TECAN.

Operating Manual for the

LS Series Scanner

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WARNING
CAREFULLY READ AND FOLLOW THE INSTRUCTIONS PROVIDED IN THIS DOCUMENT BEFORE OPERATING THE INSTRUMENT.

Notice

Every effort has been made to avoid errors in text and diagrams, however, Tecan Austria GmbH assumes no responsibility for any errors which may appear in this publication.

It is the policy of Tecan Austria GmbH to improve products as new techniques and components become available. Tecan Austria GmbH therefore reserves the right to change specifications at any time.

We would appreciate any comments on this publication.

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Declaration for EU Certificate

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About the Instructions for Use

This publication is intended as *Instructions for Use* for the LS Series Scanner instruments, which are designed to detect highly quantitative fluorescence images that have been read from microarray slides or other compatible sample formats.

This document provides information about:

- Installing the instrument
- Operating the instrument
- Cleaning and maintenance routines

Warnings, Cautions and Notes

There are four types of notices used in this publication. These notices highlight important information or warn the user of a potentially dangerous situation. The notices appear below:

	Note *Gives helpful information.*

	Caution **Indicates a possibility of instrument damage or data loss if instructions are not followed.**

	WARNING **INDICATES THE POSSIBILITY OF SEVERE PERSONAL INJURY, LOSS OF LIFE OR EQUIPMENT DAMAGE IF THE INSTRUCTIONS ARE NOT FOLLOWED.**

	WARNING **INDICATES LASER. DO NOT STARE INTO THE BEAM!**

	WARNING **INDICATES THE POSSIBLE PRESENCE OF BIOLOGICALLY HAZARDOUS MATERIAL. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.**

Table of Contents

1. Safety

1.1 Instrument Safety

1. Always follow basic safety precautions when using this product to reduce the risk of injury, fire, or electrical shock.

2. Read and understand all information in the Operating manual. Failure to read, understand, and follow the instructions in the manual may result in damage to the product, injury to operating personnel or poor instrument performance.

3. Observe all WARNING and CAUTION statements in the manual.

4. Never open the LS SERIES SCANNER while the instrument is plugged into a power source. The instrument contains class 2, 3a and 3b lasers inside.

5. Never force a slide or another sample into the instrument.

6. LS SERIES SCANNERS are intended for laboratory research use only. Observe proper laboratory safety precautions, such as wearing protective clothing and using approved laboratory safety procedures.

2. General

2.1 Instrument Features

The LS SERIES SCANNERS are laser-scanning instruments designed to detect highly quantitative fluorescence images. The scanners are designed to read microarray slides as well as other customer specific sample formats. They have exceptional flexibility with respect to sample formats and measurement modes. They can read samples up to the size of microplates as well as thick samples like gels or, for instance, liquid cells. They can read both in confocal and in non-confocal mode and a selection of up to 4 excitation wavelengths (lasers) and an almost unlimited number (28) of emission wavelengths or emission filters.

Automation friendliness is another key feature of the LS SERIES SCANNERS. The design allows the use of standard microplate equipment to load very high numbers of slides automatically. For instance, with a Connect, up to 200 slides or 50 microplates can be loaded and processed automatically within one run.

2.1.1 Samples

Slide adapters

Its special microplate/slide adapters accommodate 4 slides at once and loading is easily automated with standard microplate equipment.

Other formats

Due to its flexible design, other sample formats can be measured as well. The software allows special formats to be defined and special scanning areas to be processed. Due to an exceptionally high working distance of 6.5 mm, 'thick' samples, well structures, samples within a liquid cell or even gels can be measured. An automatic autofocus system is able to bring all of these samples into the proper focal plane. If you are in doubt whether a special sample format can be measured with the LS SERIES SCANNERS, please contact a Tecan representative.

2.1.2 Modes of operation

Confocal or non-confocal

The LS SERIES SCANNERS are equipped with 3 different user selectable pinholes for selection of the appropriate depth of focus. The smaller the pinhole the higher is the confocality of the optical system and the shorter the depth of field. A small pinhole typically gives a better signal to background ratio, because all background contributions from outside the focal plane, e.g. the glass material or the backside of the slide are rejected. However for bulkier samples or samples with an uneven or a slightly bent surface confocality can result in lower signals or in a poor reproducibility. Here a larger pinhole is more appropriate. That's why the LS SERIES SCANNERS offer the choice. Just choose the degree of confocality that is best suited for the samples.

Excitation wavelengths

- The LS Scanner can be equipped with up to 4lasers.
- Every Scanner has a HeNe laser with 633nm.
- Lasers with the wavelengths 488nm, 532nm and 694 nm are optional.

Emission wavelengths

Filters are located in special filter slides. The standard configuration comes with one filter for each excitation wavelength or laser respectively.

Additional filters can be added into the filter slides. The filter slides can be exchanged by the user. The 'identity' of each filter slide is automatically detected by the instrument when it is inserted into the slot.

Dual scanning

In order to increase the overall scan speed, two colors can be measured simultaneously with a dual channel instrument. A calibration algorithm takes care of the *crosstalk* between the two colors and corrects for it online. If you have purchased a single channel instrument, or if the wavelengths are too close to each other, the instrument can measure the two colors sequentially as well.

Resolution

The standard resolution is 10 µm.

For larger sample features there is the option to scan with 20 µm or 40 µm thus both increasing scan speed and decreasing the amount of data.

Smaller structures can be better resolved with 6 µm or 4 µm pixel resolution.

Scan widths for a single path scan are 22 mm for 10 µm, 20 µm and 40 µm, 6 µm pixels and 15 mm for 4 µm pixels.

Optimize sensitivity

For smaller scanning areas, there is a user selectable option to increase sensitivity without changing the scan time.

Another way to increase sensitivity is to scan transparent slides upside down. The reason is because surface bound molecules emit the greater part of their fluorescence into the medium with higher refractive index (e.g. glass).

2.1.3 Automation friendliness

Because the LS SERIES SCANNERS are designed to interface with microplate technology, they are very well prepared for automation using existing microplate equipment. Four slides can be loaded in an adapter with the outer dimensions of a microplate. The number of slides is limited only by the stacking capacity of the external microplate device.

2.1.4 Integrated Image Analysis Software Array Pro

The LS SERIES SCANNERS come with an integrated array analysis software package.

Caution
If the instructions given in this manual are not correctly carried out, the instrument May either become damaged or May no Longer be Able to perform Its Procedures correctly and the Accuracy of the instrument can no longer be guaranteed.

Note
In order to obtain reliable results it is strongly recommended to place reference spots onto your sample for quality control. These spots should represent every dye of your experiment and it is recommended that they are distributed in a non symmetric pattern across your sample. It is also recommended to place 'blank' spots onto your arrays in order to check for the variation of the background in your experiment.

3. Installation

3.1 Unpacking & Inspection

The delivered container includes the following:

- Instrument
- PC
- PC Monitor
- Manual
- Software: Driver LS Series Scanner (1 CD-ROM)
- Cables (RS232 cable and 1 interface cable)
- Slide Adapters
- Filter Slide
- Reference slides (histological section of mouse; spots)

- Array Pro Software
- Hardware Key Array Pro SW
- Installation CD Array Pro SW
- Array Pro User Manual
- Scattered light glass slide
- Filter Slide for Calibration
- 2 Filter Slides

Caution

Before the instrument is installed and switched on, it should be left to stand for at least three hours, so there is no possibility of condensation causing a short circuit.

Visually inspect the container for damage before it is opened.

Report any damage immediately.

Select a location to place the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents and acid vapors. Allow at least 10 cm between the instrument and the wall or any other equipment.

Remove transportation locks.

	Caution
STOP	**The LS Series Scanners are precision instruments and are very heavy. At least two people must carefully lift the instrument from the box.**

Visually inspect the instrument for loose, bent or broken parts.

Report any damage immediately.

Compare the serial number on the rear panel of the instrument with the serial number on the packing slip.

Report any discrepancy immediately.

Check the instrument accessories against the packing list.

Save packing materials and transport locks (see next section) for further transportation purposes.

	Caution **Do not try to open or close** **the instrument flap manually.**

Ensure that the flap cannot be accidentally hit when it is opened automatically. Ensure that the main switch and the main cable can be reached at all times and are in no way obstructed.

	Caution **The maximum load for the top of the housing** **of an LS Series Scanner is 3 kg.**

	Caution **Keep fingers away from the flap when it is closing** **or when a scan has been started.**

Place the PC with monitor, keyboard and mouse next to the instrument.

Ensure the computer is switched OFF and the instrument's main power switch on the back panel of the instrument is in the OFF position.

Check the voltage specifications on the computer and ensure that the voltage supplied to the computer is correct according to the specification input range selector (red button) at the power supply.

	Caution **Check voltage specification on the computer BEFORE switching ON. If computer is switched ON with the incorrect voltage supply it will be damaged.**

Connect the computer to the instrument with the delivered RS 232 interface cable.

Connect the computer to the instrument with the delivered special interface cable.

The pictures below show the connection of the interface cables to the rear of the instrument:
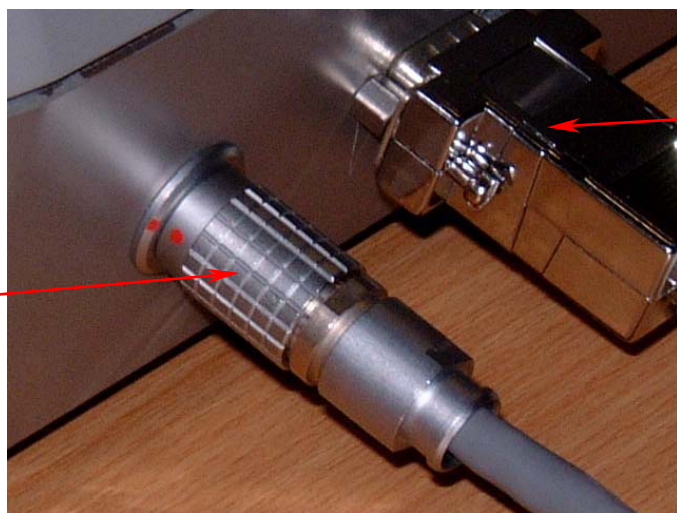
Data cable to connector box

RS232 to computer

The pictures below show the connection of the interface cables to the back of the computer:

RS232 to instrument
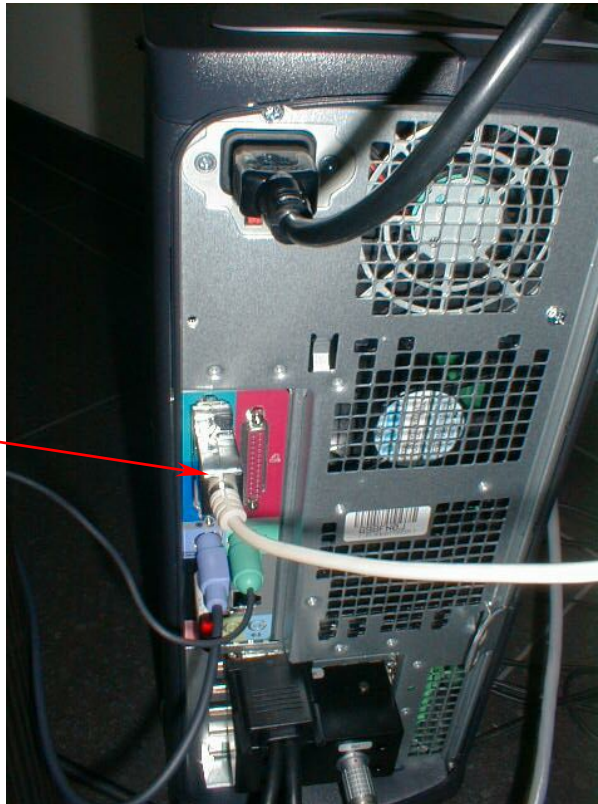
Connection for monitor cables

Connector box

Digital monitor cables
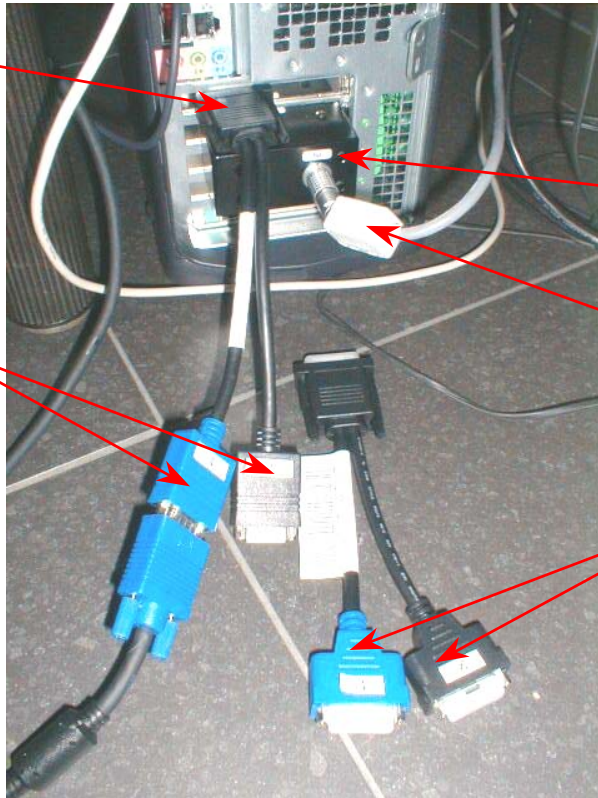
Data cable to instrument

Analog monitor cables

Insert the power cable (with protective ground connection) into the main power socket in the back panel of the instrument.

All connected devices must be approved and listed with regulations EN 60950, UL 1950 or CSA C22.2 No. 950 for Data Processing Devices.

Switch the computer ON.

Switch the instrument ON using the switch on the back panel of the instrument.

STOP	**Caution**
	Keep fingers away from the flap when it is closing or when a scan has been started.

3.2 Remove Transport Lock

Start **LS Scanner Shell**.

Select **Instrument** and click **Connect**.

A message appears that instrument is locked.

For more information on removing the transport lock see chapter 6.4.10 The Transport Unlock Wizard.

STOP	**Caution** **Do not try to open or close** **the instrument flap manually.**

STOP	**Caution** **Save packing materials and transport locks** **for further transportation purposes. The LS series scanners must be** **shipped with the original packing** **and the transport locks must be installed.**

STOP	**Caution** **Before Transportation** **Place the instrument carefully in the indicated orientation before fixing the** **transportation locks, otherwise the instrument will become damaged.**

3.3 Filter Installation

Insert the Filter Slides into Filter Channel 1(vertical orientation) and Filter Channel 2 (horizontal orientation) and make sure that the filters are oriented properly, (see pictures below).

Filter Channel 2   **Filter Channel 1**

Note
Both filter slides are only necessary in dual channel instruments.

- Close the filter compartment

3.4 Power Requirements

The instrument is auto sensing and it is, therefore, unnecessary to make any changes to the voltage range.

Check the voltage specifications on the rear panel of the instrument to ensure that the voltage supplied to the instrument is correct according to the specification.

The voltage range is 100-120 / 220-240 V.

If the voltage is not correct, please contact your distributor.

Ensure that a fuse of the correct type and rating is fitted.

Fuse = T 2.0 A / 250 V (slow blow) UL recognized

> **WARNING**
> **RISK OF FIRE!**
> **REPLACE ONLY WITH FUSES**
> **OF THE SAME TYPE AND RATING.**

> **Caution**
> **When installing or uninstalling the instrument ensure that the instrument and the computer are both switched off and disconnected from the main power supply before the RS 232 interface cable or the special connector cable is connected or removed.**

> **Caution**
> **If the instructions given in this manual are not correctly carried out, the instrument May either become damaged or May no Longer be Able to perform Its Procedures correctly and the Accuracy of the instrument can no longer be guaranteed.**

4. Technical Description

4.1 Optical System
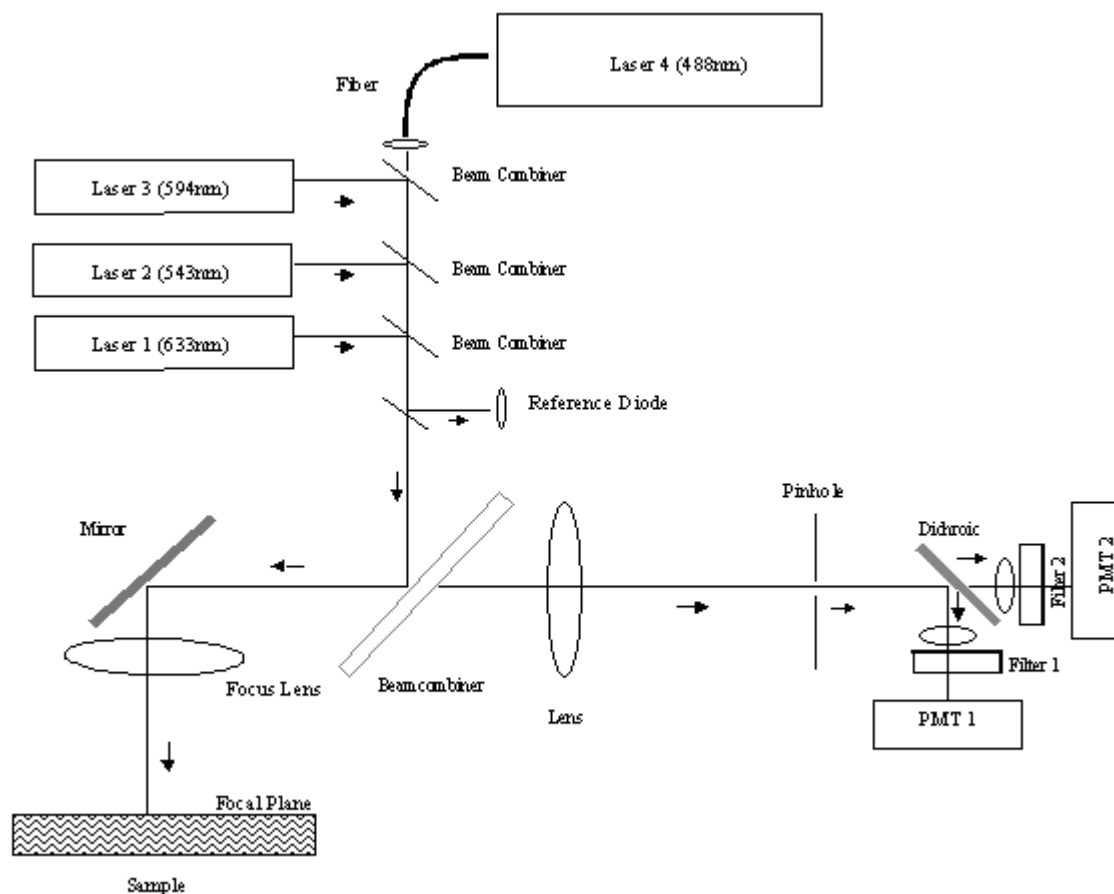
Figure 4.1

For excitation of fluorescence signals up to 4 lasers are installed. The lasers for 633 nm, and 594 nm are Helium Neon lasers; the lasers for 488 nm and 532nm are solid state lasers. Both of these laser types have excellent beam quality and stability.

Laser beams are combined with dichroic elements to enter the scanner in a collinear alignment.

Scanning is done in a first direction with the excitation beam. This very fast scanning mechanism scans 40 lines per second. The overall scanning speed and scanning time in this direction is independent from both pixel resolution and scan width. The second scanning dimension is achieved by moving the sample in a perpendicular direction. This movement is exactly synchronized with the first scan axis to create a 2-dimensional fluorescence image. For example at 10 µm resolution 4 mm are scanned within 10 s ((4 mm / 40 lines / s) * (100 lines / mm (= 10 µm pixel size)) = 10 s). For this second direction the scanning speed is reciprocal to the resolution and the overall scanning time depends linearly on the scan length.

Fluorescence light is collected with a high aperture lens with exceptional working distance (up to 6.5 mm). This long working distance is the key for the ability to measure flexible sample formats other than slides.

A second lens refocuses the collected light onto a pinhole, thus creating a confocal detection scheme.

Three pinholes of different sizes are mounted on an automatic slide carrier. The smaller the pinhole, the lower the focal depth of the detected volume. The larger pinholes typically create images with a slightly lower signal to background ratio but offer the choice to measure bulky samples or uneven surfaces as well.

Behind the pinhole the fluorescence light passes through a bandpass filter that rejects light of the excitation wavelength and all other spectral contributions that are not within the detection bandwidth of the selected filter. Filters are arranged in filter slides that can hold up to four different filters. Each filter slide has five positions, but one of them has to stay open for autofocus purposes.

Filter slides can easily be removed or exchanged; up to 8 different slides can be automatically identified by the instrument firmware when they are inserted in the filter slots.

In the dual channel instrument a dichroic beamsplitter separates the two emission wavelengths of the first and the second dye (e.g. Cy3 and Cy5) and lets them pass trough two different emission filters and to two different photomultiplier tubes (PMT's). The two different filters are located on two different filter slides in that case.

A reference diode monitors laser power before each measurement.
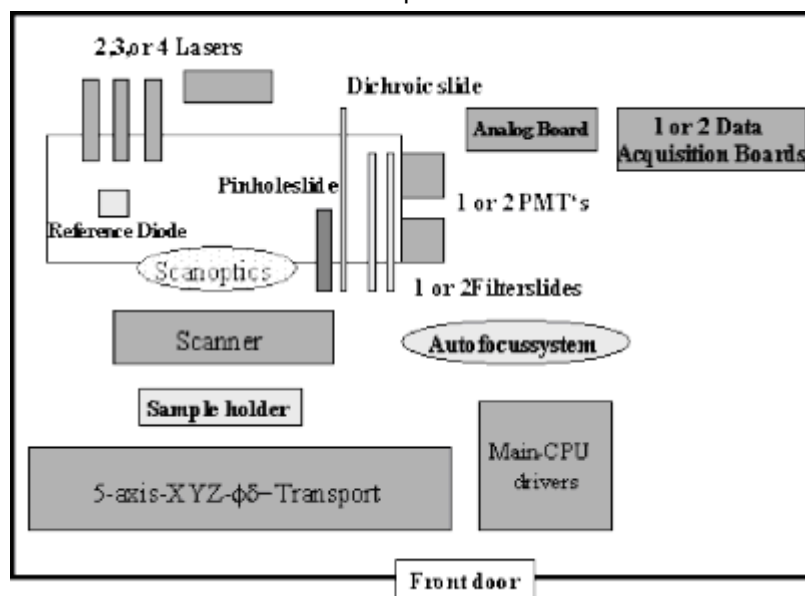
Figure 4.2

4.2 Autofocus

A key element for the flexibility of the instrument is the autofocus system. It allows every sample individually to be adjusted - exactly in the focal plane of the optics. Because this is done immediately before each scan, out of focus images due to long term drifts of the optical alignment are not an issue with the LS SERIES SCANNERS.

Before each scan two z-scans are done across user defined lines in the sample. This way two short 'vertical' images are recorded typically close to both ends of the slide or the sample. (z-axis is moved instead of x-axis). These z-scan images are taken to calculate the exact position and the orientation of the sample. Orientation of the sample is also calculated because slight amounts of tilt could give poor results with a confocal measurement scheme.

The sample is then brought into the exact focal plane both with regard to z as well as the two angles of tilt. This process is fully automated. Signals are recorded in reflection mode and with a dimmed beam (4 orders) to prevent bleaching.

To realize this functionality a high precision 5-axis transport system is implemented in the LS SERIES SCANNERS. The five axis are: X (scan axis), Y (moves sample tray between different slides or different areas on larger samples), Z (brings sample into focal position), ϑ and Φ (correct for angles of tilt of the sample or tolerances of the adapters). Precisions for the axis' are in the range of 1-2µm.

4.3 Data Processing

Further data processing is done by an analog amplifier PCB and fast 16 bit data acquisition boards. The latter ones are plugged into the PC and connected to the instrument with a special interface cable.

An internal Main-CPU takes control of all the actuators and data acquisition steps during scan. It is interfaced with a RS232 cable with the PC.

On the PC the Array Pro software is an integrated package both for instrument control and for array processing.

5. Operation

The LS SERIES SCANNER is operated using personal computer based software control. The Array Pro Software is used both for instrument control and data analysis.

This chapter describes the instrument and the instrument control software. It is intended for a general understanding of instrument parameters and operation. For details of the data analysis see the corresponding Array Pro software manual. Suggestions are made on how to optimize the instrument parameters for your applications.

Every effort has been made to ensure that the instrument will work correctly even if the default parameters are not appropriate for a particular application
- with an important exception:

STOP	**Caution** **When placing a sample into the sample carrier,** **always make sure that the correct slide and slide carrier definitions are selected in the software.** **Wrong definitions, especially with special samples can lead to damage of the instrument or the sample.**

5.1 General Operating Features

The LS Series Scanner has some general behavior and options, which are independent from particularly selected measurement techniques.

5.1.1 Instrument Start Up

Before the instrument is powered ON, ensure that *both* of the interface cables are connected.

	Caution **When the serial interface cable** **or the special interface cable** **is being plugged in or unplugged,** **the instrument and the PC should be powered off.**

5.1.2 Instrument Power On

When switching the instrument ON, the following actions are induced:

- The filter slides move into the reference position
- The dichroic filter carrier moves into the reference position
- The pinhole carrier moves into the reference position
- The microplate transport moves into the reference position
- The cooling fans start to ventilate

At the beginning of each session the user is requested to choose the lasers to be used in this session. Only the selected lasers will be switched on. This is done to increase the lifetime of the lasers.

	Note *The lasers need a warm up time of 20 minutes. The instrument is already functional before that time, but performance according to the specifications is guaranteed only after the warm up time has elapsed. Scans can be done before that time.*

5.1.3 Connect to Instrument

When the software connects to the instrument, communication is established between the instrument and the user interface. The instrument is ready for operation.

5.1.4 Finish a Measurement Session

Disconnect from Instrument

When disconnecting, communication between the instrument and the PC is terminated.

	Note
	Please remove the recently measured sample before disconnect.

Instrument Shut Down

Upon shut down, the instrument activity is stopped immediately. Normally, you should disconnect before shut down. In the rare case of an unexpected hardware error, immediate instrument shut down will reduce the risk of possible damage.

5.2 Insert Slide(s) into Slide Adapter

Insert slide(s) into slide adapter as shown below:

Figure 5.5 a

STOP

Caution
Ensure that the slide is placed tightly on its support And in the right orientation in the adapter. If it is not placed correctly in the adapter the autofocus algorithm might fail to find a focal plane.

Place slide adapter on the plate transport in the same orientation as shown below (Fig. 5.5 b): - moveable spring should be located in the front

Figure 5.5 b

i

Note
The adapter is semi-disposable. The clamps that secure the slide might wear and the slides might become too loose in the adapter. Please call your Tecan sales representative to order new adapters.

6. LSServer, Scanner Shell and Download

6.1 Introduction

6.1.1 Overview

LSServer and **Scanner Shell** are software applications designed to give the user an easy and flexible way to operate **Tecan LS SERIES SCANNER** instruments.

LSServer is called up in **Array Pro 4.5**, a special tool for analyzing microarrays, and **Scanner Shell**.

LSServer Functions:

- Connecting to LS Series Scanner
- Preparing measurement parameters in order to get the best results in scanning
- Scanning in different modes

Scanner Shell Functions:

- Connecting to Tecan LS Series Scanner
- Preparing measurement parameters in order to get the best results in scanning
- Calibrating LS Series Scanner instruments
- Creating special formats for scanning different plates and slides
- Filter Definition
- Stacker Setup

LSDownload is a software tool needed for downloading new firmware versions to LS SERIES SCANNER instruments.

For details on

- data analysis, see the corresponding **Array Pro 4.5** operating manual.
- instrument features and technical description refer to the **Tecan LS Series Scanner** operating manual.

6.1.2 Hardware Requirements

The following hardware requirements are needed for **LSServer** and **Scanner Shell**:

Hardware	Minimum	Recommend
Memory	512 Mbytes	1 Gbyte
CPU	Pentium III 1 GHz	Pentium IV 1.7 GHz
Resolution	1024 x768	1280 x 1024
Serial Ports	2	2

6.1.3 Software Requirements

The following software requirements are needed for **LSServer** and **Scanner Shell**:

Operating system	Minimum	Recommend
Microsoft Windows	Windows 2000	Windows 2000

Additional supported software:

Array Pro 4.5 for microarray image analysis.

> *Note*
> *Microsoft®Excel and Microsoft®Word must be registered by the user!*

6.1.4 Software Installation Procedure

Automatic Software Setup

LSServer / **Scanner Shell** and **LSDownload** are installed by an automatic software setup program:

> *Note*
> *Before installing LSServer components be sure that Array Pro 4.5 Software for Microarray Image Analysis is installed on your system.*

> *Note*
> *It is necessary that the person who installs the software has administrator rights on a PC with a Win 2000 operating system.*

Step	Procedure
1.	Place the LS Driver for Tecan LS V2.2 Setup CD in the CD-ROM drive.
2.	Open the CD drive folder and start **LSServer.exe**. The installation procedure begins.
3.	A **Welcome** window informs the user about the beginning of the installation process. Click **Next** to continue.
4.	A Read Me File window informs the user about the new features of the setup.
5.	The installation of the required files is carried out and files are copied to the computer. At the end of a successful installation the user can quit the installation wizard by clicking the **Finish** button.

After successful installation, installed software components for LSServer can be started via the **Window Start menu** by selecting **Start - Programs - Array-Pro Analyzer 4.5 - Array-Pro Analyzer** and installed software components for Scanner Shell and LSDownload can be started via the **Window Start menu** by selecting **Start - Programs - Tecan - ScannerShell** or **LSDownload**.

Automatic Software Removal

Scanner Shell / LSDownload can be removed using the *Windows* standard uninstall routine:

Step	Procedure
1.	Make sure the **ScannerShell / LSDownload** application is closed.
2.	Select **Add / Remove software** from **Settings** - **Control Panel** in the **Start** menu.
3.	Select the **ScannerShell** / **LSDownload** icon and click **Remove**.
4.	The Uninstall wizard is started. Select the preferred uninstall method (Automatic or Custom) and click **Next** to continue.
5.	In the next window press **Finish** to uninstall **ScannerShell / LSDownload**.
6.	A message box pops up asking if shared components should be removed. Select **Yes** to completely uninstall application. Leaving the components will not harm your system. If you are not sure what to do, it is suggested that the shared components not be removed.

The **ScannerShell** / **LSDownload** is now uninstalled.

> *Note*
> *This chapter does not apply to LSServer application.*

6.2 Tecan LS Scanner Interface

6.2.1 Overview

The **Tecan LS Scanner Interface** is a dialog box in which the options for connecting to instruments such as the **Tecan LS Series Scanner** can be set.

The next chapters describe how to:

- Set Communication port
- Choose Instrument type
- Work in Demo mode

6.2.2 Starting LS Scanner Interface
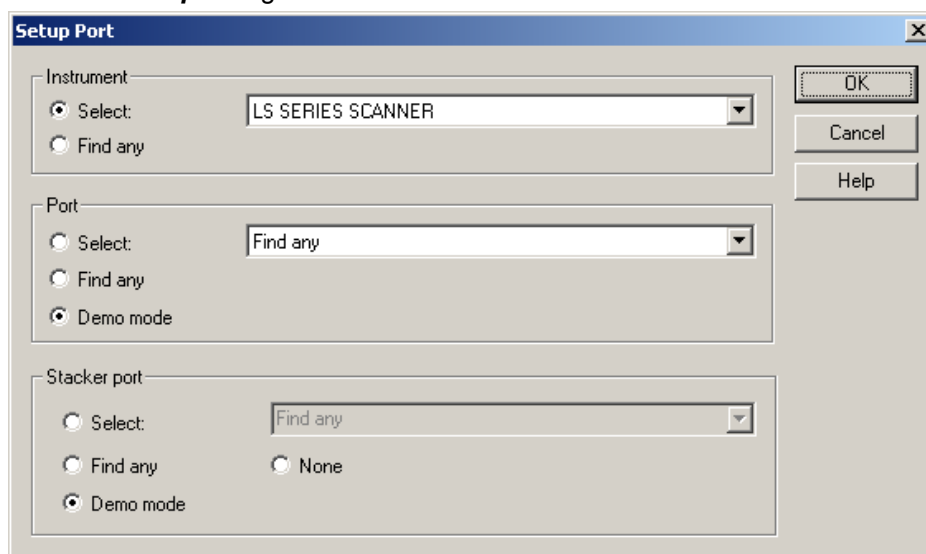
Port Setup

The **Port Setup** dialog box serves to set up options for connecting instruments: The desired **instrument** and the communication **port** are chosen. The **demo mode** provides the possibility to simulate any Tecan instrument.

