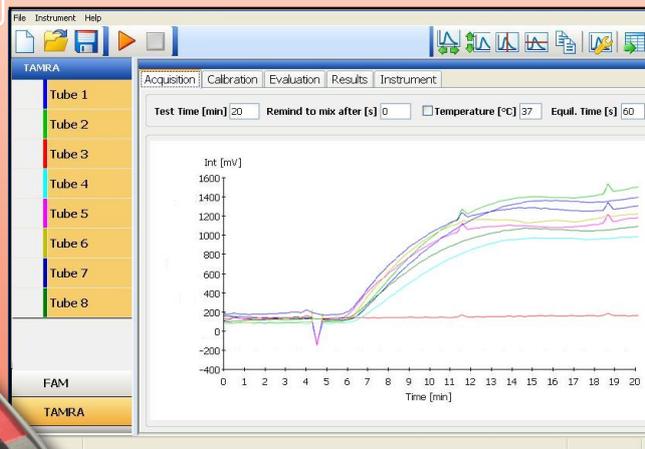




TwistDx

Twista® Studio Software Manual



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Twista® Studio Software Manual
Document Version 0.8 / March 2011

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Printed in Germany

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Table of Contents

1	Introduction	4
2	Twista® Studio Software Features	5
3	PC Requirements	5
4	Installation of the Twista® Studio Software	6
5	Test Rationale	7
5.1	Validation Tests	7
5.1.1	Baseline Validation	7
5.1.2	Threshold Validation	8
5.1.3	Slope Validation	8
5.2	Calibration	8
5.3	Test Criteria Matrices	9
5.3.1	Single Channel	9
5.3.2	Combined Result	10
6	Software Structure	11
6.1	Methods	11
6.2	Combined Result Overview	11
6.2.1	Setting Sample Names Manually	12
6.2.2	Loading Sample Names from File	12
6.2.3	Saving Sample Information to File	12
6.2.4	Setting the Sample Key Color	12
6.3	Tab Pages	13
6.3.1	Acquisition Page	14
6.3.2	Calibration Page	14
6.3.3	Evaluation Page.....	15
6.3.4	Results Page	16
6.3.5	Instrument Page	17
6.4	Menu Structure	18
6.5	Main Tool Bar	19
6.6	Graphical Data View	20
6.6.1	Graphical Tool Bar.....	20
6.6.2	Setup Graph Dialog	21
6.7	Tabular Data View	24
6.7.1	Table Tool Bar	24
7	USB Device Driver	25
7.1	First Steps	25
7.2	Installation of the USB Device Driver	26
7.3	Installation of the USB Virtual COMPort	29
8	Data Formats	32
8.1	Experimental Data	32
8.2	Sample Information	32

1 Introduction

The Twista® reader offers powerful solutions for a range of applications including

- Tests performed in clinical chemistry
- Isothermal and end-point Nucleic Acid Amplification and Testing including RPA
- End-point PCR read out
- ELISA–Tests / Immunodiagnostics
- Colorimetric and Fluorescence Bacteriological Tests / Cell-Cultures
- Colorimetric and Fluorescence Environmental Tests / Water Analysis
- DNA and Protein Quantification (Fluorescence)
- Many other Tests in Tube Format with or without thermal control

The isothermal DNA amplification process, for example Recombinase Polymerase Amplification (RPA), operates at constant temperature. Isothermal DNA/RNA amplification enables a new generation of DNA tests for a broad spectrum of end-users through a reduction in the need for hardware and sample manipulation.

Most isothermal methods lack either the necessary scalability, sensitivity, rapid time to result or specificity. Complete solutions including hardware are required to support and sustain the RPA process, especially for point-of-care and field applications.

Operating the test close to the sample source saves time and costs. It also reduces the risks of sample contamination and sample degradation due to incorrect or prolonged storage, and can therefore decrease the incidence of false-positive and false-negative results and sample mis-identification.

The Twista® reader provides sensitive, accurate, and specific results, as well as rapid turnaround. The stand-alone device provides operational and physical robustness, at an affordable cost.

Due to its mobility, the Twista® reader requires no sample storage or transportation. Due to the ability to work from clinical samples directly, most of the trouble of sample preparation is avoided.

The corresponding **Twista® Studio Software** allows the user to configure the device for the analysis of samples.

ESE GmbH is certified according to ISO 9001 and EN 13485 and is thus equipped for usual regulatory requirements.

The following document describes the functions of the Twista® Studio Software for the PC.



