dil./Conc. units	Replicate no.	Wavelength [nm]	Plate	Zoom
A	Blank_Assay	1:1	1:1	450	1	100%
B	Cal_0001	1:1	1:1	450	1	100%
C	Cal_0002	1:1	1:1	450	1	100%
D	Cal_0003	1:1	1:1	450	1	100%
E	Cal_0004	1:1	1:1	450	1	100%
F	Cal_0005	1:1	1:1	450	1	100%
G	Un_0001	1:1	1:1	450	1	100%
H	Un_0002	1:1	1:1	450	1	100%

You can view the measurement results in the plate layout and list views. Select the measurement in the **Results** tree and select either the *Table* or *List* tab, respectively.

In the table view, use the *Show options* check boxes to select the information you want to display for each well in addition to the sample name, dilution and raw measurement data. Other options are group name, sample type, dilution/concentration units and replicate number. Other viewing options are:

Results

Raw measurement data

- **Wavelength** - If the measurement was performed with multiple wavelengths, select here the wavelength for which the raw measurement data will be shown. You can use the drop-down list as well as the arrows on both sides of the box to select the wavelength.
- **Plate** - Choose here the plate to be shown, if the plate layout contains samples in more than one plate.
- **Zoom** - Select the zooming factor for optimal viewing of the wells.

For kinetic measurements and spectrum scanning, the measurement data is shown as curves. You can also show the curve enlarged in a separate **Curve** window. For more information, see “Viewing results as curves” on page 211.

You can also view the results in the list view. Click the *List* tab. In the list view, the results are shown row by row for each well.

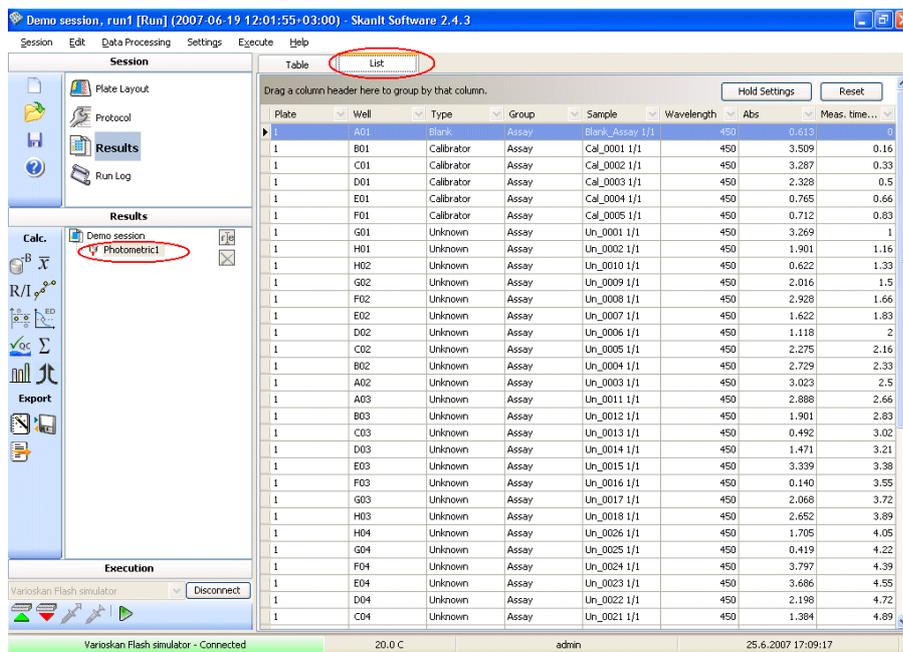


Plate	Well	Type	Group	Sample	Wavelength	Abs	Meas. time...
1	A01	Blank	Assay	Blank_Assay 1/1	450	0.613	0
1	B01	Calibrator	Assay	Cal_0001 1/1	450	3.509	0.16
1	C01	Calibrator	Assay	Cal_0002 1/1	450	3.287	0.33
1	D01	Calibrator	Assay	Cal_0003 1/1	450	2.328	0.5
1	E01	Calibrator	Assay	Cal_0004 1/1	450	0.765	0.66
1	F01	Calibrator	Assay	Cal_0005 1/1	450	0.712	0.83
1	G01	Unknown	Assay	Un_0001 1/1	450	3.269	1
1	H01	Unknown	Assay	Un_0002 1/1	450	1.901	1.16
1	H02	Unknown	Assay	Un_0010 1/1	450	0.622	1.33
1	G02	Unknown	Assay	Un_0009 1/1	450	2.016	1.5
1	F02	Unknown	Assay	Un_0008 1/1	450	2.928	1.66
1	E02	Unknown	Assay	Un_0007 1/1	450	1.622	1.83
1	D02	Unknown	Assay	Un_0006 1/1	450	1.118	2
1	C02	Unknown	Assay	Un_0005 1/1	450	2.275	2.16
1	B02	Unknown	Assay	Un_0004 1/1	450	2.729	2.33
1	A02	Unknown	Assay	Un_0003 1/1	450	3.023	2.5
1	A03	Unknown	Assay	Un_0011 1/1	450	2.888	2.66
1	B03	Unknown	Assay	Un_0012 1/1	450	1.901	2.83
1	C03	Unknown	Assay	Un_0013 1/1	450	0.492	3.02
1	D03	Unknown	Assay	Un_0014 1/1	450	1.471	3.21
1	E03	Unknown	Assay	Un_0015 1/1	450	3.339	3.38
1	F03	Unknown	Assay	Un_0016 1/1	450	0.140	3.55
1	G03	Unknown	Assay	Un_0017 1/1	450	2.068	3.72
1	H03	Unknown	Assay	Un_0018 1/1	450	2.652	3.89
1	H04	Unknown	Assay	Un_0026 1/1	450	1.705	4.05
1	G04	Unknown	Assay	Un_0025 1/1	450	0.419	4.22
1	F04	Unknown	Assay	Un_0024 1/1	450	3.797	4.39
1	E04	Unknown	Assay	Un_0023 1/1	450	3.686	4.55
1	D04	Unknown	Assay	Un_0022 1/1	450	2.198	4.72
1	C04	Unknown	Assay	Un_0021 1/1	450	1.384	4.89

The columns show the plate number, the well, the sample type, the group, the sample name and the measurement time for each well. Measurement times are counted from the first measurement of the first step which is zero. All the other measurement times are relative to that time. Other information in the list, shown in Table 11-1, depends on the measurement.

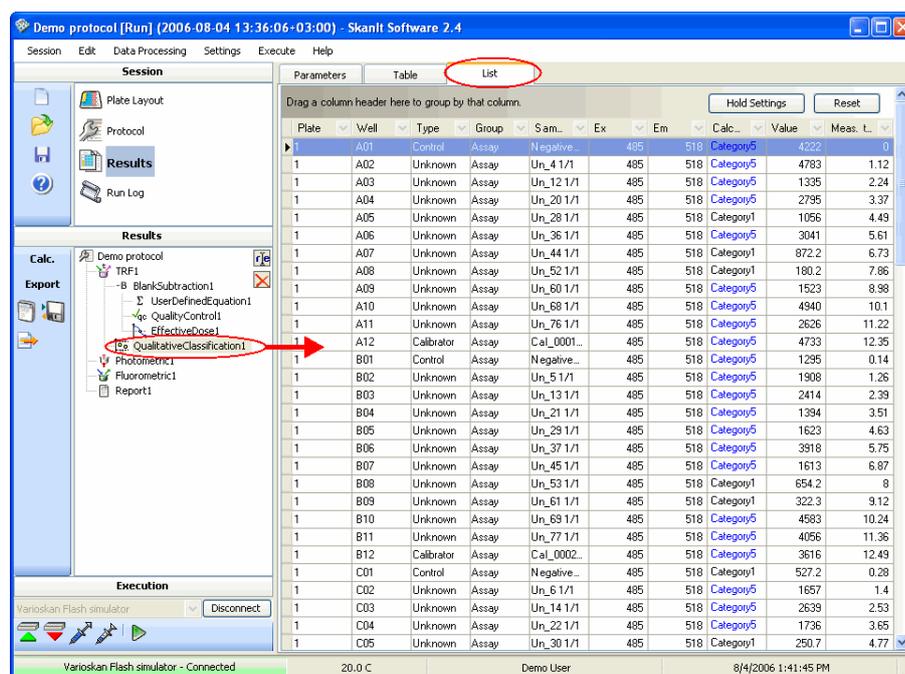
Table 11-1. Result information shown in result lists

Column header	Meaning
Abs	Measured absorbance value
Wavelength	Used wavelength
RFU	Measured RFU value
RLU	Measured RLU value
Coordinate	In a multipoint measurement, the X and Y axis of the measured point in a well. For example, $-190 : 0$ means 1.90 mm left in the X direction from the origin (0 : 0). The origin is in the center of the well. For more information, see "Using multipoint in a measurement" on page 114.
Ex Wavelength	Used excitation/absorbance wavelength
Em Wavelength	Used emission wavelength
Reading	Reading in the kinetic loop

The result list can be ordered in multiple ways. For more information, see "Lists" on page 49 and "Right-click menus" on page 50.

Calculation results

Click the calculation name in the **Results** tree on the left to view the calculation results. The results can be viewed in both list and table formats and with certain calculations as a graph. The view type is selected from the tabs.



The information shown in the table and list views depends on the calculation type. NaN = Not a number.

You can view and redefine the calculation parameters under the *Parameters* tab after you have executed the run. For example, you can change the fit type in Quantitative Curve Fit and Effective Dose calculations or change the number of readings ignored in a kinetic reduction.

Viewing results in table format

In the *Results* view, select the calculation from the **Results** tree and select the *Table* tab. The calculations results are shown in plate layout format.

Use the *Show options* check boxes to select the information you want to display for each well in addition to the sample name, dilution and calculation result data. Other options are the group name, sample type, dilution/concentration units and the replicate number. For kinetic measurements and spectrum scanning, the calculated data is shown as curves. Other viewing options are:

- **Wavelength** - If the measurement was performed with multiple wavelengths, select here the wavelength for which the calculation data

will be shown. You can use the drop-down list as well as the arrows on both sides of the box to select the wavelength.

- **Plate** - Choose here the plate to be shown, if the plate layout contains samples in more than one plate.
- **Zoom** - Select the zooming factor for optimal viewing of the wells.

You can also print the results in table format by clicking the **Print** button. The **Result Table Printing** dialog opens so you can define the printing setup and print the results with the selected printer.

Viewing results in list format

In the *Results* view, select the calculation from the **Results** tree and select the *List* tab. The calculations results are shown in list format.

The information shown in the list depends on the calculation, as can be seen in the examples below.

The cut-off results list shows a text string, for example, “Negative” or “HighPos”, for the qualitative classification result categories.

P...	W	Type	G...	Sample	Wa...	Calcul...	Value	Mea...
▶ 1	A1	Control	Assay	Neg_1 1/3	450	Negative	0.063	6.28
1	A10	Unknown	Assay	Un_68 1/1	450	Negative	0.062	11
1	A11	Unknown	Assay	Un_76 1/1	450	Negative	0.066	11.53
1	A12	Unknown	Assay	Un_84 1/1	450	Negative	0.070	12.05
1	A2	Unknown	Assay	Un_4 1/1	450	Positive	0.658	6.81
1	A3	Unknown	Assay	Un_12 1/1	450	Positive	0.137	7.33
1	A4	Unknown	Assay	Un_20 1/1	450	Positive	0.243	7.86
1	A5	Unknown	Assay	Un_28 1/1	450	Negative	0.059	8.38
1	A6	Unknown	Assay	Un_36 1/1	450	Negative	0.063	8.9

The calculated effective dose values are displayed.

Group	Sample	Type	Concentration	%	Abs
▶ Assay2	Assay2: Calib...	Calibrator	896.854	20	2.298
Assay2	Assay2: Calib...	Calibrator	4047.328	50	1.436
Assay2	Assay2: Calib...	Calibrator	7197.803	80	0.574

The text string, for example, “QC1 Succeeded”, will be displayed for the quality control categories.

Parameters List

Drag a column header here to group by that column. Hold Settings Reset

QualityControl	Result
QC1	QC1 Succeeded
QC2	QC2 Succeeded
QC3	QC3 Succeeded
QC4	QC4 Succeeded

Drag a column header here to group by that column. Hold Settings Reset

Plate	Well	Type	Group	Sample	Ex	Em	Tau [us]	Alpha	RIT [us]	R2
1	A01	Unknown	Assay	sample 1/1	340	615	722	50544.073	907	0.99991

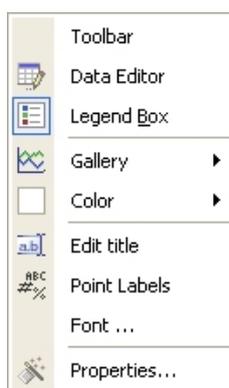
The tau (t), alpha (a), recommended integration time (RIT) and correlation coefficient (R²) values of each sample are shown for the time-resolved fluorescence decay calculation results.

The calculation result list can be ordered in multiple ways. For more information, see “Lists” on page 49 and “Right-click menus” on page 50.

Viewing results in graph format

In the *Results* view, select the calculation from the **Results** tree and select the *Graph* tab. The calculations results are shown in graph format. The graph is only available for certain calculations.

For more options on how the graph is displayed, right-click in the graph area under the *Graph* tab to display a menu.



- **Toolbar** - Display the graph toolbar.
- **Data Editor** - You can display the data points below the graph.
- **Legend Box** - You can create a legend for the curve.

- **Gallery** - Select the type of graph you want.
- **Color** - Select the color used for the curve.
- **Edit title** - You can give a title to the graph.
- **Point Labels** - You can display the value of the data points.
- **Font...** - Select the font used for displaying the data in the graph.
- **Properties...** - You can more precisely define how the graph looks like.

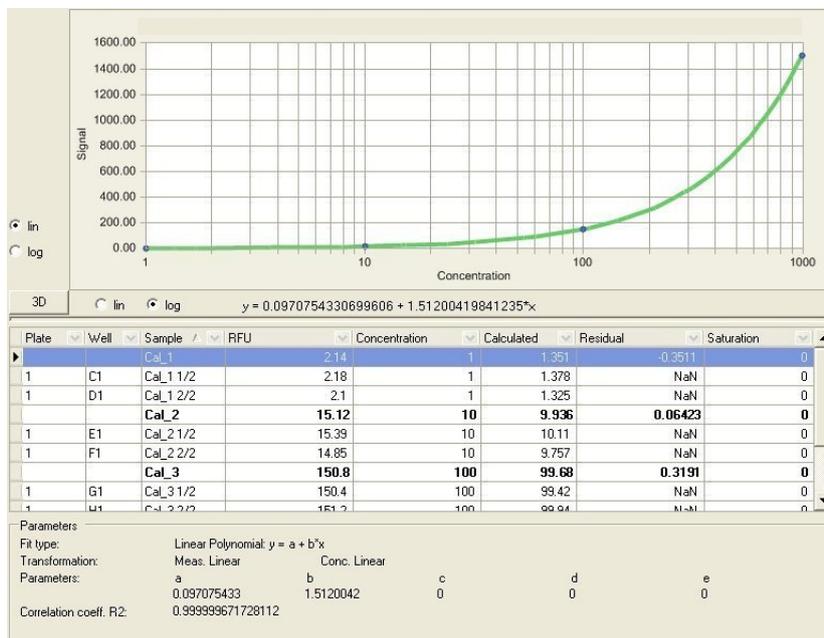
For more options on how the text and labels are displayed, right-click in the label area under the *Graph* tab to display a menu.



- **Text color** - Select the color used for the text.
- **Font...** - Select the font used for displaying the label text.
- **Edit title** - Change the title.
- **Staggered** - Stagger the label values for the graph.
- **Vertical labels** - Turn the labels vertically.
- **Grid** - Show a grid underneath the graph.
- **Interlaced** - Interlace the graph horizontally or vertically.
- **Properties...** - You can more precisely define how the graph looks like.

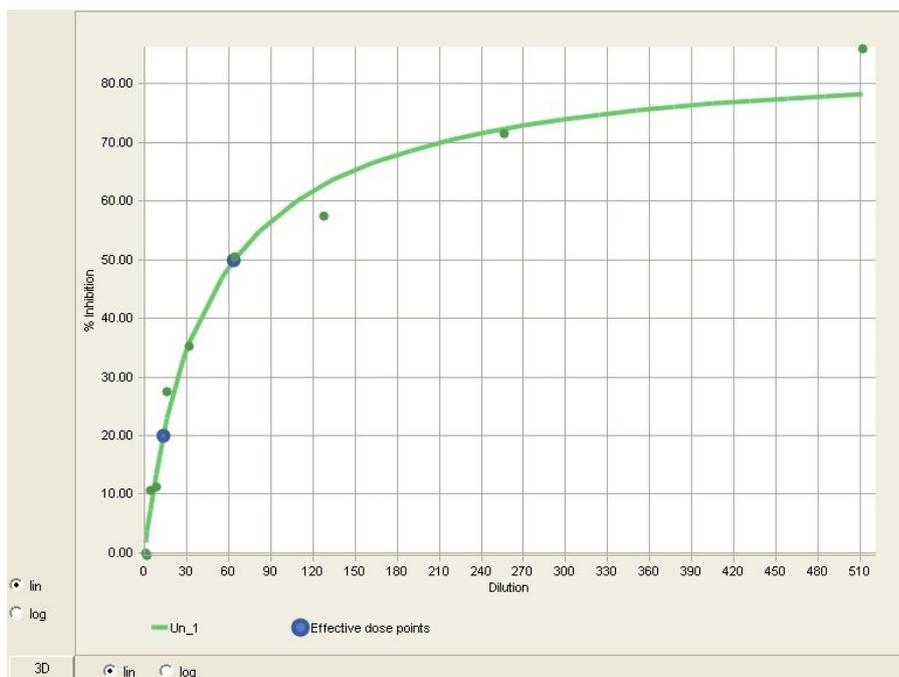
The example below shows the graph view of Quantitative Curve Fit.

Results
Calculation results



For more information on curve fit results, see “Quantitative Curve Fit results” on page 158.

The example below shows the graph view of Effective Dose calculations.

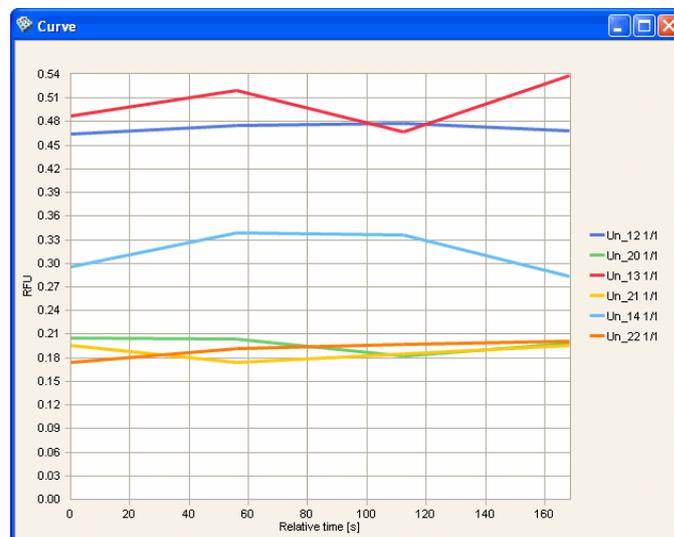


For more information on effective dose, see “Effective dose” on page 164.

Viewing results as curves

The measurement and calculation results are shown as curves for kinetic and spectrum scanning measurements. You can enlarge a single curve or several curves and show them in a separate window.

1. Select a kinetic or spectrum scanning measurement or its calculation in the *Results* view.
2. Show the results in the *Table* format.
3.
 - Select a single well.
 - Select several wells by pressing the *Ctrl* key or by painting an area. You can also paint several areas by pressing the *Ctrl* key.
4. Right-click and select **Show Graph**. The **Curve** dialog opens showing, for example, curves for several wells.



Saving measurement and calculation data

You can save and export the numeric measurement and calculation data to other systems either automatically each time the session is executed or manually afterwards.

Saving automatically

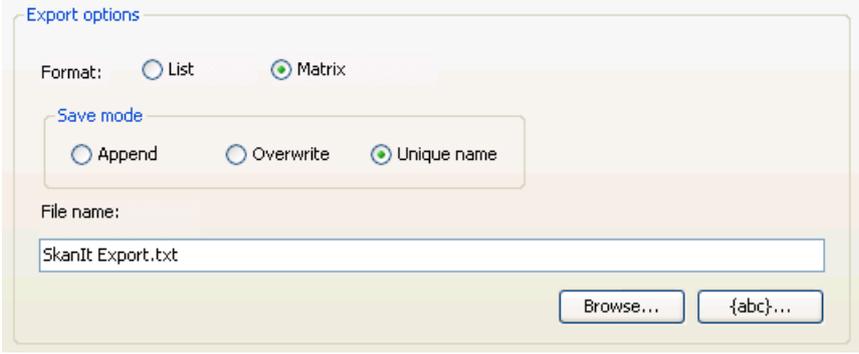
When you want to save data from a particular measurement or calculation each time the session is run, use the *Save* step. To create a more detailed report of the entire run, you can use the *Report* step as described in

Results

Saving measurement and calculation data

“Measurement and calculation reports” on page 213. In the **Results** tree, select the step containing the data you want to save.

Select Data Processing > **Save**, click the  button on the **Results** action panel, or right-click the measurement or calculation step and select **Save**. The *Save* step is added to the **Results** tree.



The image shows a dialog box titled "Export options". It contains the following elements:

- Format:** Two radio buttons: "List" (unselected) and "Matrix" (selected).
- Save mode:** A sub-dialog box containing three radio buttons: "Append" (unselected), "Overwrite" (unselected), and "Unique name" (selected).
- File name:** A text input field containing "SkanIt Export.txt".
- Buttons:** "Browse..." and "{abc}..."

- **Format** - Select the format in which the data will be laid out in the file: a list or a matrix (table view).
- *Save mode* - Select how the data is handled when the session is saved after a run:
 - **Append** - The data is added to the existing file after the data saved last.
 - **Overwrite** - The data in the existing file is overwritten.
 - **Unique name** - The file name is extended with a unique date and time string. The data is not overwritten.
- **File name** - Use the **Browse...** button to enter a name for the file and to select the folder where the file will be saved. The file can be saved as a text file (.txt).

After selecting the folder, you can use the **{abc}...** button to select a placeholder which is affixed to the file name or to the file path. Place your cursor in the **File name** field where you want the placeholder added. The placeholder is replaced with a current value when the report is created. The format of the *timestamp* placeholder is *yyyymmdd-hhmmss*. For example, you can create a folder for each year by adding the *year* placeholder to the file path and separating it with the backslash (\) character.

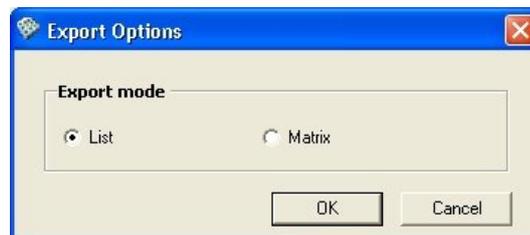
The *plate id* placeholder always uses the plate name of the first plate in naming the file or folder. The placeholder is mainly intended for use with the Automation Interface when one plate is used.



Note If you encounter problems with the measurement and calculation data files, you may need to change the number settings on your PC. The **Decimal symbol** should be determined as “.” and the **Digit grouping symbol** preferably as “,” and not “.”. For more information, see “Microsoft Windows settings” on page 20.