To open the **Port Setup** dialog box:

- In **Tecan LSA Software, Acquire menu** select **Tecan Scanner...** or
- In **Scanner Shell Software**, **Setup menu** select **Setup Port…** or
- In **LSDownload**, **Instrument menu** select **Connect…**

The **Port Setup** dialog box:

In the **Port Setup** dialog box the **instrument** type and the communication **port** are selected.

Alternatively, select **Find any** and the **LSServer** can automatically search for a connected instrument.

Click **OK** to save changes and close the dialog box.

The **Port Setup** dialog box contains the following elements:

Instrument group box	The **Instrument** group box contains the following elements:

- **Select** option button:

  A specific instrument can be selected (choose in instrument type combo box).

- **Find any** option button:

  The LSServer searches for available instruments automatically.

Port group box	The **Port** group box contains the following elements:

- **Select** option button:

  A list in which the appropriate communication port can be selected is shown.

- **Find any** option button:

  The LSServer searches for any available port.

- **Demo mode** option button:

  Allows the simulation of any instrument, e.g. for test purposes. Refer to chapter 6.4.6 Service Menu - Demo Mode Allowed for enabling the demo mode.

Stacker Port group box	The **Stacker Port** group box contains the following elements:

- **Select** option button:

  A list in which the appropriate communication port can be selected is shown.

- **Find any** option button:

  The LSServer searches for any available port.

- **Demo mode** option button:
  Allows the simulation of an instrument. Refer to chapter 6.4.6 Service Menu - Demo Mode Allowed for enabling the demo mode.

- **None:** option button

  No Stacker available for the instrument.

OK button	Click **OK** to save data and close the dialog box.
Cancel button	Click **Cancel** to close the dialog box. The selected options are not saved.
Help button	Click **Help** to display the online help for the dialog box.

6.3 LSServer

The **LSServer** is an instrument control and data scanning software specially designed for connecting to Tecan instruments such as the **Tecan LS SERIES SCANNER**. This chapter describes the parameters and operations; giving a general understanding in preparing and scanning data and in getting the best results for attached data analysis.

For details of data analysis see the corresponding **Tecan LSA Software** manual.

6.3.1 Starting the First Time

When starting LSServer for the first time, the **Tecan LS Scanner Interface** dialog box is opened. After selecting instrument type and communication port and confirming with **OK** the **Measurement Control** dialog box is displayed.

For all future starts of the **LSServer**:

In **Tecan LSA Software, Acquire Menu** select **Tecan Scanner...** and the **Measurement Control** dialog box is displayed.

6.3.2 Measurement Control

In the **Measurement control** dialog box, several groups, each containing individual buttons, are shown that offer special operations for preparing and scanning data. Measurement parameters and measurement options are defined and finally measurements are started here.

The **Measurement Control** dialog box is divided into individual groups:

- Measurement group
- Instrument group
- Measurement Parameter group
- Settings group
- Slides to Process group
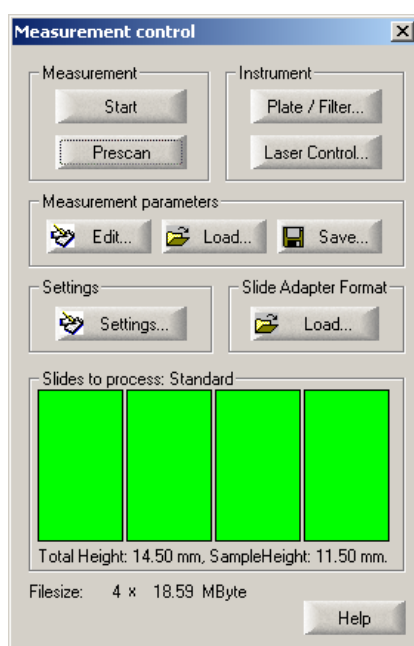
The *Measurement Control* dialog box consists of the following elements:

- The Measurement group element with the Start and Prescan button
- The Instrument group element to move plate and filters and to switch on lasers
- The Measurement Parameter group element to **Edit** parameters or to **Save** and **Load** certain predefined parameter files
- The Settings group element with the **Settings** button to enter the filename and to select the scan areas
- The Slide Adapter Format group element to load existing Slide Adapter Format (.saf) files
- The Slides to Process group element to display arrangement of the scan areas
- File size of chosen slides

Measurement

The **Measurement** group contains the following elements:

Start button	Click the **Start** button to start a measurement with the current **measurement parameter**. The **Status** dialog box is displayed.
Prescan button	Click the **Prescan** button to select three different prescan modes: o **Image prescan** o **Adapter prescan** o **Focus prescan**

Instrument

The **Instrument** group contains the following elements:

Plate / Filter button	Click the **Plate / Filter** button to open the **Movements** dialog box: Plate and filter can be moved.
Laser Control button	Click the **Laser Control** button to open the **Laser Control** dialog box: Laser type can be selected and power control can be set / changed.

Measurement Parameter

The **Measurement parameter** group contains the following elements:

Edit button	Click the **Edit** button to open the **Measurement Parameter** dialog box: Measurement parameters can be edited.
Load button	Click the **Load** button to open the **Open** dialog box: Measurement parameters can be loaded from a file.
Save button	Click the **Save** button to open the **Save As** dialog box: Measurement parameters can be saved to a file.

Settings

The **Settings** group contains the following elements:

Settings button   Click the **Settings** button to open the **Slide Settings** dialog box: Filename can be entered and scan areas can be selected.

Slide Adapter Format

The **Slide Adapter Format** group contains the following elements:

Load button   Click the **Load** button to open the **Open Slide Adapter Format** dialog box: SAF can be selected.

Slides to Process

The **Slides to process** group gives a general view of the **slide** / **plate** format used and the arrangement of available **scan areas**. Scan areas that will be scanned after pressing **Start** / **Prescan** button are **green**, those that will not be scanned are dark gray. Select / unselect slides with left mouse click. Also the calculated file size will be displayed right under the scan area window.

6.3.3 *File Types used in LSServer*

The following file types are used with **LSServer**:

File Type	File Extension	Description
Image	.tif	Scanned image files are saved in a 16bit TIF format (Tagged Image File)
Measurement Parameter	.lsp	Measurement data is saved as .lsp files.
Slide adapter format	.saf	Slide adapter format data is saved as .saf files.
Gain calibration	.dat .cvs	Gain calibration data is saved as GainCalibration.dat files. Gain calibration data is stored as a comma separated list (.cvs files). For analysis these files can be loaded in Excel or another calculation program.
Crosstalk calibration	.dat	Crosstalk data is saved as CrosstalkCor.dat files.
Focus	.tif	Focus image files are saved as • Focus1.tif for first autofocus scan • Focus2.tif for second autofocus scan • Focus3.tif for third autofocus scan (only created during MTP-autofocus)

Log	.log
	.log1 -.log5
		Log file data is saved in the following file format:
		
		• LSServer.log:
		
		  A record of current measurement data.
		
		• LSServer.log1 - LSServer.log5:
		
		  A record of earlier measurement data.

> ***Note***
> ***Each time the LSServer is started, the LSServer.log file is overwritten;***
> ***old data is saved in LSServer.logn file.***

Locating Files

LSServer Files

By default the files associated with **LSServer** are stored as subdirectories in the directory where LSServer.dll is found:

C:\Program Files\Common Files\Tecan\Reader\….

The subdirectories are displayed in the table below:

Type of File	File Extension	Directory
Measurement parameter	.lsp	Parameter
Slide adapter format	.saf	SAF
Gain calibration	.dat .csv	CalData
Crosstalk	.dat	CalData

Temporary Files

Temporary files needed for diagnostic and controlling purposes are stored in the directory:

C :\Documents and Settings*user_logged_in*\Local Settings\

The file types are displayed below:

Type of File	File Extension	Directory
Focus	.tif	Temp
Log	.log .log1 to .logn	Temp

File Management

The default paths for saving files can be changed in the ***Slide Settings*** dialog box on the ***Folder Options*** tab. See Folder Options for more information.

6.3.4　Measurement

Start

In the ***Measurement Control*** dialog box, Click **Start…**to start a measurement using the current measurement parameters. The *Status* dialog box appears and displays the current process of the scanner as static text.
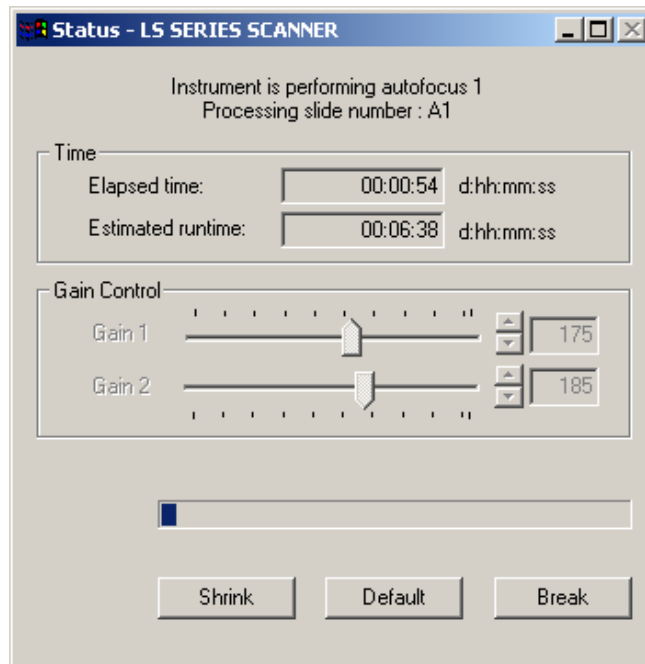
In the **Time** group box, the Elapsed time of the measurement and the Estimated run time are shown.

In the **Gain control** group box, the gain of the PMT can be adjusted online during the measurement.

The display of the *Status* dialog box can be changed using the **Shrink** or the **Default** view button.

Click **Break** to interrupt the measurement.

The *Status* dialog box contains the following elements:

Instrument is…　　This ***static text*** gives information about the current process, e.g.:

Instrument is performing autofocus

Instrument is measuring dark

Instrument is measuring image

Time group box	• **Elapsed time** static text box • **Estimated run time** static text box
Gain control group box	• **Gain 1** slider / spin control: Use the slider or spin control to online adjust the gain in Channel 1. • **Gain 2** slider / spin control Use the slider or spin control to online adjust the gain in Channel 2. (only active during dual parallel measurements).
Please wait… progress bar	**Please wait…** under the static text a progress bar indicates the progress of the ongoing process.
Shrink button	Click **Shrink** to minimize the display of the Status dialog box.
Default button	Click **Default** to maximize the display of the Status dialog box.
Break button	Click **Break** to abort the current measurement.

Prescan

Image Prescan

In the *Measurement Control* dialog box, click **Prescan** and select **Image Prescan** to start an overview measurement for the first selected scan area.

The *Image Prescan* serves to position the autofocus scan lines and to select the scan area for the final measurement. The image prescan procedure scans the complete Slide Area as defined in the selected .saf file. It is performed using the following default measurement parameters:

• Single Scan mode

• 40 µm scan resolution

• Large pinhole

• Optimize integration time selected

• No autofocus

• Scan Settings from Channel 1 only

On clicking the **Image Prescan** option the *Status* dialog box appears and an overview measurement is started.
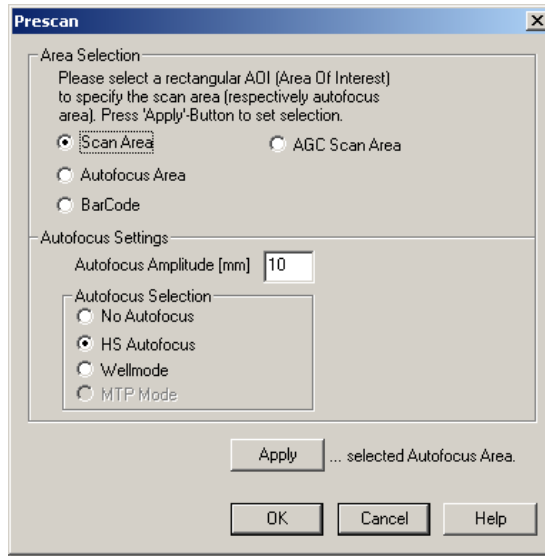

The *Status* dialog box contains the following elements:

Instrument is… static text	The static text gives information about the actual process, e.g.:
	Instrument is performing prescan
	Instrument is measuring dark
Time group box	• **Elapsed time** static text box
	• **Estimated run time** static text box
Gain control group box	• **Gain 1** slider / spin control
	Use the slider or spin control to online adjust the gain in Channel 1
	• **Gain 2** slider / spin control
	Channel 2 is disabled in Prescan mode.
Please wait… progress bar	**Please wait…** under the static text a progress bar indicates the progress of the ongoing process.
Shrink button	Click the **Shrink** button to minimize the display of the Status dialog box.
Default button	Click the **Default** button to maximize the display of the Status dialog box.
Break button	Click the **Break** button to abort the current measurement.

Image Prescan Dialog Box

When the image prescan of the first selected area is complete, the *Image Prescan* dialog box appears with the Area Selection group box and the image appears with an AOI (Area of Interest). The user is asked to set the Scan Area, the Autofocus Area and the AGC Scan Area. In Tecan LS with integrated barcode reader, it is also possible to set the Barcode Area. Click **Apply** to confirm the newly defined areas. Click **OK** to save the settings and close the dialog box.

The *Image Prescan* dialog box contains the following elements:

Area Selection group box

Scan Area option button

Select the **Scan Area** option button to adjust the boundaries of the rectangular AOI. To adjust a boundary, place the cursor along the edge or corner of the AOI and drag it when the 2-way cursor appears. To move the AOI, place the cursor in the middle of the rectangle and drag it when the 4-way cursor appears. Confirm the AOI position by clicking the **Apply** button.
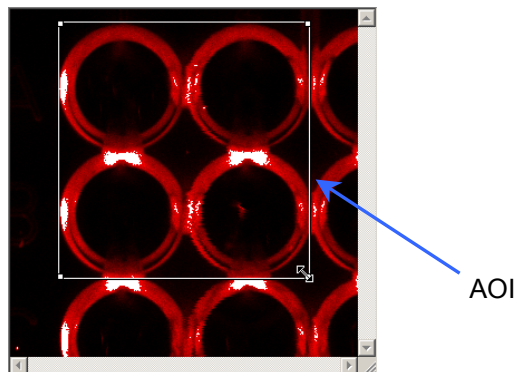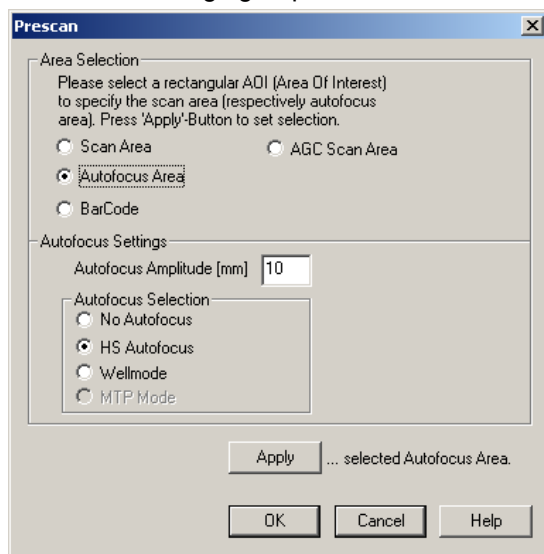

figure 6-1
Minimum scan area: 1mm x1mm.

Autofocus Area option button

Select the **Autofocus Area** option button to enable the Autofocus Settings group box.

Set the Autofocus lines by adjusting the boundaries of the rectangular AOI. To adjust a boundary, place the cursor along the edge or corner of the AOI and drag it when the 2-way cursor appears. To move the AOI, place the cursor in the middle of the rectangle and drag it when the 4-way cursor appears. Click **Apply** to confirm the newly defined areas. If HS or Wellmode Autofocus has been chosen, a green check mark appears and the Autofocus area is centered automatically to the scan area.

Legend:
barcode line
autofocus lines
scan area
AGC area
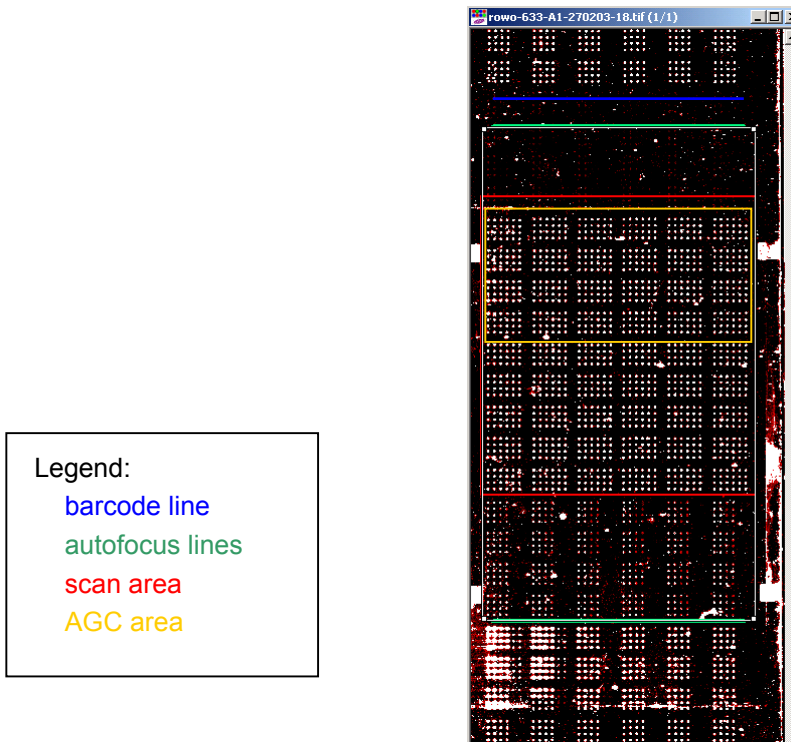
figure 6-2

Minimum distance between Autofocus lines: 3mm x 3mm.

Barcode Area option button

Select the **Barcode Area** option button to enable barcode area selection.
The barcode is always read centered to the slide width and must be set within the useable barcode area.

The **Barcode Area** button is enabled only if a barcode top reader is available to the Tecan LS Series Scanner and *Read barcode* has been selected from the *Measurement Parameter* dialog box.
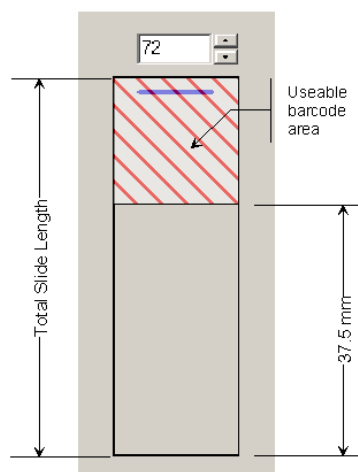

Figure 6-3

The total slide area must be at least 37.5 x 15.0 mm, otherwise there is no room for a barcode. The barcode area is from 37.5 mm to the total slide length.

If your instrument is equipped with a barcode bottom reader, the barcode area is fixed. The barcode label has to be placed at the upper end of the slide. The barcode must cover at least the area about 5mm away from the upper end.

AGC Scan Area option button

Select the **AGC Scan Area** option button to enable the AGC area selection. If the AGC feature is activated the selected area will be scanned and that image will be consulted for the automatic gain calculation.
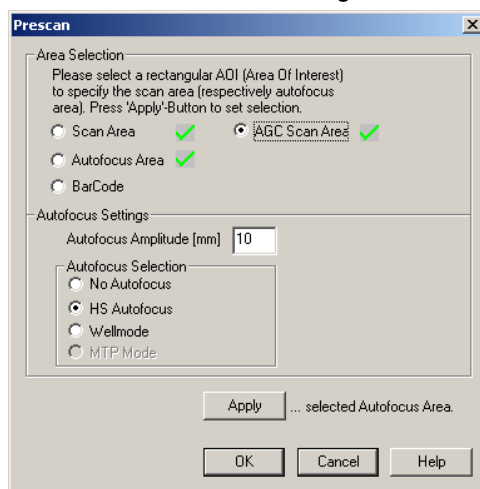

Set the AGC Scan Area lines by adjusting the boundaries of the rectangular AOI. To adjust a boundary, place the cursor along the edge or corner of the AOI and drag it when the 2-way cursor appears. To move the AOI, place the cursor in the middle of the rectangle and drag it when the 4-way cursor appears. Click **Apply**

to confirm the newly defined areas, (see figure 6-2, on page 6-15 for a picture of the AGC area). AGC is only available in:

- **Single Scan Mode**

- **Dual Sequential Scan Mode**

It is not possible in Dual Parallel Scan Mode because the Cross Talk phenomenon with special dye combinations (e.g. Cy3 and Cy5), where signal from one channel will be detected in the other channel and can therefore lead to incorrectly calculated gain values. To start a scan with the AGC feature first of all a special area for AGC must be selected. This can be done from either the *Geometry Parameters* tab of the *Measurement Parameter* dialog box or during a prescan from the *Prescan* dialog box:
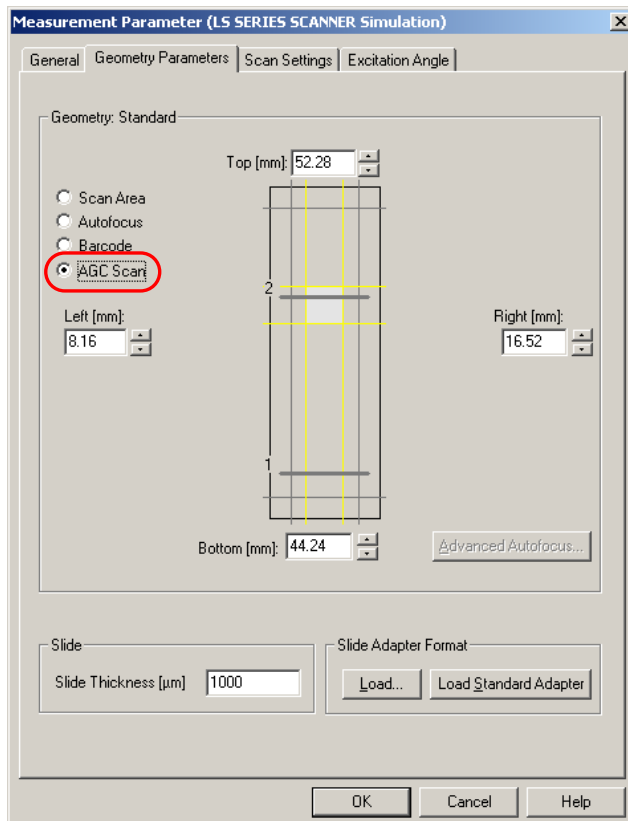

Figure 6-4 *Geometry Parameters* tab
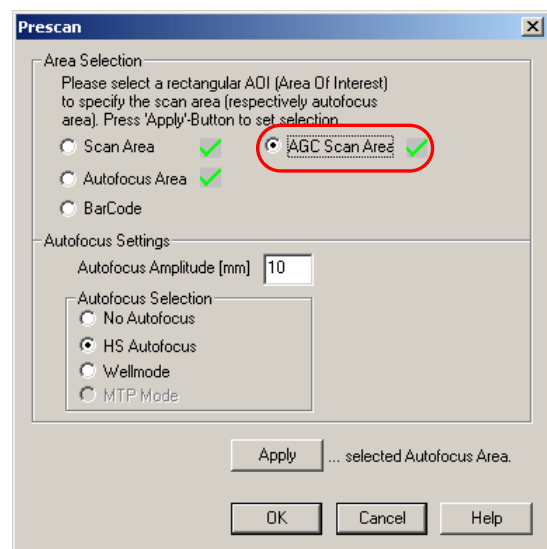
Figure 6-5 Prescan dialog box

(For more information, see AGC (Automatic Gain Control) on page 6-37).

When a scan with AGC is started, the instrument performs a scan of the selected AGC-area. After this scan, all pixels of that image will be calculated and will be virtually drawn in a histogram, where the amount of pixels against the signal values will be plotted.

On the *Scan Settings* tab of the *Measurement Parameter* dialog box, the values for calculating the AGC can be set: **Noise level** and **Signal level**.
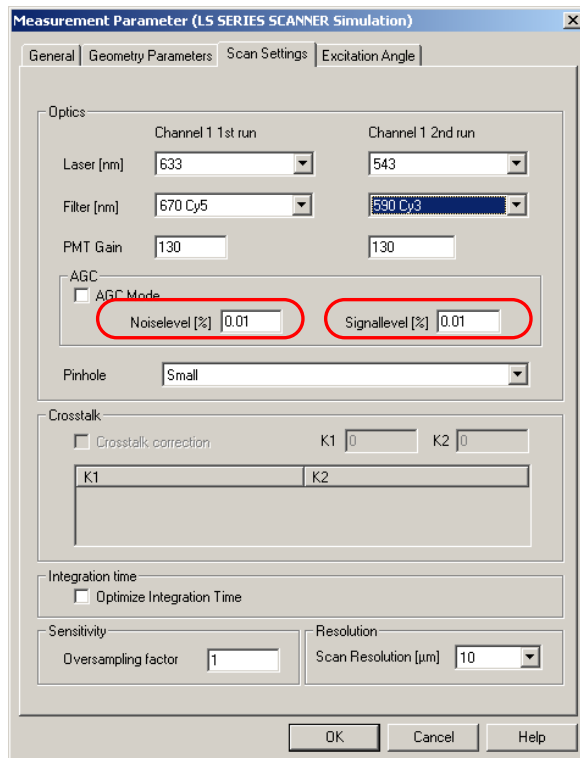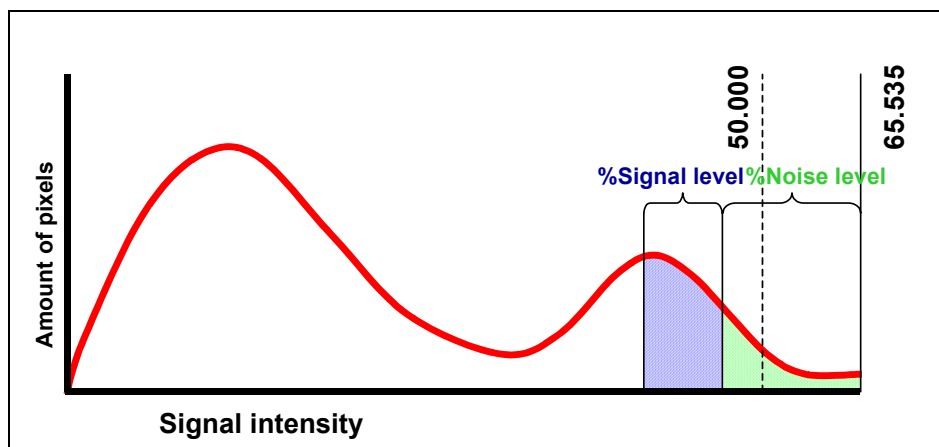

Figure 6-6 Noise level and signal level

For more information, see Scan Settings Tab on page 6-38.

Noise level: = amount of pixels in the percentage, which will be discarded **Range: 0,01% - 1%**

Signal level: = mean value of all pixels in the selected signal level range will be set to **50.000 Range: 0,01% - 1%**


Figure 6-7 Histogram of AGC image

Noise level are pixels, which have a very high signal value and derive from dust or other particles, which gave a high fluorescent signal. These pixels will be excluded for the calculation. For the right gain settings only all pixels in the signal level area will be consulted. Please keep in mind that only percentage of all pixels can be selected and not different signal values.

A good starting point to find the right settings for the AGC will be 0,1% Noise level and 0,1% Signal level. To increase the overall signal, the noise level and/or signal level must be extended.
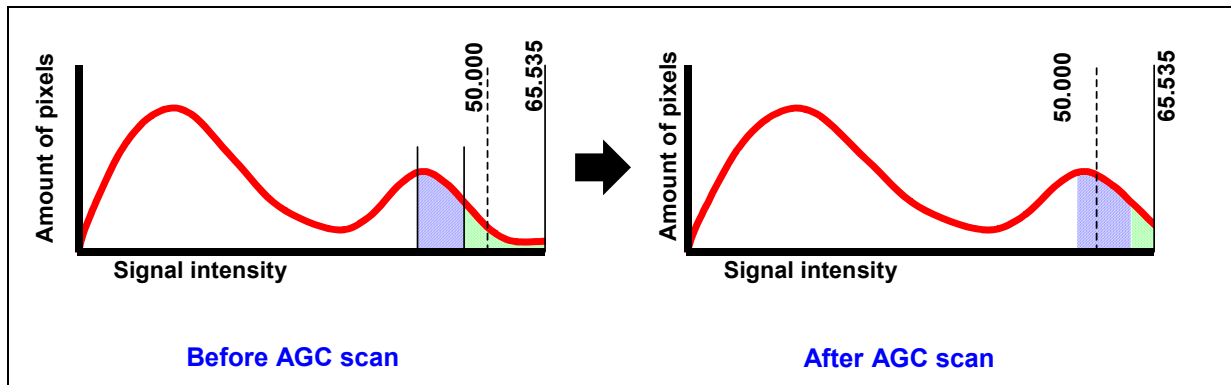
Figure 6-8 AGC Scan Functionality; Gain setting will be adjusted to the appropriate value. The result is an automatic increase of the gain value!

Autofocus Settings *group box*

Autofocus Amplitude [mm]

Enter the amplitude in mm into the **Autofocus Amplitude** text box.

Select the appropriate autofocus from the **Autofocus Selection** group box:

- No Autofocus

- HS Autofocus

- Wellmode

- MTP mode
  Select MTP in the Measurement Parameter dialog box to enable the MTP option button.

(For further information see chapter Advanced Autofocus).

Image Prescan Buttons

|	
Apply button	Click **Apply** to confirm the defined areas of the AOI.
OK button	Click **OK** to save data and close the dialog box.
Cancel button	Click **Cancel** to close the dialog box. The selected options are not saved.
Help button	Click **Help** to display the online help for the dialog box.

Adapter Prescan

In the *Measurement Control* dialog box, click **Prescan** and select **Adapter Prescan** to scan the currently used MTP adapter.

The *Adapter Prescan* serves to define the current slide adapter format (.saf) file. The adapter prescan procedure scans the complete traverse path of the Tecan LS. It is performed using the following default measurement parameters:

- Single Scan mode
- 40 µm scan resolution
- Large pinhole
- Optimize integration time selected
- No autofocus
- Scan Settings from Channel 1 only

On the displayed traverse path it is possible to define the slide areas.

Enter the correct adapter height and sample height.
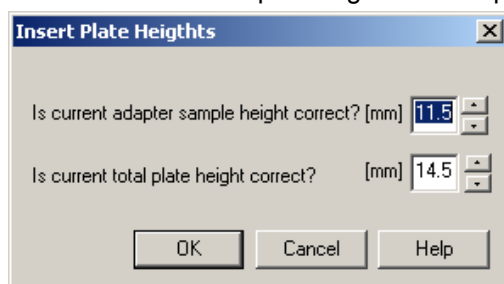
The chosen sample height of this dialog is taken as default height for the following adapter scan. The total height is only taken into account to avoid damaging the optics.

Maximum Values for total height = 16.0 mm
Maximum Value for sample height = 16.0 mm
Minimum Value for sample height = total height – 5.00 mm

Differences greater than 5.00 mm must be aborted, because of possible damage to the optics. The software produces a warning message for differences in the interval of 5.0 mm.