- Control of the Twista® reader via USB port
 - Run RPA tests
 - Display and save measurement data and results on the PC
 - Graphical display of raw data for the measurement of each tube
 - Define target temperature of the incubator
 - Save test setups in the device or on the PC
 - General device configuration
- PC with Microsoft Windows 2000, XP or Vista
 - 512 MB RAM
 - 100 MB available memory on hard drive
 - USB Interface



Figure 1: Installing Twista® Studio

4 Installation of the Twista® Studio Software

- Insert CD
- If the installation does not start automatically, open the CD within the explorer and start **autorun.exe**
- Press **Install Software**

After successfully installing the software, the icon “Twista® Studio” will appear on the desktop:



Connecting the Twista Reader

- Plug in the device
- Press the Enter key (middle button) for more than one second
- Connect the device to the PC via USB cable (For installation instructions please refer to Chapter 7: USB Driver Device)

Starting the Twista® Studio Software

- To start the Twista® Studio software simply double click on the Twista® Studio icon

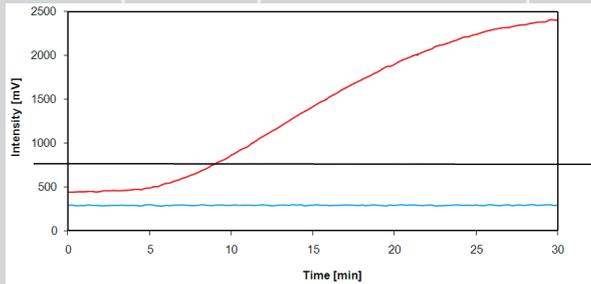


Figure 2: Typical signals from positive (red) and negative (blue) samples

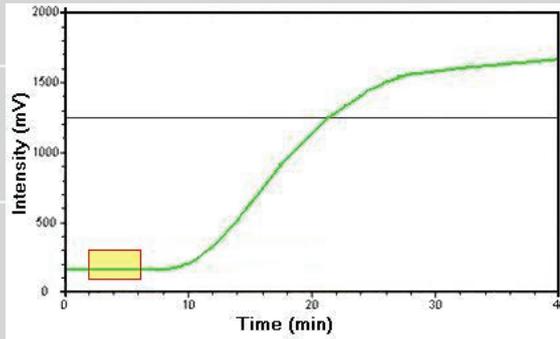


Figure 3: Baseline Validity limits

5 Test Rationale

The TwistDx test can use two fluorescent dyes to report simultaneously both the analyte concentration and the sample validity. A typical measurement takes between 10 - 30 minutes, during which time the signal can increase markedly due to successful amplification, if the sample is positive. Example time courses for positive and negative samples are shown in Figure 2. For a positive sample, the signal initially remains constant until a point where enough amplification has occurred to be detectable. The signal then rises rapidly, passing through a pre-determined cut-off level or exceeding a pre-defined slope, which defines the sample as positive. The signal continues to rise until it eventually plateaus.

5.1 Validation Tests

5.1.1 Baseline Validation

The first test criterion applied to the sample data is the baseline validity check. The baseline is deemed to be invalid if it lies outside pre-defined intensity levels within a user-defined time period. Figure 3 represents a baseline check between 100 and 300mV within the time period 2 to 6 minutes, shown by the box area lower left. If the baseline limits are exceeded within this time scale, then the sample will be reported as invalid: no Positive/Negative or quantitative results will be given for this sample.

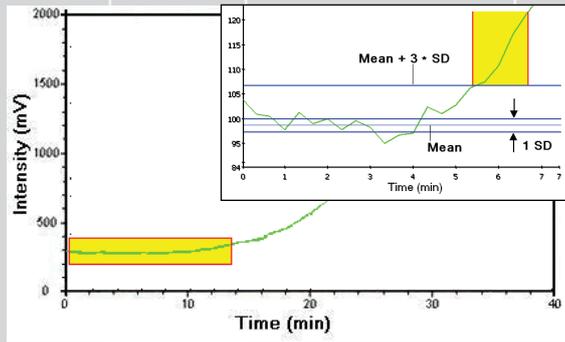


Figure 4: Threshold Validation Limits

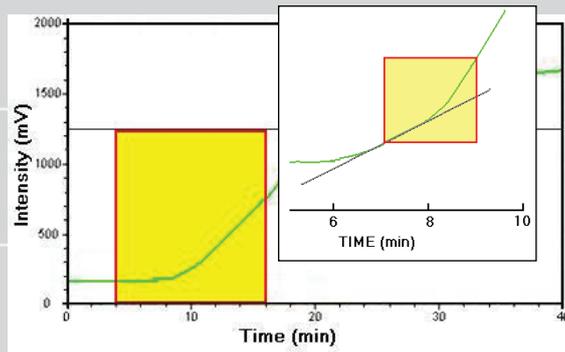


Figure 5: Slope Validation Limits

5.1.2 Threshold Validation

The Threshold Validation test is used to identify that the signal has increased sufficiently to be deemed as positive.