Saving manually

When you want to quickly export the measurement or calculation data of a particular run, use the **Quick Export** option. The Quick Export will not appear in the **Results** tree. In the **Results** tree, select the step containing the data you want to save. Select Data Processing > **Quick Export**, click the  button on the **Results** action panel, or right-click the step, and select **Quick Export**. The **Export Options** dialog is opened.



Select which columns are shown in the list.

- **Export mode** - Select the format in which the data will be laid out in the file: a list or a matrix (table view). If you select *List*, you can select the data columns from the *Columns* list. The exported list retains the selections you made in the list view, including the sorting order and filtering. If you select *Matrix*, only the measurement data is exported.

Click **OK**. Enter a name for the file and select the folder where the file will be saved. The file can only be saved in text format (.txt). The file contains the data in columns, separated by tabulators.

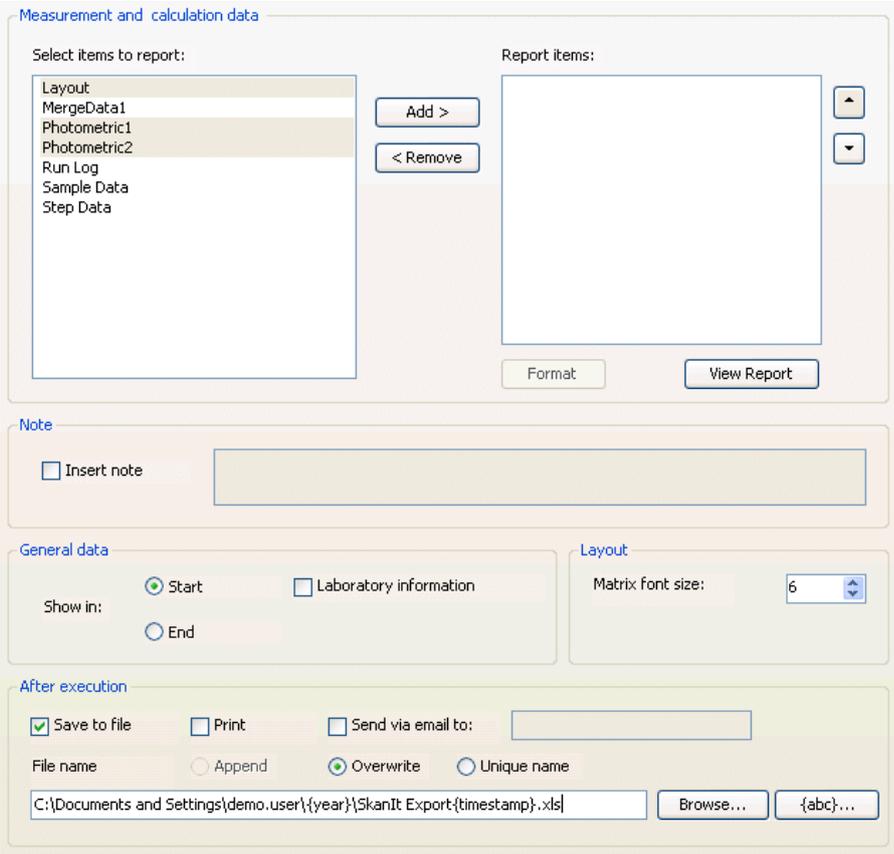
Measurement and calculation reports

You can create a more detailed measurement and calculation data report and print, save or email it each time the session is executed by adding a **Report** step to the **Results** tree after the measurements and calculations.

Results

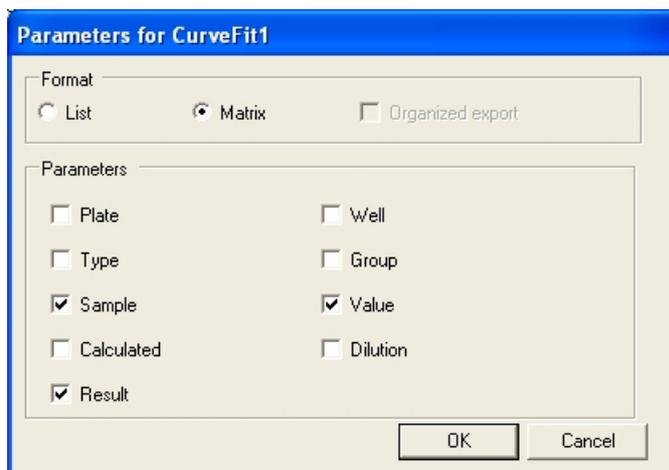
Measurement and calculation reports

Select Data Processing > **Report / Export**, click the  button on the **Results** action panel, or right-click any step, and select **Report / Export**. The report will be added as the last item on the **Results** tree.



The screenshot shows the 'Measurement and calculation data' dialog box. It has two main panes: 'Select items to report' on the left and 'Report items' on the right. The left pane contains a list of items: Layout, MergeData1, Photometric1, Photometric2, Run Log, Sample Data, and Step Data. The right pane is currently empty. Between the panes are 'Add >' and '< Remove' buttons. Below the panes are 'Format' and 'View Report' buttons. Below the panes is a 'Note' section with an 'Insert note' checkbox and a text area. Below the note is a 'General data' section with 'Show in:' radio buttons for 'Start' (selected) and 'End', and a 'Laboratory information' checkbox. To the right of 'General data' is a 'Layout' section with a 'Matrix font size:' dropdown set to '6'. Below these is an 'After execution' section with checkboxes for 'Save to file' (checked), 'Print', and 'Send via email to:'. Below that are radio buttons for 'File name' options: 'Append', 'Overwrite' (selected), and 'Unique name'. At the bottom is a 'File name' text field containing 'C:\Documents and Settings\demo.user\{year}\SkanIt Export\{timestamp}.xls', a 'Browse...' button, and a '{abc}...' button.

- **Measurement and calculation data** - Select the data items you want to display in the report. The available items are shown on the left and the selected items on the right. Click **Add>** to move the selected item(s) to the list on the right and **<Remove** to remove them from that list. You can change the order of the items in the report with the arrow buttons on the right.
- **Format** - You can define the amount of detail you want to show in the report. Select the data from the list on the right and click the **Format** button. The button is not available for Step Data, Layout, Run Log, User Defined Graphs or QC Equations.



The default selections are shown, but you can change them by selecting whether the data is shown in a *List* or table (*Matrix*) format. You can select the information shown by ticking the check boxes.

You can also select to create an **Organized export**. In this format the data is sorted in a manner that is more suitable for manipulation, such as drawing curves, in a spreadsheet program (for example, Microsoft Excel). It is also a more suitable format for use in automated systems. The selections made in the **Parameters** dialog are not applied. The *Organized export* option is only available for kinetic and spectral scanning measurements.

Click **OK** to close the dialog.

- **View Report** - You can view the report for the current run and use the Report toolbar buttons. You can also view the report by selecting the **Report** tab.
- **Note** - You can add an optional text that will be shown at the beginning of the report.
- **General data** - Select whether you want the general information to be printed at the beginning or the end of the report. The general data includes Session parameters (Session name, Session creator, Run name, Run creator, Run started, Total warnings and Total errors, as well as Electronic signature in the DDE version), Software parameters (Software version) and Instrument parameters (Instrument name, Instrument version, Instrument serial number, Actual instrument temperature and Dispenser).

You can also include laboratory information in the general data. The laboratory information includes the company contact information added

through the SkanIt Control Panel (Settings > Control Panel > **Company Settings**; see “Company settings” on page 257).

- **Layout** - You can change the font size of the text shown in matrix (table) format in the report. If you have a 384-well plate, you can reduce the font size to allow the text to be readable when printed on a larger paper. For smaller well counts, you can use a larger font size.
- **After execution** - Select the tasks that are performed after each time a session has been executed. You can save, print and/or email the report.
 - If you select **Save to file**, use the **Browse...** button to enter a name for the file and to select the folder where the file is saved. You can save the file as a Microsoft Excel file (.xls), an Adobe Acrobat Portable Document Format file (.pdf) or a text file (.txt).

After selecting the folder, you can use the **{abc}...** button to select a placeholder which is affixed to the file name or to the file path. Place your cursor in the **File name** field where you want the placeholder added. The placeholder is replaced with a current value when the report is created. The format of the *timestamp* placeholder is *yyyymmdd-hhmmss*. For example, you can create a folder for each year by adding the *year* template to the file path and separating it with the backslash (\) character.

The *plate id* placeholder always uses the plate name of the first plate in naming the file or folder. The placeholder is mainly intended for use with the Automation Interface when one plate is used.

You can also select how the data is handled when the session is saved after a run:

- **Append** - The data is added to the existing file after the data saved last. Available only when the report format is a text file (.txt).
- **Overwrite** - The data in the existing file is overwritten.
- **Unique name** - The file name is extended with a unique date and time string. The data is not overwritten.
- If you select **Print**, the measurement data is printed on the selected printer.
- If you select **Send via email to**, enter the recipient's email address in the field. The measurement data is sent to the specified email address. For more information, see “Email settings” on page 230.

The PDF file is suitable for formal record keeping and it can be printed in color.

Result Report

6/8/2006 5:58:35 PM+03:00

4

- plate 1: 1

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
A	Blank Assay 1/1 0.6361 NaN	U#_0002 1/1 0.6361 NaN	U#_0011 1/1 0.6361 NaN	U#_0019 1/1 0.6361 NaN	U#_0027 1/1 0.6361 NaN	U#_0035 1/1 0.6361 NaN						
B	Cal_0001 1/1 0.6361 NaN	U#_0004 1/1 0.6361 NaN	U#_0012 1/1 0.6361 NaN	U#_0020 1/1 0.6361 NaN	U#_0028 1/1 0.6361 NaN	U#_0036 1/1 0.6361 NaN						
C	Cal_0002 1/1 0.6361 NaN	U#_0005 1/1 0.6361 NaN	U#_0013 1/1 0.6361 NaN	U#_0021 1/1 0.6361 NaN	U#_0029 1/1 0.6361 NaN	U#_0037 1/1 0.6361 NaN						
D	Cal_0003 1/1 0.6361 NaN	U#_0006 1/1 0.6361 NaN	U#_0014 1/1 0.6361 NaN	U#_0022 1/1 0.6361 NaN	U#_0030 1/1 0.6361 NaN	U#_0038 1/1 0.6361 NaN						
E	Cal_0004 1/1 0.6361 NaN	U#_0007 1/1 0.6361 NaN	U#_0015 1/1 0.6361 NaN	U#_0023 1/1 0.6361 NaN	U#_0031 1/1 0.6361 NaN	U#_0039 1/1 0.6361 NaN						
F	Cal_0005 1/1 0.6361 NaN	U#_0008 1/1 0.6361 NaN	U#_0016 1/1 0.6361 NaN	U#_0024 1/1 0.6361 NaN	U#_0032 1/1 0.6361 NaN	U#_0040 1/1 0.6361 NaN						
G	U#_0001 1/1 0.6361 NaN	U#_0009 1/1 0.6361 NaN	U#_0017 1/1 0.6361 NaN	U#_0025 1/1 0.6361 NaN	U#_0033 1/1 0.6361 NaN	U#_0041 1/1 0.6361 NaN						
H	U#_0002 1/1 0.6361 NaN	U#_0010 1/1 0.6361 NaN	U#_0018 1/1 0.6361 NaN	U#_0026 1/1 0.6361 NaN	U#_0034 1/1 0.6361 NaN	U#_0042 1/1 0.6361 NaN						

Curve Fit Calibrator Table

Plate	Well	Sample	Value	Conc.	Calculated	Residual
1	B01	Cal_0001	0.6361	10	NaN	NaN
		Cal_0001 1/1	0.6361	10	NaN	NaN
		Cal_0002	0.6361	20	NaN	NaN
1	C01	Cal_0002 1/1	0.6361	20	NaN	NaN
		Cal_0003	0.6361	40	NaN	NaN
1	D01	Cal_0003 1/1	0.6361	40	NaN	NaN
		Cal_0004	0.6361	80	NaN	NaN
1	E01	Cal_0004 1/1	0.6361	80	NaN	NaN

The Excel file is sorted into separate worksheets.

General Info / Note / Steps Data / Run Log / Photometric1 / BlankSubtraction1 / CurveFit1

Results

Measurement and calculation reports

Chapter 12

Instrument and Software Reports

This section contains information related to instrument and software reports you can create with SkanIt Software.

Creating instrument reports

You can obtain information on the status of the instrument by running instrument status reports. These reports are useful in problem situations where the instrument does not seem to function properly, and they are used by the service personnel. You can run a status report if requested by your Thermo Fisher Scientific representative for diagnosing the problem.

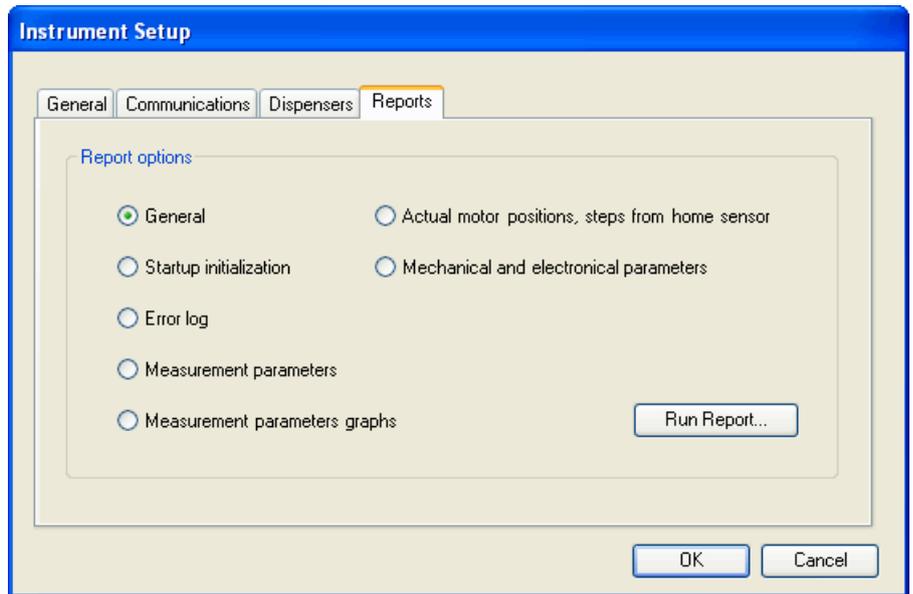
There are four types of instrument status reports: General, Start-up initialization, Error log and Service reports. Service reports include Measurement parameters, Measurement parameters graphs, Actual motor positions, steps from home sensors, and Mechanical and electrical parameters. The service reports are mainly used for diagnosing the performance of the instrument.



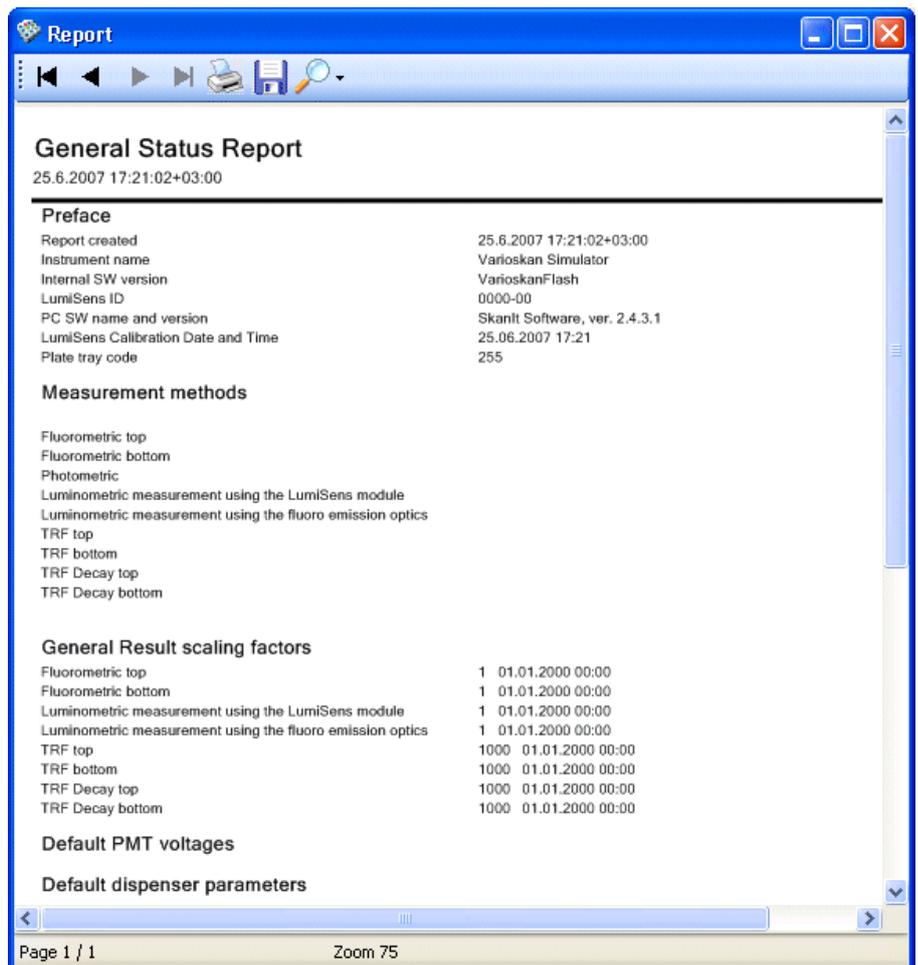
Note The processing of the *Measurement Parameters Graphs* service report may take up to fifteen minutes. The LED indicator on the instrument is orange while the reports are being processed.

The *Start-up Initialization Status Report* shows the results of the instrument's initialization tests. The instrument performs these self-tests each time the instrument is turned on. The report shows whether each test has passed or failed. The Start-up Initialization Status Report is needed for the instrument IQ/OQ/PQ.

Select Settings > Instrument... > **Setup...** and then the **Reports** tab. Select the required report type using the radio buttons and click **Run Report...**



The **Reports** window is launched showing the selected report.



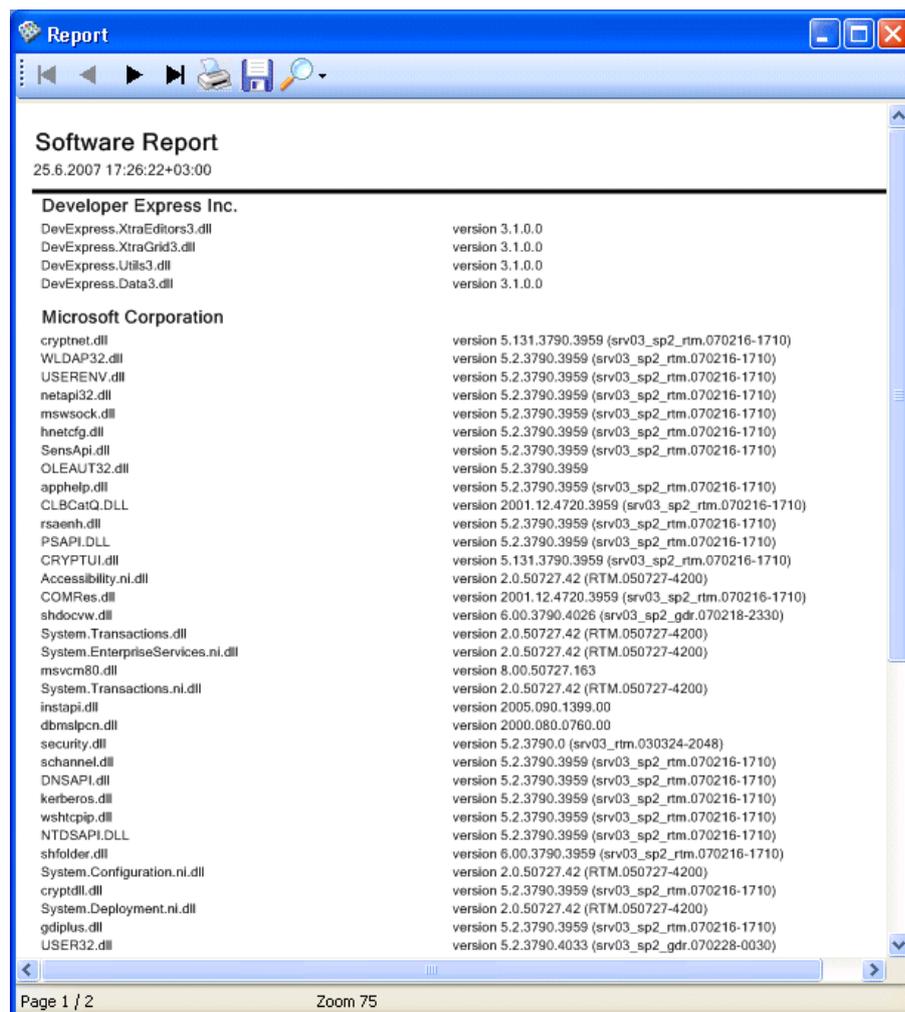
You can print the report and save it in .pdf, .xls, or .txt format. Click the  button. The **Export Report** dialog is launched. Give a name to the report, select the file type and the folder and click **Save**.

You can also print the report by clicking the  button.

Viewing software reports

You can obtain information on the software using the **Help** menu.

The software report includes information on the current version of the different software components. Select **Help > About...** and click **Details...**. The **Report** window is launched.



You can print the report and save it in an Adobe Portable Document Format file (.pdf), a Microsoft Excel file (.xls), or a text file (.txt). Click the  button. The **Save As** dialog is launched. Give a name to the report, select the file type and the folder, and click **Save**.

You can also print the report by clicking the  button.

Viewing run status reports

You can view run status reports of a session. Select the session in the **Results** tree and the run status report opens in the main panel.

The report has general run information, such as the session name and its creator, the name of the run and its creator, date and time when the run was started, and the total number of errors and warnings.

The report also has instrument-specific information including the instrument name, serial number, internal software version, installed dispensers if they are available in the instrument, LumiSens module, and the plate tray used in the measurement. The actual instrument temperature is shown also.

The report also has version information of SkanIt Software.

Run Status Report

10.09.2007 11:36:42+03:00

Run information

Session name	Multipoint in 48-well new4
Session creator	admin
Run name	Multipoint in 48-well new4
Run creator	admin
Run started	10.09.2007 11:36:24+03:00
Total warnings	0
Total errors	0

Instrument parameters

Instrument name	Varioskan Simulator
Instrument version	VarioskanFlash 4.00.37
Instrument serial number	N/A
Actual instrument temperature	20 20 20
Dispenser	1 2 3
LumiSens module	0000-00
LumiSens scaling factor	1
Plate tray	48: 6-48-well adapter for plate w lid

SW Parameters

Run Software Version	SkanIt Software 2.4.3 RE for Varioskan Flash
Current Software Version	SkanIt Software 2.4.3 RE for Varioskan Flash

Instrument and Software Reports

Viewing run status reports

Chapter 13

Settings

The available menu items in the **Settings** menu depend on the security group to which the logged-in user belongs. An administrator can view and modify all items, while other users have a more limited access to the settings. The rights defined for the different security groups are given in Table 13-1.