	Caution **If the heights inserted in this dialog are not correctly entered, the instrument may either become damaged or may no longer be able to perform Its procedures correctly and the accuracy of the instrument can no longer be guaranteed.**

After clicking **OK** on the *Insert Plate Heights* dialog box the instrument starts the prescan:
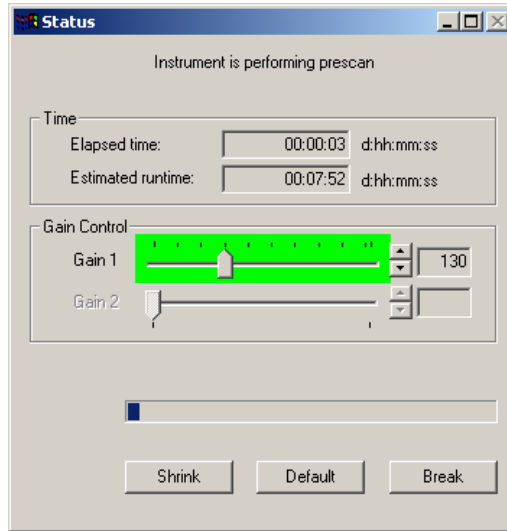
After the adapter prescan has finished, the scanned image is displayed. The currently active .saf file is displayed as an overlay on the adapter prescan image. The red rectangles represents the available scan areas. The big green rectangle represents the outer dimension of the active area of the adapter.

After the instrument has performed the prescan, the first page of the adapter format wizard appears.


Table   **MTP adapter**
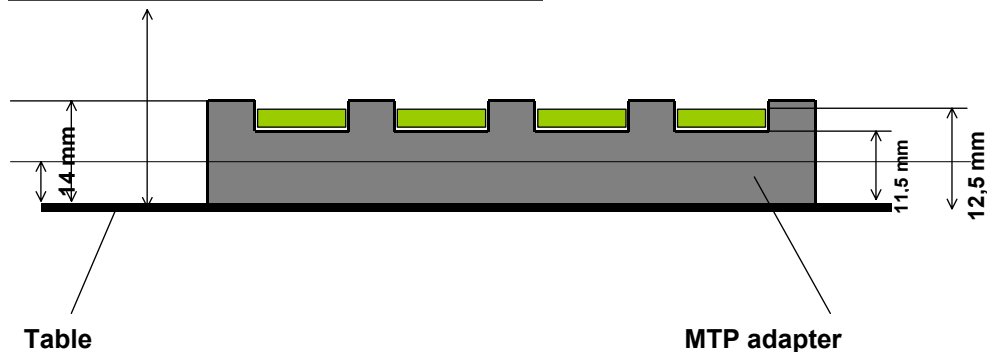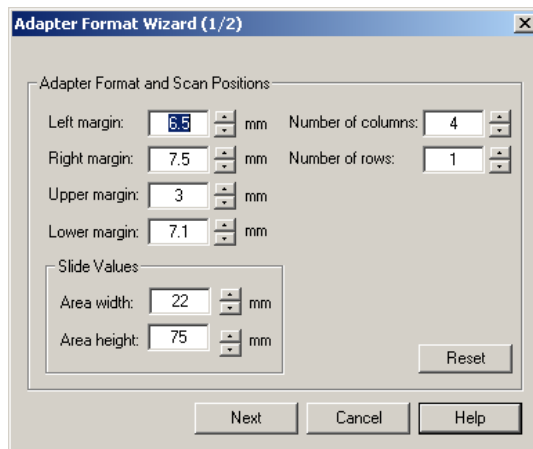
Adapter Prescan = Non confocal scan of the complete transport-table-area.

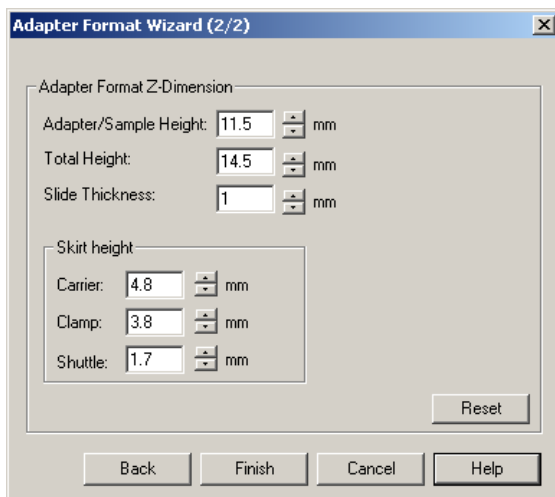The first page of the Adapter Format Wizard has the following buttons:

Reset button	Click **Reset** to return all the values to the default settings.
Next button	Click **Next** to save data temporarily and perform the second part of the wizard.
Cancel button	Click **Cancel** to close the dialog box. The selected options are not saved.
Help button	Click **Help** to display the online help for the dialog box.

After all the settings have been correctly entered click **Next** to continue to the second page of the wizard.
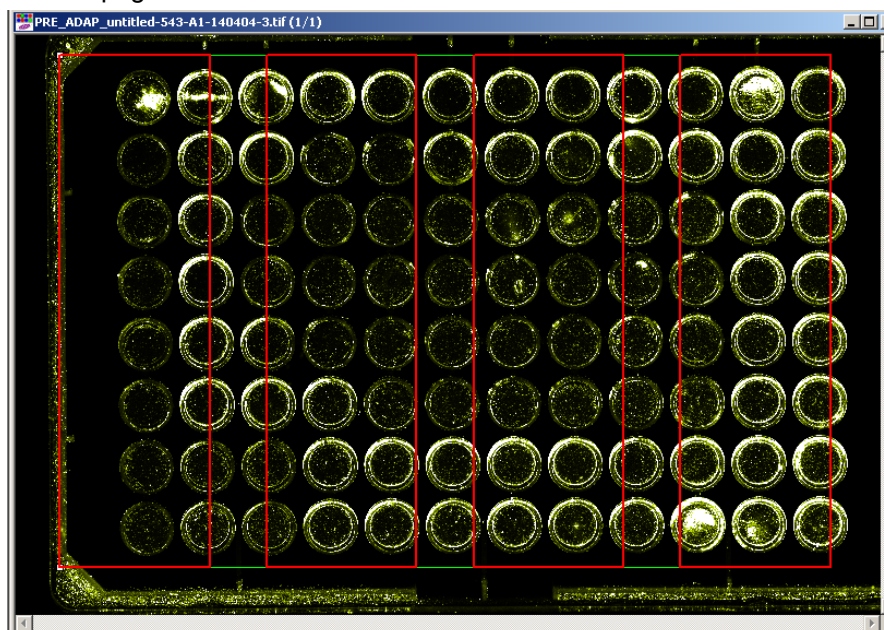

Example of current SAF parameters
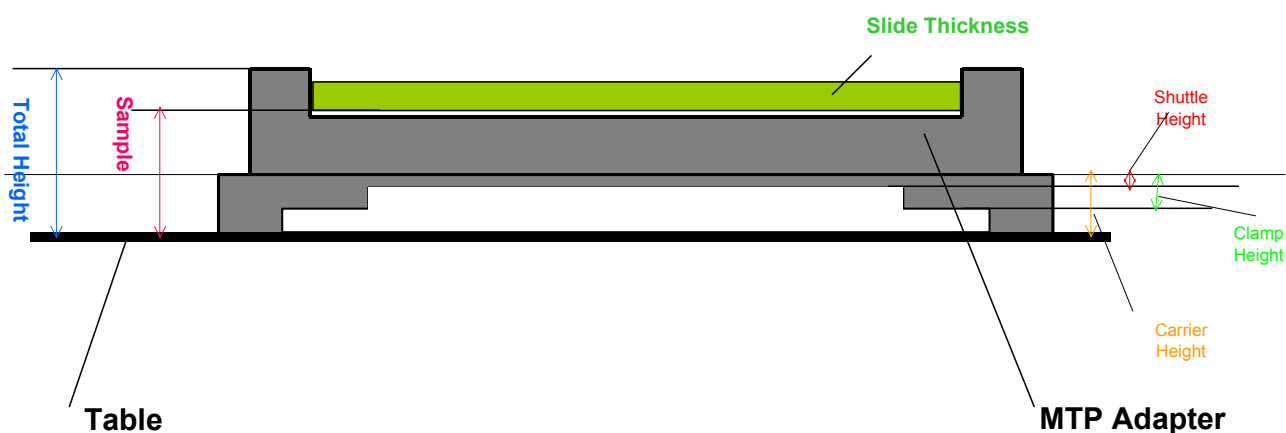
The second page of the Adapter Format Wizard has the following buttons:

Reset button	Click **Reset** to return all the values to the default settings.
Back button	Click **Back** to save data temporally and perform the first part of the wizard.
Finish button	Click **Finish** to save data and close the wizard.
Cancel button	Click **Cancel** to close the dialog box. The selected options are not saved.
Help button	Click **Help** to display the online help for the dialog box.

After all the settings have been correctly entered click **Finish** to save the settings. The *Save* dialog box will then appear.
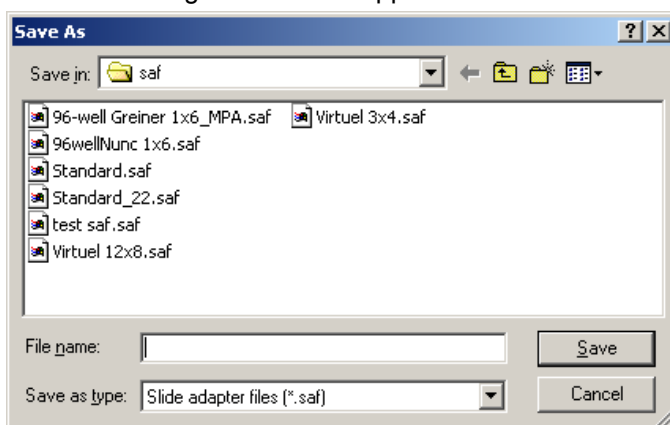

To save the Slide Adapter Format file, type in the desired filename of the newly created .saf file and click **Save**.

Focus Prescan

In the *Measurement Control* dialog box, click **Prescan** and select **Focus Prescan** to perform a focus scan of the first selected scan area of the currently used MTP adapter.

The focus prescan enables the user to define the desired focal plane and the focus offset on a z-scan image. This can also be used for probes, where the auto focus fails. The focus offset can be adjusted directly on the image, so refraction considerations are not necessary.

If no focus was previously selected, the focus prescan will be aborted with following error message: 'No focus mode selected. Please select focus mode in Advanced Autofocus dialog!'
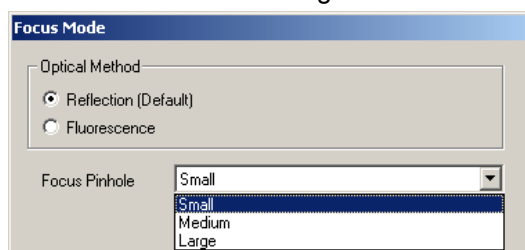
Fluorescence focus scans will be performed with the laser / filter setting of channel 1. This focus scan mode can be relevant for gel applications. The default settings are reflection modus with the red (633nm) laser.

Click **OK,** and *Focus Prescan* dialog box appears:
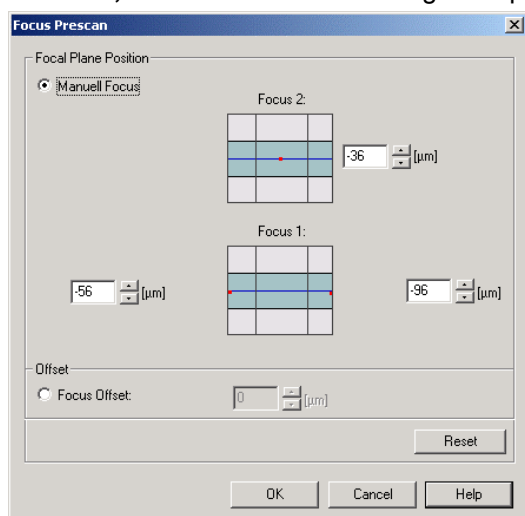
Example of HS and well mode auto focus.

The *Focus Prescan* dialog box has the following buttons:

Reset button	Click **Reset** to return all values to the default settings.
OK button	Click **OK** to save the settings. The focus values are stored for the currently scanned slide. A message appears, which asks if the focus offset should only be stored for this slide or for all slides of the adapter.
Cancel button	Click **Cancel** to close the dialog box. The selected options are not saved.
Help button	Click **Help** to display the online help for the dialog box.

Depending on the selected focus mode, two (HS and well mode) or three (MTP mode) focus scans will be performed.

The focus images will be displayed together with an overlay of lines, representing the Baseline (blue), Identified/scanning plane (red) and Focus Offset (green).

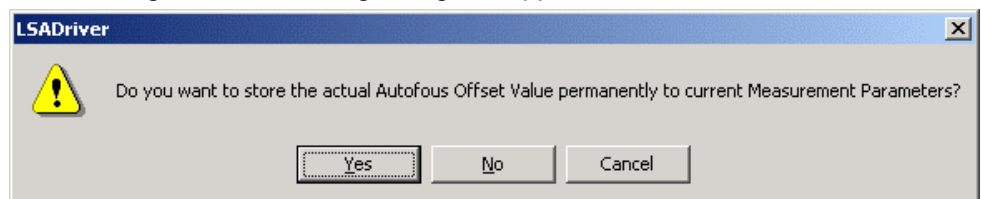*Manual Focus* radio button: Changes in the three combo boxes changes the plane that will be scanned for the next image scan. This plane will be displayed as red line in the focus images.

Focus Offset radio button: The green line represents the focus offset.
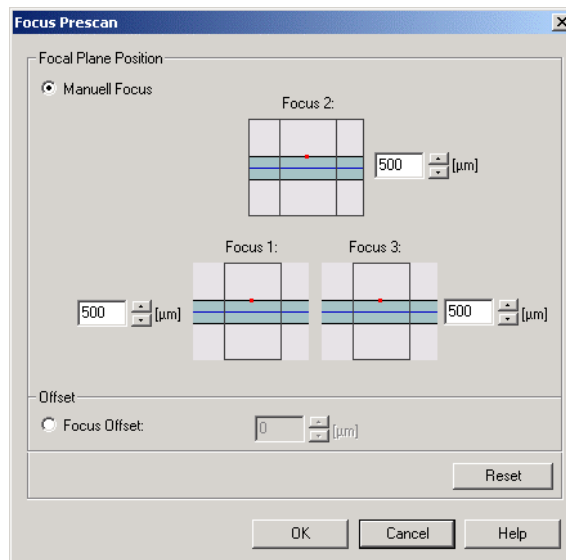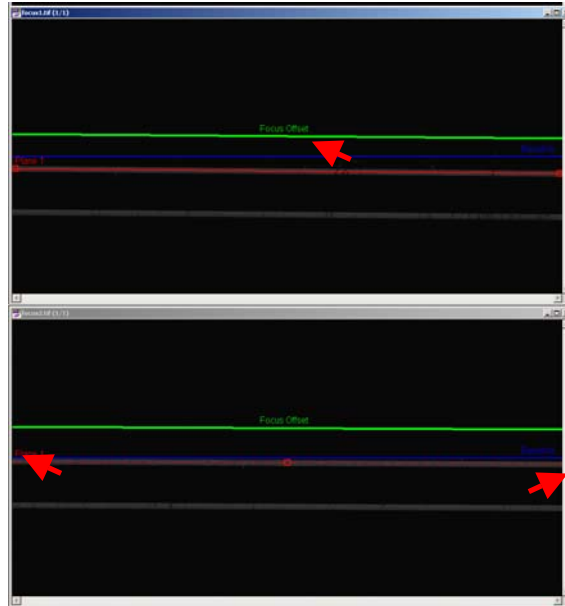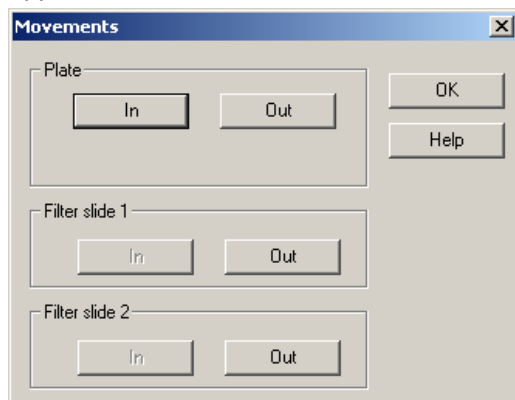

Example of focus prescan for MTP autofocus

After clicking **OK**, the following dialog box appears:

6.3.5 Instrument

Plate/ Filter

In the **Measurement Control** dialog box, click **Plate/ Filter…**to move the plate carrier and the filter slides of certain instrument. The **Movements** dialog box will appear.

In the **Movements** dialog box, the respective **In** and **Out** buttons can be used to move the plate carrier into or out of the LS Series Scanner. The respective **Out** buttons for the filter slides can be used to move the filter slides out of the LS Series Scanner.

Click **OK** to close the dialog box.

The **Movements** dialog box contains the following elements:

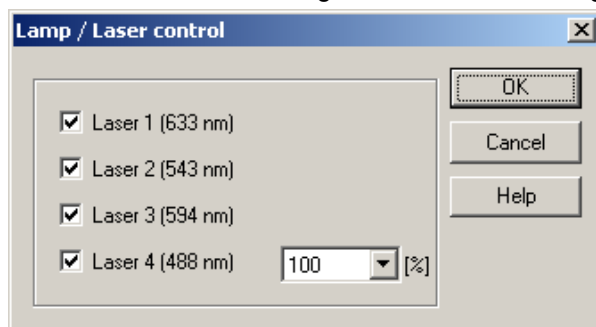|	
Plate group box	The **Plate** group box consists of the following elements: • **Plate In** button: Click the **Plate In** button to close the door and to move in the plate transport. • **Plate Out** button Click the **Plate Out** button to open the door and to move out the plate transport.
Filter Slide 1 group box	The **Filter Slide 1** group box consists of the following elements: • **In** button The **In** button is disabled. Insert the filter slide (making sure that it is properly oriented), until a slight resistance is felt, the LS Scanner will automatically move the filter slide in from this point. • **Out** button: Click the **Out** button to move out Filter Slide 1.
Filter Slide 2 group box	The **Filter Slide 2** group box consists of the following elements: • **In** button The **In** button is disabled. See the description for Filter slide 1. • **Out** button Click the **Out** button to move out Filter Slide 2.
OK button	Click **OK** to close the dialog box.
Help button	Click the **Help** button to display the online help for the dialog box.

Laser Control

In the **Measurement Control** dialog box, select **Laser Control…** and the **Lamp / Laser Control** dialog box appears.

All lasers installed in your instrument are shown in the dialog box:

Click **OK** to save the changes and close the dialog box.

Lamp / Laser control	
☑ Laser 1 (633 nm)	OK
☑ Laser 2 (543 nm)	Cancel
☑ Laser 3 (594 nm)	Help
☑ Laser 4 (488 nm) 100 ▼ [%]	

In the **Laser Control** dialog box specific lasers (1-4) can be activated / deactivated and laser power can be set (Laser 4).

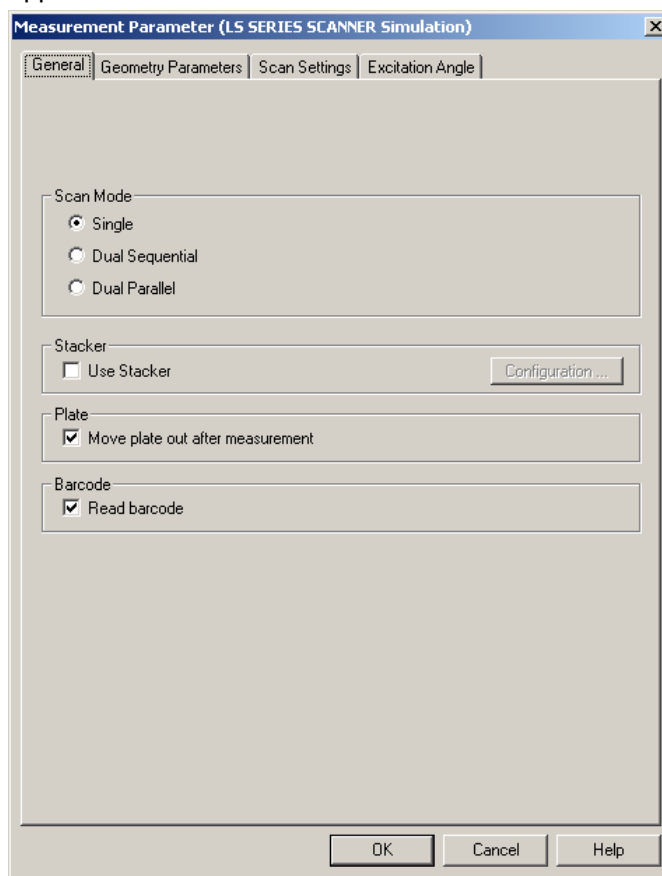The *Laser Control* dialog box contains the following elements:

Laser 1 (633 nm) check box	Select **Laser 1** check box to activate the 633 nm laser. Red LED is switched on at the rear of the instrument.
Laser 2 (532 nm) check box	Select **Laser 2** check box to activate the 532 nm laser. Green LED is switched on at the rear of the instrument.
Laser 3 check box	Select **Laser 3** check box to activate the 594 nm laser and the orange LED is switched on at the rear of the instrument.
Laser 4 check box	Select **Laser 4** check box to activate the 488 nm laser and the blue LED is switched on at the rear of the instrument. (The blue laser can be power controlled).
OK button	Click **OK** to save the selected options and close the dialog box.
Cancel button	Click the **Cancel** button to close the dialog box. The selected options are not saved.
Help button	Click the **Help** button to display the online help for the dialog box.

6.3.6   Measurement Parameter

Edit…

In the **Measurement Control** dialog box, click **Edit…** to edit general options like Scan Mode, Stacker and/or Barcode as well as define Geometry Parameters, Scan Settings and Autofocus Settings. The *Measurement Parameter* dialog box appears:

The *Measurement Parameter* dialog box contains the following elements:

- General Tab
- Geometry Parameter Tab
- Scan Settings Tab

Depending on the installed hardware features, a fourth element can also appear:

- Excitation Angle

(See 6.3.7 Excitation Angle).

OK	Click **OK** to save the selected options and close the dialog box.
Cancel	Click **Cancel** to close the dialog box without saving the options.
Help	Click **Help** to display the online help associated with the dialog box.

General Tab

In the **General tab** the type of **Scan mode** can be selected, as well as the **Barcode** reader and the **Stacker** options for automation purposes. The user can also select the option of having the plate move out of the instrument after scanning.

The **General** tab:
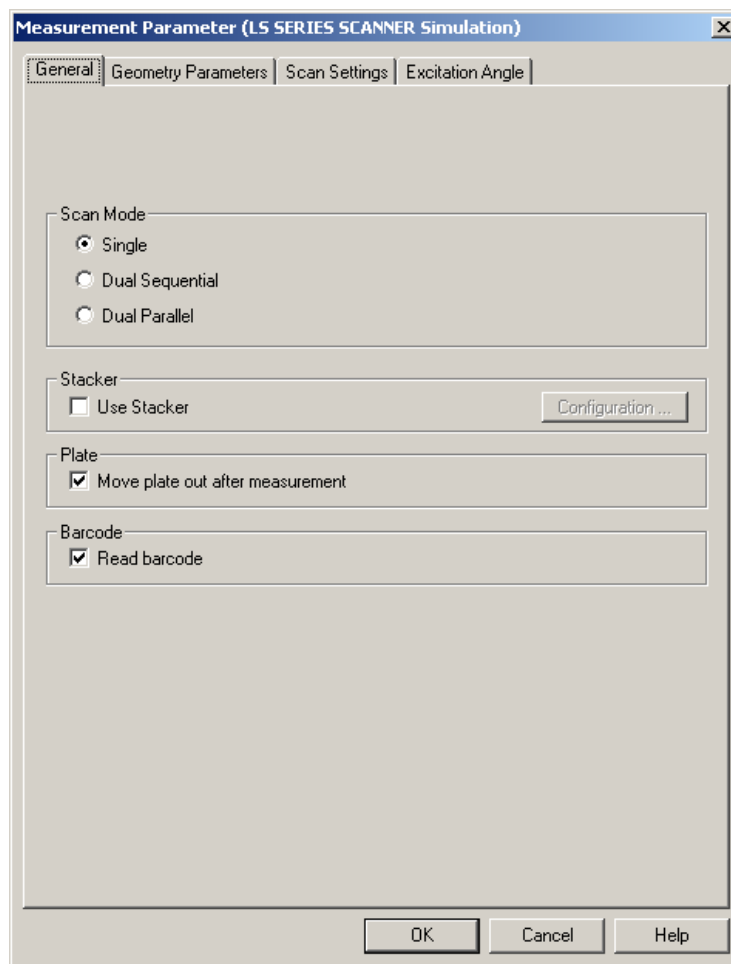
The **General** tab contains the following elements:

- **Scan Mode** group box
- **Stacker** group box
- **Plate** group box
- **Barcode** group box

Scan Mode

Scan Mode
group box

In the **Scan Mode** group box, several scan modes can be selected.

- **Single** option button:

Selects the Single Scan Mode. One excitation (= laser) and one emission (= filter) wavelength can be selected. The detection will be performed in channel 1. This is appropriate for single label experiments.

- **Dual Sequential** option button:

Selects the Dual Sequential Scan Mode. Two excitation and emission wavelengths can be selected. This is appropriate for dual label experiments in single channel instruments. The second label is measured immediately after the first and the two emissions are detected sequentially in channel 1.

- **Dual Parallel** option button:

Selects Dual Parallel Scan Mode. Two excitation and emission wavelengths can be selected and are measured simultaneously. This requires dual channel instruments as detection is performed in both channels at once and scanning is done in half the time. The Dual Parallel Scan Mode needs a Crosstalk correction (see Scan Settings tab) and a valid set of K-factors in order to compensate for spectral crosstalk.

For further information, refer to the chapter 6.4.6 Service Menu - Crosstalk… description in this manual.

Stacker

Stacker
group box

- **Use Stacker** check box:

The **Use Stacker** check box will be available, if a stacker is connected to the instrument and the instrument is turned on.

Selecting **Use Stacker** operates the instrument in an automatic mode. Autosave will be enabled automatically.

For further information about **Stacker** and **Stacker Setup** refer to the chapter 6.4.6 Service Menu -

Stacker Setup… description in this manual.

See also Tecan Connect Configuration.

Plate

Plate
group box

- **Move plate out after measurement** check box:

Selecting *Move plate out after measurement* opens the door and moves out the plate transport after the measurement is finished.

Barcode

**Barcode group box**

- **Read barcode** check box:

    Read barcode will be available, if the instrument is equipped with a barcode reader.

    By selecting **Read barcode** the barcode is read prior to each measurement at the user specified reading position. The barcode appears automatically as part of the filename (see Slide Settings).

Geometry Parameter Tab

In the **Geometry Parameters** tab the Scan area, the location of the Autofocus scan lines as well as the Barcode line are determined within a certain Slide Adapter Format (saf). Different *.safs can be loaded using the **Load…** button. The **Slide Thickness** is entered respectively.

The **Geometry Parameter** tab:
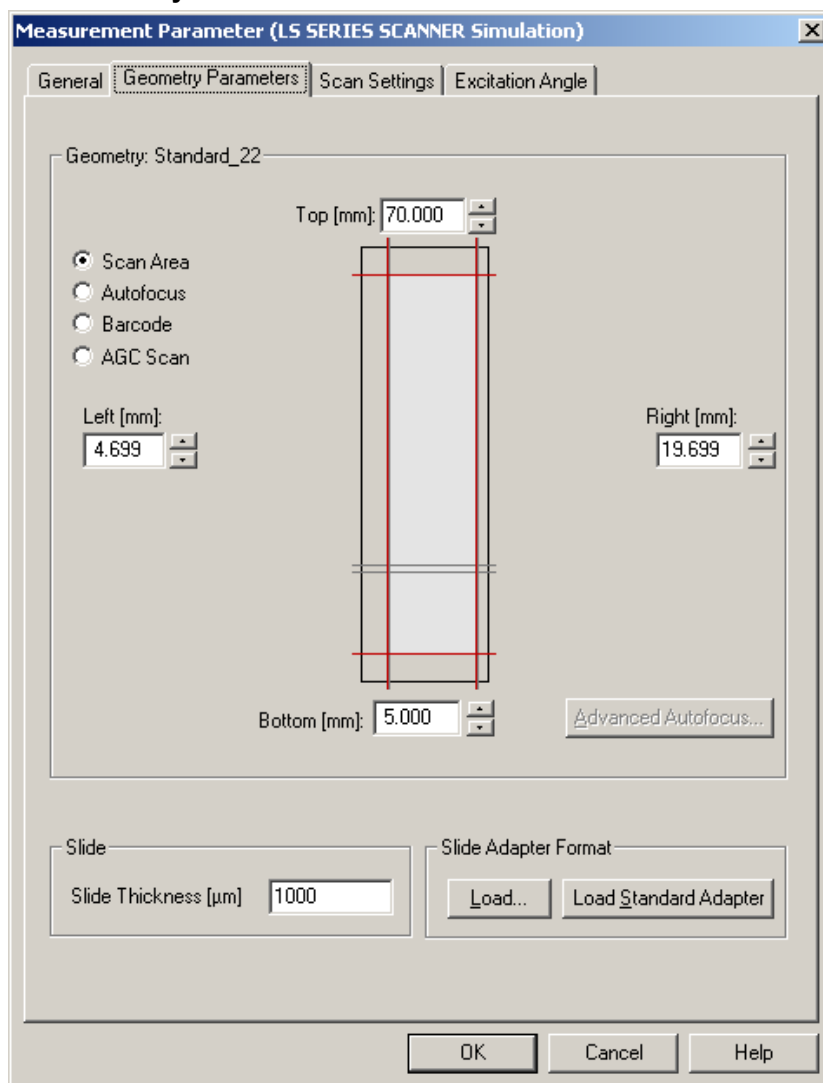
The **Geometry Parameter** tab window contains the following elements:

- **Geometry** group
- **Slide** group
- **Slide Adapter Format** group

Geometry

Geometry
group box

The **Geometry** group box contains the following elements:

- The **static text**, which appears after "*Geometry:*," displays the name of the currently loaded *.saf file.

- **Scan area** option button:

Select the **Scan area** option button to set the appropriate scan area either by dragging the four red borderlines by mouse or by typing in the distances, measured from the lower left corner.
Units are given in mm.

Minimum Scan area: 1mm x 1mm

See Scan Area on page 6-34 and Scan Area option button on page 6-14, for more information.

- **Autofocus** option button:

Select the **Autofocus** option button to specify the position of autofocus scan lines.

The Autofocus is a scan in vertical direction (= z-scan) measuring the reflection light of the 633 nm laser in order to bring the sample exactly into the right focal plane. For this purpose z-scans are performed along the autofocus scan lines that can be positioned either by dragging green lines by mouse or by typing in the distances (units in mm), measured from the lower margin.

The width of the autofocus scan lines is given by the **Amplitude** (unit in mm).

For more information, see Autofocus on page 6-35.

Select **Autofocus** to make the **Advanced Autofocus…** button available and to change autofocus parameters or to select a different autofocus and/or autofocus mode. For more information, see Advanced Autofocus.

Minimum Autofocus: 3mm x 3mm

- **Barcode** option button:

Select the **Barcode** option button to specify the reading position of the barcode by dragging the blue line by mouse or by typing in the distance in mm from the lower margin.

The total slide area must be at least 37.5 x 15.0 mm, otherwise there is no room for a barcode. The barcode area is from 37.5 mm to the total slide length.

This option is not available if your instrument is equipped with a barcode bottom reader. The reading area is fixed in that case.

See Barcode on page 6-36 and Barcode Area option button on page 6-16, for more information.

- **AGC** option button

Select the **AGC Scan Area** option button to start automatic gain control scan area selection.