The mean signal within the test time is calculated, the test then checks if the signal exceeds a user-defined multiple of the Standard Deviation of the mean signal, for at least a time defined by the user. Figure 4 represents a threshold time range of 0 - 14 minutes. Figure 4a (inset) shows a close-up of the Threshold Validation level being deemed positive: at 5.5 minutes, the signal has exceeded the threshold limit. At 6.5 minutes the signal has remained above the threshold for 1 minute so is deemed **Positive**.

5.1.3 Slope Validation

The Slope Validation test is used to identify that the signal has increased at a sufficiently high rate to be deemed as positive. Figure 5 represents a slope check within the time period 4 to 16 minutes. Figure 5a (inset) shows a close-up of the Slope Validation check: At around 9 minutes, the signal has exceeded the slope limit for 2 minutes so is deemed **Positive**.

5.2 Calibration

In addition to the Positive/Negative results generated by the Threshold and Slope tests, samples with known concentration can be measured and the results used to convert unknown sample results to absolute concentration values.

Single Channel Criterion	State									
	INV	VAL								
Threshold	XX	UNK	+	UNK	-	XX	+	IGN	XX	
Slope	XX	UNK	UNK	+	XX	-	+	XX	IGN	
Single Channel Result	INV	UNK	UNK	UNK	-	-	+	XX	XX	

Figure 6: Single Channel Evaluation Matrix

5.3 Test Criteria Matrices

5.3.1 Single Channel

The test criteria described in Section 5.1 above are all optional: one or more test criteria can be combined to give more accurate results.

For each channel (e.g. for FAM and TAMRA) an evaluation matrix exists as shown in Fig. 6, Where:

- **INV**: invalid (when the baseline threshold is exceeded, or in an over range condition)
- **VAL**: valid (when the baseline threshold is not exceeded)
- **UNK**: unknown (when the data collected have not yet fallen in the criterion's analysis time range)
- **IGN**: ignored (when the analysis criterion is not selected)
- **+**: positive
- **-**: negative
- **XX**: positive, negative, unknown or ignored

Notes:

1. When an over range condition occurs, the combined result is the one that was present immediately before the over range event if the state is either positive, negative or invalid
2. When an over range condition occurs during an unknown state the state changes to invalid
3. Once the a sample becomes **invalid** stays always invalid
4. Once a sample becomes **positive** stays always positive
5. The possible state transitions are the following:
 - From unknown to **invalid**
 - From unknown to **negative**
 - From unknown to **positive**
6. The possible states of "Single Channel Results" are: invalid, ignored, unknown, positive, negative
7. The state of a **not selected baseline** is considered always valid
8. The state of a **not selected threshold** is considered always ignored
9. The state of a **not selected slope** is considered always ignored

Single Channel Result	Condition							
Result Single Channel 1	INV	X	UNK	X	+ or -	IGN	IGN	X
Result Single Channel 2	X	INV	X	UNK	+ or -	IGN	X	IGN
Combined Result	INV	INV	UNK	UNK	Based on Evaluation Matrix	UNK	X	X

Figure 7: Combined Result Criterion Table

5.3.2 Combined Result

After results have been generated for each single channel (e.g. for FAM and TAMRA) then a combined result is generated from both channel results: This is the final reported result set for the samples. The criterion table for this combined result is shown in Figure 7, Where:

- **INV**: invalid (when the baseline conditions are not met or in over range condition)
- **UNK**: unknown (when the data collected haven't fallen yet in the criteria's analysis intervals)
- **IGN**: ignored (when the analysis criteria is not ticked)
- **+**: positive
- **-**: negative
- **X**: positive, negative, unknown

Notes:

1. The possible states of the Combined Result are: invalid, unknown, positive or negative
2. The conditions not present in the Evaluation Matrix are treated as invalid.

State Symbols

The state symbols displayed on the Twista® reader screen and on the PC interface beside the tube names have the following meaning:

- Invalid: **?**
- Unknown: **empty** tube/box
- Positive: **+**
- Negative: **-**

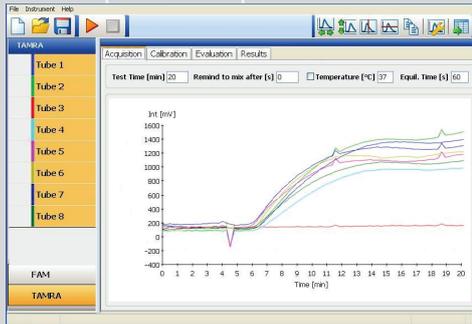


Figure 8: Software Structure

+	Patient 1a
+	Patient 1b
-	Patient 2a
+	Patient 2b
+	Patient 3a
-	Patient 3b
-	Patient 4a
-	Patient 4b

Figure 9: Combined Results Overview

6 Software Structure

This section describes the features of the Tube Scanner Studio software. Please refer to Section 5: 'Test Rationale' for a description of the evaluation types.