Table 13-1. Available Settings menu items for different security groups

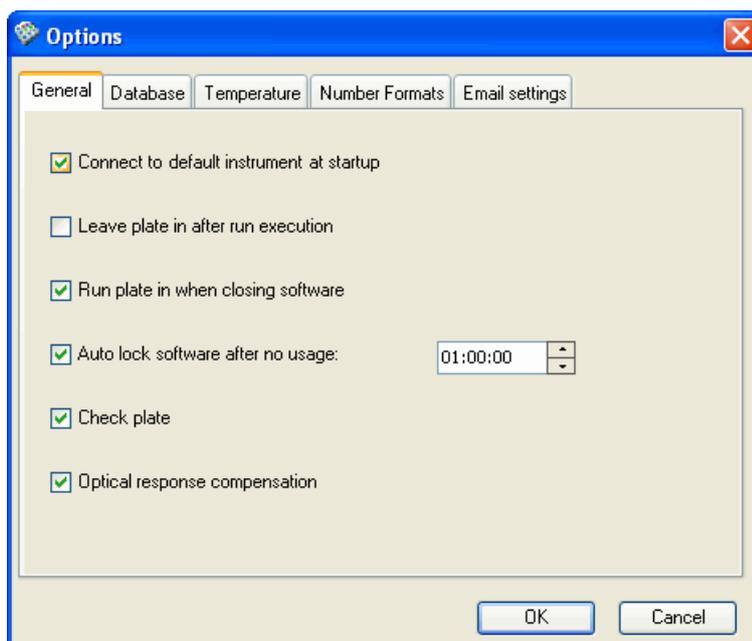
Settings menu item	Administrator	Advanced Operator	Protocol Developer	Operator	Service
Options	x	x	x	x	x
Instrument	x				x
Colors	x				x
Plate Template	x	x			
Saved Curves	x	x	x		
Plate Tray	x	x			
Pathlength Correction	x	x	x		
Protocol Template	x	x	x		
Control Panel	x	x	x	x	x

Options

Select Settings > **Options...**. The **Options** dialog is launched. The dialog has five tabs: **General**, **Database**, **Temperature**, **Number Formats** and **Email settings**.

General

The Settings > Options... > **General** tab is used for settings related to default connection, plate position, checking the plate before execution and locking the software.



- **Connect to default instrument at startup** - When selected, the software connects to the default instrument (see “Instrument” on page 231) at startup. Otherwise, the instrument is not connected. You can establish the connection later by selecting Execution > **Connect**.
- **Leave plate in after run execution** - When selected, the plate is left inside the instrument after the run. Otherwise, the plate is always run out of the instrument.
- **Run plate in when closing software** - When selected, the plate is run into the instrument when the software is closed.
- **Autolock software after no usage** - Select the time after which the user interface requires a new login, if SkanIt Software has not been used. After the user has given the correct user name and password, the software will be available in the state where it was last used.
- **Check plate** - Tick to ensure that the instrument checks that a plate is inserted in the instrument before executing the session.

The instrument reads photometrically the middle of the plate. If there is no plate present in the instrument, you will receive an error message.

If the protocol has a *Dispense* step, the plate will always be checked, regardless of the setting here.

- **Optical response compensation** - In fluorometry instrumentation, the emission reading ability is not necessarily comparable between wavelengths. This happens because the components of the optical system do not have constant responses over the emission wavelength range. Therefore this causes results which are dependent on the emission wavelength even with the same excitation conditions. This uneven emission response can cause difficulties in interpreting the spectral results. For example, the emission peaks can shift even more than 10 nm.

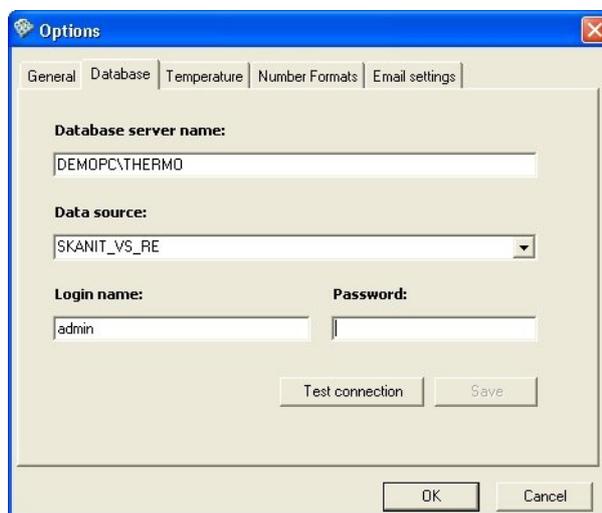
The same applies also to the excitation response.

When selected (by default), the instrument compensates for the different wavelength dependent response characteristics of the optical system in both the emission and excitation. As a result, the RFU values between different wavelengths are comparable with each other and the spectral peaks correspond to the real light emitted from the sample.

If not selected, all the optical component wavelength dependent effects influence the results.

Database

The Settings > Options... > **Database** tab is used to check and/or change the working database used by SkanIt Software (see Appendix B: “Database maintenance”).



1. Verify the used database engine. The database engine where the working database is located is shown in the *Database server name* field. The location is defined at installation (see Step 7 in “Installing the software” on page 26).

You can also switch to use another database engine. You can only connect to a database engine that has your user name defined. When you change the database engine instance, the list in the *Data source* field is updated.

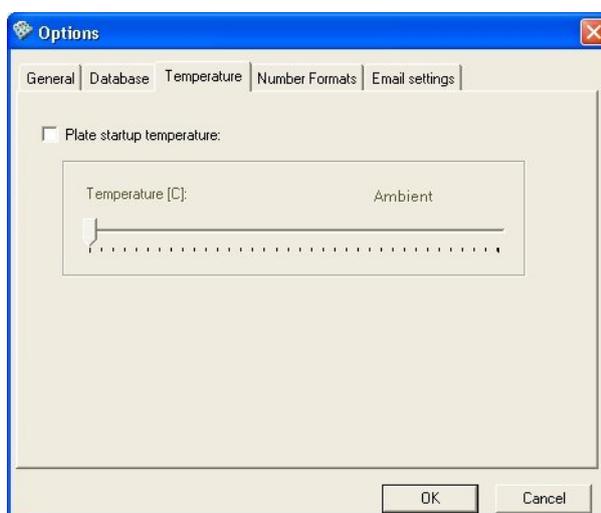
2. The working database (that is, the database currently in use by SkanIt Software), is shown in the *Data source* field. If you want to change the working database to another, select the name of the database from the drop-down menu. If you want to view archived or restored database content, the data source is the name that was given in Database Maintenance (see Appendix B: “Database maintenance”).
3. Enter the login name and login password that are defined in the database to which you are switching. The *Login name* field shows the name of the user currently logged in.
4. Click the **Test connection** button to allow the software to test the connection to the database. You will receive a notification that the connection succeeded. Click **OK**.
5. Click the **Save** button to change the database.
6. Restart SkanIt Software in order to use the new database.

If you want to view or take into use sessions or runs from an archived or restored database, first connect to that database using the procedure described

above. Restart the software. Export the sessions/runs as described in “Exporting sessions or runs” on page 65. Connect back to your working database, restart the software, and import the sessions/runs as described in “Importing sessions or runs” on page 66.

Temperature

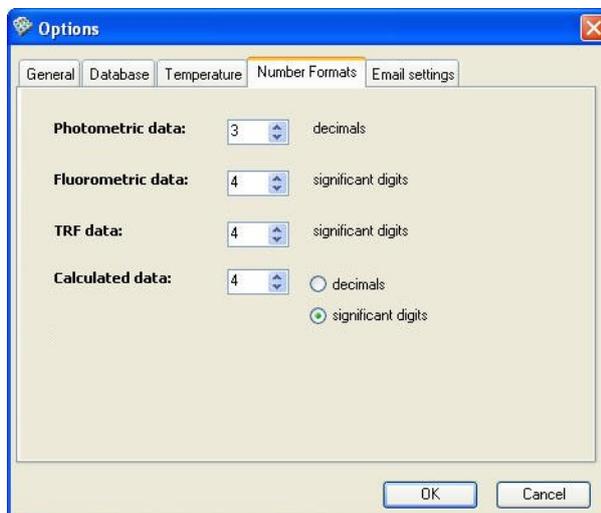
The Settings > Options... > **Temperature** tab is used for setting the plate startup temperature.



- **Plate startup temperature** - When selected, the incubator of the plate is turned on when the instrument and the software are turned on. The incubator is heated to the selected temperature and will be kept at that temperature throughout the use.
- **Temperature [°C]** - Set the startup temperature using the slider with either the mouse or the arrow buttons on the PC keyboard.

Number Formats

The Settings > Options... > **Number Formats** tab is used to select the number of decimals or significant digits that will be shown for measurement raw data and calculated data in the *Table* view and the reports. The original accuracy of the data is retained in the memory and used in the calculations.



Select the number format for presenting data. You can select:

- number of decimals for photometric data
- number of significant digits for fluorometric data
- number of significant digits for time-resolved fluorescence (TRF) data
- number of decimals or significant digits for calculated data

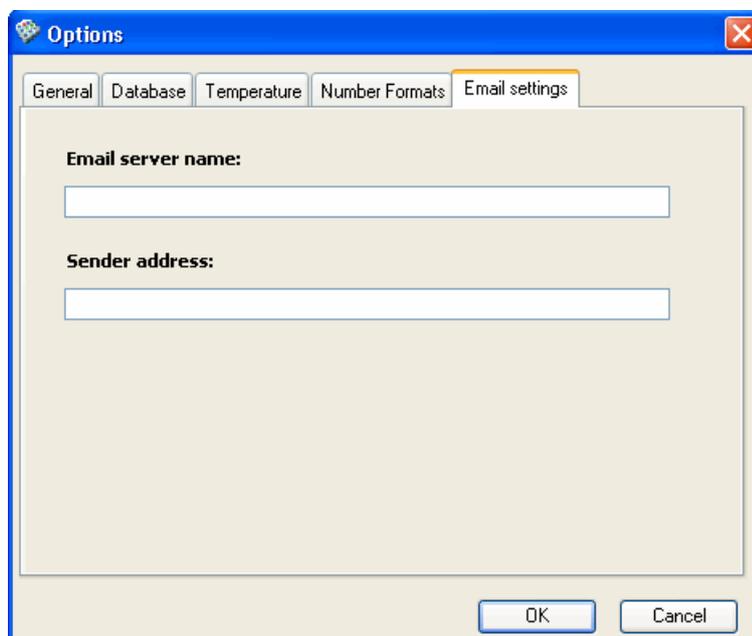
In Table 13-2 examples are shown of the way the selected number format is displayed.

Table 13-2. Number formats

Selected number	Original number	Decimals	Significant digits
5	0.0012345	0.00123	0.0012345
3	123.45678	123.457	123

Email settings

The Settings > Options... > **Email settings** tab is used to define the email settings. The settings are needed, if you want to send, for example, measurement and calculation data reports to an email address automatically after executing a session. For more information, see “Measurement and calculation reports” on page 213.



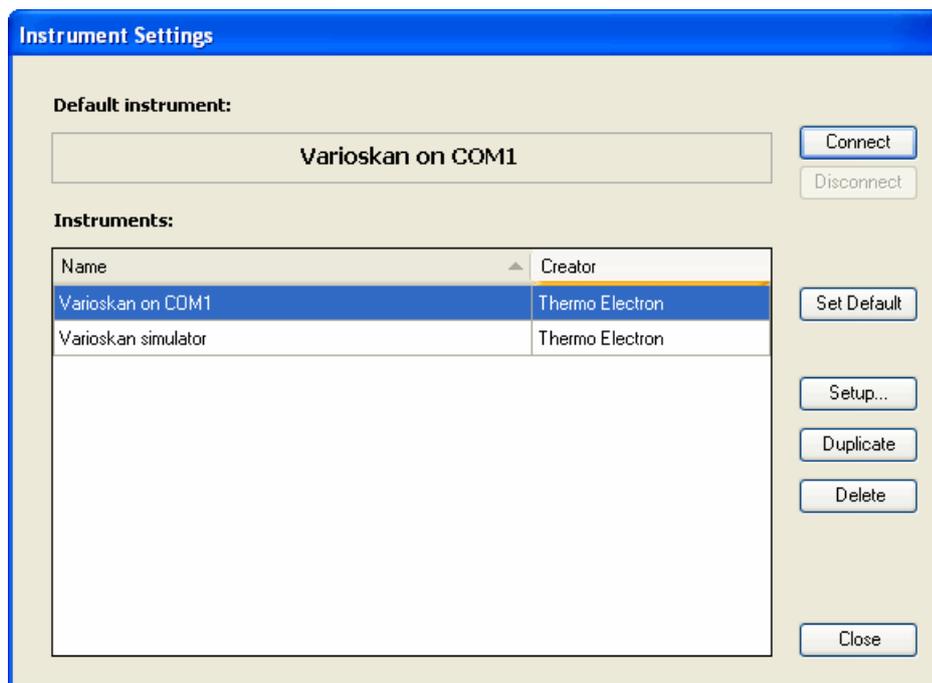
1. Enter the name of the email server.



Note If the server requires authentication, it is not possible to send email outside the server.

2. Enter the email address of the sender.
3. Click the **OK** button to save the information.

Instrument Open the **Instrument Settings** dialog by selecting Settings > **Instrument...**



- **Connect** - Opens a connection to the default instrument.
- **Disconnect** - Closes the connection to the default instrument. You must close the connection before setting another instrument as the default.
- **Set Default** - Click the button to set the selected instrument as the default. The software will try to connect to the default instrument when it is launched (see “Launching SkanIt Software” on page 54).
- **Setup...** - You can edit the setup parameters of an existing instrument and create instrument status reports.
- **Duplicate** - You can make a duplicate of an existing instrument and save it with a new name.
- **Delete** - Remove the selected instrument from the database.
- **Close** - Close the dialog.

To edit instrument-related settings, select the instrument from the list and click **Setup...**. The **Instrument Setup** dialog opens. “General” on page 233 through “Reports” on page 236 describes the **Instrument Setup** dialog. Also see “Options” on page 226.

To add a new instrument to the database, see “Adding an instrument to the PC database” on page 34.

To remove an instrument from the database, select the instrument from the *Instruments* list. Click **Delete**. The instrument is removed from the database.



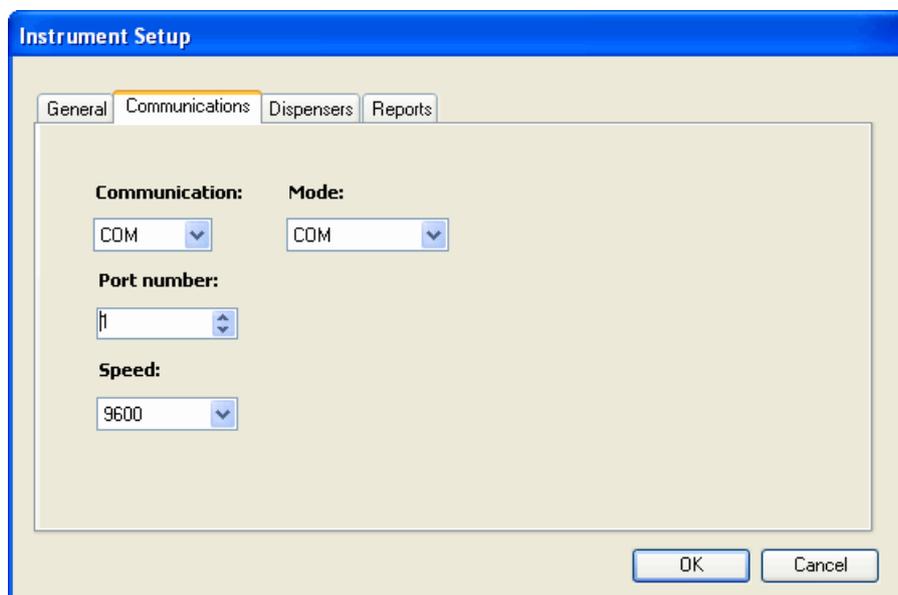
Note You cannot delete the default instrument from the database. If you want to delete the instrument that has been selected as the default instrument, first set another instrument to be the default instrument.

General Check the general information related to the instrument on the **General** tab.

The screenshot shows a window titled "Instrument Setup" with a blue header. Below the header are four tabs: "General", "Communications", "Dispensers", and "Reports". The "General" tab is selected. The main area contains three labeled input fields: "Name:" with a text box containing "Varioskan simulator"; "Instrument type:" with a dropdown menu showing "Varioskan"; and "Serial number:" with a text box containing "123". At the bottom right, there are "OK" and "Cancel" buttons.

- **Name** - The unique name of the specific instrument.
- **Instrument type** - The type of your instrument.
- **Serial number** - Check the serial number of the instrument from the type label and enter it here. The type label is on the right rear of the instrument.

Communications Define the communication settings between the instrument and the PC on the **Communications** tab.

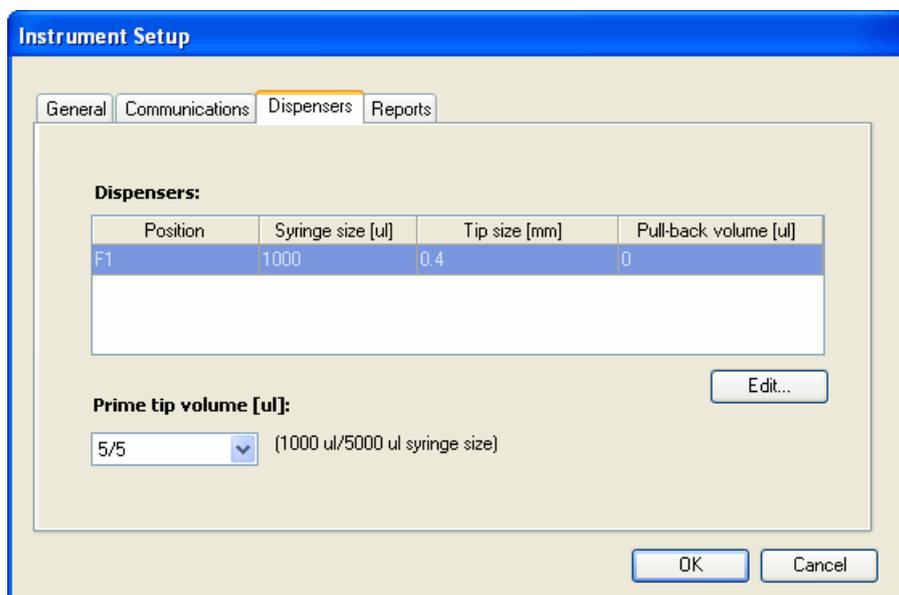


- **Communication** - Select the type of data communication port. The available port types are USB (Universal Serial Bus) and COM.
- **Mode** - Select which communication mode is used. COM or USB is used with the instrument and EMU with simulation.
- **Port number** - Select the COM port that the instrument is connected to. Allowed port numbers are 1 to 100. This selection is available only for the COM port.
- **Speed** - Select the data transfer speed. Available speeds are 9600 and 19200. The recommended speed is 9600. This selection is only available for the COM port.

Dispensers

The Varioskan can be equipped with one optional dispenser, allowing dispensing from 1 to 1,000 μ l in 1 μ l increments.

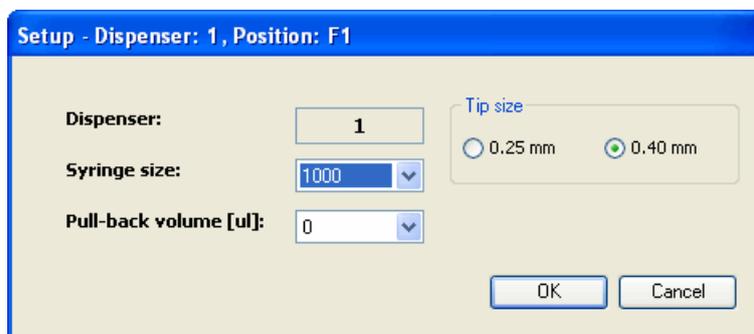
1. For configuring the dispenser of the instrument, select Settings > **Instrument...** and select the instrument from the *Instruments* list. Click **Setup....**
2. Click the *Dispensers* tab.



If the instrument is not connected, the software displays a message and you have to first connect the software to the instrument. Exit the instrument setup (click **Cancel** and then **Close**) and select Execute > **Connect**. Start over again with Step 1.

The *Dispensers* list shows the dispensing position where a dispensing head is located. It also shows the syringe and tip size as well as the pull-back volume. The information is read from the instrument. If not connected to the instrument, the last known configuration is read from the database.

3. Edit the dispenser information by selecting the dispenser in the list and clicking the **Edit...** button. The dispenser information is used in the dispensing step. For more information on using dispensing in protocols, see “Dispense” on page 120.



- **Dispenser** - The Varioskan instrument and SkanIt Software have one optional dispenser available; Dispenser 1.

- **Syringe size** - Select the volume of the dispenser syringe. 1,000 and 5,000 μl are available; 1,000 μl is the default value. The 5,000 μl syringe is available on request.
- **Tip size** - Select the tip size of the dispenser, either 0.25 or 0.40 mm in diameter; default is 0.40 mm.
- **Pull-back volume [μl]** - Select the pull-back volume for the dispensing tip in μl . The volume range is 1 to 10 μl for the 1,000 μl syringe and 5 or 10 μl for the 5,000 μl syringe. The default value is 2 μl for the 1,000 μl syringe and 5 μl for the 5,000 μl syringe.

The pull-back feature prevents liquid droplets from forming onto the dispensing tip between dispensings. Therefore the liquid is pulled slightly inward. If set, the dispenser automatically pulls back the selected amount of liquid into the syringe every time the instrument dispenses. It is always recommended to use the pull-back feature when using the 5,000 μl syringe.

Click **OK**.

4. Select the priming volume of the tip from the drop-down menu; default is 5/5 μl . The first amount is for the 1,000 μl syringe and the second for the 5,000 μl syringe. The available range is from 1 to 10 μl for the 1,000 μl syringe and 5 or 10 μl for the 5,000 μl syringe. The tip priming volume is the same for all the dispensers that use the same syringe size.

The dispenser dispenses the selected amount of reagent into the tip priming vessel every time the instrument fills the dispenser syringe. This makes the volume of the first well equal to that of the others, thus compensating for the so-called drawback phenomenon. It is recommended to use the tip priming feature to achieve greater accuracy when the dispensing volumes are small, for example, 1 to 50 μl .

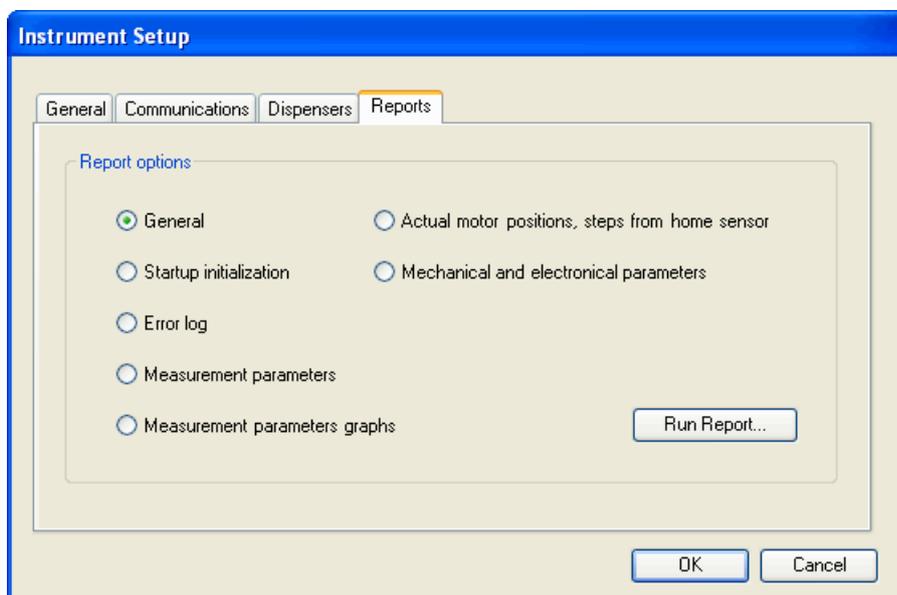


Note Tip priming is a different procedure from manual priming that must be performed when reagent bottles are installed into the dispensers and the dispenser tubes are completely empty.