AGC is only available in:

- **Single Scan Mode**
- **Dual Sequential Scan Mode**

It is not possible in Dual Parallel Scan Mode because the Cross Talk phenomenon with special dye combinations (e.g. Cy3 and Cy5), where signal from one channel will be detected in the other channel and can therefore lead to incorrectly calculated gain values.

See AGC (Automatic Gain Control) on page 6-37 and AGC Scan Area option button on page 6-16, for more information.

Slide

Slide
group box

The **Slide** group box contains the following elements:

- **Slide Thickness** box:

In the **Slide Thickness** box the thickness of a sample (i.e. a slide) must be entered in order to allow the instrument to search for the focal plane within an appropriate range.

If the Slide Thickness is entered incorrectly, the algorithm might be unable to find the right surface.

The Slide Thickness ranges from 0 to 2000 µm and must be adjusted respectively to the Adapter height defined in *.saf files (see Advanced Menu - Edit SAF… on page 6-85):

1) If the Adapter height is measured from the surface of the plate transport to the surface of the spots, the Slide Thickness must be set to 0.

2) If the Standard Tecan Slide Adapter is used (with the default height of 11.5 mm), the thickness of the slide that is inserted into the adapter must be edited as well.

Slide Adapter Format

Slide Adapter Format
group box

The **Slide Adapter Format** group box contains the following elements:

- **Load** button

Click **Load** and the ***Open Slide Adapter Format*** dialog box appears. Select a Slide Adapter Format (*.saf) file from the drop-down list and it will be loaded automatically.

- **Load Standard Adapter** button

Click the **Load Standard Adapter** button to load the Standard.saf file and to reset default Geometry Parameters.
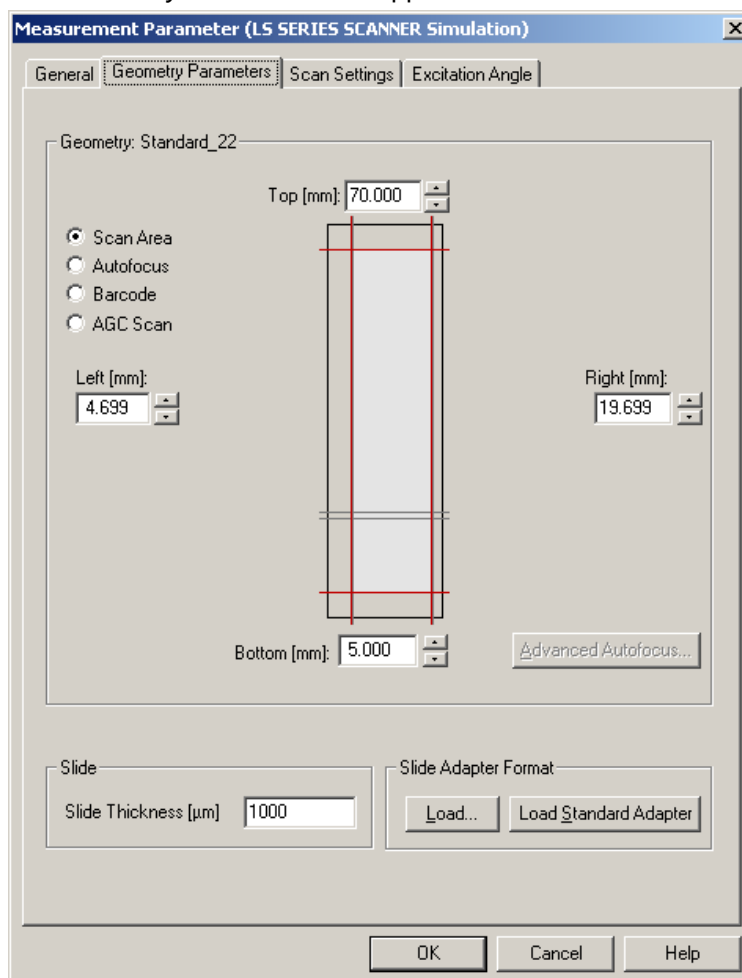
Scan Area

The **Geometry Parameter** tab appears differently depending on which option button has been selected :

Select the **Scan Area** option button to define the scan area that is displayed as a light gray area within the red borderlines.

Minimum Scan area: 1mm x 1mm anywhere on the slide.

The *Geometry Parameters* tab appears as follows:
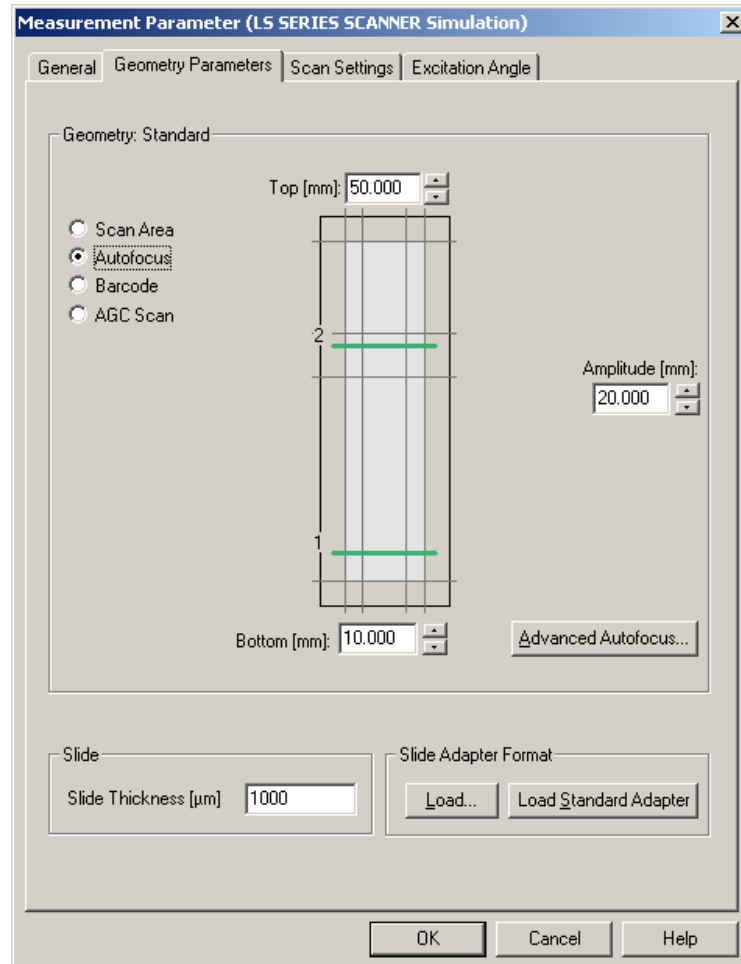

The **Scan Area** contains the following elements:

Top spin control	Use the **Top** spin control or type in a value to set the upper border of the scan area. The value is measured in mm from the lower left corner (0/0).
Left spin control	Use the **Left** spin control or type in a value to set the left border of the scan area. The value is measured in mm from the lower left corner (0/0).
Right spin control	Use the **Right** spin control or type in a value to set the right border of the scan area. The value is measured in mm from the lower left corner (0/0).
Bottom spin control	Use the **Bottom** spin control or type in a value to set the bottom border of the scan area. The value is measured in mm from the lower left corner (0/0).

Autofocus

Select the **Autofocus** option button to define the autofocus position and to make the **Advanced Autofocus…** button available.
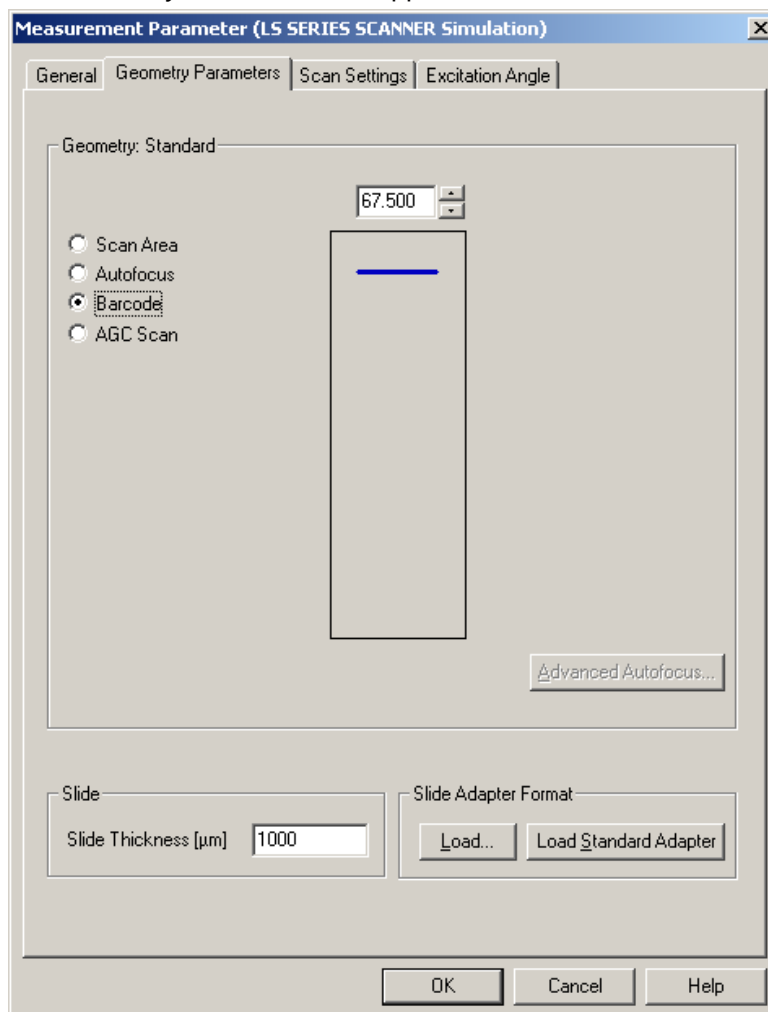
The *Geometry Parameters* tab appears as follows:

The **Autofocus** contains the following elements:

Top spin control	Use the **Top** spin control or type in a value to set the autofocus scan line for Focus 2. The value is measured in mm from the bottom line (0/0).
Bottom spin control	Use the **Bottom** spin control or type in a value to set the autofocus scan line for Focus 1. The value is measured in mm from the bottom line (0/0).
Amplitude spin control	Use the **Amplitude** spin control or type in a value to set the width of autofocus scan line for both, Focus 1 and 2.
Advanced Autofocus button	Click **Advanced Autofocus** to select a different autofocus mode or to change autofocus parameter.
	For detailed information please refer to the Advanced Autofocus description in this manual.
MTP Autofocus	Autofocus entered in Well or HS-Mode.
	Amplitude (width of Autofocus line) from 3mm to 22mm
	Distance (between Autofocus lines): from 3mm to slide length

Barcode

Select the **Barcode** option button to define the barcode reading position.

The *Geometry Parameters* tab appears as follows:

The **Barcode** window contains the following elements:

Top spin control	Use the **Top** control or type in a value to set the barcode reading position (blue line).
	The barcode position is centered on the slide and must be at least 37.5 mm from the bottom line.
	The barcode reading position can not be adjusted when using a barcode bottom reader.

> ***Note***
> ***Barcode must be defined in the upper half of the slide***
> ***due to limited traveling distances in the y-direction.***

> ***Note***
> ***If your instrument is equipped with a barcode bottom reader, the***
> ***barcode area is fixed. The barcode label has to be placed at the upper***
> ***end of the slide. The barcode must cover at least the area about 5mm***
> ***away from the upper end.***

AGC (Automatic Gain Control)

Select the **AGC Scan Area** option button to start automatic gain control scan area selection.

The *Geometry Parameters* tab appears as follows:
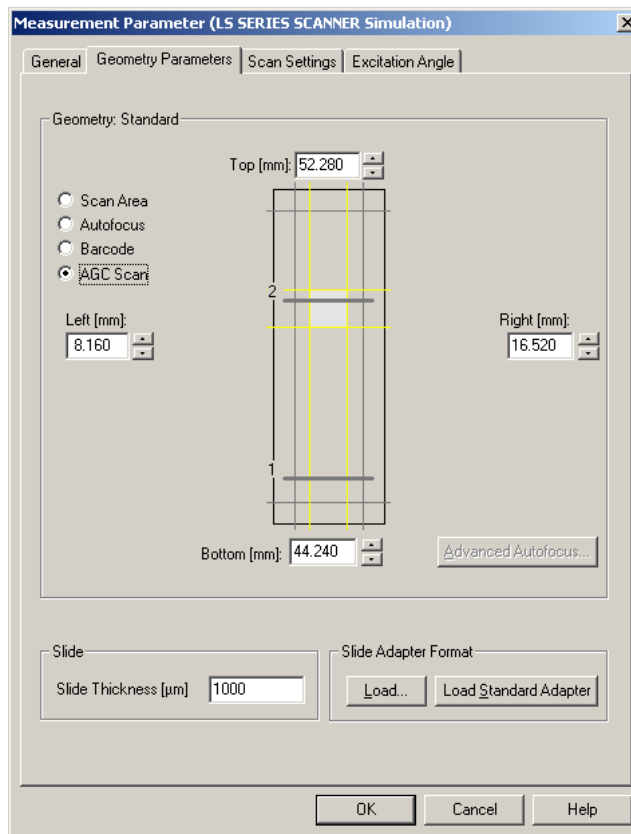
The AGC window contains the following elements:

Top spin control	Use the **Top** spin control or type in a value to set the upper border of the scan area. The value is measured in mm from the lower left corner (0/0).
Left spin control	Use the **Left** spin control or type in a value to set the left border of the scan area. The value is measured in mm from the lower left corner (0/0).
Right spin control	Use the **Right** spin control or type in a value to set the right border of the scan area. The value is measured in mm from the lower left corner (0/0).
Bottom spin control	Use the **Bottom** spin control or type in a value to set the bottom border of the scan area. The value is measured in mm from the lower left corner (0/0).

AGC is only available in:

- **Single Scan Mode**
- **Dual Sequential Scan Mode**

It is not possible in Dual Parallel Scan Mode because the Cross Talk phenomenon with special dye combinations (e.g. Cy3 and Cy5), where signal from one channel will be detected in the other channel and can therefore lead to incorrectly calculated gain values.
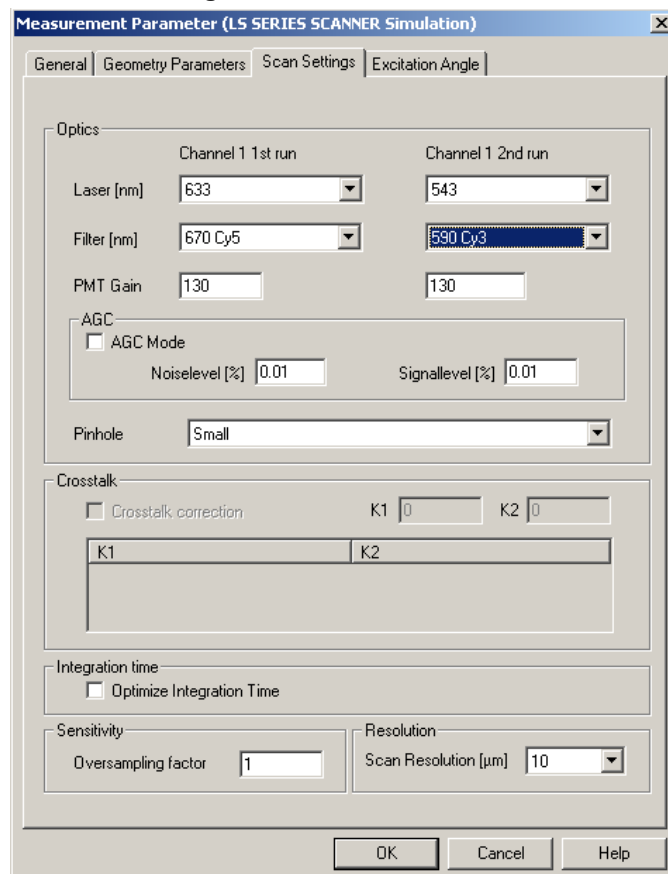
See AGC Scan Area option button on page 6-16, for more information.

Scan Settings Tab

In the **Scan Settings tab** the excitation and emission wavelengths can be selected. The gain for the PMT is set respectively in one or two channels. Pinhole, Crosstalk correction, Integration time, Sensitivity and Scan Resolution can be defined.

Click **OK** to save the changes and close the dialog box.

The **Scan Settings** tab window:

The **Scan Settings** tab window contains the following group boxes:

- Optics group box
- AGC group box
- Crosstalk group box
- Integration time group box
- Sensitivity group box
- Resolution group box

Optics

Optics group box

The appearance of the *Optics* group box is dependent on whether dual scan, single scan or dual scan sequential is selected. In dual scan sequential **Channel 1 1st Run** and **Channel 1 2nd Run** will appear instead of *Laser Channel 1* and *Laser Channel 2*.

Laser Channel 1 combo box

Select the appropriate laser from the **Laser Channel 1** combo box for excitation.

The selection of lasers is dependent on the type of instrument connected.

Laser Channel 2 combo box

Select the appropriate laser from the **Laser Channel 2** combo box for excitation.

The selection of lasers is dependent on the type of instrument connected.

Laser Channel 2 is only activated, if **Dual Parallel Scan Mode** has been selected in the **General tab**.

Filter Channel 1 combo box

Select the appropriate filter from the **Filter Channel 1** combo box to match with the selected excitation wavelength in Laser Channel 1.

All filters that have been defined in the filter slide which is inserted into Filter Channel 1, can be selected from the Filter Channel 1 combo box.

Filter Channel 2 combo box

Select the appropriate filter from the **Filter Channel 2** combo box to match with the selected excitation wavelength in Laser Channel 2.

All filters that have been defined in the filter slide which is inserted into Filter Channel 2, can be selected from the Filter Channel 2 combo box.

Filter Channel 2 is only activated, if **Dual Parallel Scan Mode** has been selected in the **General tab**.

PMT Gain Channel 1 edit box

Set **PMT Gain** to a value between 70 and 255 to regulate the detector sensitivity in Channel 1.

The PMT Gain can also be adjusted online during the measurement via the **Status control** dialog box window. The *Status* dialog box is displayed after clicking the **Start** button.

PMT Gain Channel 2 edit box

Set **PMT Gain** to a value between 70 and 255 to regulate the detector sensitivity in Channel 2.

The PMT Gain can also be adjusted online during the measurement via the **Status control** dialog box window. The *Status* dialog box is displayed after clicking the **Start** button.

PMT Gain Channel 2 ("*Channel 1 2nd Run*" when *Dual Scan Sequential* is selected) is only activated, if the instrument is a **Dual Channel Instrument** and if **Dual Parallel Scan Mode** has been selected in the **General tab**.

Unusable filter laser excitations are signaled by red exclamation marks.

- **Pinhole** combo box

Select the appropriate pinhole for measurement from the **Pinhole** combo box.

Tecan LS Series Scanners have three different pinholes (**Small**, **Medium** and **Large**) that can be changed using the software. The size of the pinhole determines the grade of **confocality** Select between **confocal** and **non-confocal** measurements:

➢ Small = confocal mode (focal depth: 70 µm)

➢ Medium = less confocal (focal depth: 300 µm)

➢ Large = non confocal (focal depth: 800 µm)

The small pinhole gives high background rejection and better signal/background ratio. If samples are bulky or have a large volume, larger pinholes and less confocality give better reproducibility and higher signals.

AGC

- **AGC Mode checkbox**

  Only available in:

  Single Scan Mode

  Dual Sequential Scan Mode

  Not available in Dual Parallel Scan Mode due to Cross-Talk problems.

- **Noise Level %** = amount of pixels in the percentage, which will be discarded; Range: 0,01% - 1%

- **Signal Level %** = Mean value of all pixels in the selected signal level range will be set to 50.000
  Range: 0,01% - 1%

See AGC Scan Area option button on page 6-16, for more information.

Crosstalk

Crosstalk group box	The **Crosstalk** group box contains the following elements:

- The **Crosstalk correction** check box

Select **Crosstalk correction** to select a correction factor (= K-factor) from the Crosstalk list box.

Dual channel instruments, operated in Dual Parallel Scan Mode, are corrected for the spectral crosstalk from the first dye into the second detection channel by applying K-factors.

The **Crosstalk correction** check box is automatically available, if a Gain Calibration file and a Crosstalk file are found for the current laser and filter combination (selected in the Laser Channel 1 / Channel 2 and Filter Channel 1 / Channel 2 combo box).

Otherwise Crosstalk elements are not enabled and no crosstalk correction factor can be set.

The defined K-factors for the current laser / filter combination are displayed in the Crosstalk list box. Clicking on a Crosstalk list item sets the K-factor (values appearing in **K1 / K2** static text box), which will be used when starting the next measurement, if the *Crosstalk correction* check box has been selected. If the *Crosstalk correction* check box has not been selected no correction is done.

- The **K1** static text box displays the current selected crosstalk factor K1 (residual signal contribution from the first dye detected in the second channel will be corrected).

- The **K2** static text box displays the current selected crosstalk factor K2 (residual signal contribution from the second dye detected in the first channel will be corrected).

- In the **Crosstalk** list box all crosstalk factor records, corresponding to selected laser / filter combination, are displayed:

Format **K1 = Dye 1**, **Name**, **Date**

Format **K2 = Dye 2**, **Name** , **Date**

For further information refer to the chapter 6.4.6 Service Menu - Crosstalk…in this manual.

![Information icon]

> ***Note***
> ***In order to obtain reliable results it is strongly recommended to place reference spots with one dye only onto your sample. These spots should represent every dye of your experiment and should be used for QC purposes.***

Integration time

Integration time group box | Select the **Optimize integration time** (OIT) check box to increase the sensitivity of the scan due to longer integration of each pixel. If the integration time is longer more photons (i.e. more signal) are gathered for each pixel resulting in higher intensity and better signal to noise ratio.

OIT not selected:

- Same Integration time for each scanning amplitude
- Comparable signal values for all scanning amplitudes in the same resolution

OIT selected:

- Increase sensitivity of scan due to longer integration time of each pixel.
- Longer integration time lead to more photons, which can gathered for each pixel.
- Higher signal to noise ratio = higher sensitivity
- Not comparable signal values between scans with different amplitudes.
- Comparable range of amplitudes:

  22 mm

  20 mm

  15 mm

  10 mm

  5 mm

Sensitivity

Sensitivity group box | **Oversampling factor** edit box.

Enter a certain **Oversampling factor** to average scan lines and to combine them into one pixel.

Oversampling factor can be a value between 1-10.

For 4 µm scan resolution: n (max) = 3

For 6 µm scan resolution: n (max) = 4

For 10 µm scan resolution: n (max) = 8

For 20 µm scan resolution: n (max) = 10

For 40 µm scan resolution: n (max) = 10

For example, with an oversampling factor of three, one pixel is scanned three times and the average of these values is calculated. Oversampling therefore reduces noise but increases scan time by the factor. The average will be calculated during scanning.

In theory, the signal to noise ratio is increased by $\sqrt{n}$ (n= oversampling factor). For example, a factor of 4 will increase the signal to noise ratio by a factor of 2.
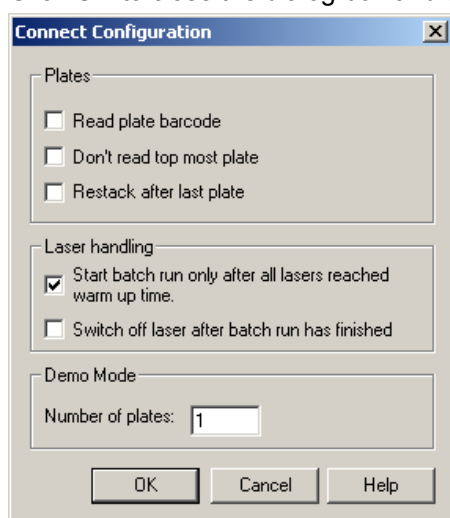
Resolution

Resolution group box	**Scan Resolution** combo box displays a selection of possible resolution of 4, 6, 10, 20 and 40 µm.
	The Scan Resolution is the number of µm per pixel in the x and y directions. The smaller the value, the higher is the resolution of the image but the more time it takes to perform a scan and the larger is the image file.

Tecan Connect Configuration

In the *Measurement Control* dialog box, select the **General** tab. Click **Configuration…** and the **Connect Configuration** dialog box appears, in which the plate settings and the *number of plates* used for the Demo Mode can selected.

Click **OK** to close the dialog box and save the selected options.

Plates group box	• **Read plate barcode** Enabled if Tecan Connect is installed with barcode reader. If the checkbox is checked, a plate barcode will be read.
	• **Don't read top most plate** If this box is checked the topmost plate will be ignored for stacking (e.g. topmost plate can be used as light shield)
	• **Restack after last plate** **If this box is checked, Tecan Connect will automatically restack all plates from output stack to input stack.**
Laser Handling	Two check boxes are available: • **Start batch run only after all lasers have reached warm-up time** • **Switch off laser after batch run has finished**
Demo Mode combo box	• **Number of plates** Enter the number of slides to be processed in Demo Mode. Maximum is 50. These setting will be ignored if the Tecan Connect instrument is connected.
OK button	Click the **OK** button to save the selected options and to close the dialog box

|	
Cancel button	Click the **Cancel** button to close the dialog box. The selected options are not saved.
Help button	Click the **Help** button to display the online help for the dialog box.

Advanced Autofocus

To edit the Autofocus parameters, select the **Geometry Parameter** tab in the *Measurement Control* dialog box, and select **Autofocus** option button to make **Advanced Autofocus…** available.

Click the **Advanced Autofocus** button and the *Advanced Autofocus* dialog box appears.

Window Elements

The **Advanced Autofocus** dialog box:
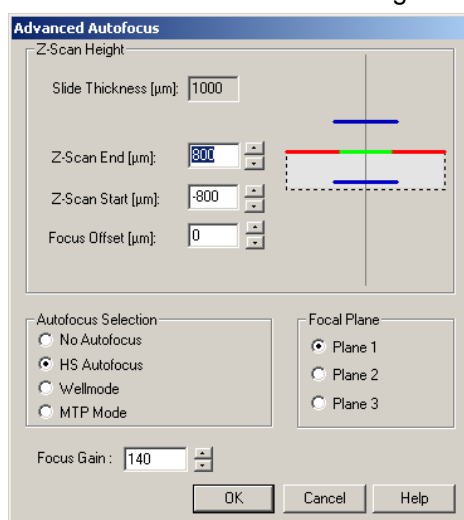
The **Advanced Autofocus** dialog box contains following elements:

- **Z-Scan Height** group box
- **Focus Offset** controls
- **Focus Gain** controls
- **Focal Plane** group box
- **Autofocus Selection** group box

The **Advanced Autofocus** dialog box contains following buttons:

OK button	Click the **OK** button to save the selected options and to close the dialog box.
Cancel button	Click the **Cancel** button to close the dialog box. The selected options are not saved.
Help button	Click the **Help** button to display the online help for the dialog box.

Z-Scan Height

Z-Scan Height group box

- **Slide Thickness** static edit box

  The **Slide Thickness** static box gives information about the current slide thickness that has been defined in the Geometry Parameters tab.

  The Slide Thickness is considered in displaying the slide scheme and needs to be defined for searching the focal plane within an appropriate range.

- **Z-Scan End** controls

  Use the **Z-Scan End** controls (µm) to enter the *End* value of the autofocus z-scan (= scan in vertical direction). Use the arrows or type in the value manually.

- **Z-Scan Start** controls

  Use the **Z-Scan Start** controls (µm) to enter the *Start* value of the autofocus z-scan (= scan in vertical direction). Use the arrows or type in the value manually.

  For a Standard.saf file, the default start and end values for the autofocus search area are +/- 800 µm. The range considers the tolerance of the slide thickness, the tolerances of positioning the slide adapter to the instrument as well as internal tolerances of the instrument itself. However, it might be necessary to increase the z-scan to guarantee that the algorithm finds the focal position reliably.

  Higher autofocus search areas (increased z-scan distances) prolong the time for autofocus determination.

Focus Offset

Focus Offset controls

- **Focus Offset** controls

  Use the **Focus Offset** controls (µm) to measure a certain value below or above a focal plane. Set the value by spinning the arrows or by typing in the offset manually.

  In certain cases the autofocus plane and the scan plane might differ, for instance: - slides with cover glasses, slides with membranes or filters. A certain value can be set as focus offset to perform the scan below (-) or above (+) the focal plane.

  E.g.: For a cover glass with a thickness of 170 µm, the correct focus offset would be 113 µm, - calculated according to following formula:

  Formula for the offset: **d-[(n-1)/n]*d**

  n= refractive index of the cover glass (= 1,5)

  d= thickness of cover glass

Focus Gain

**Focus Gain**
controls

Focus Gain controls

Use the **Focus Gain** controls to set the PMT gain for autofocus measurement.

The default value for the focus gain is 140. The gain is controlled automatically and dependent on the focus intensity profiles, but can also be adjusted manually either by using the arrows or typing in a value.

Focal Plane

Focal Plane
group box

The intensity profile from autofocus scanning in z-direction shows the different surfaces of slides (different planes). The first surface found is defined as default focal plane, but any of three can be selected.

Plane 1
Plane 2
Plane 3
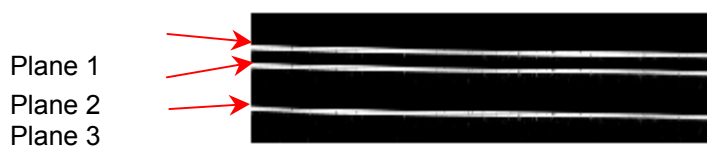
- **Plane 1** option button:

Select **Plane 1** option button to select Plane 1 (signal intensity) as focal plane calculated in the order from top to bottom from the Focus.tif images. The measurement will be performed on the selected plane 1.

- **Plane 2** option button:

Select **Plane 2** option button to select Plane 2 (signal intensity) as focal plane calculated in the order from top to bottom from the Focus.tif images. The measurement is performed on the selected second plane from the signal intensity profile in z-direction.

- **Plane 3** option button:

Select **Plane 3** option button to select Plane 3 (signal intensity) as focal plane calculated in the order from top to bottom from the Focus.tif images. The measurement is performed on the selected third plane of the signal profile in z.

Autofocus Selection

Autofocus Selection group box

- **No Autofocus** option button

Select **No Autofocus** option button, if no autofocus measurement is required.

The autofocus scan prior to the measurement is skipped and the measurement is performed at the plane that is calculated from the Adapter height, Slide Thickness and Focus Offsets.

The scan level is calculated by considering the default height of the Tecan MTP Slide Adapter **(= 11,5 mm)**, the slide thickness, as well as the Focus Offset.

No Autofocus eliminates the green autofocus scan lines from the Geometry Parameter tab.

The *No Autofocus* option is recommended to use in combination with a medium or large pinhole.

- **HS Autofocus** option button:

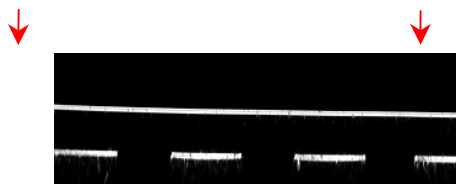Select **HS Autofocus** option button to perform the so-called five point autofocus.

To determine the right focal plane, the plate transport is moved in vertical direction (z-direction) creating a signal intensity profile from the laser reflection at the surface of the samples. From the 5 detection points a focal plane will be determined.

- **Well Mode** option button:

Select the **Well Mode** option button to perform a two point autofocus prior to measurement.

The autofocus in **Well Mode** is appropriate for microplates and all similar formats, where due to wells or other reasons a continuous focal plane is missing. The algorithm considers only the edge values in the focus.tif image.

- **MTP Mode** option button:

Select **MTP Mode** option button to perform a triangle autofocus prior to measurement.