6.1 Methods

A test consists of two simultaneous methods, each of which uses a different reporting fluorescent dye. Definition of method parameters is carried out using the Acquisition, Calibration and Evaluation pages. Note that for the Calibration and Evaluation pages, each dye has a separate parameters set. For both of these pages, setup the parameters for the first dye (in this case FAM) then select the other dye from the bottom left of the screen (in this case TAMRA) then define the second dye's parameters. When a test setup is saved, all parameters for both dyes are saved.

6.2 Combined Result Overview

Up to eight samples (as default called "tubes") can be measured. An overview of the combined results is shown which includes sample name, result (positive, negative, unknown, invalid) and a color key relating to graphical data displayed for each sample. The overview panel can be setup by a right button click on the desired sample.

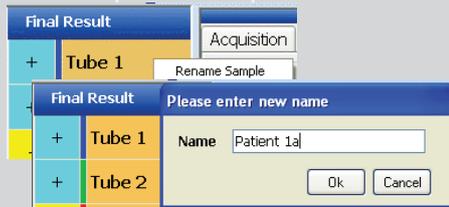


Figure 10: Setting sample names

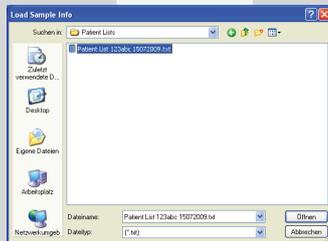


Figure 11: Loading Sample Information from file



Figure 12: Setting the Sample Key Color

6.2.1 Setting Sample Names Manually

Sample names displayed in the overview panel can be set manually or by loading a sample information file from disk. To change sample names manually, right click the desired sample name and select 'Rename Sample'. Enter the new name in the dialog that appears.

6.2.2 Loading Sample Names from File

To set sample names from a file, right click the overview panel and select 'Load Info'. Using the file selector that appears, locate and select the desired sample information file. Sample information will be loaded and displayed in the overview panel. Note that only the first 12 characters of the sample name will be visible. The required format for this file is described in Chapter 8: Data Formats.

6.2.3 Saving Sample Information to File

To save sample information to a file, right click the overview panel and select 'Save Info'. Using the file selector that appears, define the location and filename for the sample information file.

6.2.4 Setting the Sample Key Color

To set the sample key color, right click the overview panel and select 'Change Color'. Using the color selector panel that appears, select the desired key color for the sample.

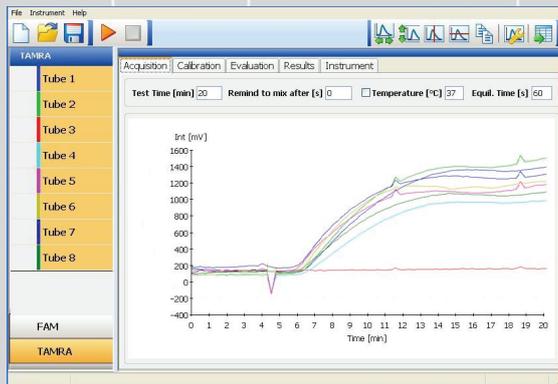


Figure 13: Tab Page ACQUISITION (Graphical Mode)

6.3 Tab Pages

The Twista® Studio software has five pages:

Acquisition

On this page, basic measurement parameters are defined. The results, i.e. raw data, are shown in graphical or tabular format. During measurement, data display is continuously updated.

Calibration

On this page, the concentration curve can be determined by measuring two standard samples: one for low signal and one for high signal. A linear concentration curve is calculated automatically and shown in graphical format. The calibration curve is used for the calculation of the results in the evaluation and result tabs.

Evaluation

On this page, the parameters for evaluation are defined. Raw or derivative data are shown in graphical or tabular format.

Results

On this page the individual results are shown together with evaluation criteria to produce final quantitative results.

Instrument

This page allows the user to upload and download data to the Twista® reader.

Figure 14: ACQUISITION Page Details

Standard ID	Nominal: Int [mV]	Measured: Int [mV]
Standard 1	100	
Standard 2	0	

Figure 15: CALIBRATION Page Details

6.3.1 Acquisition Page

Test Time :

Duration time for the measurement. Allowed range is 1 - 60 minutes.

Remind to mix after :

Time in seconds after which the user is reminded to mix the sample. The beeper will stop as soon when the lid is opened. A value of "0" disables the beeper. Allowed range is 0 or integer 1 - 999.

Temperature:

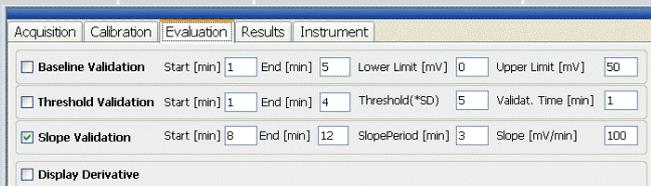
Temperature at which the test is carried out. Allowed range is 35 - 42°C.