5. Click **OK**.

Reports

You can create instrument status reports on the **Reports** tab. For more information, see “Creating instrument reports” on page 219.

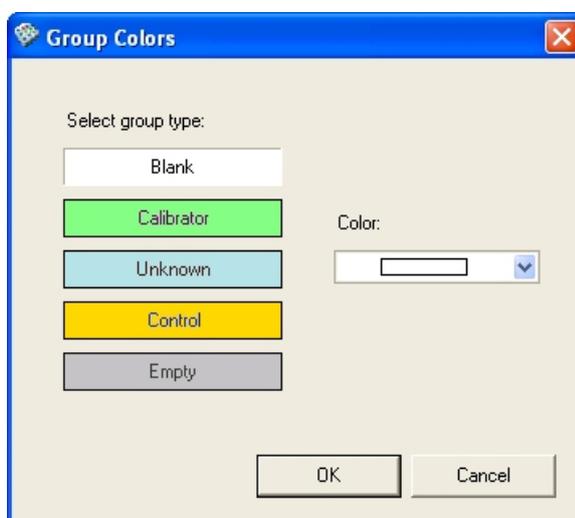


1. Select the report option you want to create.
2. Click **Run Report....** The report is created and you can view, print or save it.

Colors

You can change the default colors set for the sample types (blanks, calibrators, unknowns, controls, and empty wells) to display them in the plate layout and in the Results table view.

Select Settings > **Colors....** The **Group Colors** dialog is launched.



Click the sample type and then select a new color from the drop-down list.

Plate template settings

You can change the default plate template, create new templates in the plate template database, modify existing plate templates, and delete plate templates from the database. Select Settings > **Plate Template...** The **Plate Template Settings** dialog is opened.



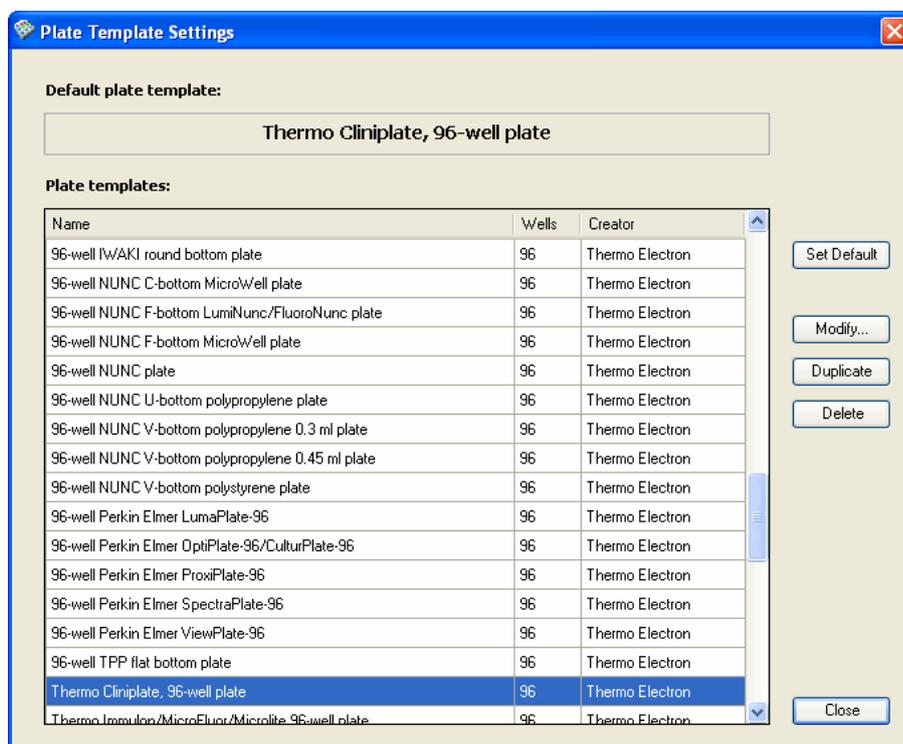
Caution Verify the plate template settings each time you start using a new batch of plates. The dimensions of the plate may have changed slightly and thus the instrument performance may be jeopardized. The instrument and software may give incorrect data and even cause damage to the instrument. Thermo Fisher Scientific takes no responsibility for any incorrect data or damage to the instrument if caused by using incorrect plate template settings.



Note You cannot modify plate templates created by Thermo Fisher Scientific. You can only view the settings. If you want to modify an existing plate template, make a duplicate of it and save it with a new name.



Note To use a newly created plate template, you have to first associate the plate template with one or several plate trays. For more information, see “Associating plate templates with plate trays” on page 245.

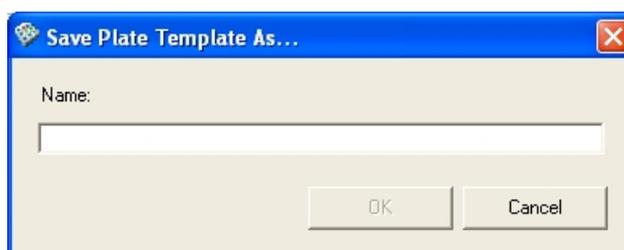


- **Set Default** - Click the button to set the selected plate type as the default. The default plate type is used when creating a new session and in the Protocol Properties (see “Creating a new session” on page 61 and “Editing a protocol” on page 81).
- **Modify...** - You can view the settings of an existing plate template and edit them. See “Modifying plate templates” on page 240.
- **Duplicate** - You can create a new plate template by making a duplicate of an existing plate template. See “Creating plate templates” on page 239.
- **Delete** - Remove the selected plate type from the database. The software requires you to confirm the deletion.
- **Close** - Close the dialog.

Creating plate templates

You can create new plate templates by duplicating existing plate templates.

1. Select Settings > **Plate Template...** The **Plate Template Settings** dialog opens.
2. Select a plate template from the list. Select a plate template that is as similar as possible to the one you want to create.
3. Click **Duplicate**.



4. Give a name to the new plate template and click **OK**. The duplicate plate template is saved.
5. Select the new plate template from the list of plate templates and click **Modify...** The **Plate template editor** opens.
6. Modify the dimensions of the plate template as necessary (see “Modifying plate templates” on page 240).

7. Click **Save**.

Modifying plate templates

You can modify the dimensions of a plate template, for example, after creating the plate template by duplicating an existing plate template.

1. Select Settings > **Plate Template....** The **Plate Template Settings** dialog opens.
2. Select the plate template from the list and click **Modify....** The **Plate template editor** opens.

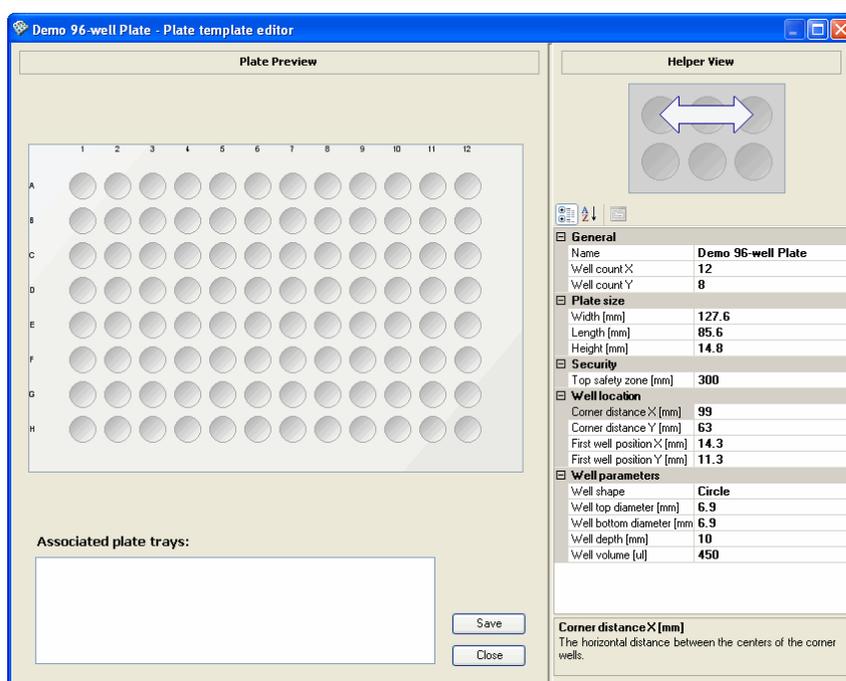


Plate Preview shows a model of the plate.

Helper View is a visual aid showing the selected distance on a 6-well plate.

The plate template definition has the following parameters. The unit of the distances is given in mm at a precision of 0.1 mm.

General:

- **Name** - The name of the plate.
- **Well count X** - The number of wells in the X-direction.
- **Well count Y** - The number of wells in the Y-direction.

Plate size:

- **Height [mm]** - The height of the plate.
- **Length [mm]** - The length of the plate (vertical).
- **Width [mm]** - The width of the plate (horizontal).

Security:

- **Top safety zone [mm]** - The top safety zone of the well in multipoint measurements.

Well location:

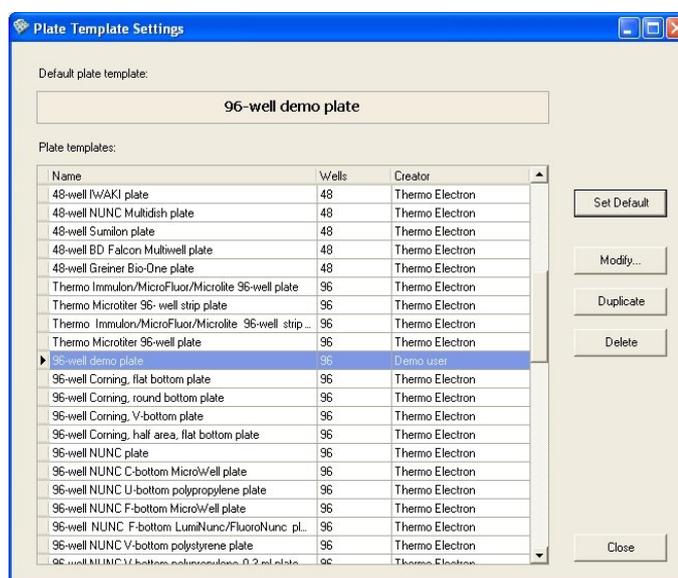
- **Corner distance X [mm]** - The horizontal distance between the centers of the corner wells.
- **Corner distance Y [mm]** - The vertical distance between the centers of the corner wells.
- **First well position X [mm]** - The distance of the center of the top-left well (A1) from the left edge of the plate.
- **First well position Y [mm]** - The distance of the center of the top-left well (A1) from the top edge of the plate.

Well parameters:

- **Well shape** - The shape of the well is usually a circle or a square, but may also be an ellipse or a rectangle, if the height and width of the well are not equal.
- **Well top diameter [mm]** - The well top diameter.
- **Well bottom diameter [mm]** - The well bottom diameter.
- **Well depth [mm]** - The well depth.
- **Well volume [µl]** - The maximum filling volume of the well in µl.

The *Associated plate trays* list displays the plate trays that are compatible with the plate template. If you are adding a new plate template to the database, you have to associate it with plate trays before it can be used. The plate trays are associated with plate templates using the **Plate Tray Editor** (see “Associating plate templates with plate trays” on page 245).

3. Click on appropriate fields and enter the new dimensions in them.
4. Save your changes by clicking **Save**.
5. Close the editor by clicking **Close**. If you have unsaved changes, you can save or ignore them here.
6. If needed, set the plate template as the default plate template:
 1. Select the plate template from the list.



2. Click **Set Default**.

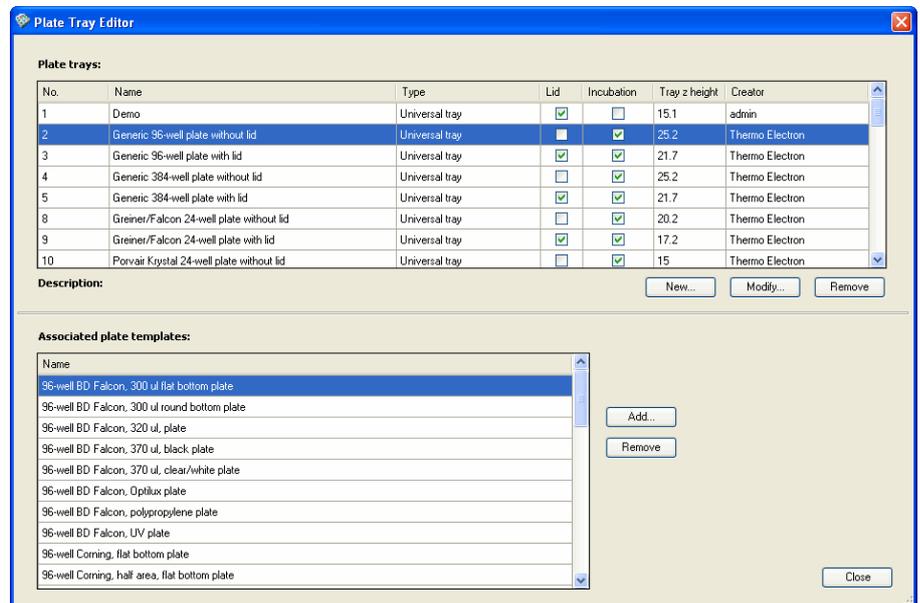
Plate tray

The software performs compatibility checks each time the instrument and the software are started up to ensure that the used plate type and the used plate tray are compatible. Only compatible plates are allowed.

If the plate tray is changed while the instrument and the software are on, you can manually identify the tray by selecting **Execute > Identify Plate Tray**. The name of the plate tray and the plate tray identification number are shown.

The database includes association links between plate types and plate trays. You can add new plate trays to the database and include compatibility information. You can also connect new plate templates to compatible plate trays.

Select Settings > **Plate Tray...** The **Plate Tray Editor** dialog is displayed.



The **Plate Tray Editor** includes the following parameters.

Plate trays:

- **No.** - The plate tray recognition number.
- **Name** - The name of the plate tray.
- **Type** - The type of plate tray: Universal tray, Robot tray or Specific incubation tray.
- **Lid** - Indicates if the plate tray type can be used with plates with a lid.
- **Incubation** - Indicates whether the plate tray type can be used with incubation.
- **Tray z height** - The tray z height.
- **Creator** - The name of the user who has added the plate tray to the database.
- **Description** - An optional description of the plate tray, as given in the **Add New Plate Tray** or **Modify Plate Tray** dialog.
- **New...** - Add a new plate tray to the database (see “Adding new plate trays to the database” on page 244).
- **Modify...** - Modify existing plate tray information (see “Modifying plate trays” on page 246).

- **Remove** - Remove the selected plate tray from the database.

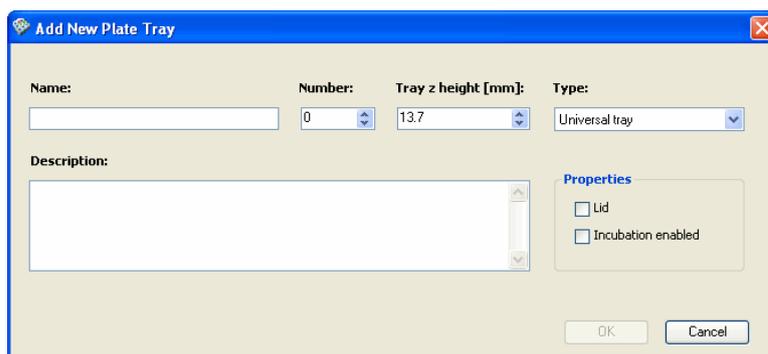
Associated plate templates:

- **Add...** - Add an associating link between a plate template and a compatible plate tray. The plate can thus be used when the plate tray is in the instrument (see “Associating plate templates with plate trays” on page 245).
- **Remove** - Remove the link between the selected plate template and the selected plate tray.

Adding new plate trays to the database

You can add a new plate tray to the database.

1. Select Settings > **Plate Tray...** The **Plate Tray Editor** dialog opens.
2. Click **New....** The **Add New Plate Tray** dialog opens.



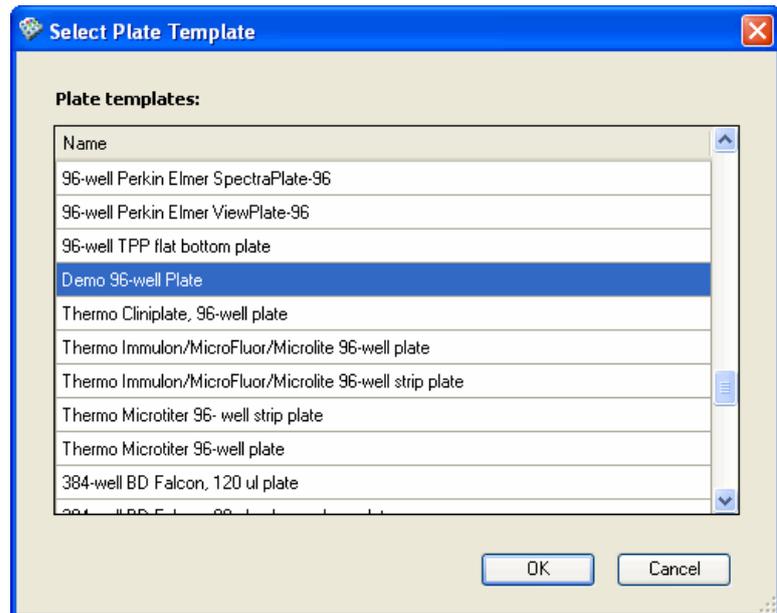
3. Define the following settings:
 - **Name** - Enter the name of the plate tray. The name must be unique and it should be descriptive.
 - **Number** - Enter the plate tray identification number. You can only use numbers that are not already in use (the **OK** button is otherwise not available).
 - **Tray z height [mm]** - Enter the tray z height provided with the adapter.
 - **Type** - Select the type of plate tray: Universal tray, Robotic tray or Specific incubation tray.
 - **Lid** - Tick the check box if the plate tray can be used with plates with a lid.

- **Incubation enabled** - Tick the check box if the plate tray can be used with incubation.
 - **Description** - Enter an optional description of the plate tray.
4. Click **OK**.

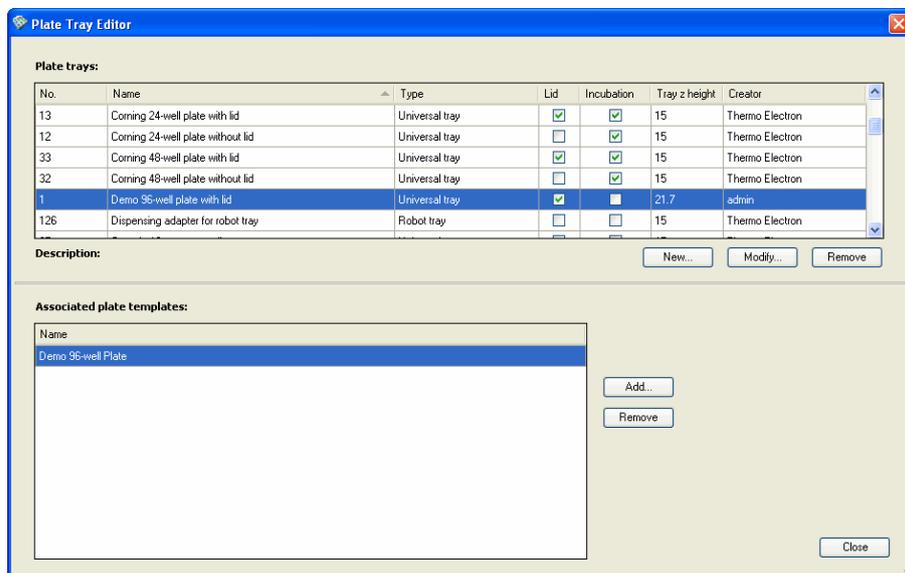
Associating plate templates with plate trays

You must associate a plate template with one or several plate trays before you can use the plate template. An associating link between a plate template and a plate tray is created.

1. Select Settings > **Plate Tray...** The **Plate Tray Editor** dialog opens.
2. Select the plate tray from the list and click **Add...** The **Select Plate Template** dialog opens.



3. Select the plate template from the list and click **OK**.

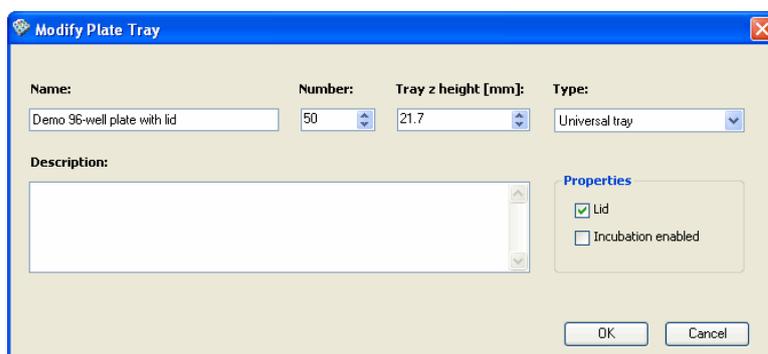


The plate template is now associated with the plate tray.

Modifying plate trays

You can modify the information of an existing plate tray.

1. Select Settings > **Plate Tray...** The **Plate Tray Editor** dialog opens.
2. Select the plate tray from the list.
3. Click **Modify...** The **Modify Plate Tray** dialog opens.



4. Modify the information as necessary. For description of the settings, see “Adding new plate trays to the database” on page 244.

5. Click **OK**.

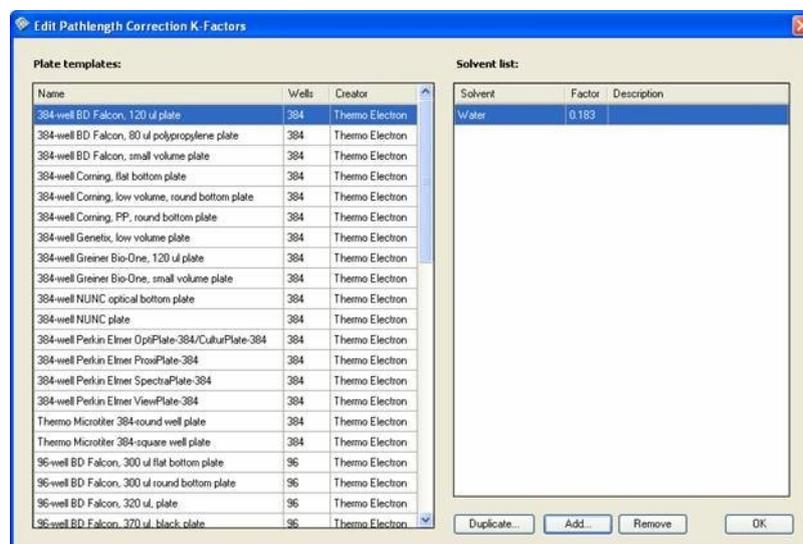
Editing pathlength correction K-factors

The **Edit Pathlength Correction K-Factors** dialog can be used for checking the pathlength correction factors already in the database and for adding known values (from literature) to the database. For more information on pathlength correction and how to define new pathlength correction factors, see “Pathlength correction” on page 72 and “Measuring new pathlength correction K-factors” on page 73. For information on using pathlength correction factors in measurements, see “Using pathlength correction in a measurement” on page 97.



Note The pathlength correction is dependent on the material used to manufacture the plate, and should be redetermined if the plate type is changed.

Select Settings > **Pathlength Correction...** The **Edit Pathlength Correction K-Factors** dialog is launched.



- **Plate templates** - Select the plate template for which you want to check the pathlength correction factor or add a new factor.



Note The pathlength correction factor can only be used with 96- and 384-well plates.