The autofocus in **MTP Mode** performs three z-scans (Focus1, 2 and 3) on three positions in a triangle order onto a microplate. Between these three points the overall focal area is calculated.
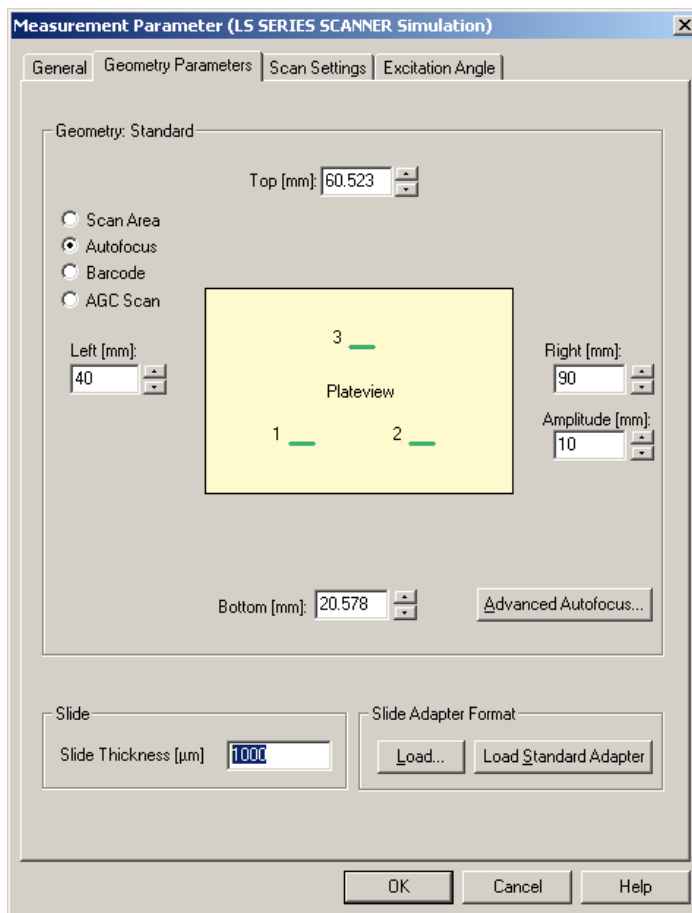
> **Note**
> *MTP Autofocus is aligned to the adapter,*
> *i.e. all dimensions are oriented to the table.*
> *In contrast, HS and Well Autofocus are slide oriented .*

6.3.7　Excitation Angle Tab

The excitation beam of the laser scanner usually strikes the sample with an angle of approximately 25° (see *Figure 6-10*). This improves the confocality and minimizes unwanted stray light effects due to back reflections. Therefore this configuration (='Default') gives the best performance for standard slides and other flat sample formats.

However there might be applications where the default angle of incidence is not optimal or the samples cannot be measured at all. A typical example is a microplate with printed arrays at the bottom of the wells. When measured from the top an inclined laser beam would usually be blocked by the walls and would not reach the bottom of the well. The variable beamshift feature of the laser scanner allows these samples to be measured as well. The angle of incidence for the fluorescence excitation can be varied between 0° and 25° in steps of 0.1° via the software.

The geometry of the sample determines the angle of incidence. The angle should be set to a value that allows the excitation beam to excite the full area of interest at the bottom of your sample without hitting the wall of the MTP before reaching the measurement surface.

If the angle for the scan is changed, the angle used for autofocus should also be adjusted. Because the autofocus does not work with normal incidence, the lowest angle accepted by the scanner is 10°. If your sample has high walls we recommend using the lowest possible value (=10°).

If 'Wellmode' is selected (in the advanced autofocus settings menu), except for the image scan, it is not necessary that the full area covered with spots is hit by the laser beam for the autofocus. In that case some shadowing effect can be tolerated. A minimum of 2.0 mm at the center part of the bottom is required however.

Default

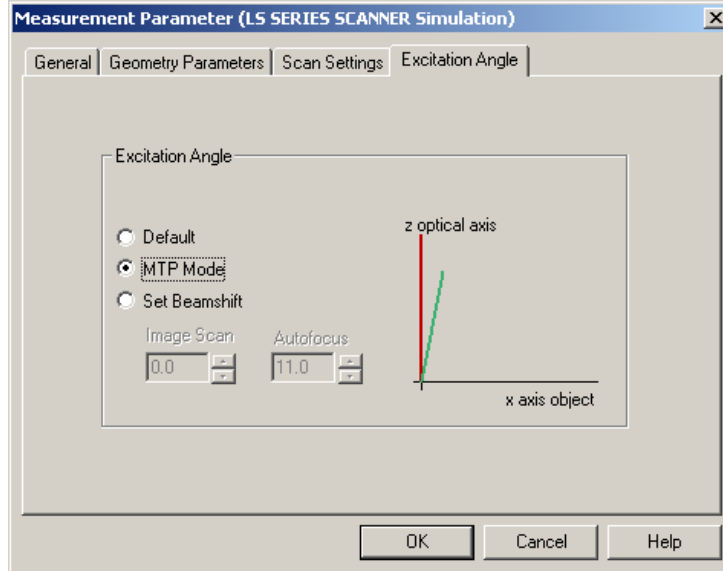It is recommended to select **Default** for all 'flat' samples (e.g. slides).

Angle is set to 25° for both the image scan and for autofocus.
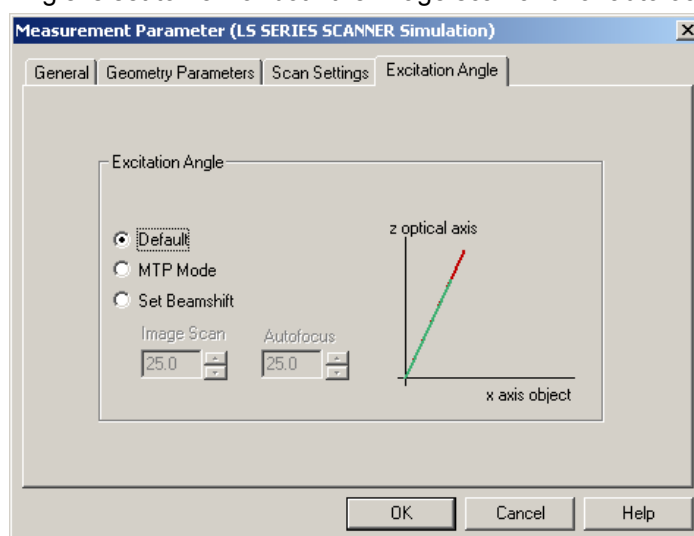
MTP Mode

Select **MTP Mode** for most MTP applications.

Angle is set to 0° (normal incidence) for image scan and 10° for autofocus.
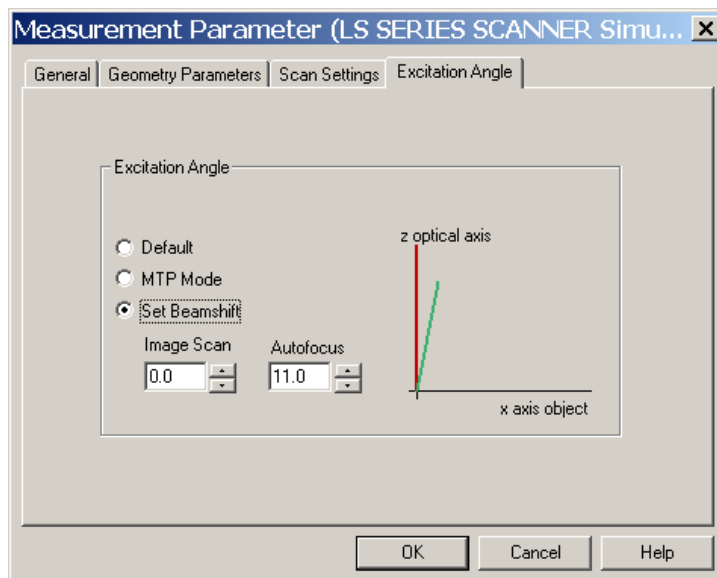

Set Beamshift

Set Beamshift may be a useful optimization parameter if your sample has only 'flat' walls with a relatively low aspect ratio.

The excitation angle for the image scan can be set to any value between 0° and 25° in 0.1° increments

The angle of incidence for the autofocus scan can be set to any value between 10° and 25° in 0.1° increments

Increasing the angle of incidence might reduce the background in your images. However, if it is too high for your kind of sample only a part of your sample area could visible on the scanned image. If the autofocus angle is set too high autofocus might fail. Use simple geometry according to figure below to calculate the appropriate angles for your samples.
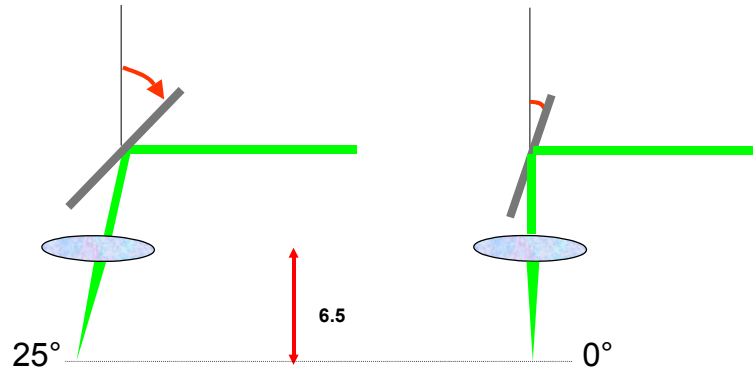
Figure 6-9

Adjustable laser beam angle (0.1° increments)
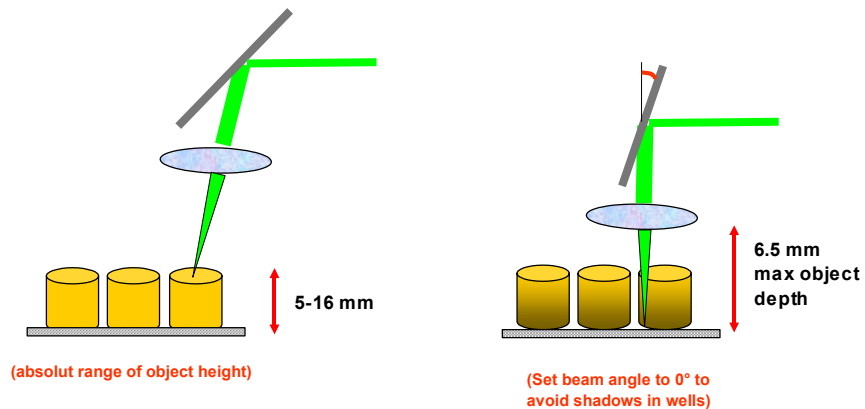
High working distance (6.5 mm)

Figure 6-10

> ***Note***
> ***If your microplates have a transparent bottom and your process allows them to be measured upside down it is still a good idea to compare an upside down measurement with default settings with an upright measurement with the angles set to MTP mode or another value determined as optimum for your samples.***

Load…

In the *Measurement Control* dialog box, click **Load…** to open existing *Measurement Parameter (.lsp)* files. The standard *Windows* **Open** dialog box appears.

In the **Open** dialog box all existing *Measurement Parameter* files will be shown. After selecting the requested file it will appear in the filename line. Click **OK** to load the new measurement parameters and close the dialog box.
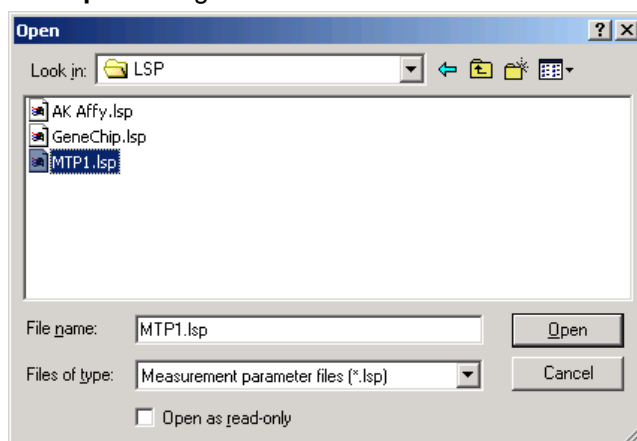
When a Measurement Parameter file is opened the defined measurement parameters of the file will be loaded, which include all of the settings in the Measurement Parameter dialog box on the General, Geometry Parameter, and Scan Settings tabs.

Note
The selection of the slides to be processed will use the default settings in which all slides are selected (see Slide Settings).

The **Open** dialog box:

The **Open** dialog box is the standard *Windows* file open dialog box. It contains the following elements:

Look in combo box	In the **Look in** combo box, the folder from which the measurement parameter file can be opened, is selected.
Workspace list box	The **Workspace** list box shows the files and folders selected in the **Look in** combo box.
File name edit box	In the **File name** edit box the measurement parameter file can be selected. Either type the name of the file or use the **Look in** selection to obtain a list of file names from which the measurement parameter file can be selected.
Files of type combo box	All Tecan measurement parameter files have the file extension ***.lsp**.
Open as read only check box	Select **Open as read only** to view the file and protect it from being changed.
Open button	Click the **Open** button to load a selected measurement parameter file (*.lsp).
Cancel button	Click the **Cancel** button to close the dialog box without loading a file.

Save…

In the *Measurement Control* dialog box, click **Save…** to save the current measurement parameters under the selected filename. The standard *Windows* file **Save As** dialog box appears. Click **OK** to save the new parameters as a **Measurement Parameter (.lsp)** file and close the dialog box.

When a *Measurement Parameter* file is saved the currently defined measurement parameters will be saved, which include all of the settings in the Measurement Parameter dialog box on the General, Geometry Parameter, and Scan Settings tabs.
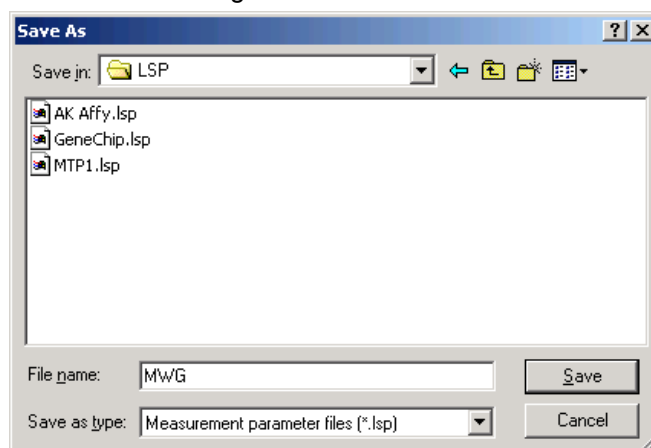
> *Note*
> *The selection of the slides to be processed will not be saved.*

If a measurement parameter file with the same name already exists it will be overwritten.

The **Save As** dialog box:

The **Save As** dialog box is the standard *Windows* file Save As dialog box. It contains the following elements:

|	
Save in combo box	In the **Save in** combo box, select the drive and the folder in which the measurement parameter file should be saved.
Workspace list box	The **Workspace** list box shows the files and folders selected in the Save in combo box.
File Name edit box	Enter the **File Name** for your measurement parameter file or select one from the list.
Save as type combo box	All Tecan measurement parameter files are saved with the file extension ***.lsp**.
Save button	Click the **Save** button to save the current measurement parameters in a file with the extension *.lsp.
Cancel button	Click the **Cancel** button to close the dialog box without saving.

6.3.8 Settings

Slide Settings

In the *Measurement Control* dialog box, click **Settings …** to display the slide setting options:

- • The filename(s) of the scanned image(s)
- • The slides to be processed
- • If the scanned image(s) should be saved automatically or not
- • If the images should be stitched or not
- • If the stitched image should be flipped or not

The **Filename** can be either a standard name or an individual **Filename Format**. **AutoSave, Stitching** of resulting images or **Flipping** can be selected, if desired. Under **Measure,** select the slides to process.

The **Slide Settings** dialog box:
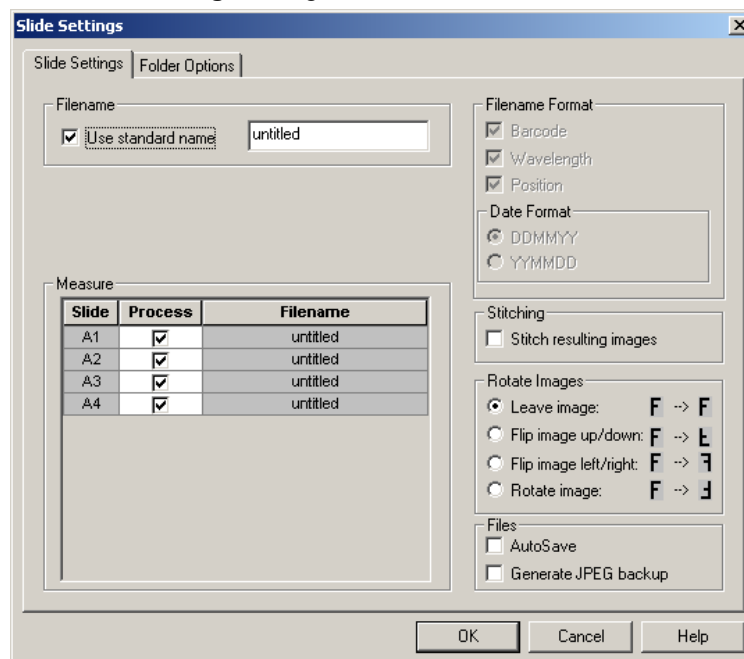
The **Slide Settings** tab contains the following elements:

- • **Filename** group box
- • **Measure** grid box
- • **Filename Format** group box
- • **Date Format** group box
- • **Stitch resulting images** check box
- • **Rotate image** group box
- • **Files** group box

The **Slide Settings** tab also contains the following buttons:

OK button — Click **OK** to save the selected options and close the dialog box.

Cancel button — Click **Cancel** to close the dialog box without saving the options.

Help button — Click **Help** to display the online help for the dialog box.

Filename

Filename group box

In the **Filename** group box a standard name for the scanned image can be selected. This name will be part of the image filename shown later as the title of the image window.

The **Filename** group box contains the following elements:

- Select the **Use standard name** check box to enter the name in the **standard name** edit box. The name gets part of the image filename. Normally, the standard image filename is grouped as follows:

  Standard name + Filename Format

  The **Filename Format** cannot be changed. It is fixed to:

  - Barcode - excitation wavelength - slide position - current date - image counter, - if a barcode reader is installed and if the barcode option is selected.

  - Excitation wavelength - slide position - current date - image counter, - if no barcode reader is installed.

- In the **standard name** edit box the image name must be entered. The following limits must be considered:

  - Maximum name length: 50 characters

  - Input data allowed:

  Alphanumerical data A -Z, a-z, digits 0-9 and underline.

Clear the **Use standard name** box to enable the **Filename** grid column (see **Measure** grid box / **Filename** grid column)

Measure

Measure
grid box

Before starting a scan, the slides to be processed and name(s) of the image(s) must be defined (all slides selected is the default setting). This is done in the **Measure** grid box consisting of 3 columns and a variable number of rows:

- The **Slide** grid column displays the slide position in alphanumerical order. The first character represents the row, the second character the column position in the defined slide adapter.

For example, using a slide adapter with slide positions arranged in 2 columns and 2 rows the **Slide** grid column shows:

A1 = slide on position column 1 / row 1

B1 = slide on position column 1 / row 2

A2 = slide on position column 2 / row 1

B2 = side on position column 2 / row 2

- The **Process** grid column displays check boxes for each slide defined. All slides selected are processed during next measurement.

Select the check boxes of the corresponding slides to be processed.

Select several rows and press **Insert Key** / **Delete Key** to mark the selected slides **to be processed** / **not to be processed**.

Click the heading of the Process column to select all of the rows in this column. Press **Insert Key** / **Delete Key** to mark all of the slides **to be processed** / **not to be processed**.

- In the **Filename** grid column the image name must be entered. The following limits should be noted:

  - Maximum name length: 50 characters

  - Input data allowed:

Alphanumerical data A -Z, a-z, digits 0-9 and underline.

The **Filename** grid column is disabled if **Use standard name** check box is selected. The name entered in **standard name** edit box is used creating image filename.

The **Filename** grid column is enabled if **Use standard name check box** is cleared. The name entered in **Filename** grid column is used creating the image filename.

Filename Format

Filename Format group box	The **Filename Format** group box contains the following elements: • The **Barcode** check box is automatically selected, if a barcode reader is connected to the instrument and is enabled for use. If barcode reading fails, barcode is set to "xxx". • The **Wavelength** check box must be selected, if the excitation wavelength is to be part of the image filename. • The **Position** check box must be selected, if the slide position is to be part of the image filename. The **Counter** check box is automatically selected and cannot be reset by the user. When using standard name (**Use standard name** check box is selected) the filename format is fixed to: • Barcode - excitation wavelength - slide position - current date - image counter, if a barcode reader is installed and the barcode option is selected. • Excitation wavelength - slide position - current date - image counter, if no barcode reader is installed. If no check boxes are enabled, the **Filename Format** cannot be changed. Clear **Use standard name** to enable the following check boxes / option buttons and the **Date Format** group box: • **Wavelength** check box • **Position** check box **Filename Format** is user-definable.

Date Format

Date Format group box	• The **Date Format** is automatically selected and cannot be reset by the user, but the type of date format can be selected: ▪ DDMMYY - Day Month Year ▪ YYMMDD - Year Month Day

Stitching

|	
Stitch resulting images check box	Select the **Stitch resulting images** check box to combine all scanned slides together into one image. The filenames used for parts of the stitched images are automatically set to standard name. The filename of the stitched image is set to: **Standard name** + fixed **Filename Format**, where barcode option and slide position are set to "xxx".

Rotate Images

Leave Images	Image is not changed
Flip image up/down	Flips image vertically.

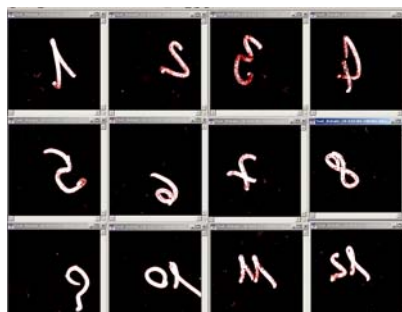For example, if **Flip image up/down** is selected, the result will be as follows:

When **Flip image up/down** and **Stitch resulting images** are selected, the result will be as follows:
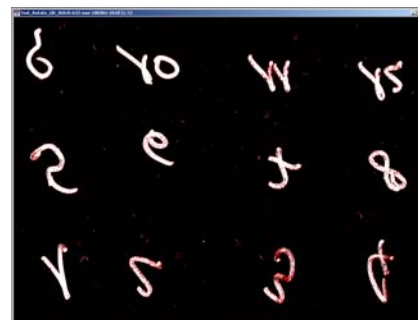
Flip image left/right   Flips image horizontally.

For example, if **Flip image left/right** is selected, the result will be as follows:

When **Flip image left/right** and **Stitch resulting images** are selected, the results will be as follows:
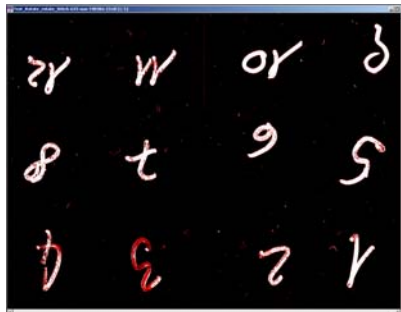
Rotate image   Rotates image clockwise 180°.

For example, if **Rotate image** is selected, the result will be as follows:

When **Rotate image** and **Stitch resulting images** is selected the results will be as follows:

Files

AutoSave check box	Select the **AutoSave** check box to save every scanned image automatically:
	AutoSave on: all files will be stored in designated folder,

AutoSave off: all files will be stored in temp folder,
(see chapter Folder Options)

Filename = title of the image window = standard or special name according to the selected options in the **Slide Settings** tab

Directory = **Tecan Array Pro Software** subdirectory **= Array Pro 45 \ Images \...**

The images are saved as 16bit TIF format.

Scanning of microplates or other slide adapter formats will open a lot of image windows in the **Tecan Array Pro Software**.

AutoSave is automatically enabled in *Stacker Mode*; no user action necessary.

> *Note*
> *To save memory and user time the first window will be closed after 10 scans.*
> *(10 active pictures is the default setting). So the user won't see all the scans at once, just the last 10.*

Generate JPEG backup check box

The image will also be saved as a .jpg file and saved in the same folder as the .tif file.

> *Note*
> *There must be an equal number of pictures in each column and in each row, (i.e. the pictures must form a complete rectangle,*
> *e.g. 2 x 3, 4 x 6, etc.)*
> *Improper selection of images to be stitched can cause an unexpected stitched image result.*

Folder Options

In the *Measurement Control* dialog box, click **Settings …** to open the *Slide Settings* dialog box:
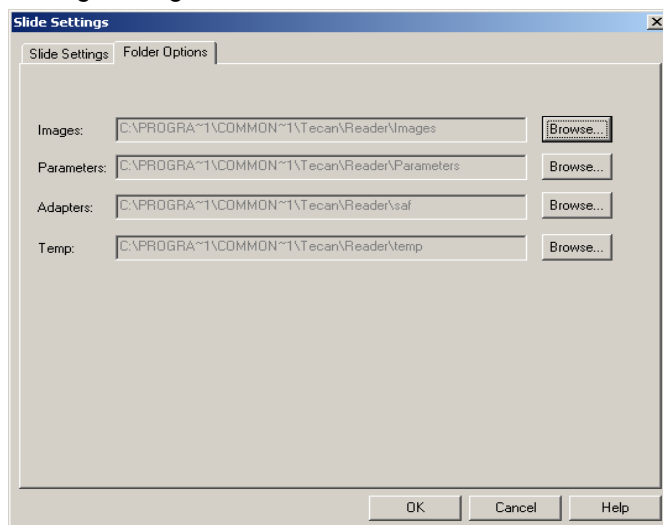
Select the ***Folder Options*** tab to view the current file storage paths.

By clicking the **Browse** button, paths can be defined for the following file types:

- Images (*.tif; *.jpg)
- Parameters (*.lsp)
- Adapter (*.saf)
- Temp: all files (.tif, .jpg, .lsp, .saf, autofocus images) when **AutoSave** has not been selected, (see Files on page 6-60).
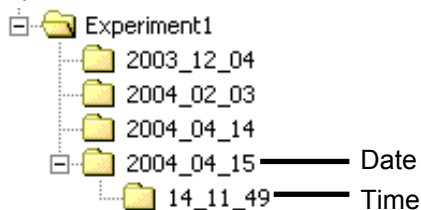
Example:

After a scan is finished:
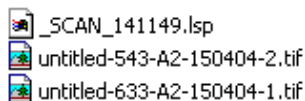
A folder with the current date is created, (Y_M_D).

In the date folder, a folder with the current time is created, (h_m_s).

The current .lsp file will be saved as _SCAN_Time.lsp) (e.g. _SCAN_141149.lsp; 2 pm, 11 minutes and 49 seconds).

Experiment Folders:

Scan Parameter and Image files:

See also Filename Format on page 6-57.

6.3.9   Slide Adapter Format

The **Slide Adapter Format** group contains the following elements:

Load button   Click the **Load** button to open the **Open Slide Adapter Format** dialog box: SAF can be selected.

See Slide Adapter Format for more information.

6.3.10   Slides to Process

The **Slides to process** group gives a general view of the **slide** / **plate** format used and the arrangement of available **scan areas**. Scan areas that will be scanned after pressing **Start** / **Prescan** button are green, those that will not be scanned are dark gray.

The selection can be changed in the *Measurement* dialog box. Click on a slide to toggle its selection. For *Prescan,* only the first selected slide will be scanned.

Scanlist

A scan list is a text file (.txt) for batch processing:
(e.g.: created with Notepad or Microsoft Text Editor)

Example1:

   S,160,0,633,670,0,0

Scan Mode: S (Single), DS (Dual Sequential), DP (Dual Parallel)

Gain Channel1:160, Gain Channel2: 0

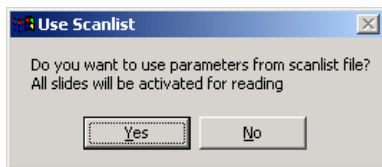Laser Channel1:633

Filter Channel1:670
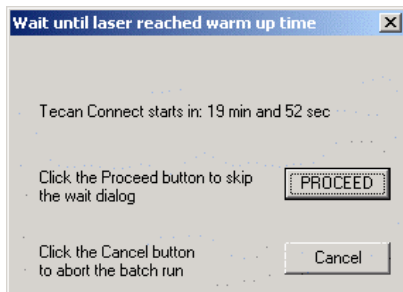
Laser Channel2:0

Filter Channel2:0

In this example, a single scan is performed with a gain of 160, with red laser and red filter.

The text file must be located in the same folder as ArrayPro32.exe file. (Default folder **C:\ArrayPro4.5**).

When using a scanlist file the following dialog box will appear after clicking **Start**:

Click **Yes** and the following dialog box will appear:

Click the **Proceed** button to skip the warm up time of the laser. If the **Proceed** button is not clicked the scan starts automatically after the wait time has expired.

Example2:

S,180,0,633,670,0,0

DP,220,190,594,635,488,535

DS,120,100,633,670,488,535

Scan Mode: S (Single), DS (Dual Sequential), DP (Dual Parallel), Scan Mode, Gain Channel1, Gain Channel2, Laser Channel1, Filter Channel1, Laser Channel2, Filter Channel2

In this example, three different scans are performed:

First a single scan is performed on each scan area of the adapter with a gain of 180, with red laser and red filter.

Next a dual parallel scan is performed with a gain of 220 in the first channel with orange laser and orange filter and a gain of 190 in the second channel with blue laser and blue filter.

Then a dual sequential scan is performed with a gain of 120 in the first channel with red laser and red filter and a gain of 100 in the second channel with blue laser and blue filter.

6.4 LS Scanner Shell

6.4.1 General

The following chapters contain the description of the special **LSServer** options, which can be viewed and utilized in the **Scanner Shell** program. The Scanner Shell software is required for the following:

- Defining filter slides
- Locking and unlocking the instrument for transportation purposes
- Calibrating the gain of PMTs
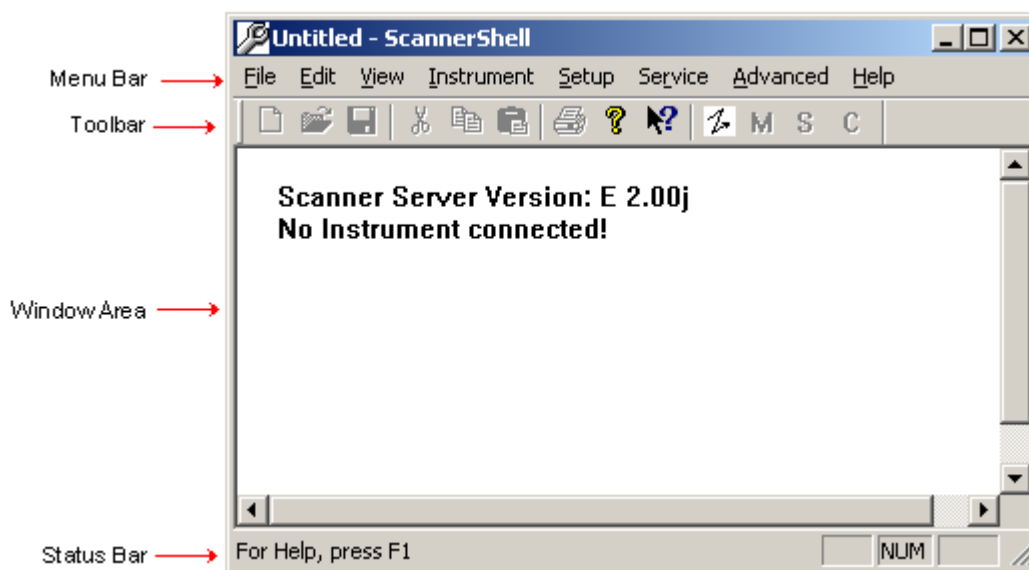- Defining crosstalk
- Setting up and calibrating the stacker

6.4.2 Starting Scanner Shell

Close all programs using Tecan LSServer.

Click the **Scanner Shell** icon in the **Tecan** program group.

The **Scanner Shell** window is displayed.

6.4.3 Window Elements

The **Scanner Shell** main window contains a **menu bar**, a **toolbar**, the **window area** and the **status bar**.

The **window area** displays the type of the connected **LSServer**. For example: **LS SERIES SCANNER** Simulation.