Equilibration Time:

Wait time in seconds after the target temperature is reached, to allow efficient equilibration of the temperature within the sample. Allowed range is 0 or integer 1 - 999.

6.3.2 Calibration Page

After the user-definable calibration parameters have been entered (title, units and number format for the ordinate axis), the graphical data display on the calibration and evaluation pages are updated with these new parameters by clicking on the Update button.



Validation Type	Start [min]	End [min]	Lower Limit [mV]	Upper Limit [mV]	Threshold (*SD)	Validat. Time [min]	SlopePeriod [min]	Slope [mV/min]
<input type="checkbox"/> Baseline Validation	1	5	0	50				
<input type="checkbox"/> Threshold Validation	1	4			5	1		
<input checked="" type="checkbox"/> Slope Validation	8	12					3	100
<input type="checkbox"/> Display Derivative								

Figure 16: EVALUATION Page Details

6.3.3 Evaluation Page

Baseline Validation:

The measurement is considered valid if all values in the measurement time are within the range of the upper and the lower limits.

Threshold Validation:

The threshold is defined as the mean value of the signal within the measurement time plus a multiple of the standard deviation of that mean value. The sample is deemed positive if the measured data are higher than the defined threshold for at least the validation time.

Slope Validation:

The sample is deemed positive if the slope within the time window is higher than the slope threshold for a given time period. The slope period defines the number of points that are used to determine the slope. The calculated slope can be displayed in the derivative window.

Display Derivative:

Switches display between raw data and slope of the reaction (1st derivative).

For more detailed information about using the Data Validation and Evaluation options, please see the TwistDx website www.twistdx.co.uk under the section titled Technical Resources.

Acquisition Calibration Evaluation Results Instrument								
Final Outcome Test								
Tube1	Tube2	Tube3	Tube4	Tube5	Tube6	Tube7	Tube8	
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
Result Details								
Tube1	Tube2	Tube3	Tube4	Tube5	Tube6	Tube7	Tube8	Info
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	FAM Baseline
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	FAM Threshold
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	FAM Slope
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	FAM
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	TAMRA Baseline
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	TAMRA Threshold
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	TAMRA Slope
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	TAMRA
Evaluation Matrix <input type="button" value="Add result"/> <input type="button" value="Remove result"/>								
Final Outcome	FAM	TAMRA						
Positive	Positive	Positive						
Negative	Negative	Positive						

Figure 17: RESULTS Page Details

6.3.4 Results Page

Final Outcome Test

Displays the final test result per sample (tube)

Result Details

Displays the result of each validation check for each sample

Evaluation Matrix

Defines post-calibration validation to give a final test result

Add / Remove Results

Adds or removes an entry in the Evaluation Matrix

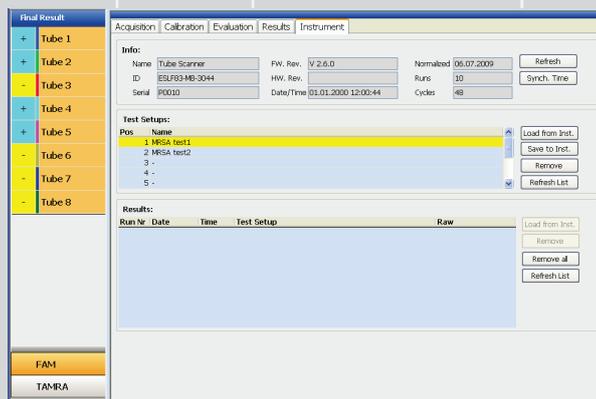


Figure 18: INSTRUMENT Page Details

6.3.5 Instrument Page

Info:

Refresh

Loads information from instrument

Synch. Time

Synchronises the time in the instrument to the time of the PC

Test Setups:

Load from Inst.

Loads test setup from the instrument

Save to Inst.

Saves current test setup to the instrument

Remove

Removes test setup from the list

Refresh List

Refreshes list with test setup(s) currently in memory.

Results:

Load from Inst.

Loads test result(s) from the instrument

Remove

Removes the selected result set(s)

Remove all

Removes all result set(s)

Refresh List

Update list with new results in memory

Raw data

If this is ticked, this means that time course data is resident in the instrument's memory, and can be uploaded to the PC software



Figure 19: Main Tool Bar (left side of the Menu Bar)

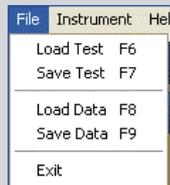


Figure 20: FILE Menu



Figure 21: INSTRUMENT Menu



Figure 22: HELP Menu

6.4 Menu Structure

File

Load Test Setup	Loads a test setup from file
Save Test setup	Saves the current test setup to file
Load Data	Loads data from file into the software
Save Data	Saves current data to file
Exit	Exits the program