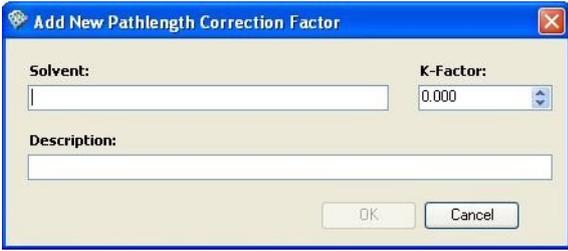
- **Solvent list** - List of solvents and their pathlength correction factors with descriptions.
- **Duplicate...** - Make a copy of an existing pathlength correction factor and save it with a new name.

Settings

Editing pathlength correction K-factors

- **Add...** - Add a new known pathlength correction factor to the list. See below.
- **Remove** - Remove the selected pathlength correction factor from the database.

When you click the **Add...** button, the **Add New Pathlength Correction Factor** dialog is opened. Make sure that you have selected the correct plate type.



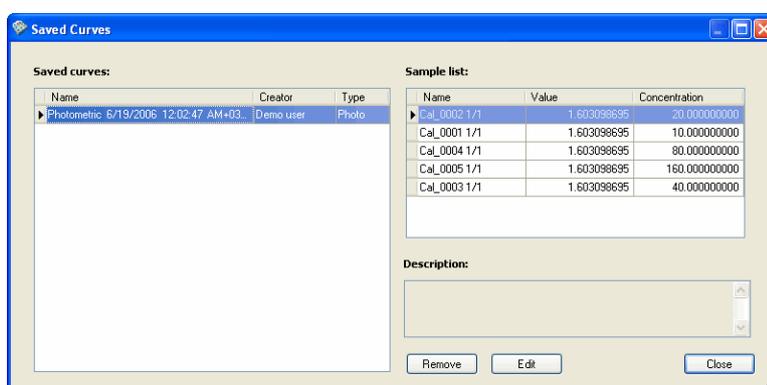
- **Solvent** - Enter the name of the solvent.
- **K-Factor** - Enter the pathlength correction factor value (from literature or the measurement).
- **Description** - An optional description.

Click **OK** to enter the new pathlength correction factor to the database.

Saved curves

You can view the saved standard curves used in quantitative analysis and edit their descriptions. You can also remove them through the **Saved Curves** dialog. The dialog opens when you select Settings > **Saved Curves....** For more information about quantitative analysis, see “Quantitative curve fit” on page 153.

When you export sessions or runs, the saved standard curves related to them are also exported. For more information, see “Exporting sessions or runs” on page 65.



Saved curves:

- **Name** - Name of the standard curve given when the quantitative curve fit calculation was defined. Select a saved standard curve to view its properties in the sample list.
- **Creator** - Name of the creator.
- **Type** - Measurement technique used.

Sample list:

- **Name** - Name of the samples of the selected standard curve.
- **Value** - Value of each sample.
- **Concentration** - Concentrations used in the measurement for each sample.

Description - Shows the description of the selected curve.

- **Remove** - Removes a curve from the list of saved curves. Select the curve you want to delete and click the button.

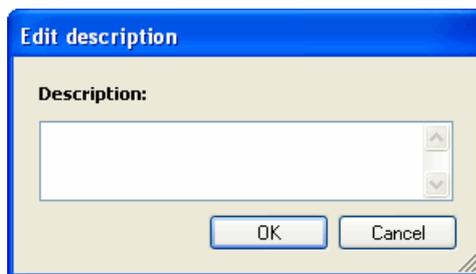


Note You can only delete curves that are not in use.



Note Before deleting curves, close your session. Save your session before closing, if necessary.

- **Edit** - Opens the **Edit description** dialog. Enter or change the description and click the **OK** button.

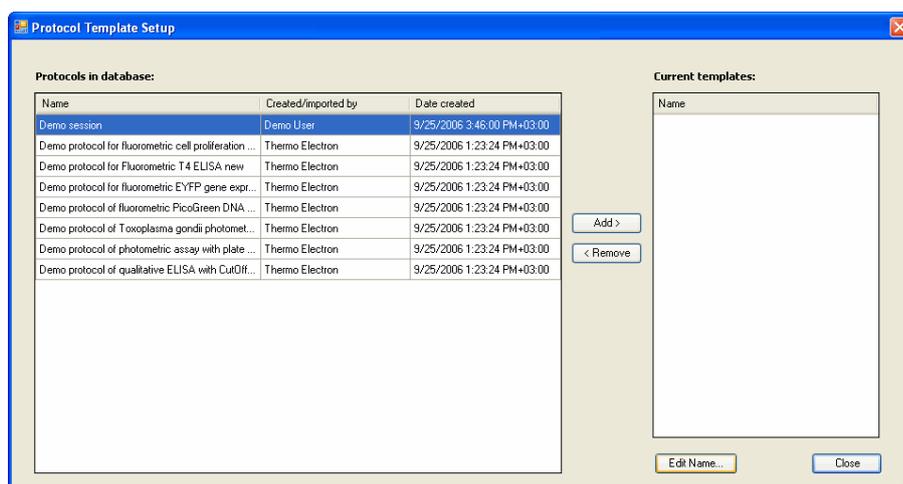


- **Close** - Saves the changes and closes the dialog.

Protocol template setup

You can use an existing protocol as a template that can be used later to facilitate protocol creation. The protocol template will be available in the **Create New Session** dialog under *User-defined protocol* types. See “Creating a new session” on page 61.

Select Settings > **Protocol templates...** The **Protocol Template Setup** dialog is launched.

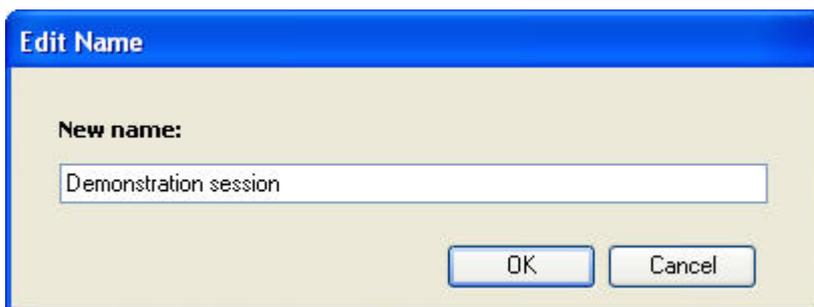


- **Protocols in database** - List of the protocols currently in the database.
- **Current templates** - List of the protocol templates. These protocol templates are available when creating new sessions and selecting *User-defined protocol* (see “Creating a new session” on page 61).
- **Add >** - Add the selected protocol to the template list.



Note When you set the protocol as a protocol template, the multipoint selections are disabled to prevent problems with plates that do not allow multipoint measurements.

- < **Remove** - Remove the selected template from the template list.
- **Edit Name...** - Click to change the name of the protocol template.



Enter a new name for the protocol template and click **OK**.

SkanIt Control Panel

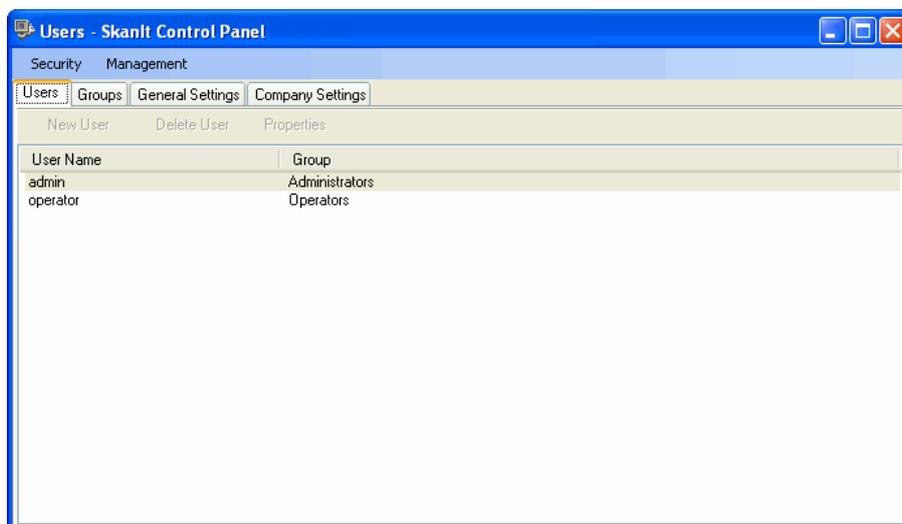
Users are managed through **SkanIt Control Panel**. The rights in the software and the Control Panel depend on which user group the logged-in user belongs to. Only the administrator may register and remove users and user groups and assign rights for them. The administrator can also change passwords for other users (see Appendix A: “Administrators”). Users with more limited access rights can use the Control Panel to change their logon and signature passwords (only in the DDE version) as well as the company information.

Select Settings > **Control Panel**. You can also click the **SkanIt Control Panel** icon on the desktop or select Programs > Thermo SkanIt Software > **SkanIt Control Panel** from the Start menu. The **Log On To SkanIt Control Panel** dialog is displayed.



Only the user currently logged onto SkanIt Software can log onto SkanIt Control Panel. Enter your password and click **OK**.

SkanIt Control Panel is launched. A list of the users and the security groups they belong to is displayed.



SkanIt Control Panel has the following views: **Users** (see “Users” on page 253), **Groups** (see “Groups” on page 256), **General Settings** (see

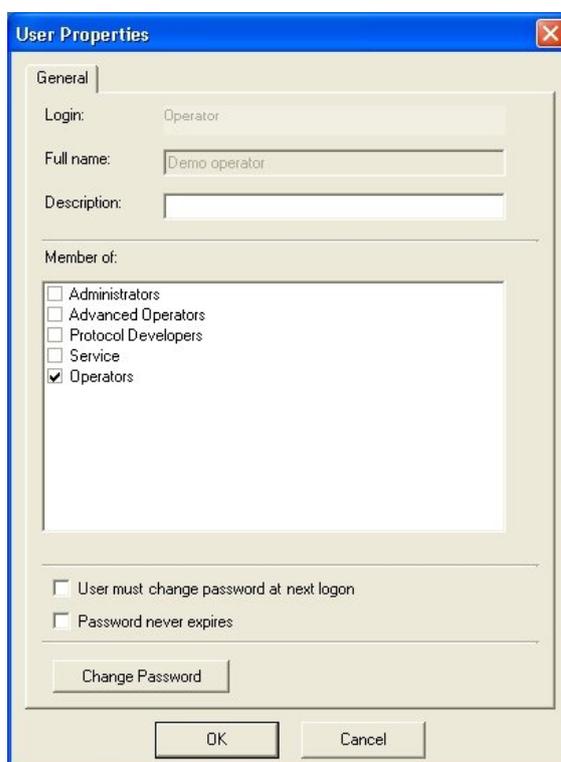
“General settings” on page 256) and **Company Settings** (see “Company settings” on page 257) plus **Audit** in the Drug Discovery Edition (see “Audit trail” on page 300). These views can be selected by clicking the appropriate tab at the bottom of the window or by selecting them from the **Management** menu.

Users In the **Users** view it is possible to change logon and signature passwords, and users with administrator rights can create new users, edit user properties and remove users.

The dialog lists the current users and the user groups they belong to that are defined in the database.

Changing your own logon password

1. Ensure that no session is open before you change your password.
2. Select your own user name from the list.
3. Click **Properties** or double-click your user name. The **User Properties** dialog is opened.



4. Click the **Change Password** button to launch the **Change Logon Password** dialog. The button is only available, when the *Password never expires* check box is not selected.



5. Enter your current password in the *Old password* text box and the new password in both the *New password* and the *Confirm password* text boxes.



Note The logon password is not case sensitive.

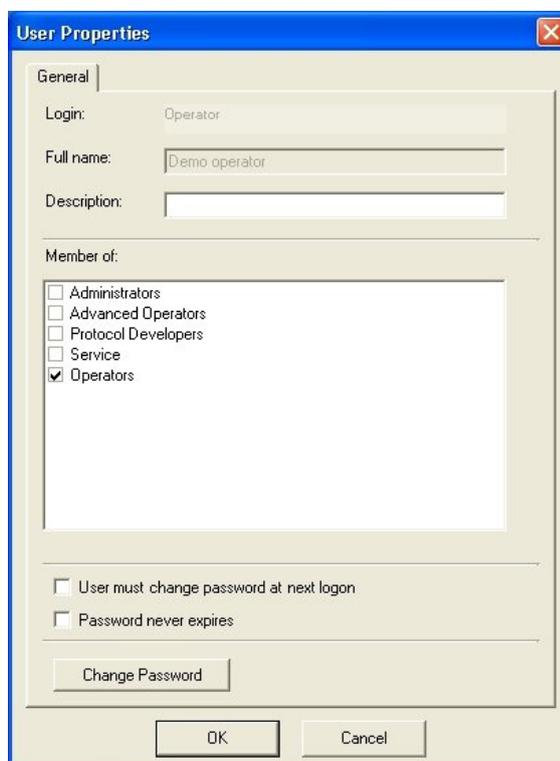
6. Click **OK**. Your new password is valid the next time you log onto SkanIt Software.

Viewing user information

Only an administrator can view and modify the user information of other users (see “Users” on page 275).

In **SkanIt Control Panel** select your own user name and click **Properties**.

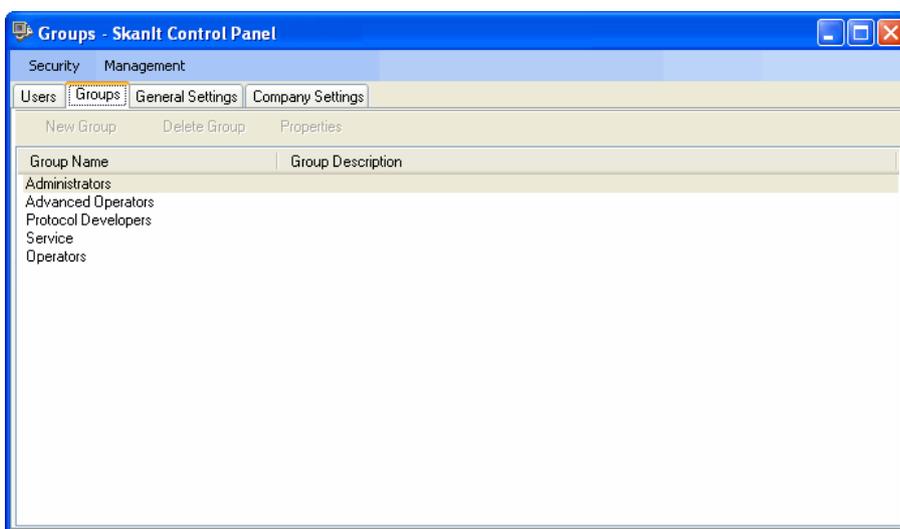
The **User Properties** dialog is launched.



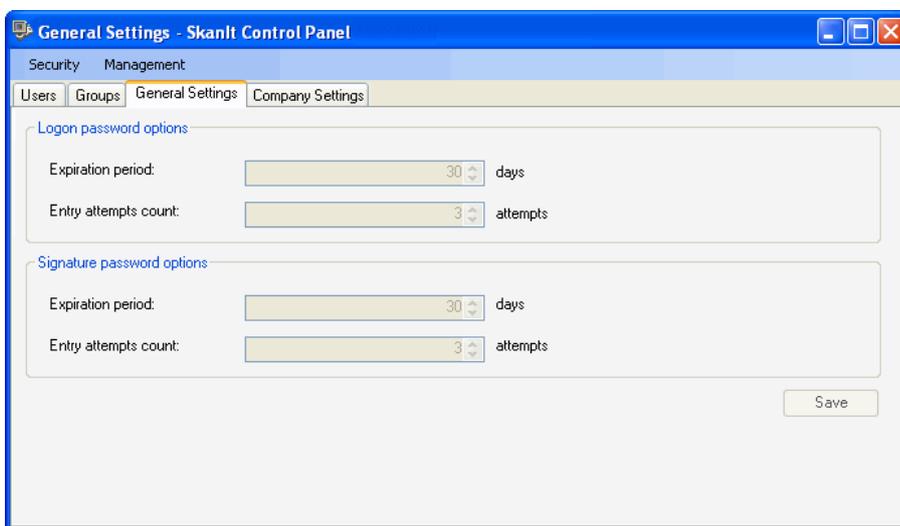
- **Login** - The login name of the user. This is the name that is entered when logging onto SkanIt Software and the Control Panel.
- **Full name** - The full name of the user. It is displayed as the creator name in session, protocol and plate layout lists and is shown on the status bar. The Audit trail (DDE only) also records the full name.
- **Description** - An optional description of the user.
- **Member of** - The security group(s) the user is a member of. The rights of the different security groups are listed in Table A-1.
- **User must change password at next logon** - If this is checked, the user is prompted to change the password the first time (s)he logs onto the software.
- **Password never expires** - If this is checked, the password does not expire.
- **Change Password** - Click the button to launch the **Change Logon Password** dialog. The button is only available, when the *Password never*

expires check box is not selected. See “Changing your own logon password” on page 253

Groups Only the administrator can add new user groups and modify their rights (see “Groups” on page 279). Other users may only view the group list.



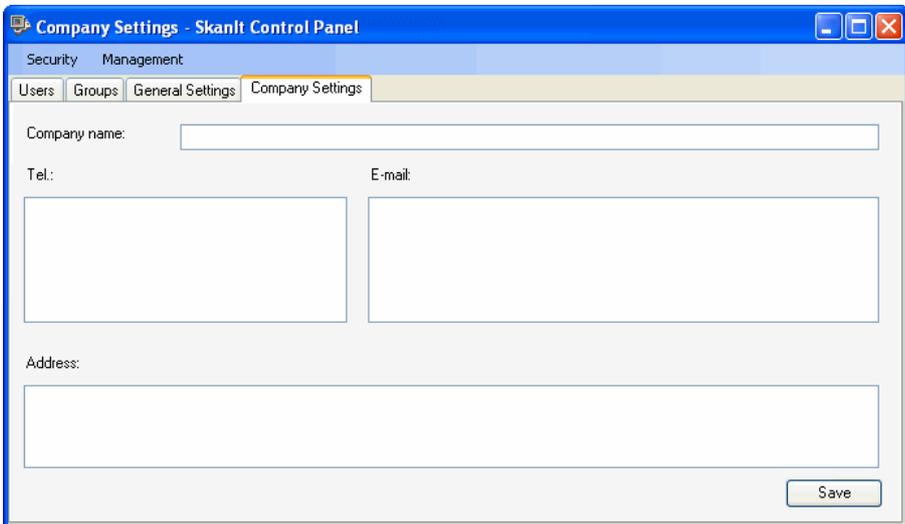
General settings Use the general settings to view and modify general logon and signature password settings. Only an administrator can modify these settings (see “General settings” on page 282), other users may only view them.



- **Expiration period** - The number of days the password is valid.
- **Entry attempts count** - The number of failed password entry attempts. If the user fails to enter the correct password within the allowed number of times, the user account is locked and only an administrator can unlock it, and if necessary, change the user's password.

Company settings

You can give information here on the company (laboratory) performing the measurements. This information will be displayed in the reports (see “Measurement and calculation reports” on page 213).



- **Company name, Tel., E-mail and Address** - Enter the name and contact information of the company.
- **Save** - Click the button to save the information.

Automation interface

The Remote Control Interface for SkanIt Software (SkanIt Automation Server) is developed to provide a standard interface for automation systems to manage instruments connectable to SkanIt Software. The main idea is to hide the communication protocol, complexity, state behavior and differences that exist in these instruments.

The technology approach is an event-based messaging system built on the .NET Framework version 2.0.

SkanIt Automation Server is started by the Automation System and it has no graphical user interface.

Settings

Automation interface

The Automation System will act as a master and SkanIt Automation Server as a slave. The normal case is that these two software components may exist in one computer, but optionally, the remote control can be run over a TCP/IP network, too.

A separate document, *Thermo Scientific Automation Interface for SkanIt Software ver. 2.4 User Manual* (Cat. no. N05302), is available upon request from Thermo Fisher Scientific.

Chapter 14

Menus and Action Panels

Figure 14-1 shows the location of the menu bar and action panels in the main window of SkanIt Software.

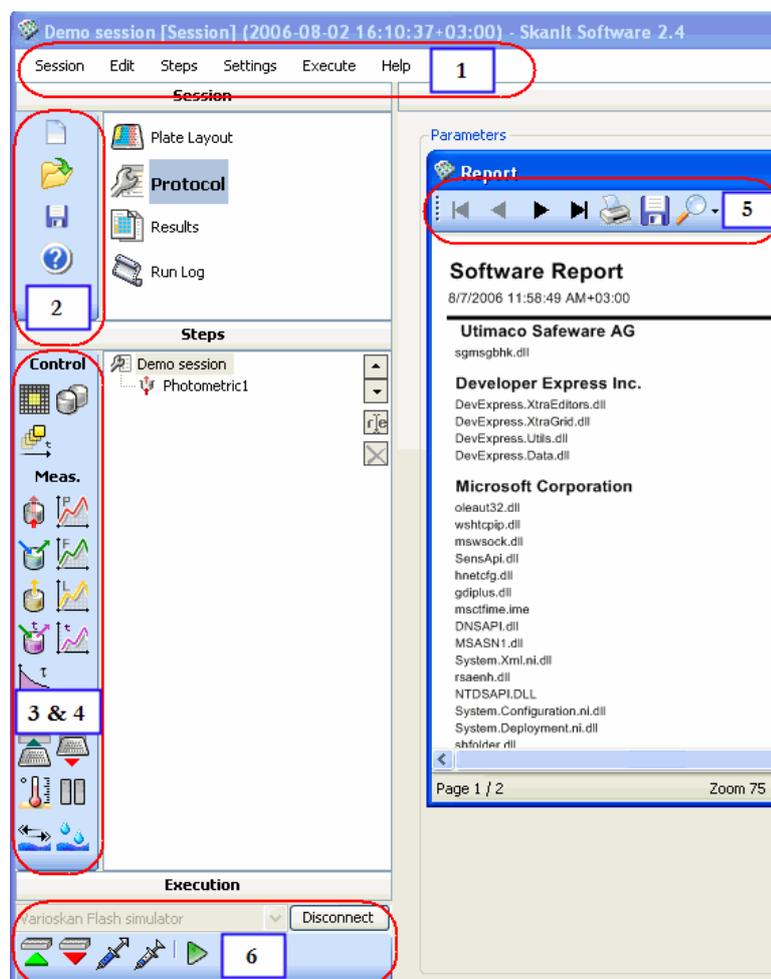


Figure 14-1. Menus and action panels of SkanIt Software

- **1** - Menu bar. For more information about the menu items, refer to “Menu commands” on page 260.
- **2** - Session action panel. For more information about the buttons, refer to “Session action panel” on page 264.
- **3** - Steps action panel. It is available when **Protocol** is selected. For more information about the buttons, refer to “Steps action panel” on page 264.

- **4** - Results action panel. It is available when **Results** is selected. For more information about the buttons, refer to “Results action panel” on page 265.
- **5** - Report toolbar. It is available when a report has been created. For more information about the buttons, refer to “Report toolbar” on page 266.
- **6** - Execution action panel. For more information about the buttons, refer to “Execution action panel” on page 266.

Menu commands

This section includes descriptions of all the menu items in SkanIt Software for Varioskan.