Menu Bar

Refer to chapter 6.4.5 Scanner Shell Menus for information of the **menu** options.

Toolbar

The following buttons are available on the toolbar:

Click the **About** button to show the **About** dialog box.

It contains the following elements:

- Program information
- Version number
- Copyright

Click the **Help** button to display the online **Help** for elements such as:

- Buttons
- Menus
- Windows

Click the **Connect** button to directly connect to an instrument.

Click the **Measurement** button to display the **Measurement Parameter** dialog box**.**

The **Start Measurement** button appears gray, because measurements cannot be started from the Scanner Shell software.

Click this button to show the **Measurement Control** panel.

Status Bar

The **Status bar** displays the following information:

- Help access: Press **F1** for **Help**
- Current status of the keyboard toggles: **CAP** (caps lock), **NUM** (Numeric block lock), **SCRL** (Scroll lock).

6.4.4   *File types used in Scanner Shell*

The following file types are used with **Scanner Shell**:

File Type	File Extension	Description
Measurement Parameter	.lsp	Measurement data is saved as .lsp files.
Slide adapter format	.saf	Slide adapter format data is saved as .saf files.
Gain calibration	.dat	Gain calibration data is saved as GainCalibration.dat files.
	.cvs	Gain calibration data is stored as a comma separated list (.cvs files). For analysis these files can be loaded in Excel or another calculation program.
Crosstalk calibration	.dat	Crosstalk data is saved as CrosstalkCor.dat files.

Log	.log
	.log1 - .log5
		Log file data is saved in the following file format:
		• LSServer.log:
		  A record of current measurement data.
		• LSServer.log1 - LSServer.log5:
		  A record of earlier measurement data.

> *Note*
> *Each time the Scanner Shell is started, the LSServer.log file is overwritten; old data is saved in LSServer.logn file.*

Locating Files

Scanner Shell Files

By default, the files associated with **Scanner Shell** are stored as subdirectories in the directory where LSServer.dll is found:

C:\Program Files\Common Files\Tecan\Reader\….

The subdirectories are displayed in the table below:

Type of File	File Extension	Directory
Measurement parameter	.lsp	Parameter
Slide adapter format	.saf	SAF
Gain calibration	.dat .csv	CalData
Crosstalk	.dat	CalData

Temporary Files

Temporary files needed for diagnostic and controlling purposes are stored in the directory:

C :\Documents and Settings*"user name"*\Local Settings\

The file types are displayed below:

Type of File	File Extension	Directory
Focus	.tif	Temp
Log	.log .log1 to .logn	Temp

Note
To see the user name of the person logged on,
press Ctrl + Alt + Delete and the user name will be shown
in the Logon dialog box.

File Management

The default paths for saving files can be changed in the *Slide Settings* dialog box on the *Folder Options* tab. See Folder Options.

6.4.5   Scanner Shell Menus

File Menu

Exit

In the **File** menu, select **Exit** to exit the Scanner Shell software.

Edit Menu

Not implemented in this version of the Scanner Shell software.

View Menu

Toolbar

In the **View** menu, select the **Toolbar** option to show / hide the Scanner Shell software Toolbar.

When the Toolbar option is selected (√) the Scanner Shell software Toolbar will be shown.

When the Toolbar option is cleared the Scanner Shell software Toolbar will be hidden.

Status Bar

In the **View** menu, select the **Status Bar** option to show / hide the Scanner Shell software Status Bar.

When the Status Bar option is selected (√) the Scanner Shell software Status Bar will be shown.

When the Status Bar option is cleared the Scanner Shell software Status Bar will be hidden.

Instrument Menu

Connect

In the **Instrument** menu, select **Connect** or on the toolbar click the **Connect** button or press **Shift + F5**.

This option allows the connection to an instrument. Before connecting the desired instrument the communication port must be chosen. To do this select **Setup Port…** from the **Setup menu**. For further information refer to the chapter Port Setup on page 6-4 in this manual.

The desired instrument will be connected using the selected communication port. A message in the **window area** of **Scanner Shell** software shows that the connection is successful or unsuccessful:

Working in Scan Mode

Connection	Message in window area of Scanner Shell
Unsuccessful	*Scanner Server Version: V 2.20* *No instrument connected!*
Successful	Scanner Server Version: V 2.20 LS SERIES SCANNER (+LS) 12902000004 V 3.00 08/03 IMAGING V1.00 08/01 MEAS IMAGE iV 1.10……………….

Working in Demo Mode

Connection	Message in window area of Scanner Shell
Unsuccessful	*Scanner Server Version: V 2.20* *No instrument connected!*
Successful	*Scanner Server Version: V 2.20* *LS SERIES SCANNER Simulation (+LSSim) 12345Sim* *V 2.20 12 / 02 SIMULATION* *V2.20 (RV1) iV 2.20……………….*

Measurement Control Panel…

In the **Instrument** menu, select **Measurement Control Panel…** and the *Measurement control* dialog box appears.

In the *Measurement control* dialog box several groups, each containing individual buttons, are shown that offer special operations for preparing and scanning data. Measurement parameters and measurement options are defined. The Start and Prescan buttons are not available in the Scanner Shell software.

For further information refer to the chapter 6.3 LSServer description in this manual.

Move Plate and Filter…

In the **Instrument** menu, select **Move Plate and Filter…** and the **Movements** dialog box appears.

In the **Movements** dialog box, specific **In** and **Out** buttons can be used to drive the plate carrier or filter slides into or out of the LS Series Scanner. Click **OK** to close the dialog box.

For further information refer to the chapter Plate/ Filter on page 6-26 description in this manual.

Edit Measurement Parameters…

In the **Instrument** menu, select **Edit Measurement Parameters…** or on the toolbar click the **Edit Parameter** button or press **Ctrl + F5** and the **Measurement Parameter** dialog box appears.

In the **Measurement Parameter** dialog box general options like Scan Mode, Stacker and/or Barcode as well as Geometry Parameters and Scan Settings can be defined.

For further information refer to the chapter Edit… on page 6-28 in this manual.

Start Measurement

The *Start Measurement* option is not available in the Instrument menu of the Scanner Shell software.

Disconnect

In the Instrument menu, select the **Disconnect** option to disconnect the currently connected instrument. Another instrument can then be connected.

> *Note*
> *This option is only available, if an instrument is already connected.*

Setup Menu

Setup Port…

In the **Setup** menu, select **Setup Port…**.

This option allows an instrument to be connected. The **Setup Port** dialog box is displayed, where the desired instrument and communication port can be selected.

For more information refer to the chapter 6.2 Tecan LS Scanner Interface description in this manual.

Define Filter Slides…

In the **Setup** menu, select **Define Filter Slides…**

The **Define Filter Slides** option is used to define the filter slides found in the currently connected instrument.

For example in **Dual Channel Instruments,** two filter slides can be loaded simultaneously. Parameters and comments for one / two **filter slides** (channel 1 / channel 2) must be set before starting measurement (The first time is done by a service engineer):

1. Load the selected filter(s) into channel 1 slot by hand.

	Caution
STOP	**You cannot define a filter slide that has been loaded into the channel 2 slot! All filters must be defined in channel 1 slot.**

2. In the **Setup** menu select **Define Filter Slides…** The **Define current loaded filter slides** dialog box is displayed.

KEEP IN MIND: THIS OPTION IS NOT FOR GENERAL USE!

	Caution
STOP	**The instrument can recognize predefined filter slides and the filter values should not be changed. However, if the filter in the filter slide have been changed (by a service engineer) or if a new undefined customized filter slide is to be used, the filter slides need to be defined.**

The **Define current loaded filter slides** dialog box represents all filter slides found and defined in the currently connected instrument. For each slide that is loaded a different tab-dialog box is displayed. When connected to a **Tecan LS SERIES SCANNER** instrument, two tabs **Filter slide 1** (filter in channel 1 slot) and **Filter slide 2** (filter in channel 2 slot) are shown.

The **Wavelength**, **Bandwidth** and a **Name** for the specific dye used for detection can be defined. Click **OK** to save the filter definition changes and close the dialog box.

Each filter slide contains 6 filter positions:

- Position 1 is reserved for focus measurement.

- Positions 2 - 5 are free for filter definition.

- Position 6 is reserved.

Note
Position 1 is always reserved for focus measurement and cannot be changed.

Note
Position 6 is reserved for future functions and cannot be changed.

Note
Empty filter slide positions (Wavelength / Bandwidth) must be set to 0.
No spaces are allowed in the filter name.

The **Define current loaded filter slides** dialog box is divided into individual tabs:

- **Filter Slide 1** tab
- **Filter Slide 2** tab

The **Define current loaded filter slides** dialog box contains the following buttons:

OK   Click the **OK** button to save the selected options and to close the dialog box.

Cancel   Click the **Cancel** button to close the dialog box. The selected options are not saved.

Help   Click the **Help** button to display the online help for the dialog box.

Filter Slide 1 tab

The **Filter Slide 1** tab window contains all data definitions for the filter slide loaded in channel 1 slot.

The **Filter Slide 1** tab window:

The **Filter Slide 1** tab window consists of the following elements:

Pos. 1 - Pos. 6 static text	This static text corresponds to the filter positions on the filter slide loaded in filter slot 1: • **Pos. 1** is reserved for focus measurement • **Pos. 2 - 5** are free for filter definition • **Pos. 6** is reserved
Wavelength edit box	In this field the **Wavelength** of corresponding filter must be entered.
Bandwidth edit box	In this field the **Bandwidth** of corresponding filter must be entered.
Name edit box	In this field the **Name** for a specific dye used for detection of corresponding filter must be entered. No spaces are allowed in the name.
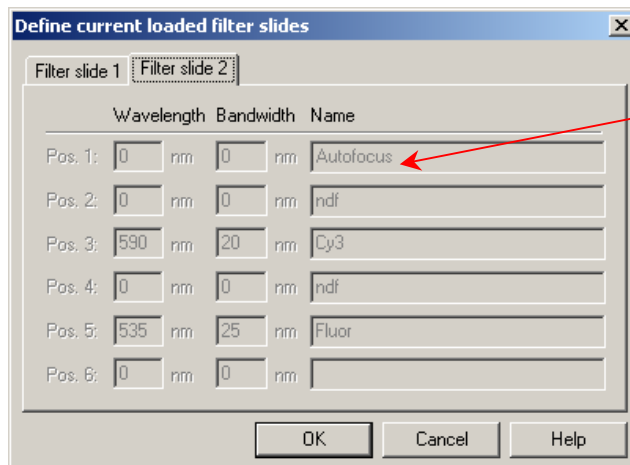
Filter Slide 2 tab

The **Filter Slide 2** tab contains all data definitions for the filter slide in channel 2. The tab is disabled in this version of the Scanner Shell software.

The **Filter Slide 2** tab window:

Pos. 1 must be defined as Autofocus otherwise this operation will fail

The **Filter Slide 2** tab window consists of the following elements:

Pos. 1 - Pos. 6 static text — This static text corresponds to the filter positions on the filter slide loaded in filter slot 2:

- **Pos. 1** is reserved for focus measurement
- **Pos. 2 - 5** are free for filter definition
- **Pos. 6** is reserved

Instrument Options

The **Instrument Options** option is not available in this version of the Scanner Shell software.

Language

In **Setup** menu, select **Language…**

The **Language** option shows the available languages of the **LS Scanner Shell** software.

When the **Language** is selected (√) the **LS Scanner Shell** software (menus, windows, comments) will be displayed in this **language**.

6.4.6   Service Menu

Test Commands…

In the **Service** menu, select **Test Commands…** and the **Test Command** dialog box is displayed:
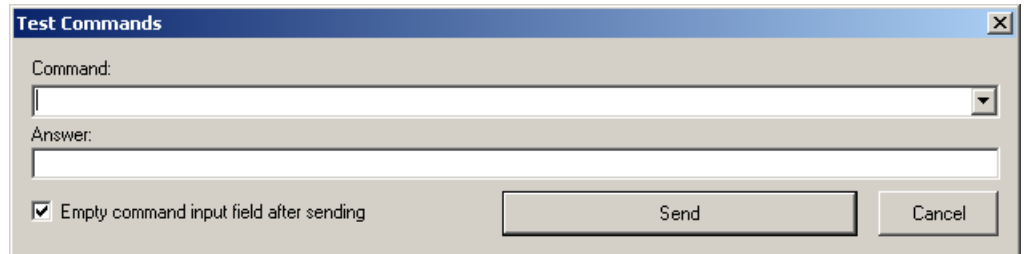
In the **Test Command** dialog box, commands can be sent to the connected instrument. These commands must be typed in the **Command** edit box. Click **Send** to transmit the commands to the instrument. The instrument answer is immediately displayed in the **Answer** edit box. Click **Cancel** to close the dialog box.

The **Test Command** dialog box is used in case of errors and for testing purposes.

Including but not limited to the following:

- No or faulty communication between PC ↔ INSTRUMENT.
- No correct instrument function.
- Testing new instrument features.
- Setting new instrument parameter.

Sending commands directly to the instrument is an easy way of changing / viewing instrument parameters and testing nearly all functions independent of special driver applications such as **LSServer** software.

KEEP IN MIND: THIS OPTION IS NOT FOR GENERAL USE!

	Caution
[STOP sign]	**Sending wrong data to an instrument can cause crashes of transports or damage the instrument. Only service engineers should use this option.**

The **Test Command** dialog box contains the following elements:

Command combo box	Use the **Command** combo box edit field to type in the command that should be sent to the instrument.
	Each command entered is automatically added to the **Command** combo list: All commands sent can be repeated by clicking on the corresponding list item.
Answer edit box	The **Answer** edit box shows answers received from connected instrument.
Empty command input field after sending check box	Selecting this box deletes commands typed in **Command** combo box after they have been sent to the instrument. New commands can be entered immediately.
	If the same command needs to be repeated several times this box should not be selected.
Send button	Click the **Send** button to send the command, which is in **Command** combo box edit field, to the connected instrument.
Cancel button	Click the **Cancel** button to close the dialog box.

Demo Mode Allowed

To enable the **Demo Mode** option button in the **Setup Port** dialog box**,** select **Demo Mode Allowed** from the *Service* menu.

In **Demo Mode** the **Tecan LSServer** can be simulated, no instrument needs to be connected. This option is used for training purposes.

For further information refer to the chapter 6.2 Tecan LS Scanner Interface description in this manual.

Transport Lock…

To start the *Transport Lock wizard,* select **Transport Lock…** from the Service menu. A wizard guides the user through the transport lock procedure..

The **Transport Lock** option is implemented in **Scanner Shell** as a wizard. It represents workflow modules, which define a sequential procedure and guide the user step-by-step to perform a specific complex activity.

For transportation purposes it is recommended to lock the connected instrument. A transportation shock absorber pad is inserted between the plate transport and the optic chassis to avoid damage to the optical parts.

For further information refer to the chapter 6.4.9 The Transport Lock Wizard description in this manual.

During the **Transport Lock** procedure:

1. The instrument door is opened.
2. The plate transport is moved into the correct position.
3. The user is asked to insert the transportation shock absorber pad.
4. Instrument is locked.
5. The instrument door is closed.

As long as the instrument is locked, the **LSServer Measurement Control** cannot be started. Thus scanning is not possible.

For information unlocking instrument see 6.4.10 The Transport Unlock Wizard description in this manual.

Gain Calibration…

To start the *Gain Calibration wizard*, select **Gain Calibration…** in the Service menu. A wizard guides the user through the gain calibration procedure.

The **Gain Calibration** option is implemented in **Scanner Shell** as a wizard. It represents workflow modules, which define a sequential procedure and guide the user step-by-step to perform a specific complex activity.

The **Gain Calibration** is needed to take into account differences in sensitivity between PMTs used in **Tecan LS SERIES SCANNER** instrument. It is NOT only necessary for dual channel instruments. The deviation between PMTs can be up to 500 %.

The **LS SERIES SCANNER** is provided with calibrated PMTs. Data from the PMTs becomes less accurate over the course of its life-span, so gain calibration should be repeated regularly: After **100 days** the user will be requested to start gain calibration again.

> *Note*
> *Gain calibration no longer valid after 100 days.*

Tools needed for gain calibration are delivered with each instrument:

- Scattered light glass slide
- Calibration filter slide with different OD filters

For further information refer to the chapter The Gain Calibration Wizard on page 6-77 in this manual.

During the **Gain Calibration** procedure:

1. Door opens and filter slides are moved out.
2. The user is asked to prepare the instrument for **Gain Calibration**.
3. Instrument parameters are set for **Gain Calibration**.
4. **Gain Calibration** PMT1 is done.
5. **Gain Calibration** PMT2 is done.
6. **Gain Calibration** data PMT1 / PMT2 is saved as GainCalibration.cvs in the CalData folder.
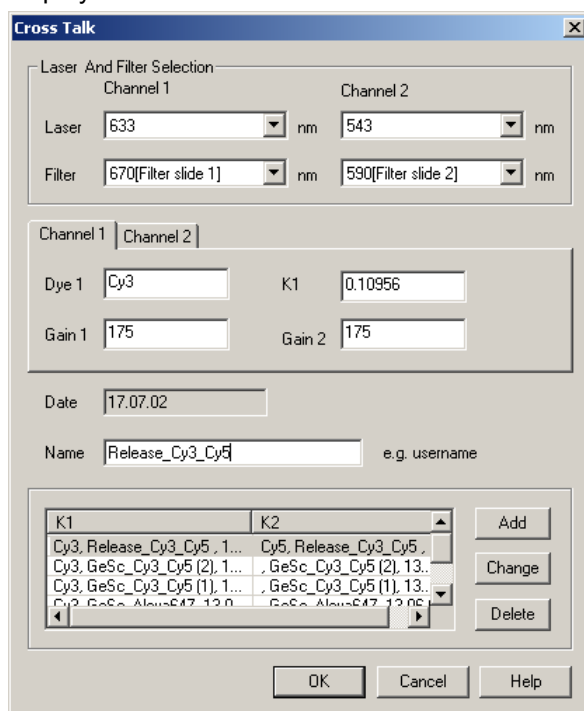7. Door is closed and filter slides are moved out.

> *Note*
> *The gain calibration must be done for all PMTs found in instrument.*

Crosstalk…

In the **Service** menu, select **Crosstalk…** and the **Crosstalk** dialog box is displayed:

Spectral crosstalk can occur in dual label experiments when residual signal contributions from the first dye can be detected in the second detection channel.

For example if there is a strong signal of dye with shorter emission wavelength (e.g. Cy3) there will typically be a weak, but detectable, contribution of this dye's signal in the dye with longer emission wavelength (e.g. Cy5) detection channel as well. This is especially true for simultaneous dual color measurements.

In principle, crosstalk can occur in both directions. For example a Cy3 contribution can be found in the Cy5 channel and vice versa a Cy5 contribution can be found in the Cy3 channel.

Typically the shorter wavelength dye into the longer wavelength dye's detection channel is much stronger. In our example of Cy3 and Cy5 there is a significant contribution from Cy3 to Cy5 that needs to be calibrated, but there is a negligible contribution from Cy5 to Cy3. The latter one needs no calibration.

The **LS SERIES SCANNERS** have an automatic correction algorithm implemented for simultaneous dual color measurements. Correction takes place on a pixel level during data acquisition.

In order to function correctly, this algorithm needs a valid set of K-factors for the dye combination to be measured. The quantitative amount of crosstalk depends on the filters / laser used → the K-factors are specific for individual filter / laser combination.

The signals measured for crosstalk correction depend on PMT gains. If a valid **Gain Calibration** has been performed, the instrument takes the PMT gains automatically into account. For further information refer to the chapter 6.4.6 Service Menu - Gain Calibration…description in this manual.

All data needed for calculating crosstalk correction must be entered / selected in the **Crosstalk** dialog box.

For information about how to get special data needed for crosstalk correction and how to perform the correction, refer to the **LSServer Beginners Guide** manual**.**

In the **Crosstalk** dialog box, all data needed for crosstalk correction must be set.

The requested options must be entered in the corresponding edit boxes, selections must be made in combo and list boxes.

The **Crosstalk** dialog box is divided into four areas:

Channel 1 / Channel 2 tab:

In the **Channel 1 / Channel 2** tab, data must be entered, which has been previously determined through special measurement (e.g. using Tecan LSA Software for microarray analysis): Dye 1, Dye 2, K1, K2, Gain 1 and Gain 2.

Laser / Filter selection:

In the **Laser / Filter** combo box, laser and filter combinations for crosstalk must be selected.

Current Date and Name edit box:

In the **Date** field the current date is displayed. In the **Name edit** box name or initials must be entered

K1 / K2 data list box:

All data selected / entered in the **Crosstalk** dialog box is stored as a crosstalk factor record (CrosstalkCor.dat). This can be added to **K1 / K2 data** list box. Clicking on an item in the list box displays corresponding data. Crosstalk factor records can be added, changed or deleted at any time.

Click **OK** to save all **K1 / K2** list box data in a special file **CrosstalkCor.dat** and close the dialog box.

The first part of **Crosstalk** dialog box is divided into individual tabs:

1. **Channel 1** tab

   Before calculating crosstalk correction a so-called K-factor must be determined. This is done during a measurement without taking into account crosstalk correction. Adjusted values in this measurement session must be entered in the **Channel 1** tab.

The **Channel 1** tab contains the following elements:

Dye 1 edit box	In the **Dye 1** edit box the description of used dye must be entered.
K1 edit box	In the **K1** edit box the correction factor for contribution of dye with shorter emission wavelength into the detection channel of dye with longer emission wavelength must be entered.
	The **K1** default value is set to 0. This means no crosstalk correction will be done, no other parameters (Dye, gain) must be specified.
Gain 1 edit box	In the **Gain 1** edit box PMT Gain channel 1 must be entered.
Gain 2 edit box	In the **Gain 2** edit box PMT Gain channel 2 must be entered.

2. Channel 2 tab

Before calculating crosstalk correction a so-called K-factor must be determined. This is done during a measurement without taking into account crosstalk correction. Adjusted values in this measurement session must be entered in **Channel 2** tab.

The **Channel 2** tab contains the following elements:

Dye 2
edit box

In the **Dye 2** edit box the description of used dye must be entered.

K2
edit box

In the **K2** edit box the correction factor for contribution of dye with longer emission wavelength into the detection channel of dye with shorter emission wavelength must be entered.

The **K2** default value is set to 0. This means no crosstalk correction will be done, no other parameters (Dye, gain) must be specified.

Gain 1
edit box

In the **Gain 1** edit box PMT Gain channel 1 must be entered.

Gain 2
edit box

In the **Gain 2** edit box PMT Gain channel 2 must be entered.

Next part of **Crosstalk** dialog box contains the following elements:

Channel 1 Laser
combo box

In **Channel 1 Laser** combo box all lasers found in current connected instrument are displayed. The one used for crosstalk correction must be selected.

Channel 2 Laser
combo box

In **Channel 2 Laser** combo box all lasers found in current connected instrument are displayed. The one used for crosstalk correction must be selected.

Channel 1 Filter
combo box

In **Channel 1 Filter** combo box all filters found in filter slide, loaded in slot 1 of current connected instrument, are displayed. The one used for crosstalk correction must be selected.

Channel 2 Filter
combo box

In **Channel 2 Filter** combo box all filters found in filter slide, loaded in slot 2 of current connected instrument, are displayed. The one used for crosstalk correction must be selected.

Date static box

The **Date** static box shows the current date.

Format = DD.MM.YY

Name edit box

In the **Name** edit box the user can type in his name or initials or some other comment.

K1 / K2 list box	The **K1 / K2** list box displays all data needed for crosstalk correction as a crosstalk factor record.
	Format **K1** = **Dye 1**, **Name**, **Date**
	Format **K2** = **Dye 2**, **Name** , **Date**
	Special operations can be performed on a crosstalk factor record:

	Add button	Click the **Add** button to collect all data entered by the user in a crosstalk factor record, calculate the crosstalk correction factor and add a new item to the list box.
	Change button	Click the **Change** button to change the current selected crosstalk factor record in list box. Old record data is overwritten.
	Delete button	Click the **Delete** button to delete the crosstalk factor record of the selected list item.

OK button	Click the **OK** button to save the selected options in a data file called CrosstalkCor.dat and close the dialog box.
Cancel button	Click the **Cancel** button to close the dialog box. The selected options are not saved.
Help button	Click the **Help** button to display the online help for the dialog box.

Stacker Setup…

Stacker Setup is only used when **Tecan LS Scanner** is connected to a special autoloader, the **Twister**. If the Tecan **Connect** is associated with the LS Scanner, than a special XConnect software, which is delivered with the instrument, is responsible for the calibration and configuration of the connected autoloader **Connect**.

For further information about using **Connect**, refer to the **Connect** manual.

In the *Service* menu, select **Stacker Setup…** and the **Stacker Setup** dialog box is displayed:
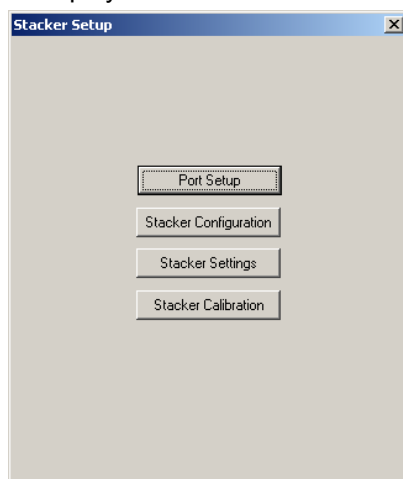
The **Tecan LS SCANNER** instrument can operate in manual and automatic mode. For automation purposes, a stacker instrument such as the **Twister** must be connected.

For further information about using **Twister** refer to the **Twister** manuals: **The Twister User's Guide and The Twister Service Manual**.

The **Stacker Setup** dialog box helps the user select, calibrate and configure the installed stacker instrument.

For further information about **Stacker Setup** refer to the chapter 6.4.7 Stacker Setup - Twister description in this manual.

In the **Stacker Setup** dialog box the user can choose between several options setting up the connected stacker instrument:

Click the **Port Setup** button to display the **Port Setup** dialog box: Stacker type and communication port can be selected.

Click the **Stacker Configuration** button to display the **Stacker Configuration** dialog box, the corresponding to the connected stacker instrument.

Click the **Stacker Settings** button to display the **Stacker Settings** dialog box, the corresponding to the connected stacker instrument.

Click the **Stacker Calibration** button to display the **Stacker Calibration** dialog box which corresponds to the connected stacker instrument.

The **Stacker Setup** dialog box contains the following elements:

|	
Port Setup button	Click the **Port Setup** button to display the **Port Setup** dialog box.
Stacker Configuration button	Click the **Stacker Configuration** button to display the **Stacker Configuration** dialog box.
Stacker Settings button	Click the **Stacker Settings** button to display the **Stacker Settings** dialog box.
Stacker Calibration button	Click the **Stacker Calibration** button to display the **Stacker Calibration** dialog box.

Advanced Menu

Open SAF…

In the Instrument menu, select **Connect** to connect the instrument.

In the Advanced menu, select **Open SAF…** and the **Open Slide Adapter Format** dialog box is displayed:
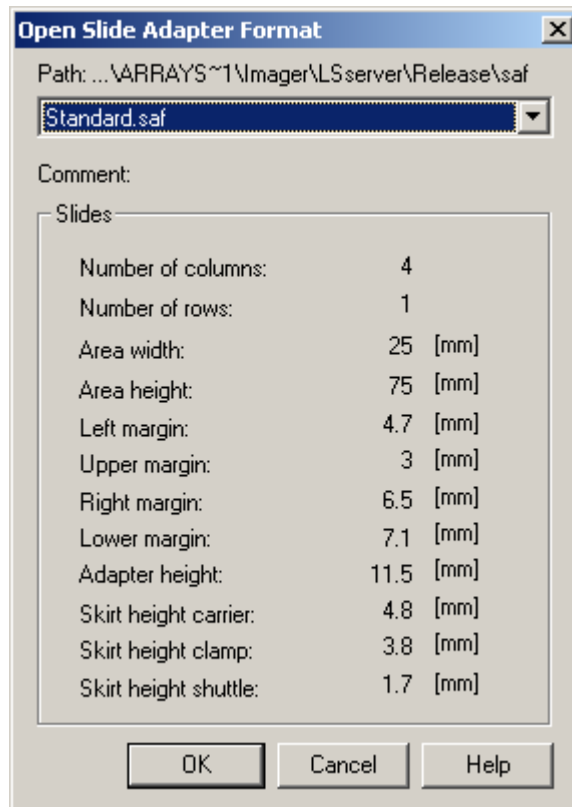
The **Open SAF** dialog box is used to load special slide adapter files. For further information about creating and editing Slide Adapter Format files refer to the chapter Advanced Menu - Edit SAF… on page 6-85 description in this manual.

In the **Open Slide Adapter Format** dialog box the last loaded adapter file and its contents are displayed:

A drop-down list contains all of the available **Slide Adapter Format** files.

Selecting a file shows all file data in the **Comment:** text and **Slide** group box.

Click **OK** to set the selected **Slide Adapter Format** file as the current file format and close dialog box.

The **Open Slide Adapter Format** dialog box contains the following elements:

File combo box	In the **File** combo box all Slide Adapter Format files found are displayed. File data is shown as **Comment** in the **Slide** group box.
Comment: static text	In the **Comment** static text box a comment, describing the slide adapter format, is displayed.
Slides group box	In the **Slide** group box slide format data corresponding to selected files in **File** combo box are shown:

- **Number of columns**
- **Number of rows**
- **Area width**
- **Area height**
- **Left margin**
- **Upper margin**
- **Right margin**
- **Lower margin**
- **Adapter height**
- **Skirt height carrier**
- **Skirt height clamp**
- **Skirt height shuttle**

OK button	Click the **OK** button to set the selected file as current file and close the dialog box.
Cancel button	Click the **Cancel** button to close the dialog box. The selected options are not saved.
Help button	Click the **Help** button to display the online help for the dialog box.

Edit SAF…

The ***Slide Adapter Format*** dialog box is used to edit or create Slide Adapter Format files. Each file consists of data which represents a copy of a standard slide adapter or microplate (MTP), for example: GREINER 96 well or NUNC 96 well. To enter and save adapter parameters the ***Slide Adapter Format*** dialog box must be displayed.

In the *Advanced* menu, select **Edit SAF…** and the ***Slide Adapter Format*** dialog box appears:
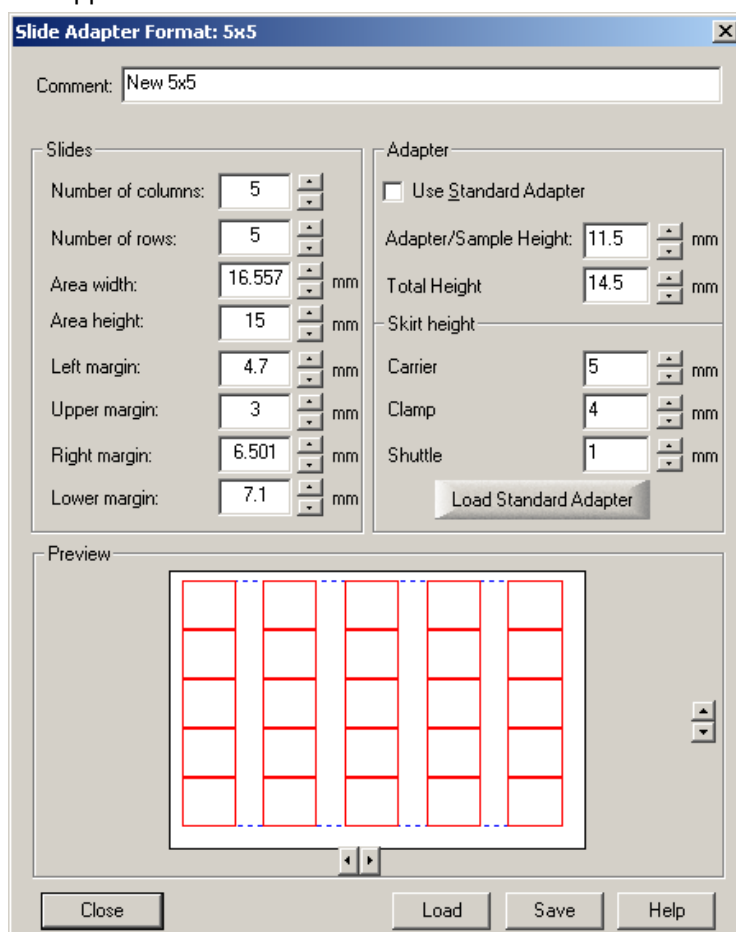
In the ***Slide Adapter Format*** dialog box all parameters, defining a standard slide adapter or a microplate, must be entered:

- Number of slides (columns and rows – max. 14 x 9).
- Geometry settings (width [min. 9 mm], height [min. 9 mm], margins):

  For determining squares and distances keep in mind that the point of origin is top left. Enter values of distances in mm (2 digits may be included) for the specific parameters.