Instrument

Update Firmware	Opens a file dialog which allows selecting an update of the firmware (software for the device)
Reset ComPort	Communication between PC and the Twista® reader runs internally via a ComPort. The external USB port automatically connects with a free internal ComPort after start-up. For some computers, it will be necessary to manually connect to the ComPort used by the instrument

Help

About	Shows the “About” dialog with information about the Twista® readerSoftware and Firmware
-------	---



6.5 Main Tool Bar

New: Removes the currently loaded data

Load: Loads data from file into the software

Save: Saves current data to file

Start: Starts the measurement

Stop: Stops the measurement immediately

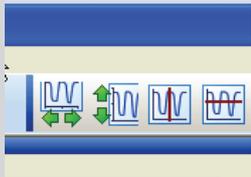


Figure 23: Graphical Tool Bar (visible if graphic data displayed)



Auto Scale X / Y



Cursor X / Y



Display as Graphic/Table



Copy

6.6 Graphical Data View

Where graphical data is displayed, the graphic tool bar appears, offering a range of relevant functions.

Zoom

Click and hold the left mouse button to define the desired zoom region.

To cancel the zoomed area, double click the left mouse button.

6.6.1 Graphical Tool Bar

Auto Scale X / Y:

Automatically expands the X-axis / Y-axis

Cursor X / Y:

Displays the X-cursor / Y-cursor into the graph to identify specific values.

Display as Graphic/Table:

Switches between tabular and graphical data display.

Copy:

Copies graphical or tabular data to the clip board.

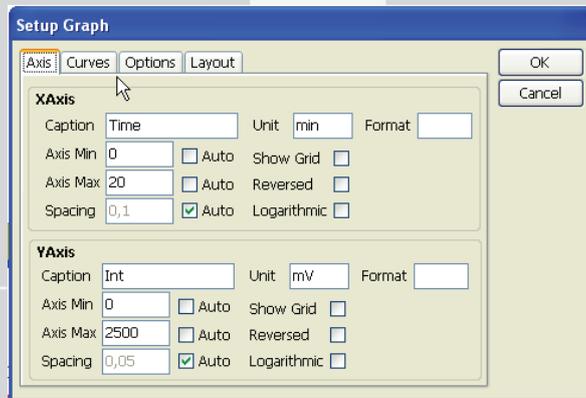


Figure 24: Axis Parameters Setup Page

6.6.2 Setup Graph Dialog

This allows the user to setup graph options. To open, click on the following button:



Axis

Caption: Defines the caption for the X and Y axes.

Unit: Defines the units for the X and Y axes.

Format: Defines the numerical format of numbers displayed.

Axis Min: Defines the start value for the X and Y axes.

Axis Max: Defines the end value for the X and Y axes.

Spacing: Defines the labelling for the X and Y axes.

Auto: Uses detail from the data to define Axis Min, Axis Max and/or Spacing.

Show Grid: Defines grid lines for the X and Y axes.

Reverse: Reverses the Start and End for the X and Y axes.

Logarithmic: Converts the axis to logarithmic scaling for the X and Y axes.

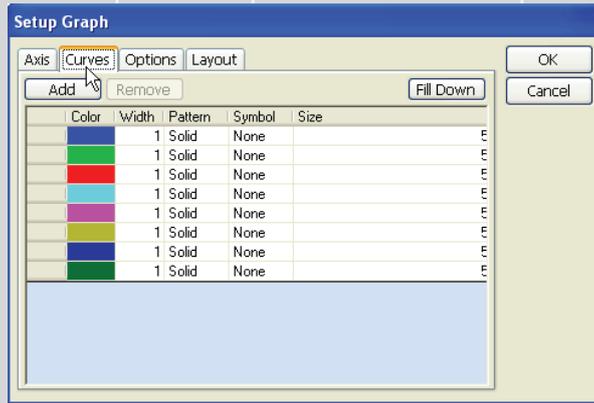


Figure 25: Curves Parameters Setup Page

Curves

Add: Adds a new line (curve appearance definition) to the table.

Remove: Removes the currently selected line (curve appearance definition) from the table.

Fill Down: Copies the data from the currently selected cell to the bottom of the table.

Color: Defines the curve colour for the currently selected curve definition.

Width: Defines the curve line width for the currently selected curve definition.

Pattern: Defines the curve pattern for the currently selected curve definition. Options are: None, Solid, Long dash, Dotted, Short dash.

Symbol: Defines the symbol for the currently selected curve definition. Options are: None, Dot, Box, Triangular, Diamond, Start, Vline, Hline, Cross, Circle.

Size: Defines the size of the symbol for the currently selected curve definition. Permitted values: 1 – 20.