Session

- **New...** - Launches the **Create New Session** dialog (see “Creating a new session” on page 61).
- **Open...** - Launches the **Open Existing Session/Run** dialog for opening sessions and runs from the database (see “Opening an existing session” on page 59).
- **Save** - Saves the current session in the database (see “Saving a session” on page 63).
- **Save As...** - Launches the **Save As** dialog for saving the current session, protocol or plate layout with a new name.
- **Import/Export** - Saves (exports) selected sessions and/or runs in separate files that can be imported to a database of another PC with SkanIt Software for Varioskan. Imports selected data from another PC with SkanIt Software for Varioskan (see “Exporting and importing sessions” on page 65).
- **Close All** - Closes all open items (session, run, protocol, and plate layout).
- **Lock Software** - Prevents the use of the software without first logging in with a user name and a password. Only the user who has locked the software or the administrator can unlock the software.
- **Exit** - Exits SkanIt Software.

Edit

The part of the session selected in the **Session** action panel affects the available menu commands.

- **Cut** - Cuts the selected protocol step in the protocol step list or the selected item in the plate layout to the clipboard.
- **Copy** - Copies the selected protocol step in the protocol step list or the selected item in the plate layout to the clipboard.
- **Paste** - Pastes the protocol step or item from the clipboard to the protocol step list or the plate layout. The step or item will automatically be renamed.
- **Rename** - Allows you to rename the selected protocol step or calculation step.
- **Delete** - Deletes the selected protocol step from the protocol step list or the selected item from the plate layout.

Steps

- **<Step type>** - Adds the chosen step type to the current protocol after the selected step. If no step is selected, the new step is added after the last step. For more information, see “Adding new protocol steps” on page 83 and Chapter 7: “Step Parameters”.

Data Processing

- **<Calculation type>** - Adds the chosen calculation to the selected measurement results. For more information, see Chapter 10: “Data Processing Parameters”.
- **Report / Export** - You can create a custom-made report (see “Measurement and calculation reports” on page 213).
- **Save** - You can automatically save and print the measurement or calculation results directly after a measurement (see “Saving measurement and calculation data” on page 211).
- **Quick Export** - You can manually export the measurement and calculation data (see “Saving manually” on page 213).

Settings

- **Options...** - Launches the **Options** dialog. You can set general default parameters, such as the startup temperature, and others (see “Options” on page 226).
- **Instrument...** - Launches the **Instrument Setup** dialog. You can configure the instrument connected to SkanIt Software and set the

default instrument. You can also define a new instrument (see “Instrument” on page 231) and create instrument status reports (see “Creating instrument reports” on page 219).

- **Colors...** - Launches the **Group Colors** dialog. You can change the colors with which the groups (blank, calibrator, unknown, control, and empty) are displayed in the *Plate Layout* view (see “Colors” on page 237).
- **Plate Template...** - Launches the **Plate Template Settings** dialog. You can set a plate template as default, modify a plate template (e.g., well sizes and positions), create new plate templates by making plate duplicates and modifying the data, and delete plate templates (see “Plate template settings” on page 238).
- **Plate Tray...** - Launches **Plate Tray Editor** for adding new plate trays and for adding association links between plate types and plate trays (see “Plate tray” on page 242).
- **Pathlength Correction...** - Launches the **Edit Pathlength Correction K-Factors** dialog. You can check the pathlength correction factors in the database and add pathlength correction factors from literature (see “Editing pathlength correction K-factors” on page 247).
- **Saved Curves...** - Launches the **Saved Curves** dialog for viewing saved standard curves and for editing their descriptions (see “Saved curves” on page 249).
- **Protocol Templates...** - Launches the **Protocol Template Setup** dialog. You can add an existing protocol to the protocol template list displayed in the **Create New Session** dialog (see “Protocol template setup” on page 250).
- **Control Panel** - Launches the **SkaniT Control Panel** window. You can change your login password and modify the company contact information. The administrator can manage user and user group information and password settings (see “SkaniT Control Panel” on page 252). In the Drug Discovery Edition, the audit trail can be checked (see “Audit trail” on page 300) and signatures can be defined (see “Electronic signature” on page 293).
- **Sign...** - Sign a document electronically (see “Signing a session or a run” on page 298).

Electronic Signatures (DDE only)

- **View and Verify Signatures...** - Check the electronic signatures (see “Viewing signatures” on page 299).

Execute

- **Session** - Starts the session execution (see Chapter 5: “Executing a Session”).
- **Prime...** - Launches the **Prime** dialog for priming the dispenser to fill the dispenser tubing (see Chapter 5: “Executing a Session”).
- **Empty...** - Launches the **Empty** dialog for emptying the dispenser tubing after use (see Chapter 5: “Executing a Session”).
- **Wash...** - Launches the **Wash Dispensers** dialog for washing the dispensers after use (see “Washing dispensers” on page 77).
- **Run Plate In** - Drives the plate tray into the instrument.
- **Run Plate Out** - Drives the plate tray out of the instrument.
- **Identify Plate Tray** - Obtains the plate tray information from the instrument (see “Plate tray” on page 242).
- **Connect** - Connects the PC to the instrument.
- **Disconnect** - Disconnects the PC from the instrument.

Help

- **Contents...** - Launches the Help application (see Chapter 15: “Using Help”).
- **About...** - Contains information about SkanIt Software and lets you create software reports (see “Viewing software reports” on page 222).

Action panels

This section includes descriptions of all the action panel buttons in SkanIt Software for Varioskan.

Session action panel

The action panel has the following buttons:

-  New session (“Creating a new session” on page 61)
-  Open session (“Opening an existing session” on page 59)
-  Save session (“Saving a session” on page 63)
-  Open the Help application (Chapter 15: “Using Help”)

Steps action panel

The action panel has the following buttons:

-  Kinetic Loop (“Kinetic loops” on page 91)
-  Well Loop (“Well loops” on page 87)
-  Area Definition (“Area definition” on page 85)
-  Photometric measurement (“Photometric measurement” on page 93)
-  Photometric Scanning (“Photometric spectrum scanning” on page 95)
-  Fluorometric measurement (“Fluorometric measurement” on page 99)
-  Fluorometric scanning (“Fluorometric spectrum scanning” on page 101)
-  TRF measurement (“TRF measurement” on page 103)
-  TRF Scanning (“TRF scanning” on page 107)
-  TRF Decay (“TRF decay” on page 109)
-  Incubate (“Incubate” on page 116)
-  Pause (“Pause” on page 122)

 Shake (“Shake” on page 117)

 Dispense (“Dispense” on page 120)

 Plate In (“Plate In” on page 123)

 Plate Out (“Plate Out” on page 123)

Results action panel

The action panel has the following buttons:

 Blank Subtraction (“Blank subtraction” on page 147)

 Basic Statistics (“Basic statistics” on page 150)

 Data Normalization (“Data normalization” on page 151)

 Multipoint (“Multipoint calculation” on page 152)

 Quantitative Curve Fit (“Quantitative curve fit” on page 153)

 Qualitative Classification (“Qualitative classification” on page 160)

 Effective Dose (“Effective dose” on page 164)

 Quality Control (“Quality control (QC)” on page 167)

 User-Defined Equation (“User-defined equation” on page 169)

 Spectral Analysis (“Spectral analysis” on page 174)

 Kinetic Calculations (“Kinetic calculations” on page 183)

 Merge Data (“Merge data” on page 198)

 Graph (“Graph” on page 198)

 TRF Decay Calculation (“TRF decay calculation” on page 201)

 Report / Export (“Measurement and calculation reports” on page 213)

 Save results (“Saving automatically” on page 211)

 Quick Export (“Saving manually” on page 213)

Report toolbar

The toolbar has the following buttons:

 Go to the first page of the report

 Go to the previous page

 Go to the next page

 Go to the last page of the report

 Print the report

 Save the report

 Zoom the report view

Execution action panel

The action panel has the following buttons:

 Open the connection to the instrument selected from the list

 Close the connection to the instrument

 Run the plate into the instrument

 Run the plate out of the instrument

 Prime the dispenser tubing

 Empty the dispenser tubing

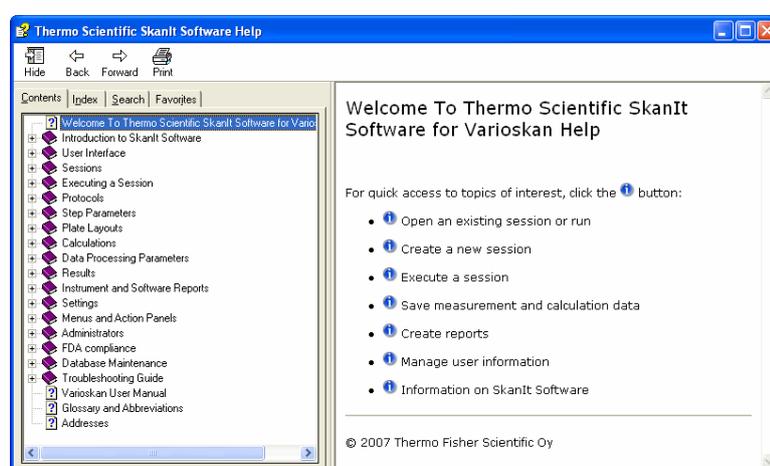
 Start the session execution (Chapter 5: “Executing a Session”)

Chapter 15

Using Help

You can launch the Help application by selecting Help > **Contents...** from the menu bar, pressing the **F1** key on your keyboard, or clicking the  button on the action panel.

The following **Thermo Scientific SkanIt Software Help** window appears.



The Help toolbar buttons are:

- **Hide** – Hides the left-hand side navigation pane. To display the navigation pane again, click the **Show** button that appears instead of the **Hide** button.
- **Back** – Takes you back to the previous view in your view history.
- **Forward** – Takes you to the next view in your view history.
- **Print** – Prints a single topic or multiple topics.

You can access the help content in different ways by selecting one of the following tabs:

- **Contents** – Browse the help topics by subject.
- **Index** – Type in a keyword or browse all keywords to find a specific topic.
- **Search** – Find a specific help topic by entering words to search in the help content.

- **Favorites** – Add shortcuts to help topics of your choice.

Chapter 16

Troubleshooting Guide



Note Do not use the instrument if it appears that it does not function properly.

Note that the instrument does not verify the logic flow of the received commands.

When an error is detected, the current operation is terminated. After an error, it is best to abort the current run and restart from the beginning after the problem is fixed.

If the computer cannot communicate with the instrument, check that the serial connector or USB cable is securely connected to the computer and instrument and that the instrument is defined in the database (see “Communicating with instruments” on page 34).

You can also try switching the instrument OFF and ON again.

The run log shows the error and warning messages that occur during the run. For their explanations, see:

- “Error messages” on page 269
- “Warning messages” on page 273

Error messages

Error messages reported

Code	Explanation	Suggested action
1	Internal firmware error. When this error occurs, the internal software halts. This error can only be seen in the instrument error log.	Contact service.
2	The instrument did not recognize the command it received.	Contact service.
3	The arguments of the received command are not valid.	Contact service.
4	The X carriage position is incorrect.	Contact service.
5	The Y carriage position is incorrect.	Contact service.

Troubleshooting Guide

Error messages

Code	Explanation	Suggested action
6	The 1st excitation grating position is incorrect.	Contact service.
7	The 2nd excitation grating position is incorrect.	Contact service.
8	The 1st emission grating position is incorrect.	Contact service.
9	The 2nd emission grating position is incorrect.	Contact service.
10	Excitation diffraction order filter position is incorrect.	Contact service.
11	Emission diffraction order filter position is incorrect.	Contact service.
12	Excitation selector position is incorrect.	Contact service.
13	Emission selector position is incorrect.	Contact service.
14	Varioskan LumiSens filter position is incorrect.	Contact service.
15	Excitation bandwidth selector position is incorrect.	Contact service.
16	Excitation beam diameter selector position is incorrect.	Contact service.
17	The distance between measurement points is too short for scan measurement. The plate cannot be moved as slowly as the distance requires.	Contact service.
18	The sampling time for a single result is too long for scan measurement. The plate cannot be moved as slowly as the sampling time requires.	Use normal measurement instead of scan. Use a shorter sampling time.
19	The requested plate position is outside the mechanical limits of the tray movements.	Check the plate template.
20	The offset voltage of the temperature measurement electronics is too high.	Contact service.
21	The offset voltage of the A/D converter board is too high.	Contact service.
22	The background noise of the A/D converter board is too high.	Contact service.
23	Error when checking the A/D converter board reference voltage.	Contact service.
24	Calibration of the A/D converter board gain steps failed.	Contact service.

Code	Explanation	Suggested action
25	Analog signal outside measurement range. This situation is an error during startup and autocalibration.	Contact service.
26	Flash lamp failure.	Contact service.
27	Attempt to set the instrument serial number when it already has been set.	Do not try to set the serial number.
29	The dispenser is not responding.	Contact service.
30	Non-volatile parameters lost.	Contact service.
31	The D/A converters check of the A/D converter board failed.	Contact service.
32	The requested measurement method is not available.	Contact service.
33	Dispensing or priming was attempted when there was no plate inserted into the tray.	Insert a plate into the tray.
34	Dispenser tip priming was attempted when there was no tip priming vessel inserted into the tray.	Insert the tip priming vessel.
35	This is only reported in response to the factory calibration.	Contact service.
36	This is only reported in response to the factory calibration.	Contact service.
37	This is only reported in response to the factory calibration.	Contact service.
38	This is only reported in response to the factory calibration.	Contact service.
39	This is only reported in response to the factory calibration.	Contact service.
40	This is only reported in response to the factory calibration.	Contact service.
41	This is only reported in response to the factory calibration.	Contact service.
42	This is only reported in response to the factory calibration.	Contact service.
43	This is only reported in response to the factory calibration.	Contact service.
44	This is only reported in response to the factory calibration.	Contact service.
45	XY table position calibration failed.	Contact service.
46	No factory calibration for the current measurement method.	Contact service.
47	This is only reported in response to the factory calibration.	Contact service.

Troubleshooting Guide

Error messages

Code	Explanation	Suggested action
48	Varioskan LumiSens module calibration failed.	Contact service.
49	No tray attached to the instrument. The tray carriage will not move unless there is a tray.	Attach a tray to the tray holder.
50	Too high background level. The background level is checked during internal calibration.	Clean off any possible liquid spills inside the measurement chamber. Contact service if the error persists.
51	The dispenser has failed to initialize properly.	Contact service.
52	The dispenser received an unknown command.	Contact service.
53	Invalid dispenser command operand.	Contact service.
54	Invalid sequence of dispenser commands.	Contact service.
56	Dispenser parameter memory error.	Contact service.
57	Attempt to use a dispenser before it is set up.	Contact service.
59	Dispenser plunger overload.	If you are trying to dispense a viscose liquid with high speed, reduce the dispensing speed. Contact service if the error persists.
60	Dispenser valve overload.	Contact service.
61	The valve position does not allow moving the plunger.	Contact service.
65	Dispenser command buffer overflow.	Contact service.
66	Attempt to dispense when the dispenser is not primed.	Prime the dispenser before dispensing.
67	The dispenser is not installed.	Do not try to use a nonexistent dispenser. Contact service if the dispenser exists.
68	The drift compensation factor is too far from the nominal value of 1.0.	Contact service.
69	Not enough memory for a new user-defined parameter.	Delete some unused user parameters to make room for new parameters.
75	Default PMT voltages calibration failed.	Contact service.
76	Automatic dynamic range PMT voltages calibration failed.	Contact service.
77	The dark level signal on some of the A/D converter board input channels is too high.	Contact service.
78	PMT linearity calibration failed.	Contact service.

Code	Explanation	Suggested action
79	Bandwidth factors calibration failed.	Contact service.
80	The measurement chamber door is open.	See that nothing is preventing the measurement chamber door from closing when the plate is driven in. Do not open the door while the instrument is measuring.
81	Such an error during startup that the execution of some functions is not possible.	The measurement chamber door must be closed during startup. If it is not, contact service.
82	Invalid TRF measurement command.	Contact service.
83	Varioskan LumiSens crosstalk shield position is incorrect.	Clean the Varioskan LumiSens base. Contact service if the error persists.
84	Varioskan LumiSens reference light failure.	Contact service.
85	Dispensing head is not in the correct position.	Move the dispensing head into the correct position.
86	Wrong plate type.	Use a suitable plate type. Dispensing is only possible with up to 384-well plates.
88	Varioskan LumiSens base installed, measurement module not present.	Install the Varioskan LumiSens measurement module.

Warning messages

Warning messages reported

Code	Explanation	Suggested action
100	Unable to comply with the defined lag time.	The minimum timing depends on the combination of measurement parameters, plate movement parameters, dispensing parameters and volume, number of wavelengths and number of measured points.
101	Unable to comply with the defined well interval.	
102	Unable to comply with the defined wavelength interval.	
103	Unable to comply with the defined kinetic interval.	
104	Unable to comply with the defined group interval.	
105	The timer referenced in the command is not running (anymore). Your timing requirement is not met.	
	Related to step duration.	

Troubleshooting Guide

Warning messages

Code	Explanation	Suggested action
106	The lamp lifetime has reached its end.	Arrange for the replacement of the lamp as soon as convenient. Contact service.
107	Protocol time settings did not allow internal runtime calibration.	The accuracy of the measurement results may have suffered. If there is no waiting time in the assay, then you have to accept the possible accuracy reduction.
108	The command has no effect.	This just informs that a command has been used which has no effect in the current measurement method.
109	The synchronization of measurements failed. The second measurement started later than requested.	The minimum timing depends on the combination of measurement parameters, plate movement parameters, dispensing parameters and volume, number of wavelengths and number of measured points.
110	Dark level interpolation buffer overflow.	Reduce well group size, use dark level interpolation between wells or do not use interpolation at all.

Appendix A

Administrators

SkaniIt Control Panel

Users are managed through the **SkaniIt Control Panel**. For information on launching the SkaniIt Control Panel, see “SkaniIt Control Panel” on page 252. The rights in the Control Panel depend on which user group the logged-in user belongs to. Only the administrator may register and remove users and user groups and assign rights for them. The administrator can also change passwords for other users. Users with more limited access rights can use the Control Panel to change their logon and signature passwords as well as company information (see “SkaniIt Control Panel” on page 252 and “Electronic signature” on page 293 for the DDE version).

The Control Panel is also used to view the audit trail in the DDE version (see “Audit trail” on page 300).

Users

In the **Users** view the administrator users can create new users, edit user properties, such as passwords, and remove users.

The dialog lists the current users and the user groups they belong to that are defined in the database.

Adding a new user

Only an administrator can add new users.

1. Click **New User**.

The **Add User** dialog is launched.

The screenshot shows a Windows-style dialog box titled "Add User". It is divided into several sections. The top section, "User Properties", contains three text input fields: "Login:" with the value "Demo", "Full name:" with "Demo user", and "Description:" with "Demonstration user". Below this is a "Member of:" section featuring a list box with five items: "Administrators" (checked), "Advanced Operators", "Protocol Developers", "Service", and "Operators". Underneath the list box are two checkboxes: "User must change password at next logon" (unchecked) and "Password never expires" (checked). The bottom section contains two text input fields for "Password:" and "Confirm password:", both filled with "xxxxx". At the bottom of the dialog are "OK" and "Cancel" buttons.

2. Enter the information in the text boxes.
 - **Login** - The user ID that is used when logging onto the software.
 - **Full name** - The full name of the user. It is displayed as the creator name in session, protocol and plate layout lists and is shown on the status bar. The Audit trail (DDE only) also records the full name.
 - **Description** - An optional description of the user.
 - **Member of** - Select which user group(s) the user is a member of. The rights of the different user groups are listed in Table A-1.
 - **User must change password at next logon** - Tick this check box to prompt the user to change the password the first time (s)he logs onto the software. You can also use this if you are giving a temporary password to a user who has forgotten the password.
 - **Password never expires** - Tick the check box to avoid password expiration (defined in General Settings, “General settings” on page 282).
 - **Password** - Enter the password. The user can later change the password.

- **Confirm Password** - Enter the same password again.



Note The logon password is not case sensitive.

3. Click **OK**. The new user can log onto SkanIt Software the next time the software is launched. The user will be visible in the **Users** list the next time you log onto the software.

Modifying user information

Only an administrator can view and modify the user information of other users. Other users may modify their own user information.

1. In **SkanIt Control Panel** select the user you want to modify and click **Properties**.

The **User Properties** dialog is launched.

2. Edit the user properties.
 - **Full name** - The full name of the user. It is displayed as the creator name in session, protocol and plate layout lists and is shown on the status bar. The Audit trail (DDE only) also records the full name. The full name cannot be edited after the user has been created.

- **Description** - An optional description of the user.
- **Member of** - Select which security group(s) the user is a member of. The rights of the different security groups are listed in Table A-1.
- **User must change password at next logon** - Tick this check box to prompt the user to change the password the first time (s)he logs onto the software. You can also use this if you are giving a temporary password to a user who has forgotten the password.
- **Password never expires** - Tick the password to avoid password expiration (defined in General Settings, see “General settings” on page 282).
- **Change Password** - Click the button to launch the **Change Logon Password** dialog. The button is only available, when the *Password never expires* check box is not selected.



3. Enter the new password in both the *New Password* and *Confirm Password* fields.



Note The logon password is not case sensitive.

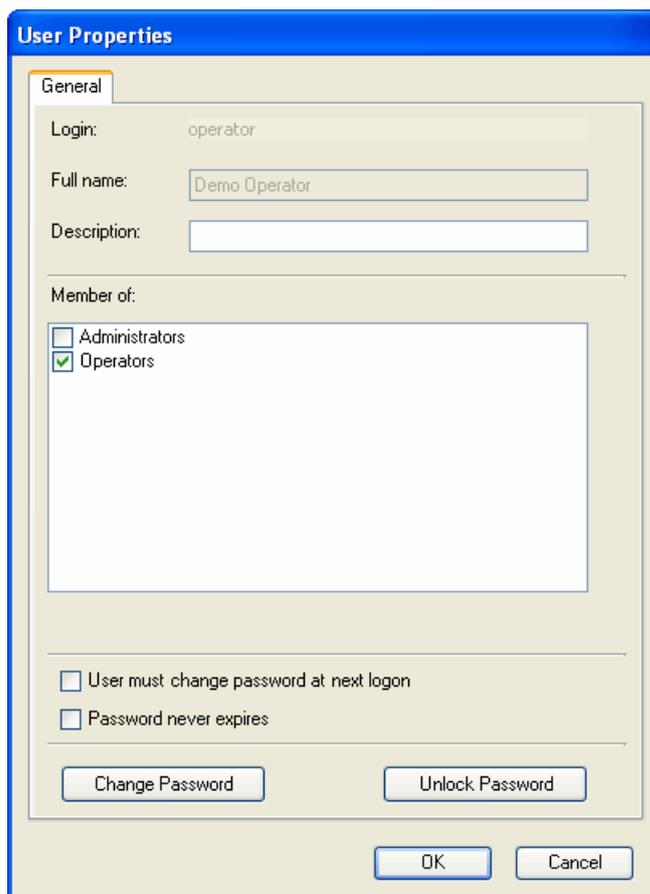
4. Click **OK**.

Unlocking user accounts

Only an administrator can unlock a user account, and if necessary, change the user's forgotten password.

1. In **SkaniT Control Panel** select the user whose account is locked and click **Properties**.

The **User Properties** dialog opens.



2. Click **Unlock Password**. The account is unlocked.
3. If the user has forgotten the password, click **Change Password** to change it. For more information, see “Modifying user information” on page 277.
4. Click **OK**.

Groups

Only the administrator can add new user groups and modify their rights. Other users may only view the group list. The security rights defined for the different user groups are given in Table A-1.