The **Slide Adapter Format** dialog box contains the following elements:

Title bar   In the **Title** bar the name of the loaded adapter file is displayed.

Comment edit box   In the **Comment** edit box the user can enter special information relating to the provided slide adapter.

Slides group box   In the **Slide** group box the slide adapter format must be defined.

Rectangular areas must be partitioned in rows and columns. The number of elements per row and columns must be typed in the:

- **Number of columns** controls
- **Number of rows** controls

The dimensions of individual areas are determined in the:

- **Area width** controls
- **Area height** controls

The overall dimensions of the scanned surface can be entered:

- **Left margin** controls
- **Upper margin** controls
- **Right margin** controls
- **Lower margin** controls

Adapter   Select **Use Standard Adapter** to set all adapter height data to default values (equivalent to the Standard Tecan Slide Adapter).

Adapter / Sample Height spin control

In the **Adapter / Sample Height** spin control the adapter height must be entered:

The height of the Standard Tecan Slide Adapter is 11.5 mm.
The thickness of the slide that is inserted into the adapter must be edited as well.

Total Height spin control

In the **Total Height** spin control the total adapter height must be entered.

Caution
A Total Height outside of the recommended range can damage the sample and/or the instrument.
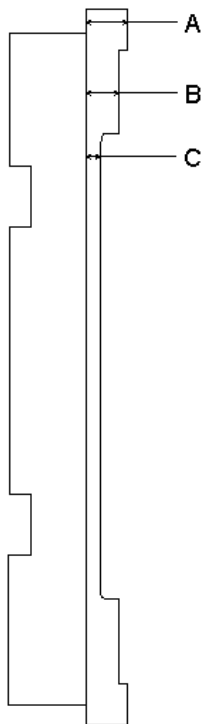
Figure 6-11 Skirt heights

Skirt height   In the **Skirt height** group box the skirt dimensions of the slide adapter must be entered.

Carrier controls

Enter the carrier skirt height (**A** in *Figure 6-11 Skirt heights*).

Clamp controls

Enter the clamp skirt height (**B** in *Figure 6-11 Skirt heights*).

Shuttle controls

Enter the shuttle skirt height (**C** in *Figure 6-11 Skirt heights*).

Click the **Load Standard Adapter** button to set all adapter data to default values (equivalent to the Standard Tecan Slide Adapter).

Preview group box   In the **Preview** group box the adapter format which corresponds to the entered parameters is displayed.

The **Preview** window displays the adapter format according to the data entered: A frame is generated with single sub-areas called *scan areas*. The parameters of a single scan area are represented in the *Geometry Parameter Tab* of the *Measurement parameter* dialog box of LSServer interface.

Close   Click the **Close** button to save the entered slide data and close dialog box.

Load   Click **Load** to open the standard *Windows* **Open** dialog box. *Slide Adapter Format* (.saf) files already available are shown in the file area. To select a file to be loaded, click on the item or enter its name into the **File name** edit box. Click **OK** to load the SAF file.

One *Adapter Format* file, corresponding to the Standard Tecan Slide Adapter, is already installed: **standard.saf**.

All special adapter format files are loaded from / saved in:

- the default directory created after the installation of the LSServer software: C:\Program Files\Common Files\Tecan\Reader\SAF
- or in a user defined directory

Save   Click the **Save** button to open the standard *Windows* **Save** dialog box. After entering the file name and clicking **OK** all adapter data is saved.

Help   Click the **Help** button to display the online help for the dialog box.

Log Ole Interface

In the Instrument menu, select **Connect** to connect the instrument.

In the Advanced menu, select **Log Ole Interface**.

The **Log Ole Interface** option disables / enables the logging of LSServer data.

When the **Log Ole Interface** option is selected (√), the **LSServer** data log will be activated.

When the **Log Ole Interface** option is cleared, the **LSServer** data log will be deactivated.

Show Logfile

In the Instrument menu, select **Connect** to connect the instrument.

In the **Advanced** menu, select **Show Logfile**.

The **Show Logfile** option starts the standard Windows editor (i.e. notepad) and shows the LSServer log file.

When the **Show Logfile** option is selected (√), the **LSServer.log** file will be opened**.**

When the **Show Logfile** option is cleared, the **LSServer.log** file will not be opened**.**

Lamp Control…

In the Instrument menu, select **Connect** to connect the instrument.

In the Advanced menu, select **Lamp Control…**

The **Lamp Control** dialog box is used to select lasers and laser power.

For further information refer to the chapter Laser Control description in this manual.

In the **Lamp Control** dialog box specific lasers (1-4) can be activated / deactivated and laser power can be set (Laser 4).

Help Menu

About

In the **Help** menu, select **About…**.

Information about the Scanner Shell program, the version number and Copyright are displayed in the **About** dialog box.

6.4.7   Stacker Setup - Twister

General

The **Tecan LS SCANNER** instrument can operate in manual and automatic mode. For automation purposes a stacker instrument such as the **Twister** must be connected.

For further information about using the Twister, refer to Twister manuals: **The Twister User's Guide and The Twister Service Manual**.
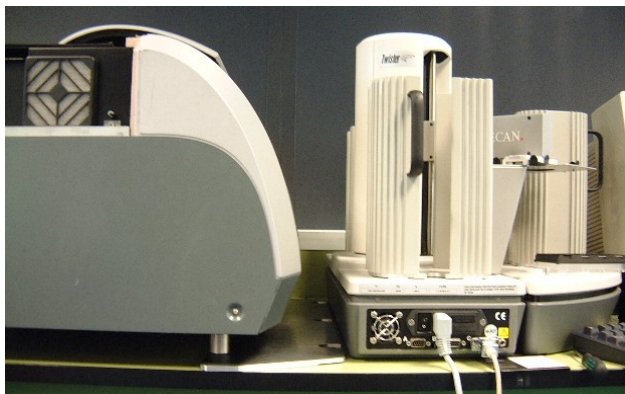
Twister connected to LSx00 scanner

The following modifications have to be performed:

1) Set up the Twister Bottom Plate as described in the Twister Manual.

2) Unscrew the instrument feet from bottom of the scanner; add spacers to instrument feet (see figure below) and mount both back into the original position.
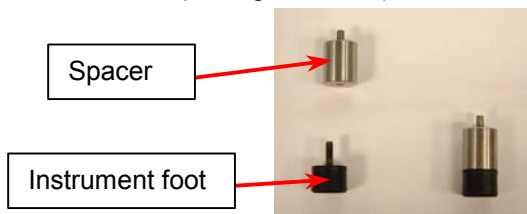
3) The Twister arm has to be slightly modified by adding Extended fingers to the Twister arm (see figure below).
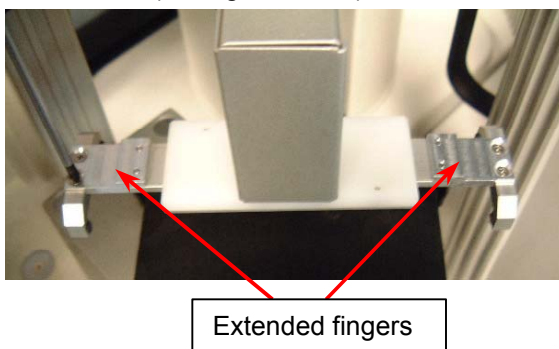
Before starting the Twister, the user must adjust some settings in order to ensure that the Twister is the stacker instrument used and that it functions optimally. The **Stacker Setup** dialog box offers all of the necessary options for setting up the Twister.

Port Setup - Twister

In the *Instrument* menu, select **Connect** to connect the instrument. In the Service menu, select **Stacker Setup…** and then click **Port Setup**. The **Port Setup** dialog box appears in which the stacker instrument and communication port can be selected:
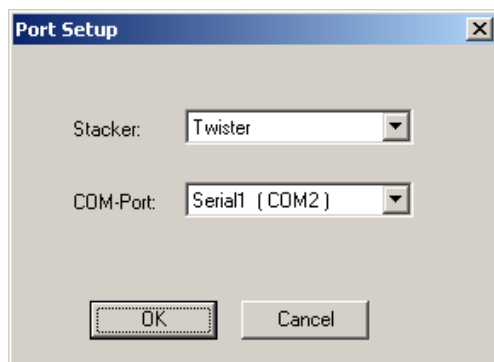
In the **Port Setup** dialog box select **Twister** from the **Stacker** combo box.

Select the communication port which is used to connect the Twister from the **COM Port** combo box. Normally the Twister is connected using COM 2, because COM 1 is reserved for other instruments such as the **Tecan LS SERIES SCANNER**.

Clicking **OK** saves all entered data and closes the **Port Setup** dialog box.

The **Port Setup** dialog box contains the following elements:

Stacker combo box	In the **Stacker** combo box the **Twister** must be chosen as the connected stacker instrument.
COM Port combo box	In the **COM Port** combo box the COM Port, into which the Twister is connected, must be selected:
	When using the **Tecan LS SERIES SCANNER** instrument, the LS Scanner instrument is normally connected to COM 1 and the **Twister** is connected to COM 2.
OK button	Click **OK** to save the selected options and close the dialog box.
Cancel button	Click **Cancel** to close the dialog box. The selected options are not saved.

6.4.8   Scanner Shell Wizards

General

The **Scanner Shell** is equipped with a number of wizards. These wizards represent workflow modules, which define a sequential procedure and guide the user step-by-step to perform a specific activity.

Standard Elements of a LS Scanner Shell Wizard

Every wizard consists of a number of windows that are displayed in succession. They provide the user with the necessary information and options to enter data.

To help the user navigate from one window to another, the following buttons are used in the individual windows of a wizard:

Back button	The **Back** button can be used to navigate back to the previous window within a wizard. In the first window of a wizard, the **Back** button is not active.
Next button	The **Next** button can be used to navigate forward to the next window within a wizard. In the last window of a wizard, there is no **Next** button.
Finish button	The **Finish** button is only found in the last window of a wizard. It must be used to close the wizard, save all changes made and finish the procedure.
Cancel button	The **Cancel** button can be clicked to close wizard. All changes made in the wizard are cancelled.
Help button	The **Help** button can be clicked to display the online help of the wizard.

The **Status Control** bitmaps display the status of operation:

 Operation in progress: Wait until finished.

 The operation has finished with no errors.

 During operation an error occurred. The user is informed about the reason for the error in a message box.

6.4.9 The Transport Lock Wizard

General

The **Transport Lock** wizard is designed to quickly guide the user through the transportation lock procedure.

From the **Service** menu select **Transport Lock…** and the **Transport Lock** wizard is started and the following dialog boxes appear in succession:

- The **Start Setting Transport Lock** window gives a short overview of the main focus of the wizard.

- In the **Position Transport** window the plate is moved in and transports are initialized.

- In the **Apply Absorber Pad** window the user is asked to insert the shock absorber pad.

- In the **End Setting Transport Lock** window, the transport lock procedure is completed.

Once the instrument is locked no further measurement is possible. To unlock instrument, the **Transport Unlock** wizard must be started. This is done automatically at every attempt to connect to a locked instrument.

For further information about **Transport Unlock** wizard refer to 6.4.10 The Transport Unlock Wizard in this manual.

The Start Setting Transport Lock window

The **Start Setting Transport Lock** screen gives a short overview of the main focus of the wizard. The static text displays information about starting **Transport Lock.** This is a purely informational window and no actions must be carried out in this window.

The **Start Setting Transport Lock** window:
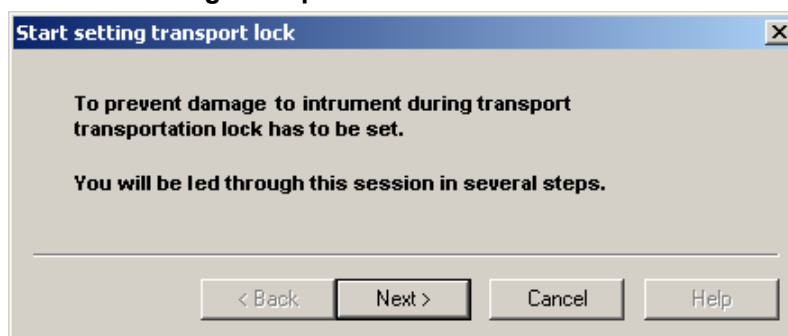
The **Next** button guides the user to the next screen of the wizard: the **Position Transport** window.

The Position Transport window

In the **Position Transport** window instrument transport is initialized and plate is moved into the **Transport Lock** position.

Click **Next** to move the plate in and initialize transports.

Status Control bitmaps show the results of the operations.

The **Position Transport** window:
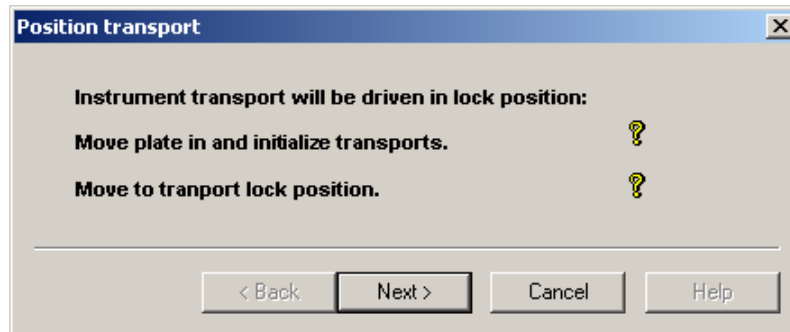
The **Next** button guides the user to the next screen of the wizard: **the Apply Absorber Pad** window.

The Apply Absorber Pad window

In the **Apply Absorber Pad** window the user is instructed to put the transportation shock absorber pad into the instrument and informed that the transportation lock will be set.

The **Apply Absorber Pad** window:
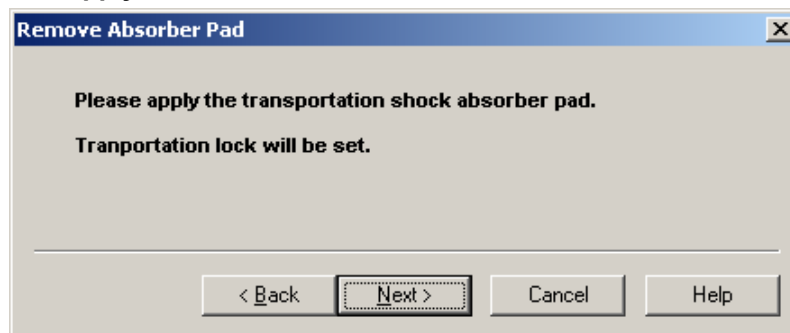
The **Next** button guides the user to the next screen of the wizard: the **End Setting Transport Lock** window.

The End Setting Transport Lock window

The **End Setting Transport Lock** window informs the user that the transportation lock has been set and instructs the user how to complete the process.

The **End Setting Transport Lock** window:
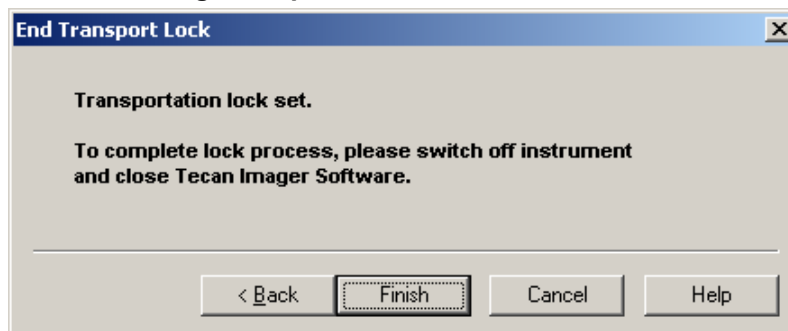
Click **Finish** to complete the **Transport Lock** procedure and close the **Transport Lock** wizard.

6.4.10   The Transport Unlock Wizard

General

The **Transport Unlock** wizard is designed to quickly guide the user through the transportation unlock procedure.

If the user tries to connect to a locked instrument, or by selecting **Instrument - Connect …** from **LS Scanner Shell** menu, the **Transport Unlock** wizard is displayed.

- The **Start Remove Transport Lock** window gives a short overview of the main focus of the wizard.
- In the **Remove Absorber Pad** window the user is asked to remove the shock absorber pad from the instrument.
- In the **Reset Transport Lock** window, lock status is removed and transports are initialized.
- In the **End Remove Transport Lock** window, the transport unlock procedure is completed.

Measurements are possible only after the transport lock has been successfully removed.

The Start Remove Transport Lock window

The **Start Remove Transport Lock** screen gives a short overview of the main focus of the wizard. A static text displays information about starting the **Remove Transport Lock** procedure. This is a purely informational window and no actions must be carried out.

The **Start Remove Transport Lock** window:
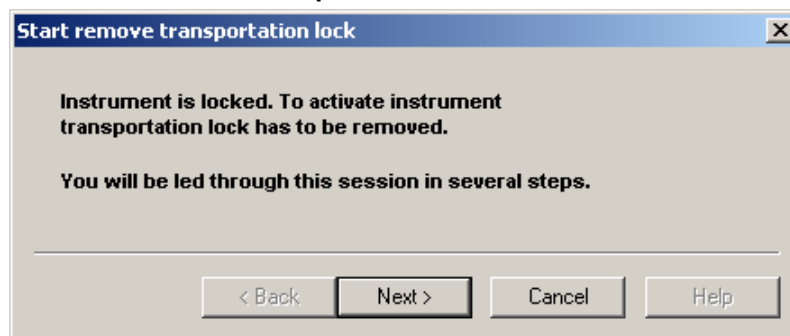

The **Next** button guides the user to the next screen of the wizard: the **Remove Absorber Pad** window.

The Remove Absorber Pad window

In the **Remove Absorber Pad** window the user is instructed to remove the transportation shock absorber pad found in instrument.

The **Remove Absorber Pad** window:
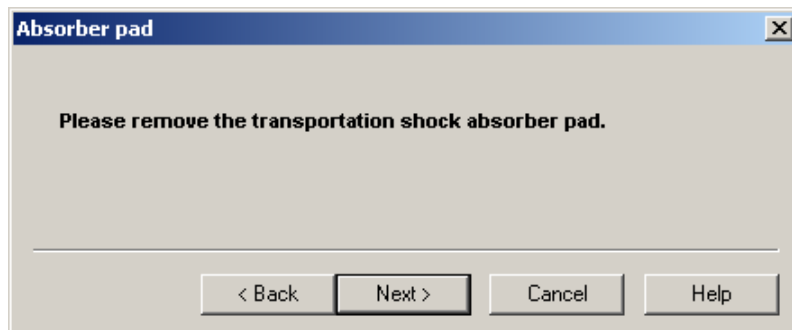
The **Next** button guides the user to the next screen of the wizard: the **Reset Transport Lock** window.

Remove the transportation shock absorber pad (see picture below)

Confirm that the transportation shock absorber pad has been removed.

Flap is closed automatically.

STOP

Caution
Caution **Do not try to open or close** **the instrument flap manually.**

Instrument is initialized and Tecan Scanner Control is now available .

STOP

Caution
Caution **Save packing materials and transport locks** **for further transportation purposes. The LS series scanners must be** **shipped with the original packing** **and the transport locks must be installed.**

STOP

CAUTION
CAUTION **BEFORE TRANSPORTATION** **Place the instrument carefully in the indicated orientation before fixing the** **transportation locks, otherwise the instrument will become damaged.**

The Reset Transport Lock window

In the **Reset Transport Lock** window lock status is removed from the instrument, transportation lock is reset and transports are initialized.

Click **Next** to start the removal of the lock status and to initialize the transports.

A **Status Control** bitmap shows the result of the operation.

The **Reset Transport Lock** window:

The **Next** button guides the user to the next screen of the wizard: **the End Remove Transport Lock** window.

The End Remove Transport Lock window

In the **End Remove Transport Lock** window the static text informs the user that transport lock has been successfully removed and that the instrument is ready for use.

The **End Remove Transport Lock** window:
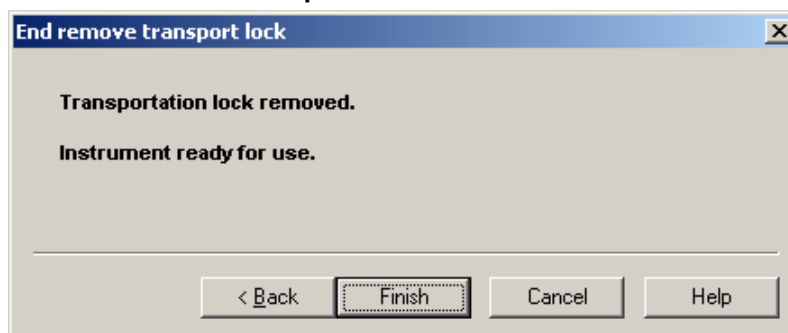
Click **Finish** to complete the **Remove Transport Lock** procedure and close **Transport Unlock** wizard.

The Gain Calibration Wizard

The **Gain Calibration** is needed to take into account the differences in sensitivity between PMTs used in **Tecan LS SERIES SCANNER** instrument. For each PMT found in instrument the **Gain Calibration** must be done.

Tools needed for gain calibration are delivered with each instrument:

- Scattered light glass slide
- Calibration filter slide with different OD filters
- Normal filter slide

Pay attention when loading the filter slides:

- Gain Calibration PMT 1: Put the calibration filter slide in filter slot 1 and the normal filter slide in filter slot 2.

- Gain Calibration PMT 2: Put the calibration filter slide in filter slot 2 and the normal filter slide in filter slot 1.

> *Note*
> ***To prevent damage to a PMT which has not been calibrated, a normal filter slide must be loaded in the corresponding filter slot.***

The **Gain Calibration** wizard is designed to quickly guide the user through the gain calibration procedure. The following dialog boxes appear in succession:

- The **Start Gain Calibration** window asks the user to put special slide(s) into the instrument.

- The **Prepare Gain Calibration** window prepares the instrument for performing the gain calibration.

- The **Measurement Gain Calibration** window carries out the gain calibration.

- The **Start Gain Calibration** window, the **Prepare Gain Calibration** window and the Measurement **Gain Calibration** window are repeated during gain calibration depending on the number of PMTs available in instrument.

- The **End Gain Calibration** window saves the gain calibration data and finishes the gain calibration.

For further information refer to the chapter 6.4.6 Service Menu - Gain Calibration… description in this manual.

The Start Gain Calibration window

The **Start Gain Calibration** screen asks the user to insert specific special slide(s) into the instrument:

- Scattered light glass slide
- Calibration filter slide with different OD filters

The **Start Gain Calibration** window:
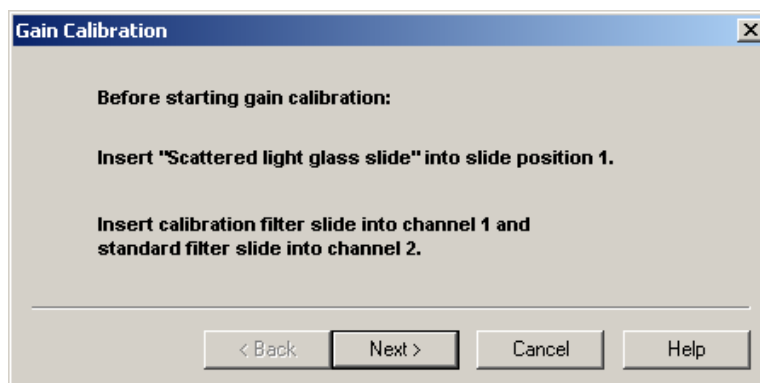
Click **Next** to confirm that the special slides have been loaded and the user is guided to the next screen of the wizard: The **Prepare Gain Calibration** window.

The Prepare Gain Calibration window

In the **Prepare Gain Calibration** window the **Tecan LS SERIES SCANNER** instrument is prepared for gain calibration.

The following steps are carried out:

- Check for the correct filter slides.
- Set special device parameters.
- Perform an Autofocus scan.
- Prepare for gain calibration measurement.
- Perform test scan.

A **Status Control** bitmap shows the result of the operation.

The **Prepare Gain Calibration** window:
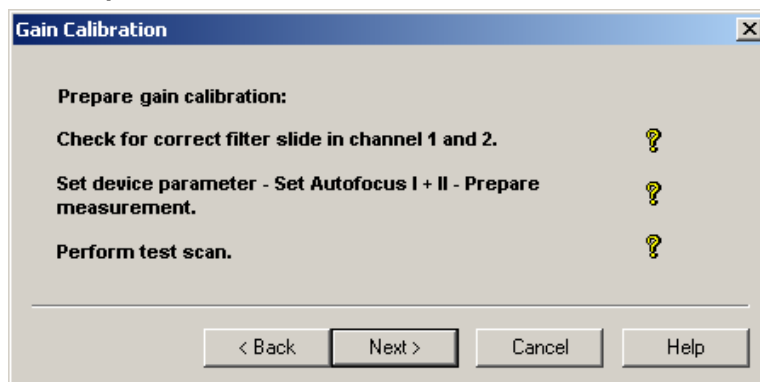
Click **Next** to start the procedure and the user is guided to the next screen of the wizard: The **Measurement Gain Calibration** window.

The Measurement Gain Calibration window

In the **Measurement Gain Calibration** window the gain calibration is done: Intensity measurements are performed with gains between 255 and 70 and the calibration data is calculated.
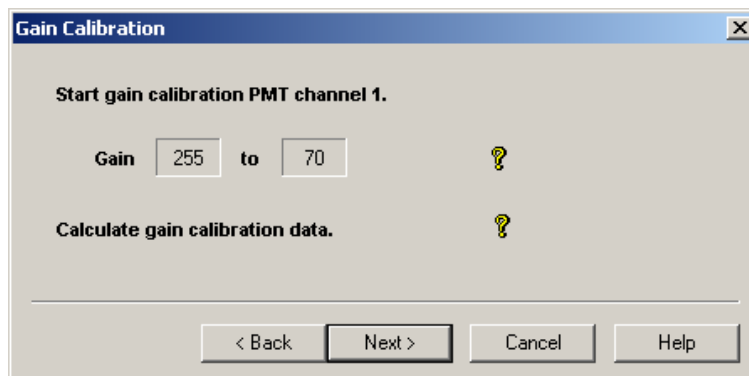
Gain static text box	The **Gain** static text box shows the maximum gain value. Gain is measured from 255 to 70.
to static text box	The **to** static text box shows the current gain value just measured. It finishes at value 70.
Measurement Progress Bar	The **Measurement** progress bar indicates the progress of the ongoing process.
Stop button	Click **Stop** to stop the measurement. A message box is shown. Click **Cancel** to continue the measurement.

Clicking **Next** starts gain calibration and guides the user to the next screen of the wizard: The **End Gain Calibration** window.

The End Gain Calibration Window

When the **End Gain Calibration** window appears, gain calibration has been finished successfully. All calculated gain data is saved in files called GainCalibration.dat and Gaincalibration.cvs.

To view the gain calibration file, load the Gaincalibration.cvs file in a spreadsheet program such as Microsoft Excel.

The **Gain Calibration** window:
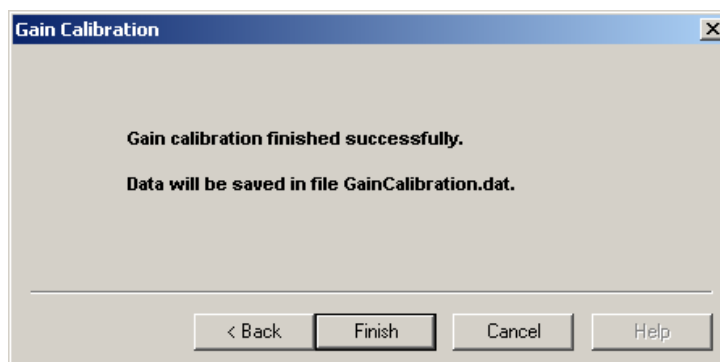
Click the **Finish** button to complete the **Gain Calibration** procedure and close the **Gain Calibration** wizard.

6.5 LSDownload

The LSDownload Software is used to download firmware to the instrument.

6.5.1 Starting LSDownload

Close all programs using Tecan LSServer.

Click the **LSDownload** icon in the **Tecan** program group.

The **LSDownload** window is displayed:
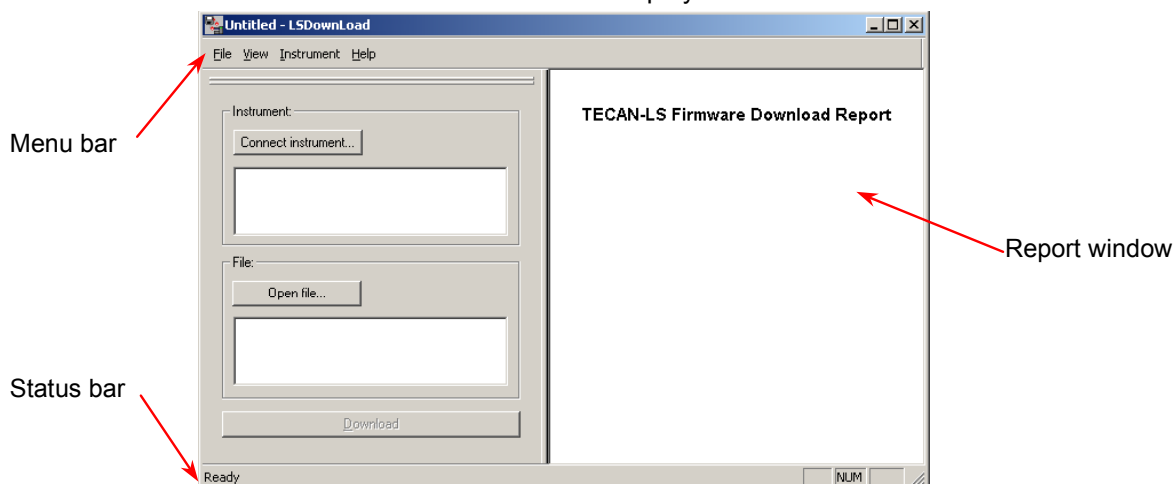
The **LSDownload** *Report Window* contains a menu bar, the report area and the status bar.

The window area displays the **Tecan Firmware Download Report**.
For example: **LS SERIES SCANNER Download**.

User interactions are performed in separate windows that are displayed if a menu is selected or if a toolbar button is clicked.

The **LSDownload** dialog box contains the following elements:

Instrument group box	• **Connect instrument…** button
	Click the **Connect instrument** button to open the **Setup Port** dialog box.
	The Setup port dialog box serves to set up options for connecting instruments: - the desired **instrument** and the communication **port** are chosen.
	• **Status** text box
	In the **Status** text box the currently connected instrument is displayed. The Firmware Version and the Serial number of the instrument are shown as well.
File group box	• **Open File…** button
	Click **Open File…** to select existing Download files (files with the extension ***.upd**) from the *Open* dialog box.
	Confirm by clicking **Open**.
	• **Open File…** text box
	The **Open File** text box displays the selected Download file (*.upd file), as well as the instrument name and the version number.
Download button	Click the **Download button** to download the selected download file.

6.5.2   LSDownload Menus

File Menu

New

In **File** menu, select **New** or press **Ctrl + N** to clear all fields.