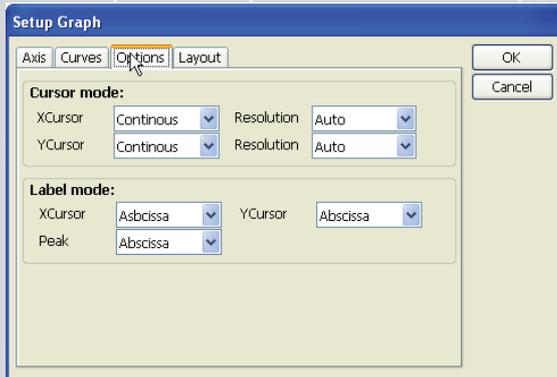


Figure 26: Options Parameters Setup Page

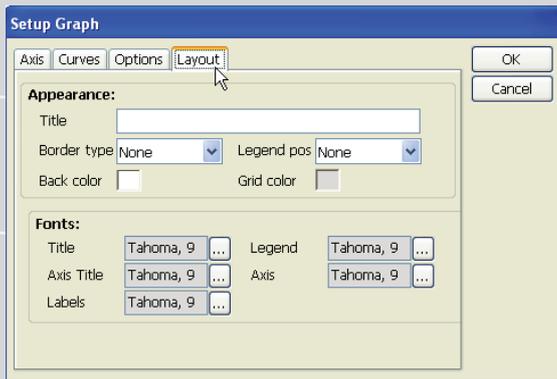


Figure 27: Definition of "Layout" in the Graphics Setup

Options

- Cursor Mode:** Defines the function of the X and Y cursors
 XCursor / YCursor: Defines the step resolution for the X / Y cursor. Options are Continuous or point.
 X / Y Resolution: Defines the resolution for the X / Y cursor. Options are: Auto, 10, 1, 0.1, 0.01, 0.001.

Label Mode: Defines the functionality of labels

- XCursor / YCursor: Defines information to be displayed with the cursors. Options are Abscissa, Ordinate or both.
 Peak: Defines peak information to be displayed. Options are Abscissa, Ordinate or both.

Layout

- Appearance:** Defines the graphical appearance
 Fonts: Defines fonts for text displayed within the graphic.



Figure 28: Tabular Tool Bar



Display as Graphic/Table



Copy

6.7 Tabular Data View

This toolbar appears if tabular data is displayed.

6.7.1 Table Tool Bar

Display as Graphic/Table:

Switches between tabular and graphical data display.

Copy: Copies table to clip board.

7 USB Device Driver

The exact sequence and appearance of the installation procedure depends on the version of Microsoft Windows ®.

The following documentation describes the installation using Windows® XP.

The operating system must provide full USB support.

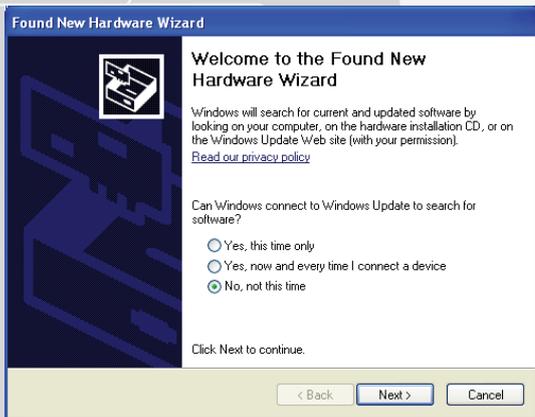
Ensure that you have sufficient rights for the installation of the drivers (e.g. administrator rights).

If problems should arise during installation, you can de-install the current USB device driver using the Windows control panel.

Installation can be re-started by re-connecting the USB connector.

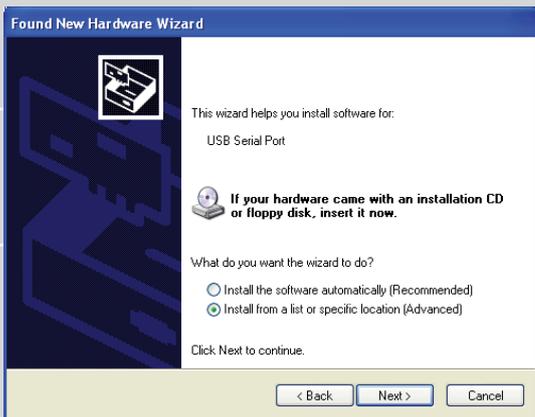
7.1 First Steps

- Put the CD-ROM into the CD-ROM drive.
- Copy the folder 'USB Driver' to the computer's desktop
- Connect the Twista® reader with the USB cable.
- Windows® automatically detects the new USB device. The new hardware assistant appears.