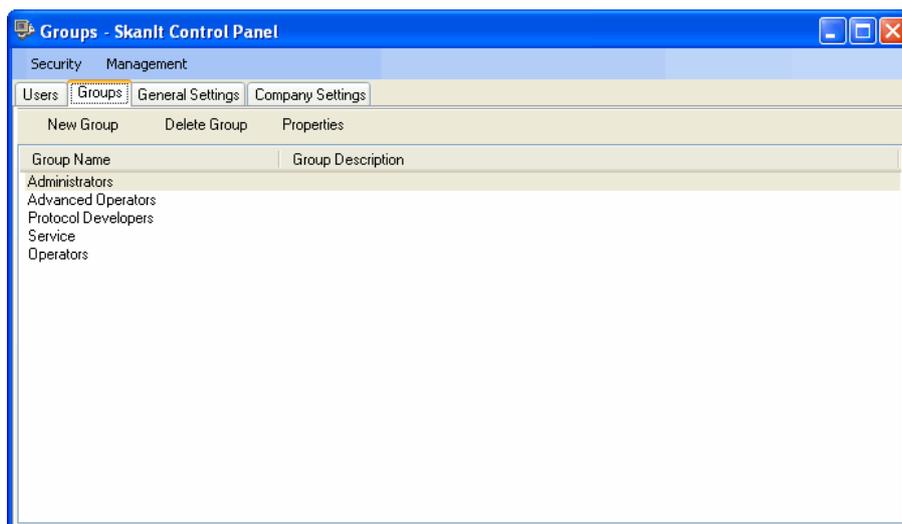
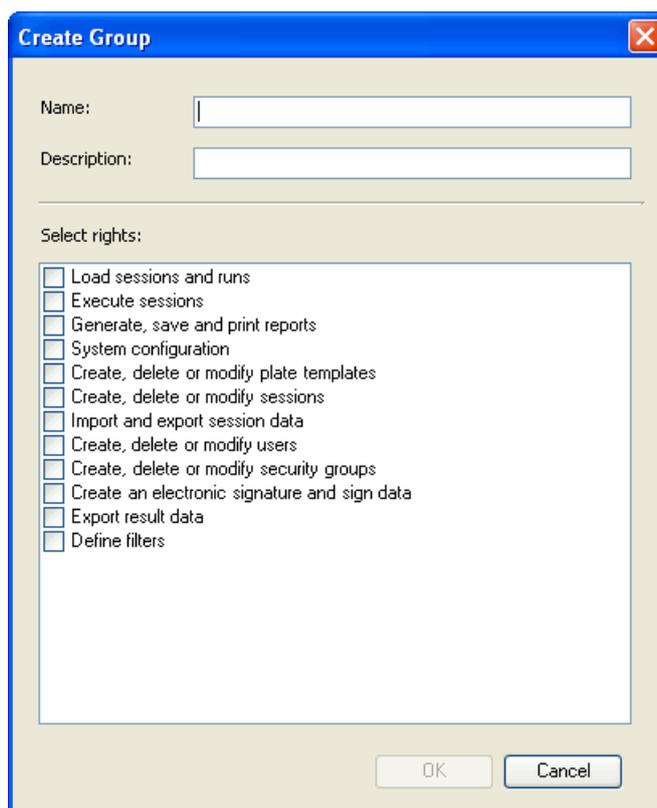


Table A-1. Rights of the different security groups

Rights	Administrator	Advanced Operator	Protocol Developer	Service	Operator
Load sessions and runs	X	X	X	X	X
Execute sessions	X	X	X	X	X
Generate, save and print reports	X	X	X	X	X
System configuration	X			X	
Create, delete or modify plate templates	X	X			
Create, delete or modify sessions	X	X	X		
Import and export session data	X	X	X		
Create, delete or modify users	X				
Create, delete or modify security groups	X				
Create an electronic signature and sign data	X	X	X		X
Export result data	X	X	X		X

To add a new user group, click **New Group**. The **Create Group** dialog opens.

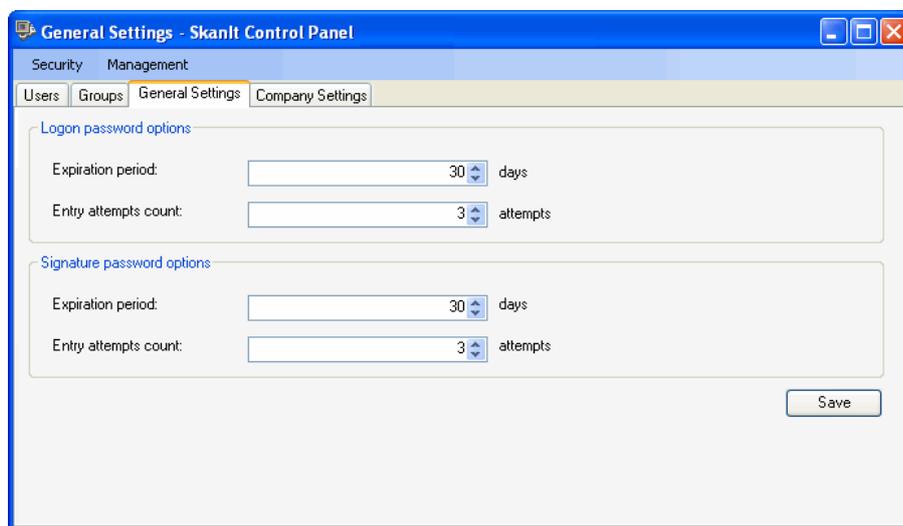


Give a name to the new user group and provide it with a description. Select the rights the group will have from the list.

When a group is selected from the Control Panel *Group* list and the **Properties** button is pressed, the rights defined for the group can be viewed and modified.

General settings

Use the general settings to view and modify general logon and signature password settings. Only an administrator can modify these settings, other users may only view them.



- **Expiration period** - Select the number of days the password is valid. The range is 1 to 100 days; default is 30 days. You can override this selection for individual users by selecting the *Password never expires* option in the **User Properties** dialog (see “Modifying user information” on page 277).
- **Entry attempts count** - Select the number of failed password entry attempts. The range is 1 to 100 attempts; default is 3 attempts. If the user fails to enter the correct password within the allowed number of times, the user account is locked and only an administrator can unlock it, and if necessary, change the user's password.
- **Save** - Click the button to save the new settings.

Company settings

You can give information here on the company (laboratory) performing the measurements. This information will be displayed in the reports (see “Measurement and calculation reports” on page 213).

The screenshot shows a software window titled "Company Settings - SkanIt Control Panel". The window has a blue title bar and a menu bar with "Security" and "Management". Below the menu bar are tabs for "Users", "Groups", "General Settings", and "Company Settings". The "Company Settings" tab is selected. The form contains the following fields:

- Company name: [Text input field]
- Tel.: [Text input field]
- E-mail: [Text input field]
- Address: [Text input field]

A "Save" button is located at the bottom right of the form area.

- **Company name, Tel., E-mail and Address** - Enter the name and contact information of the company.
- **Save** - Click the button to save the information.

Appendix A Administrators

Company settings

Appendix B

Database maintenance

SkanIt Software uses the Microsoft SQL Server Desktop Engine (MSDE) as the database engine. The database names have to comply with MSDE name conventions. For more information, visit the Microsoft website (www.microsoft.com).

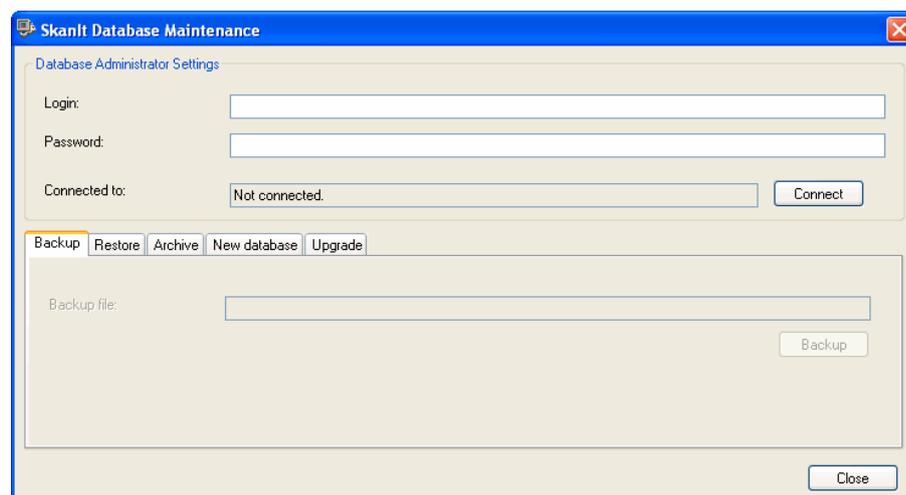
The database administrator can archive past database content, make a backup file of the database, restore a backup file as a working database, create a new empty database or upgrade a database of an earlier SkanIt Software version. The *Backup / Restore* pair is used to make backup copies of databases and to restore them.



Note Make sure that the database you want to maintain is currently selected in SkanIt Software. See “Changing the working database” on page 290.

Close SkanIt Software before you perform any database maintenance operations.

Select Start > Programs > Thermo SkanIt Software > **SkanIt RE/DDE for Varioskan Database Maintenance**.



- **Database Administrator Settings** - Give the database administrator login name, usually “sa” and password as they were given during installation (see Step 1 in “Installing software prerequisites” on page 23).
- **Connected to** - When you launch the SkanIt Database Maintenance, it is not connected to any database engine.

Click **Connect**. The SkanIt Database Maintenance program connects to the working database engine and database.

The database engine name is displayed in the *Connected to* field. You can now use the *Backup*, *Restore*, *Archive*, *New database* and *Upgrade* tabs.

To use a restored, archived or new database, you have to change that database to be the working database within SkanIt Software. See “Changing the working database” on page 290.

Backup

You can make a backup file of the database. The *Backup* function creates a copy file of the working database used by SkanIt Software. The backup file should be stored in a permanent and secure media.

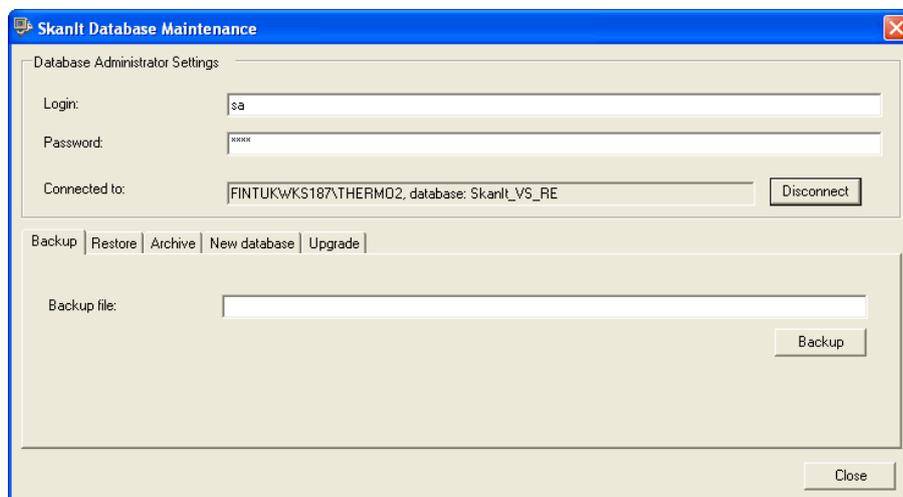


Caution You must make a separate backup file of the SkanIt database. A general company backup does not backup the SkanIt database because the MSQL server does not give access to the SkanIt database.



Note It is the responsibility of the user to make a backup file of the database.

Make sure the working database is correct. See “Changing the working database” on page 290.



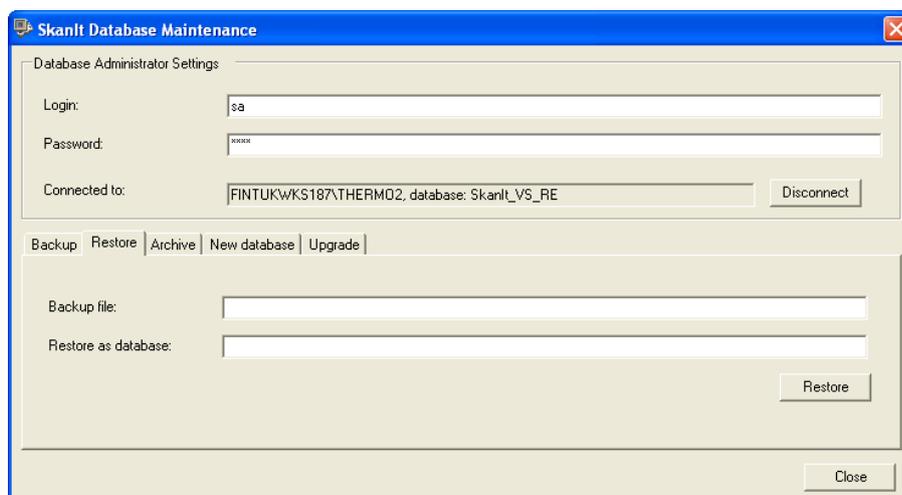
- **Backup file** - Give a name to the backup file with a full file path and a file extension, for example, **C:\SkanIt_backupfile.bak**.

Click the **Backup** button. You will be notified when the backup file is ready.

A backup file can be restored to a database using the *Restore* function.

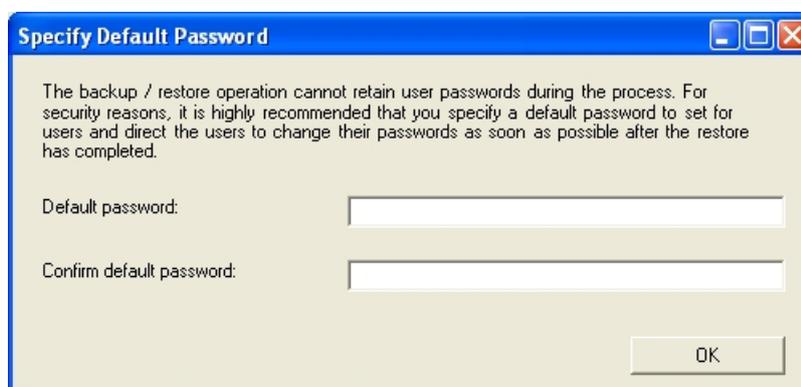
Restore

You can restore a backup file as a database. The *Restore* function returns a file created with the *Backup* function to a new database.



- **Backup file** - Give the name of the backup file and its full file path.
- **Restore as database** - Give a name to the new database. The new database name could, for example, be **SkanIt_VS_RE_2**. If the name is the same as an existing database, or the database name does not comply with Microsoft's naming conventions, you will be notified, and you are asked to give a new name.

Click the **Restore** button. The **Specify Default Password** dialog is launched.



The database backup file includes the SkanIt Software login user names, but the login passwords are not retained. For this reason, you must provide the users with a new login password. This password will be the same for all users, and must therefore be changed by each user as soon as possible using the SkanIt Control Panel (see “Changing your own logon password” on page 253).

However, if any of the user names is found from the working database engine instance, its password will not be replaced. This new login password will only be given to those users that are currently not defined in any of the databases of the working database engine instance. That is, when you are

restoring a backup file to a computer that is not the one where the backup was taken from or where the database has not been installed before, the passwords must be changed. The passwords must not be changed if you are restoring the file to the originally backed up computer.

- **Default password** - Give a default password to all users.
- **Confirm default password** - Type the password again.

Click **OK**. You will be notified when the database has been restored.

To use the restored database, change the working database in SkanIt Software. Select Settings > Options... > **Database** (see “Changing the working database” on page 290).

Archive

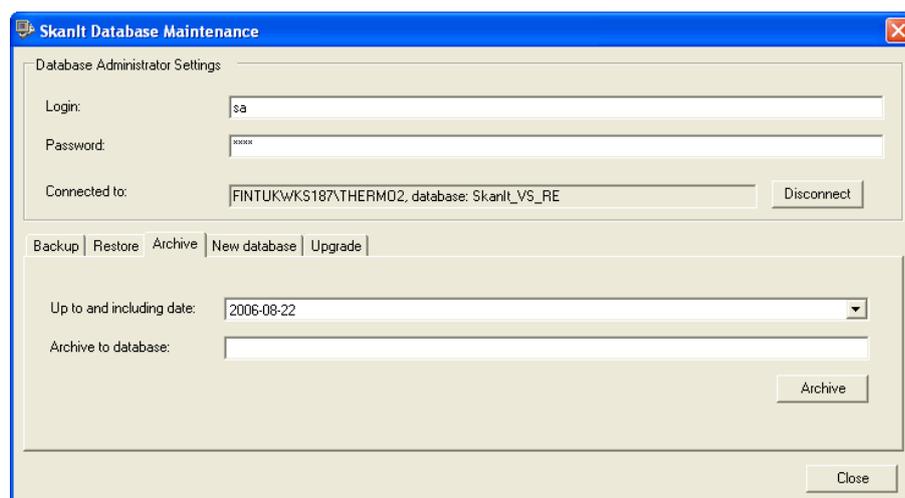
In the archiving process, the runs executed before the specified date will be transferred from the working database to the archive database.

All runs executed after the specified date will remain in the working database. The sessions for the archived runs will also be present in both databases, and can be manually deleted from the working database, if desired. The archive database is read-only and cannot be altered. Runs can be exported from the archive database and imported into the working database, if needed.



Note Archiving does not delete sessions from the working database due to FDA requirements, such as audit trails.

Make sure the working database is correct. See “Changing the working database” on page 290.



- **Up to and including date** - Choose the date up to which all the data will be archived (the selected date included).



- **Archive to database** - Give a name to the archive database. If the name is the same as an existing database, or the database name does not comply with Microsoft's naming conventions, you will be notified, and you are asked to give a new name. Write down the name you gave to the archive database, so that you can retrieve it at a later time.

Click the **Archive** button.

To use the archive database, change the working database in SkanIt Software. Select Settings > Options... > **Database** (see “Changing the working database” on page 290).

New database

You can create a new database locally on the computer for use with SkanIt Software.



- **Database name** - Give a name to the new database. The new database name could, for example, be **SkanIt_VS_RE_2**. If the name is the same as an existing database, or the database name does not comply with Microsoft's naming conventions, you will be notified, and you are asked to give a new name.

Click the **Create** button. The database creation may take up to 15 minutes depending on your computer. During this time, the Database Maintenance may appear to stop working but you should wait patiently. You will be notified when the database has been created.

To use the new database, you have to change the working database in SkanIt Software. Select Settings > Options... > **Database** (see “Changing the working database” on page 290).

Upgrade

You can upgrade a database of an earlier software version to make it compatible with a later software version. You are notified if the database is not compatible with the current software version when you are selecting or changing the database in SkanIt Software. You can upgrade a database starting from SkanIt Software version 2.0.



- **Database name** - Enter the name of the database you want to upgrade.



Note You can only upgrade databases within the same instrument type.

Click the **Upgrade** button. You are notified when the upgrade has been completed.

To use the upgraded database, you have to change the working database in SkanIt Software. Select Settings > Options... > **Database** (see “Changing the working database” on page 290).

Changing the working database

Make sure that the database you want to maintain is currently selected in SkanIt Software. Follow the procedure below.

1. Close the Database Manager, if it is running.
2. Launch SkanIt Software.
3. Select Settings > Options... > **Database**.
4. Verify the used database engine. The database engine where the working database is located is shown in the *Database server name* field. The location is defined at installation (see Step 7 in “Installing the software” on page 26).

You can also switch to use another database engine. You can only connect to a database engine that has your user name defined. When you change the database engine instance, the list in the *Data source* field is updated.

5. The working database (that is, the database currently in use by SkanIt Software), is shown in the *Data source* field. If you want to change the working database to another, select the name of the database from the drop-down menu.

If the database you select is not compatible with the current software version, you can upgrade the database (see “Upgrade” on page 290).

6. Enter the login name and login password that are defined in the database to which you are switching. The *Login name* field shows the name of the user currently logged in.
7. Click the **Test connection** button to allow the software to test the connection to the database. You will receive a notification that the connection succeeded. Click **OK**.
8. Click the **Save** button to change the database.
9. Exit SkanIt Software.
10. Launch the Database Manager. When you now click the **Connect** button, the Database Manager is connected to the database of your choice.

Appendix B Database maintenance

Changing the working database

Appendix C

Drug Discovery Edition with 21CFR11 Compliance

In SkanIt Software, the features related only to the Drug Discovery Edition offering FDA's Rule 21 CFR Part 11 are:

- Electronic signature (see “Electronic signature” on page 293).
- Prompting to provide a reason for any changes made that affect the information content of the database.
- Audit trail (see “Audit trail” on page 300) for viewing the log of operations made by the users.

The date and time formats comply with FDA's Rule 21 CFR Part 11 and they are explained in “Date and time format” on page 300.

Electronic signature

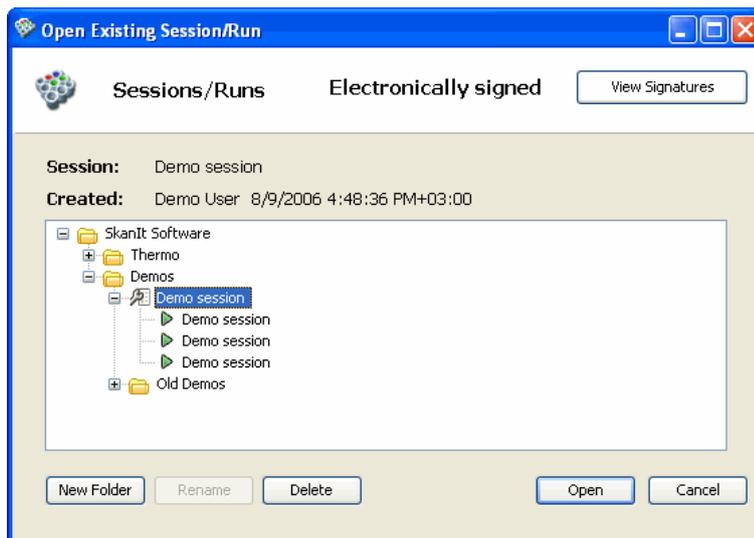
An electronic signature is used for signing sessions and runs. A session or run is signed by providing a signature password.

The signature is always printed in all custom-made reports.

Run information

Session name	Demo session
Session creator	Demo User
This session has been signed electronically	Approved By Demo User 8/17/2006 1:54:28 PM+03:00
This session has been signed electronically	Reviewed By Demo User 8/15/2006 3:24:21 PM+03:00
Run name	N/A

The signature is shown in the **Open Existing Session/Run** dialog.



Creating a signature password

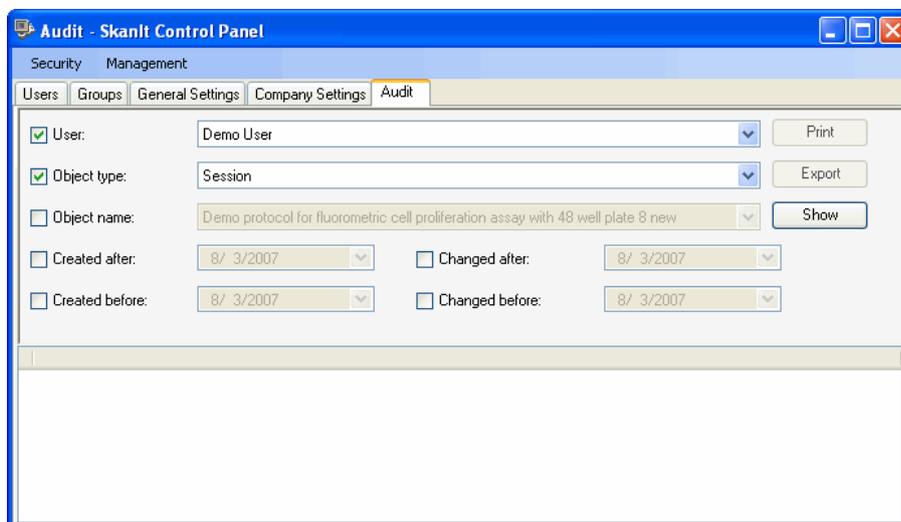
Only an administrator can create an electronic signature for a user.