Save Report

In the **File** menu, select **Save Report…** or press **Ctrl + S** and the **Save as** dialog box appears.
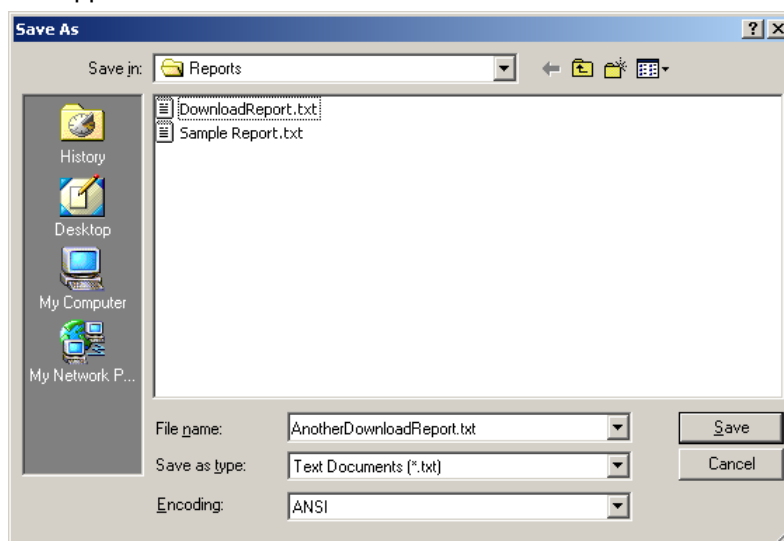
The **Save As** dialog box is the standard *Windows* file Save As dialog box. It contains the following elements:

Save in combo box	In the **Save in** combo box select the drive and the folder, in which the download report should be saved.
Workspace list box	The **Workspace** list box shows the files and folders selected in the Save in combo box.
File Name edit box	Enter the **File Name** for the download report or select one from the list.
Save as type combo box	The download report will be saved as a text file (*.txt)
Save button	Click the **Save** button to save the current download report as a text file (extension *.txt).
Cancel button	Click the **Cancel** button to close the dialog box without saving.

Print Report

To print the report of the current download select the **Print Report…** option in the **File** menu or press **Ctrl + P**.

The following dialog box is displayed:
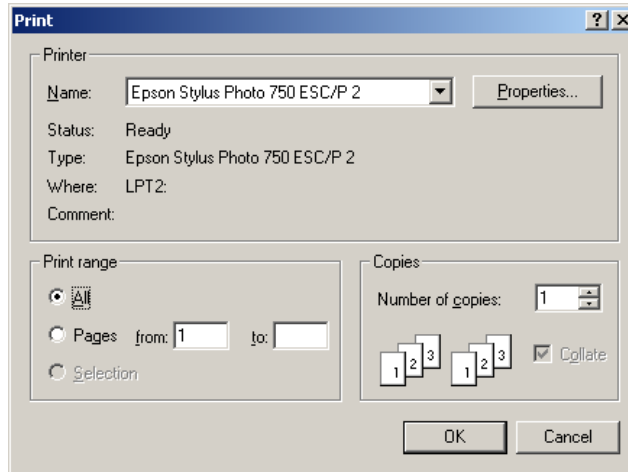
This is the standard *Windows* Print dialog box.

Click **OK** and the printing of the report of the current download is started.

Print Setup

The **Print Setup** option in the **File** menu is used to select the desired printer and set the options for the printer.
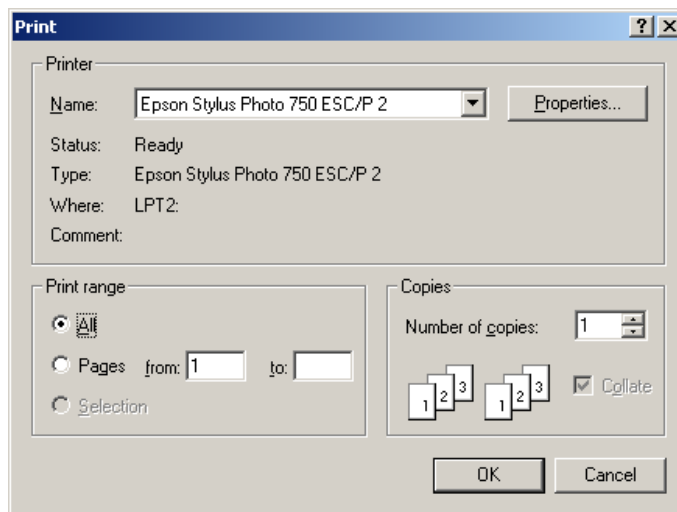
See the *Microsoft Windows* manual for more details about the *Print Setup* dialog box. When the required options are set, click **OK** to close the dialog box.

Print Preview

In the **File** menu, select **Print Preview** and the program displays the Print preview screen and the first page of the report is shown.
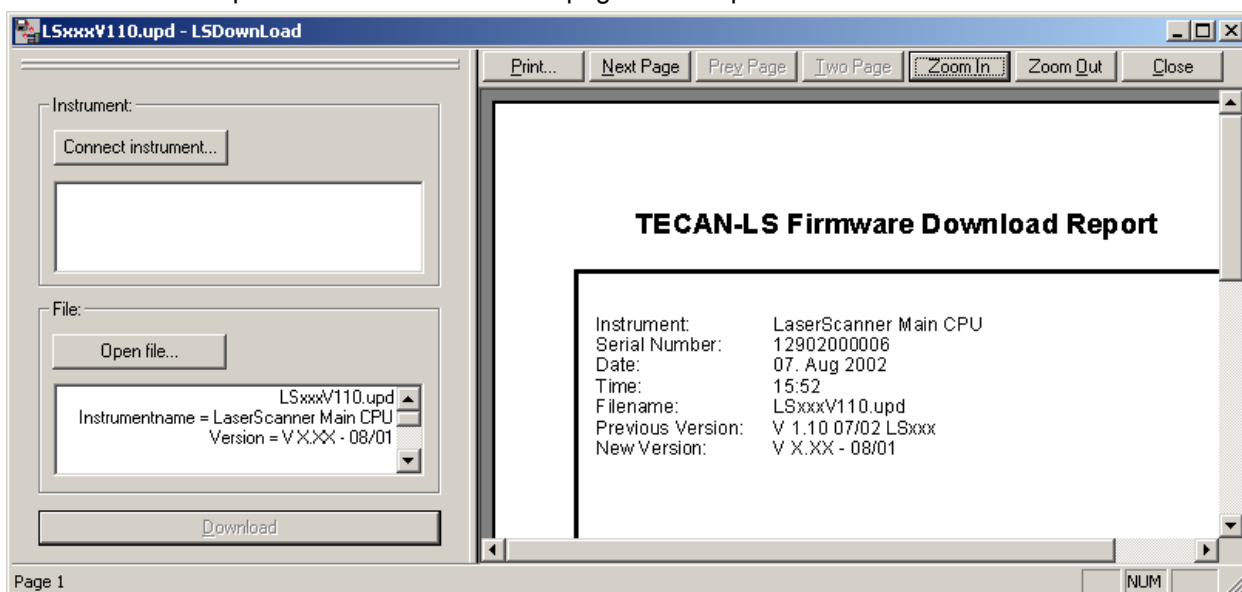
The **Print Preview** option has the following buttons:

Button	Description
Print	Selects the print option to print the report
Next Page	Displays the next page of the report
Prev Page	Displays the previous page of the report
Two Page	Displays two pages of the report simultaneously
Zoom In	Zooms in so that a part of the report is displayed larger
Zoom Out	Zooms out so that a part of the report is displayed smaller
Close	Closes the **Print Preview** option and returns to the main screen

Exit

In the **File** menu, select **Exit** and the **LSDownload** software will be exited.

View Menu

Status Bar

In the **View** menu, select **Status Bar** to show / hide the **LSDownload** software **Status Bar**.

When the **Status Bar** option is selected (√) the **LSDownload** software **Status Bar** will be shown.

The status bar displays following information:

- Help access: Press **F1** for Help
- Activity of the keyboard toggles: CAP (caps lock), NUM (Numeric block lock), SCRL (Scroll lock).

When the **Status Bar** option is cleared the **LSDownload** software **Status Bar** will be hidden.

Instrument Menu

Connect

This option allows an instrument to be connected.

In the **Instrument** menu, select **Connect** or in the *Instrument* group box click **Connect Instrument…**or press **Shift + F5** and the **Setup Port** dialog box appears. Select the desired instrument and communication port from the drop-down lists.

For further information refer to the chapter 6.2 Tecan LS Scanner Interface description in this manual.

The desired instrument will be connected using the selected communication port. A message in the **window area** of the **LSDownload** software shows that the connection is successful or unsuccessful:

Connection	Message in report area of LSDownload
Unsuccessful	*No instrument connected!*
Successful	*LS SERIES SCANNER ….* *V…* *V…*

Open Download File

In the **Instrument** menu, select **Open Download File** to browse for a .upd file. The standard *Windows* Open dialog box appears.
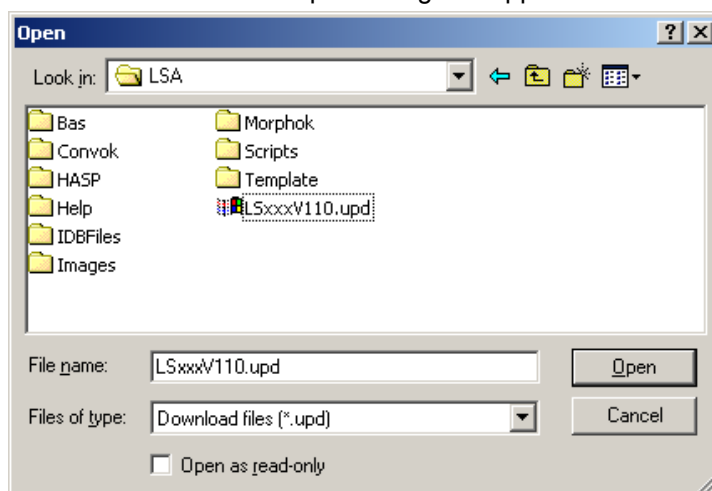
In the **Open** dialog box all existing Download files files will be shown. After selecting the requested file it will appear in the filename line. Click **OK** to load the new firmware and close the dialog box.

The **Open** dialog box is the standard *Windows* file open dialog box. It contains the following elements:

Look in combo box	In the **Look in** combo box, the folder from which the Download files can be opened, is selected.
Workspace list box	The **Workspace** list box shows the files and folders selected in the **Look in** combo box.
File name edit box	In the **File name** edit box the Download files can be selected. Either type the name of the file or use the **Look in** selection to obtain a list of file names from which the Download files can be selected.
Files of type combo box	All Tecan Download files have the file extension ***.upd**.
Open as read only check box	Select Open as read only to view the file and protect it from being changed.
Open button	Click the **Open** button to load a selected Download file (*.upd).
Cancel button	Click the **Cancel** button to close the dialog box without loading a file.
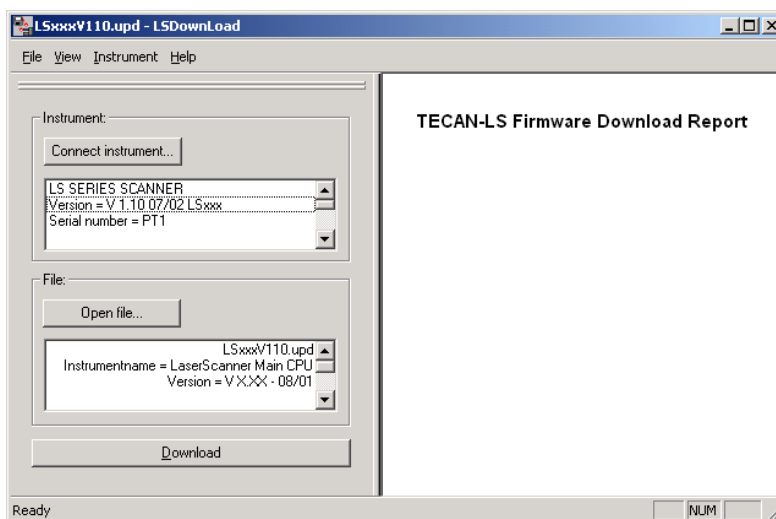
The **Open File** text box displays the selected Download file (*.upd file), as well as the instrument name and the version number.

Download

This option allows firmware to be downloaded to an instrument.

In the **Instrument** menu, select **Download** and a dialog box appears which recommends to stop all programs that are currently running.

The desired data will be sent to the connected instrument. After the download has successfully finished a dialog box appears instructing the user to switch the instrument off. The result is shown in the report area of **LSDownload** software:
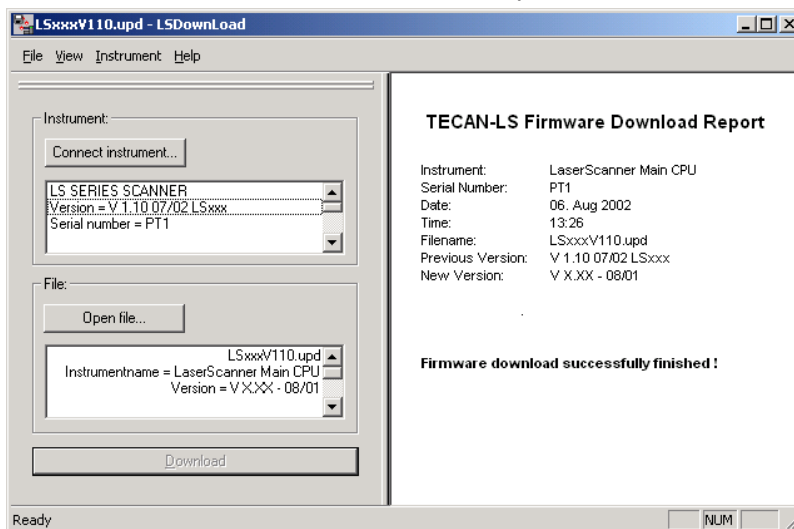
A message in the **window area** of the **LSDownload** software shows that the download was successful or unsuccessful:

Download	Message in report area of LSDownload
Unsuccessful	Firmware download failed!
Successful	Firmware download successfully finished!

Disconnect

In the Instrument menu, select **Disconnect** to disconnect the currently connected instrument. Another instrument can then be connected.

This option is only available, if an instrument is already connected.

Help Menu

Contents

In the **Help** menu, select **Contents** and the **Online Help for LSServer, Scanner Shell & Download** is displayed. Or press **F1** to view the online help for the current dialog box.

About

In the **Help** menu, select **About** and the **About** dialog box appears:
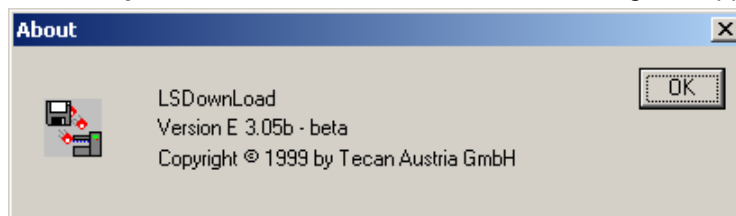
The software version and copyright information can be viewed.
Click **OK** to close this dialog box.

7. Instrument Features

7.1 Specifications

The table below lists the technical specifications for the instrument.

Parameters	Characteristics
Measurement	Software controlled by external PC
Image processing	Integrated Software package Array Pro
PC	Delivered together with the instrument
Operating system	Windows 2000
Interface	Serial interface RS 232 and high speed data acquisition connector
Data acquisition	High speed 16 bit A/D conversion
Samples to be measured	Biochips on microscope slides and user defined substrates up to the size of microplates
Slide adapter	Holds 4 slides and allows automatic loading with microplate handling equipment

Parameters	Characteristics
Optical	
Light sources	2-4 Lasers
	633 nm HeNe, 532 nm solid state, 594 nm HeNe, 488 nm solid state
Fluorescence Detector(s)	Low dark current photo-multiplier tube(s)
Gain adjustment	70-255 units
Optics	High aperture collection optics (NA 0.6) with high working distance
Working distance	max. 6.5 mm
Emission Filters	User selectable
	Held in exchangeable filter slides
	Up to 28 different filters supported by the instrument
	Additional filters can be defined and added by the user
Dichroic Beamsplitter	Two different beamsplitters (automatically selected)
(Dual channel instruments only)	An additional beamsplitter can be implemented
Pinholes	3 pinholes of different sizes, user selectable by software
Reference Detector	Monitors laser power
Autofocus	Automatic, user selectable parameters
Barcode reader	Optional
Mechanical	
Scanner	Scans 40 rows per second
Scan table	5-axis high precision module for scanning and automatic alignment of exact focus position
Front door	Opens automatically
Performance	
Sensitivity	< 0.1 Fluoequivalent/ μm^2 (Cy5)
Dynamic Range	5 orders of magnitude
Pixel resolution	40 µm, 20 µm, 10 µm, 6 µm, 4 µm
Reading speed	40 lines per second
	< 4 minutes for full slide (two colors for dual channel instruments)

Parameters	Characteristics
Electrical	
Power supply	Auto-sensing: 100-120 V / 220-240 V 50/60 Hz
Power consumption	250 VA
Main fuse	T 2.0 A / 250 V (slow blow)
Approvals	DIN EN 61010-1ff, UL 3101-1, C22.2 No. 1010.1
Physical	
Outer dimensions	Width: 812 mm 31.7 inches Height: 355 mm 13.9 inches Depth: 566 mm 22.1 inches
Weight	approx. 60 kg
Environmental	
Ambient temperature	
Operation	+15°C - +30°C +59°F - +86°F
Non-operation	-20°C - +60°C -4°F - +140°F
Relative humidity	
Operation	90 % non-condensing
Overvoltage category	II
Pollution degree	2
Usage	Commercial
Noise level	< 75 dBA
Method of disposal	Electronic waste (infectious waste)

7.1.1 Lasers

The LS SERIES SCANNERS are rated as a Class 1 laser product.

The Instrument contains up to four lasers rated as Class 3 laser products.

Therefore it is strictly prohibited to open the housing when power is switched on.

STOP	**Caution** **Never open the instrument when power is switched on**

⚠	**WARNING** **LASER RADIATION - DO NOT STARE INTO BEAM!** CLASS 3B LASER PRODUCT INSIDE

⚠	**WARNING** **CAUTION – THE USE OF CONTROLS OR ADJUSTMENTS OR PERFORMANCE PROCEDURES OTHER THAN THOSE SPECIFIED HEREIN MAY RESULT IN HAZARDOUS RADIATION EXPOSURE**

7.1.2 Optional Barcode Scanner

The barcode laser scanner (optional) is for the identification of barcode labels on a slide. This should not be confused with the automatic identification of filter slides (standard). The barcode device is able to read more than 20 different barcodes as Code 128, EAN-8 and EAN-13. For detailed information on barcodes please consult the corresponding manufacturer.

Note: Depending on whether your instrument is configured for barcode top or barcode bottom reading the bar code label needs to be placed on the upper or the lower side of the slide.

7.1.3 Reproduction of labels placed on the instrument

Labels placed on the rear front of the instrument

Labels placed inside the instrument when housing is opened.
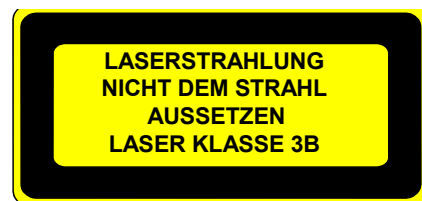
Label placed underneath lid

8. Cleaning & Maintenance

8.1 Introduction

> **Caution**
> **Ensure that the sample is removed from the instrument before it is prepared for shipment. If a sample is left in the instrument, liquids may spill onto the optical parts and damage the instrument.**

The cleaning and maintenance procedures are important in order to prolong the instrument's life and to reduce the need for servicing.

This section contains the following procedures:

- Liquid Spills
- Replacing the Fuse
- Instrument Disinfection

8.2 Liquid Spills

1. Wipe up the spill immediately with absorbent material.
2. Dispose of contaminated material appropriately.
3. Clean the instrument surfaces with a mild detergent.
4. For biohazard spills, clean with a 5-10 % solution of bleach in de-ionized water.
5. Wipe cleaned areas dry.

8.3   Replacing the Fuse

- Turn the instrument OFF and unplug the main power cable from the instrument. The fuse is located to the right of the main power cable connection in the rear of the instrument.
- Open the fuse compartment by inserting a screwdriver into the slot in the top of the cover and push the fuse holder out.
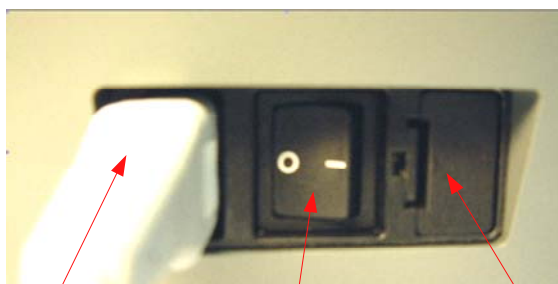
Main power socket   On/Off Switch   Fuse compartment
                           1 = ON
                           0 = OFF

- Pull the fuse out and replace the defective fuse(s) with the spare fuse(s).
- Ensure that the fuse(s) has/have the correct rating: **T 2.0 A / 250 V (slow blow)**

	WARNING
⚠	**RISK OF FIRE!** **REPLACE ONLY WITH FUSES** **OF THE SAME TYPE AND RATING.**

- Replace the fuse holder.
- Reconnect the mains cable.
- Turn the instrument on.

	Caution
🛑 STOP	**If the fuse continues to blow, call for service.**

8.4 Remote Control and Data Exchange

8.4.1 FTP up / download

For exchange of large data sets between Tecan and your company, Tecan offers an ftp site. It is accessible through

ftp://ftp.tecan.com

Contact your local dealer or email helpdesk-at@tecan.com to receive login information (user name and password) to access the ftp server.

8.4.2 Working with pcAnywhere:

When pcAnywhere is activated for the first time, the software asks you to register the software. Please follow the step-by-step wizard registration (refer also to chapter 2, page 25 of the pcAnywhere 10.5 User's Guide).

Setting up host connection

STEP	PROCEDURE
1.	Connect your analog phone line with the Tecan LS PC internal modem. The connector is on the rear of the Tecan LS.
2.	Start pcAnywhere. The software is installed in <programs -> Symantec pcAnywhere>.
3.	The pcAnywhere *Manager* dialog box appears. Click the *Hosts* button. Double click *Modem* in the Hosts window.

4. The fist time the software is started, the following warning appears:
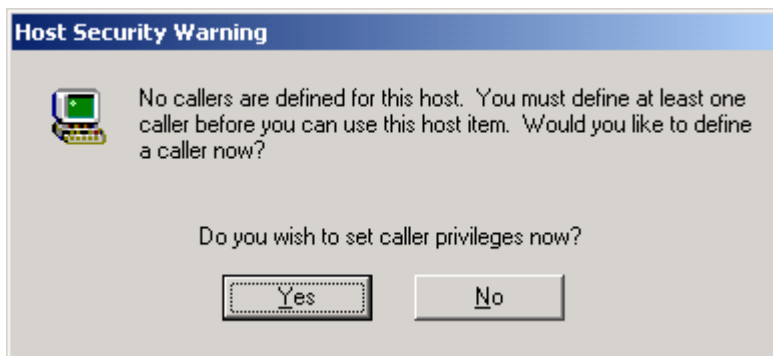
Click **Yes** and proceed with step 5.

If this message isn't displayed, continue to step 7.

5. Create a new caller. On the *Callers* tab, click the *Create* button in the *Caller list* (button is marked by the red circle on the image below).
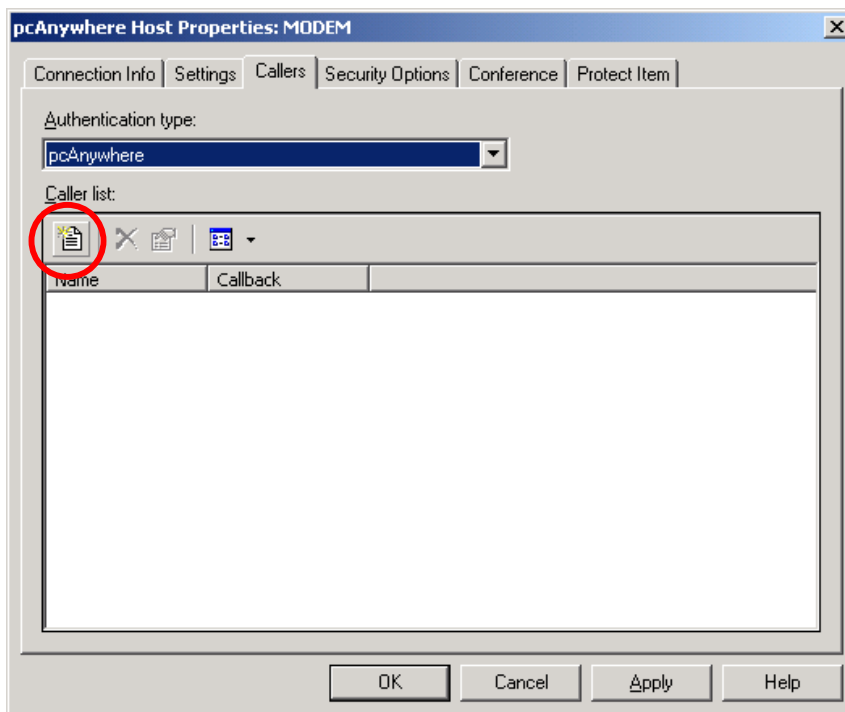
6.	A new message box pop's up. For new caller identification type in: **Login Name**: tecan **Password**: tecanls and confirm the password.
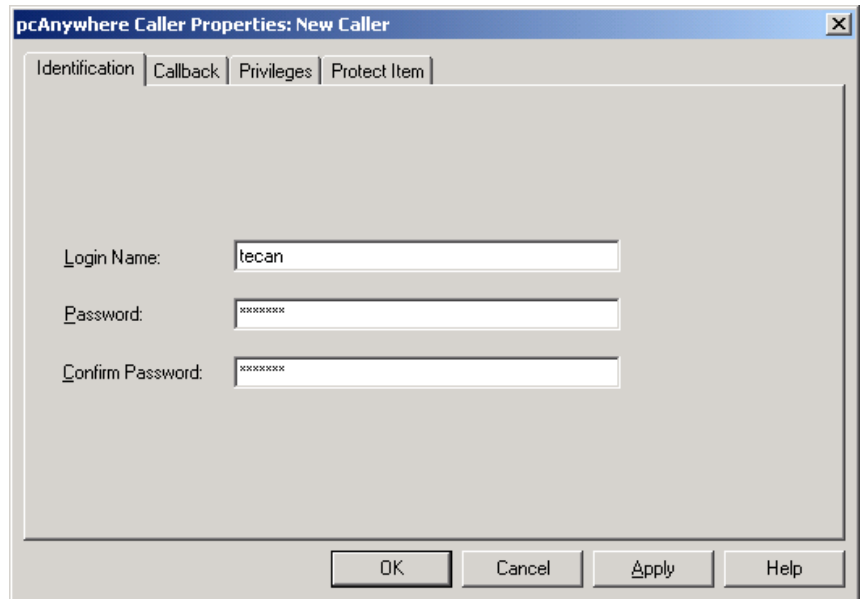
PcAnywhere's login identification is case sensitive. Please use lower case letters. If you use a different login and password as suggested, provide Tecan service with this information, before each planned start of a remote session.

7.	Click **OK** and the pcAnywhere icon appears in taskbar.

pcAnywhere is launched and is waiting for a remote call from Tecan service.

8.4.3 *Working with CITRIX web conferencing manager:*

For online demo, training, and troubleshooting between Tecan and your company, Tecan offers an ftp site. It is accessible through

http://travel.tecan.ch

Please download

Citrix ICA web client for 32-bit windows

Contact your local dealer or email helpdesk-at@tecan.com to receive login information (user name and password) to access the conference ftp server.

When Citrix conferencing manager is activated and you have entered the meeting organized by the Tecan administrator please make sure that you have shared information about the according telephone connection on both sides for voice conversation. At the very beginning of the application meeting the cursor on your screen have to be moved once by yourself in order to show it online on everybody desktop.

8.5 Instrument Disinfection

All parts of the instrument that come into contact with biological samples, or other hazardous material must be treated as potentially infectious areas.

<table>
<tr><td></td><td>Caution
It is very important that the instrument is thoroughly disinfected before it is removed from the laboratory or any servicing is performed on it.</td></tr>
</table>

Before the instrument is returned to the distributor for servicing, it must be disinfected and a disinfection certificate completed. If a disinfection certificate is not supplied, the instrument may not be accepted by the servicing center or it may be held by the customs authorities.

8.5.1 Disinfection Solutions

If the laboratory has no specific disinfection procedure, the following procedure should be used to disinfect the instrument.

The instrument should be disinfected using a solution such as:

Lysetol   Manufacturer: Schülke & Mayr Ges.m.b.H.

Aseptisol   Manufacturer: Bode Chemie Hamburg

If neither of these solutions are available 70 % ethanol should be used as an alternative.

<table>
<tr><td></td><td>Caution
The disinfection procedure should be performed by authorized trained personnel in a well-ventilated room wearing disposable gloves, protective glasses and clothing.
Please note that the disinfectant can influence the performance of your instrument if it is applied inside the instrument.</td></tr>
</table>

8.6 Disinfection Procedure

The following procedure should be used to disinfect the outside surfaces of the instrument.

1. Disconnect the instrument from the main power supply.
2. Disconnect the instrument from any accessories that are used.

   Ensure that you are wearing disposable gloves.
3. Carefully wipe all the outside surfaces of the instrument with a wad of cotton wool that has been soaked in the disinfecting solution.
4. Ensure that the same disinfection procedure is performed with the plate carrier.
5. Repeat the disinfection procedure on any accessories that are also being moved or returned.
6. After the disinfection procedure has been performed, ensure that the disinfection certificate is completed.

> ***Note***
> ***See Disinfection Certificate below***
> ***for an example of the disinfection certificate that must be completed***
> ***before the instrument is returned to the distributor for servicing.***

8.7 Disinfection Certificate

This disinfection certificate must be completed before the instrument is returned to the distributor for servicing.

The certificate must be attached to the top of the outer package in which the instrument is returned. ***It must be visible from the outside of the shipping container!***

> I declare that the instrument in this package has been decontaminated or disinfected to remove or inactivate any biological material, which could be dangerous to the service personnel, or that it has never been exposed to any hazardous biological material.
>
> Name:...
> Firm: ....
> Address: ....
> ....
> ....
> Country:..
> Signature:...

8.8   Disposal of Instrument

Because the LS SERIES SCANNERS are exposed to potentially infectious chemical samples, toxic or corrosive chemicals or radioactive chemicals, waste management of the complete instrument must be carried out to ensure that there is no risk of contamination.

WARNING
DEPENDING ON THE APPLICATIONS, PARTS OF THE LS SCANNER MAY HAVE BEEN IN CONTACT WITH BIOHAZARDOUS MATERIAL.
• **MAKE SURE TO TREAT THIS MATERIAL ACCORDING TO THE APPLICABLE SAFETY STANDARDS AND REGULATIONS.**
• **ALWAYS DECONTAMINATE ALL PARTS BEFORE DISPOSAL.**

Follow laboratory and country specific procedures for bio-hazardous or radioactive waste disposal.

Pollution degree	2
Method of Disposal	Electronic waste
	Contaminated Waste
	(infectious waste)

8.8.1   Disposal of Packing Material

According to Directive 94/62/EC on packaging and packaging waste, the manufacturer is responsible for the disposal of packing material.

Returning Packing Material

If you do not intend to keep the packing material for future use, e.g. for transport and storage purposes:

Return the packaging of the product, spare parts and options via the field service engineer to the manufacturer.

8.8.2   Disposal of Operating Material

WARNING
BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) .
OF THE PROCESS RUN ON THE SAFIRE² ABSORBANCE READER.
TREAT THE USED MICROPLATE, OTHER DISPOSABLES, AND ALL SUBSTANCES USED, IN ACCORDANCE WITH GOOD LABORATORY PRACTICE GUIDELINES.
INQUIRE ABOUT APPROPRIATE COLLECTING POINTS AND APPROVED METHODS OF DISPOSAL IN YOUR COUNTRY, STATE, OR REGION.

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