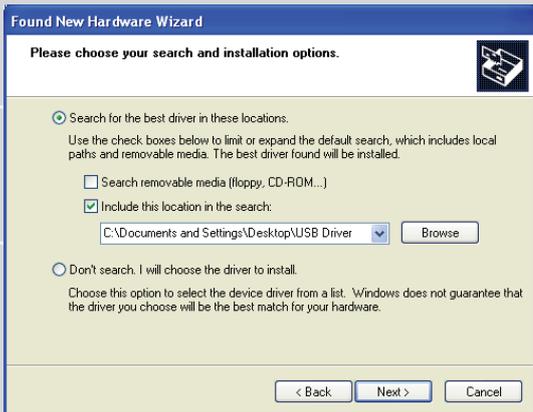
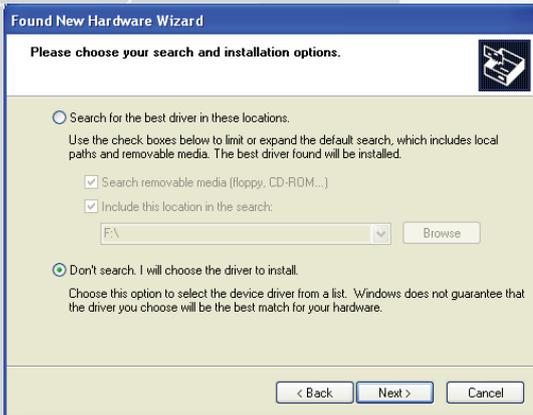


7.2 Installation of the USB device driver

Select the last button “No, not this time” to ignore a connection with Windows Update and press “Next”.



Select the option “Install from a list or specific location” and press “Next”.



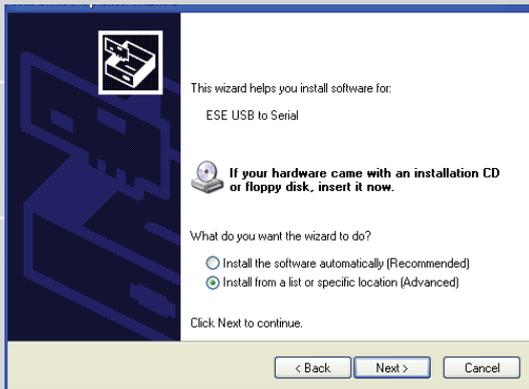
Select the option “Don't search, I will choose the driver to install” and press “Next”.

Press the “Browse” button to locate the USB Driver folder on the desktop and press “Next”.



Confirm the next screen by pressing the button “Continue Anyway”.

On completion of the installation process, press “Finish”

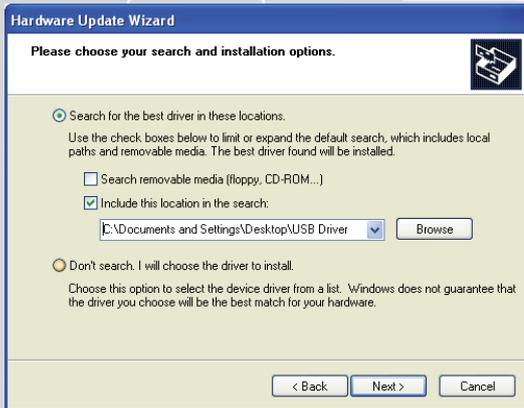


7.3 Installation of the USB virtual COM Port

Immediately after the installation of the USB device driver the installation of the virtual COMPort will be initiated .

Select the last button “No, not this time” and press “Next”.

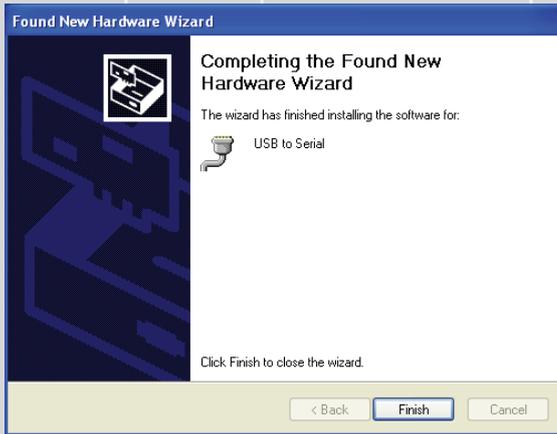
Select the option “Install from a list or specific location” and press “Next”.



Click the “Browse” button to locate the USB Driver folder on the desktop and press “Next”.



Confirm the next screen by pressing the button “Continue Anyway”.



On completion of the installation process, press "Finish"

8 Data Formats

8.1 Experimental Data

Experimental Data is saved in two formats:

- Binary saved with extension “.dat” (automatically saved in the default directory). This binary format is proprietary, and can be supplied on request.
- TAB separated text (TAB separated ASCII) saved with extension “.txt”

8.2 Sample Information

Sample Information can be saved to- and loaded from- disk as a file. This file must have a title line followed by a separating line, followed by 8 lines of text, one line for each tube. An example is as follows (note the inclusion of non-alphanumerics in sample info line 1):

Sample Info

Patient#<"^?+' 1a

Patient 1b

Patient 2a

Patient 2b

Patient 3a

Patient 3b

Patient 4a

Patient 4b



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the quality standards in
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