Select Settings > **Control Panel**. You can also click the **SkaniT Control Panel** icon on the desktop or select Programs > Thermo SkanIt Software > **SkaniT Control Panel** from the Start menu. The **Log On To SkanIt Control Panel** dialog is displayed.

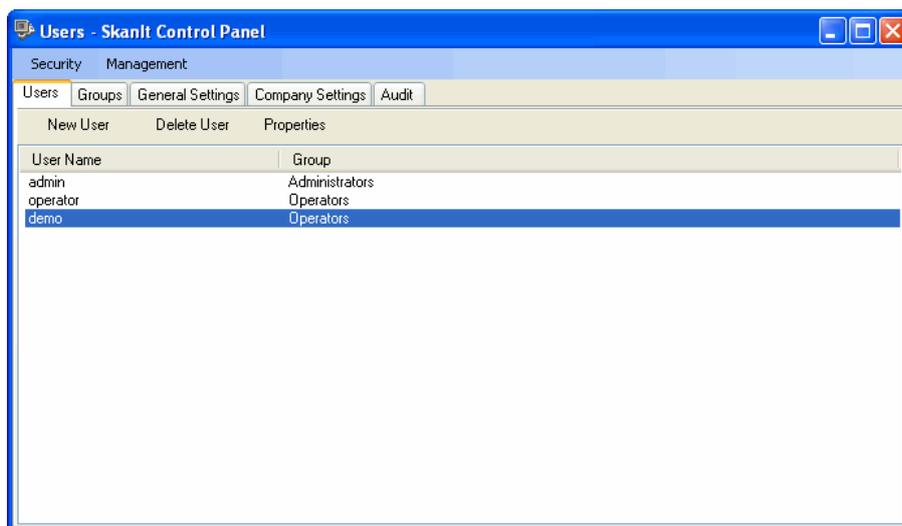


Enter your current user name and password and click **OK**.

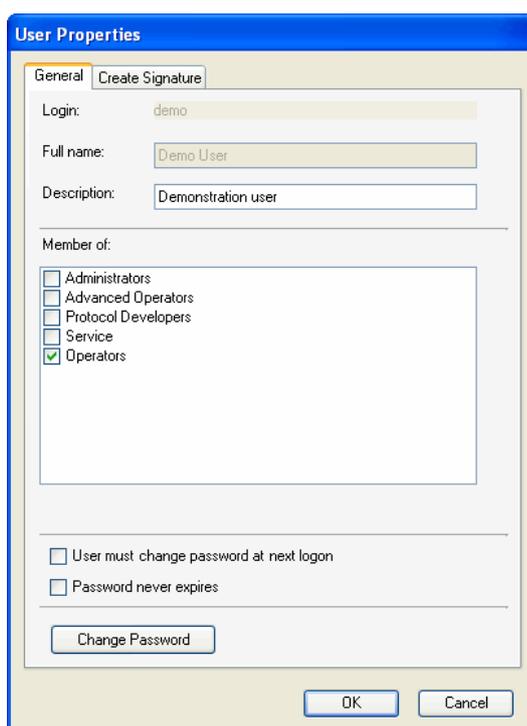
The **SkaniT Control Panel** is launched.



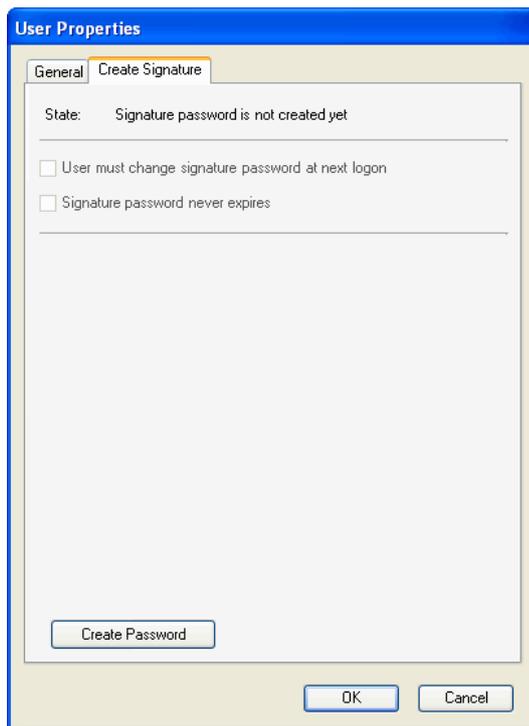
Select the **Users** tab.



Select the user you want to create the signature password for and click **Properties**.



Click the **Create Signature** tab.



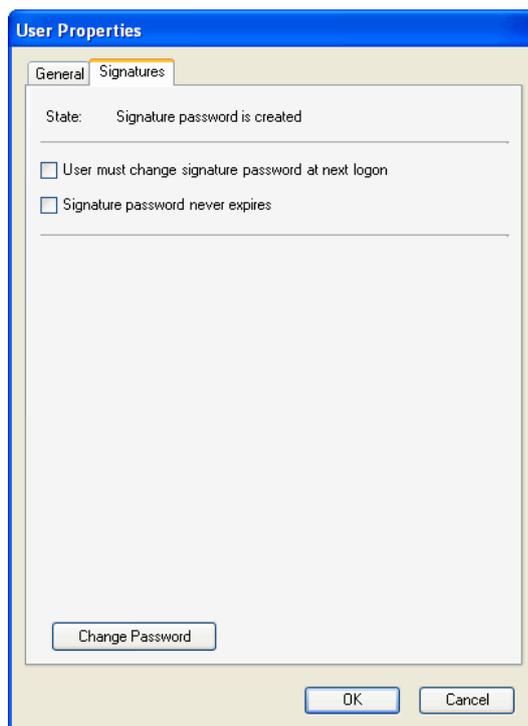
Click the **Create Password** button.



Enter the new signature password in both the *Signature Password* and *Confirm signature password* fields. The signature password must have a minimum of eight characters and it must contain at least one small letter (a – z), one capital letter (A – Z), one number (0 – 9) and one special character (such as +, %, &, *, and so on).

Click **OK**. You will receive a confirmation message.

The **Create Signature** tab has changed into the **Signatures** tab, the state is now *Signature password is created* and the **Create Password** button has changed into **Change Password**.



- **State** – State of the signature password.
- **User must change signature password at next logon** – Tick this check box to prompt the user to change the signature password the first time (s)he signs a session or a run. You can also use this if you are giving a temporary signature password to a user who has forgotten the password.
- **Signature password never expires** – Tick the check box to avoid signature password expiration (defined in General Settings, “General settings” on page 256).

Click **OK**. Before you can use the signature password, both the software and the control panel need to be restarted.

Changing the signature password

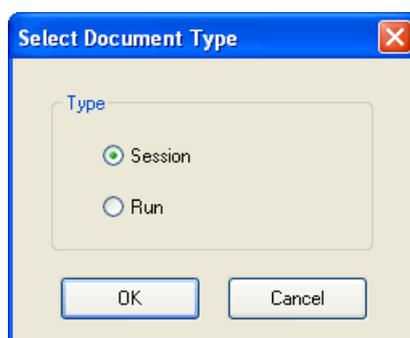
In Control Panel, select the user whose signature password you want to change and click **Properties**. All users can change their own signature passwords. Select the **Signatures** tab and click the **Change Password** button. The **Change Signature Password** dialog is opened.



- **Options** – Select whether you want to change the existing signature password or whether the user has forgotten the password and needs a new one. The *Recover signature password* option is only available for the administrator.
- **Signatures** – Enter the old signature password if you are changing the signature password. If you are recovering a forgotten signature password, the *Old signature password* field is not available. Enter the new signature password in both the *Signature Password* and *Confirm signature password* fields. The password must have a minimum of eight characters and it must contain at least one small letter (a – z), one capital letter (A – Z), one number (0 – 9) and one special character (such as +, %, &, *, and so on).

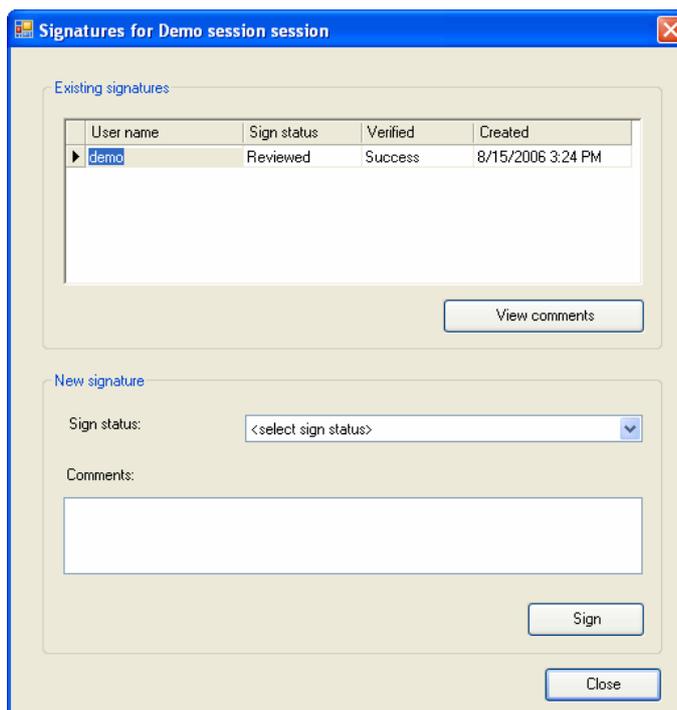
Signing a session or a run

Only the user currently logged on to SkanIt Software may sign sessions and runs. Select Electronic Signatures > **Sign...** The **Select Document Type** dialog opens.



Select whether you want to sign the session or the run. Click **OK**.

The **Signatures for <session name> session/run** dialog is opened.



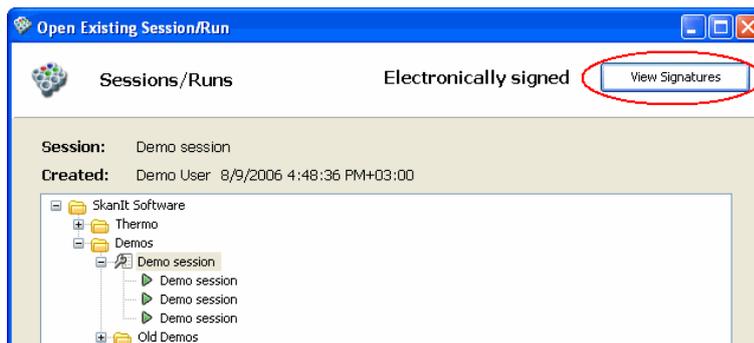
- **Existing signatures** – If the session or run has been signed, the signature information is shown here.
- **New signature** – Select the type of signature from the *Sign status* box: “Reviewed”, “Approved” or “On behalf of”. Enter any further comments in the *Comments* field. Click the **Sign** button. The **Enter Signature Password** dialog is opened.



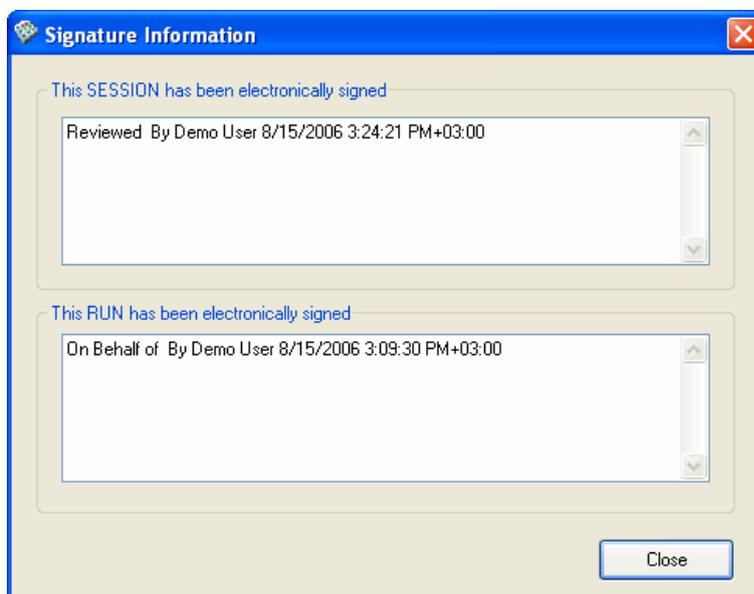
Enter the signature password and click **OK**.

Viewing signatures

When you open a session or a run, click on the **View Signatures** button to view which user has signed the session or the run.



The **Signature Information** dialog opens showing whether the session or the run has been signed, the signature status, the user name of the person who has signed the session or run, the date and time of the signature, and the comments.



If you have a session or a run already open, the **Signature Information** dialog also opens when you select Electronic Signatures > **View and Verify Signatures....**

Information about the signature status of a session, a run or both is shown in the title bar of the SkanIt main window.

Date and time format

The date and time format used in SkanIt Software follows the UTC (Universal Coordinated Time) convention:

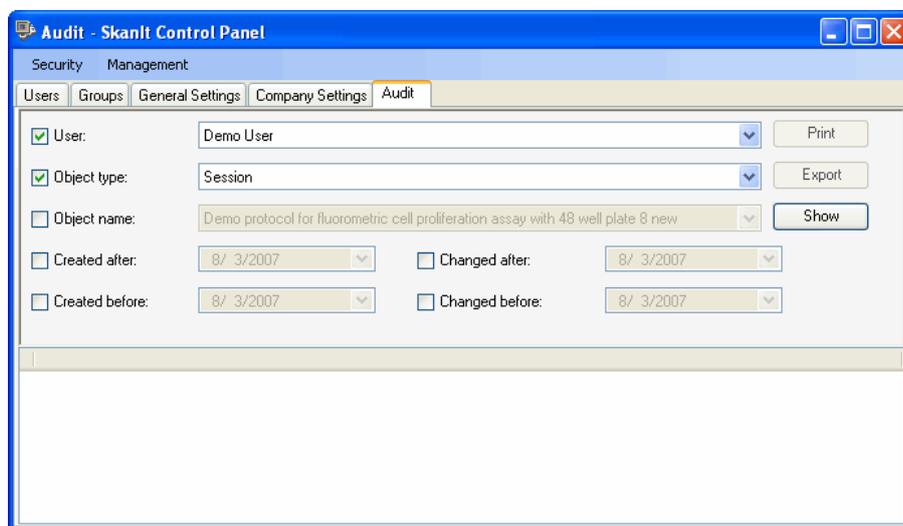
Date format: *dd.mm.yyyy*, for example, 23.07.2006.

Time format: *hh:mm:ss +hh:mm*, where +hh.mm means UTC+hh.mm, for example, 13:05:59 +02:00.

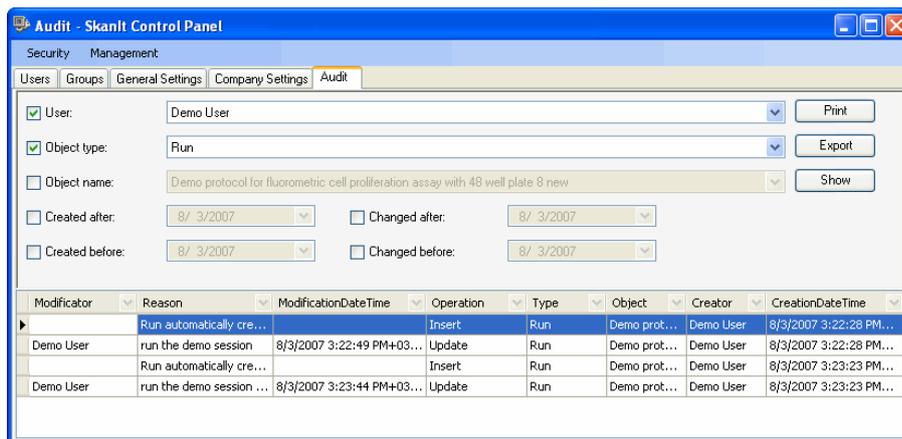
Audit trail

The audit trail shows who did what and when and what kind of operation was carried out in the system. The following operations are audited: creation,

update, and deletion of an object. The objects that are audited are sessions, runs, plate layouts, protocols, users and their rights, user groups, instruments, instrument types, and plate templates.



- **User** – Tick the check box if you want to limit the number of objects shown in the audit trail according to the user name. Choose the user from the list.
- **Object type** – Tick the check box if you want to limit the number of objects shown according to the object type. The available object types are Session, Run, Protocol, Layout, Instrument type, Instrument, User's access rights and Plate Template.
- **Object name** – Tick the check box if you want to limit the number of objects shown according to the object name, for example, a specific session name.
- **Created After, Created Before, Changed After and Changed Before** – Tick one or more of the check boxes if you want to limit the number of objects shown according to the creation and/or modification time.
- **Show** – Click the button to show the audit trail information.



The following buttons become available:

- **Print** – Click to print the audit trail information. The **Page Setup** dialog is opened. Modify the settings if needed and click **OK**. The **Print** dialog is opened. The default printer of the PC is suggested. Change the printer if needed and click **OK**. Click the  icon on the **Print Preview** dialog.
- **Export** – Click to open the **Save As** dialog. Give a name to the file and select a folder. The audit trail information will be saved in .txt format.

The audit trail information is provided as a table with the following columns:

- **Modifier** – The modifier of the object.
- **Reason** – The reason given by the user for the modification.
- **ModificationDateTime** – The date and time of the modification.
- **Operation** – The operation made, such as Insert, Update and Delete.
- **Type** – The type of object.
- **Object** – Which object was modified (Session, Run, Protocol, Layout, Instrument type, Instrument, User's access rights and Plate Template).
- **Creator** – The creator of the original object.
- **CreationDateTime** – The date and time of the creation of the original object.

Glossary

a (alpha) The peak of the measured signal in a time-resolved fluorescence measurement.

Abs See absorbance (optical density).

absorbance (optical density) (Abs) A logarithmic function of the transmission of a wavelength of light through a liquid.

$\log(I/I_0)$, dimension [A]

bioluminescence Naturally occurring chemiluminescence from light-emitting organisms, e.g., glowworms, some deep-sea fish, some bacteria and some fungi.

biometrics A method of verifying an individual's identity based on a measurement of the individual's physical feature(s) or repeatable actions where those features and/or actions are both unique to that individual and measurable.

chemiluminescence Luminescence as a result of pure chemical reactions.

CFR See FDA Code of Federal Regulations.

closed system An environment in which system access is controlled by persons who are responsible for the content of electronic records that are in the system.

COM port Serial RS-232 port.

computer system validation Confirmation by examination and provision of objective evidence that computer system specifications conform to user needs and intended uses, and that all requirements can be consistently fulfilled.

crossover point The point where the excitation and emission spectra meet.

crosstalk Interfering signal from neighboring wells.

DDE See Drug Discovery Edition.

Drug Discovery Edition (DDE) Drug Discovery Edition of SkanIt Software. The DDE includes features needed for compliance with FDA's 21CFR11. See Also FDA Code of Federal Regulations.

decade Order of magnitude. A logarithmic value that is used for presentation of dynamic range.

diffraction Spreading of light transmitted through a narrow slit or reflected from a narrow groove.

digital signature An electronic signature based on cryptographic methods of originator authentication, computed by using a set of rules and a set of parameters such that the identity of the signer and the integrity of the data can be verified.

dose-effect curve This is a graph drawn to show the relationship between the dose of a drug or other chemical and the magnitude of the graded effect that it produces.

dynamic range Dynamic range refers to the range of signals an instrument can read, from the minimum to the maximum detectable. For example, dynamic range of seven decades means that the difference between the lowest and highest signals that can be measured is 10^7 .

electronic record Any combination of text, graphics, data, audio, pictorial, or other information representation in digital form that is created, modified, maintained, archived, retrieved, or distributed by a computer system.

electronic signature A computer data compilation of any symbol or series of symbols executed, adopted, or authorized by an individual to be the legally binding equivalent of the individual's handwritten signature.

emission The release of light from a fluorochrome when an electron falls from an excited state to a lower energy state of the molecule.

EMU Emulation mode.

error message Indication that an error has been detected.

excitation The absorption of light energy by a fluorochrome, during which electrons in the fluorochrome molecule are boosted to a higher energy level.

FDA United States Food and Drug Administration.

FDA Code of Federal Regulations (CFR) The FDA Code of Federal Regulations. 21CFR11 is Title 21 Part 11 of the CFR.

fluorescence The emission of light from a fluorochrome, the wavelength of the light generally being of longer wavelength than that of the absorbed light.

fluorometry Fluorometry is the measurement of fluorescence.

FRET Fluorescence resonance energy transfer.

handwritten signature The scripted name or legal mark of an individual, handwritten by that individual and executed or adopted with the present intention to authenticate a writing in a permanent form. The act of signing with a writing or marking instrument, such as a pen or stylus, is preserved. The scripted name or legal mark, while conventionally applied to paper, may also be applied to other devices that capture the name or mark.

initialization Initialization tests are so-called self tests, which are carried out before operation to ascertain that the necessary instrument adjustments have been carried out.

kinetic measurement Continuous or frequent monitoring of the readings in a chemical reaction to determine its rate.

median effective dose The dose that produces the desired effect. When followed by a subscript (generally "ED₅₀"), it denotes the dose having such an effect on a certain percentage (e.g., 50%) of the test animals.

monochromator Transmits certain wavelengths of the incoming light. Consists of a rotatable diffraction grating and entrance/exit slits (in this context).

MSDE Microsoft SQL Server Desktop Engine.

off-the-shelf software (OTS software) A generally available software component for which the user cannot claim complete software life cycle control.

open system An environment in which system access is not controlled by persons who are responsible for the content of electronic records that are in the system.

photometry The measurement of the properties of light, particularly (luminous) intensity.

photomultiplier tube (PMT) A photoelectric cell that converts light into electric current and amplifies the current.

plate A plate containing microwells.

PMT See photomultiplier tube.

predicate rule Requirements set forth in the Act, the PHS Act, or any FDA regulation, with the exception of Part 11.

priming Completely filling the dispenser tubing and syringe with bubble-free fluid to allow sustained, reproducible dispensing action. The air in an unprimed line acts as a spring, adversely affecting accuracy and precision.

protocol A sequence of steps that performs desired function(s).

reliability The ability of a system or component to perform its required functions under stated conditions for a specified period of time.

relative fluorescence/fluorometric units (RFU)
The arbitrary units in which fluorescence intensity is reported.

RFU or rfu See relative fluorescence/fluorometric units.

sa See system administrator.

self tests Initialization tests and adjustments that the instrument performs prior to operation as well as autocalibration.

spectrum analysis The measurement of the amplitude of the components of a complex waveform throughout the frequency range of the waveform.

spm Shakes per minute. In orbital shaking, it is equivalent to revolutions per minute (rpm).

step A protocol consists of a number of steps. One step performs a specific function, such as measuring, dispensing, shaking, and so on. Each step also has a number of parameters according to which the step is carried out.

Stokes shift The difference between the wavelengths of the excitation and emission peaks.

strip A strip of wells in a row.

system administrator (SA) The user having the administrator privileges for database management (also called sys admin).

t (τ) Fluorescence lifetime of the TRF label.

time-resolved fluorometry/fluorescence (TRF)
Fluorescence intensity measurement using special labels.

transmittance The ratio of transmitted (I) and incident light (I_0), I/I_0 .

tray A plate carrier into which the microplate is loaded for measurement and/or dispensing.

TRF See time-resolved fluorometry/fluorescence.

TRF delay Waiting period between the excitation flash end and the beginning of the emission light measurement.

USB Universal serial bus.

well An individual reaction vessel in a plate.

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