

GE Healthcare

ImageScanner III LabScan 6.0

User Manual



Important user information

All users must read this entire manual to fully understand the safe use of ImageScanner III and LabScan 6.0.

WARNING!



The WARNING! sign highlights instructions that must be followed to avoid personal injury. Do not proceed until all stated conditions are clearly understood and met.

CAUTION!

The CAUTION! notice highlights instructions that must be followed to avoid damage to the product or other equipment. Do not proceed until all stated conditions are met and clearly understood.

Notes

Note: *A note is used to indicate information important for trouble-free and optimal use of the product.*

CE Certifying

This product meets all requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

Recycling



This symbol indicates that the electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

WARNING!

This is a Class A product. In a domestic environment, it might cause radio interference, in which case the user might be required to take appropriate measures.

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1 Introduction

This user manual is intended for both beginning and expert users of ImageScanner™ III and LabScan™ for acquiring images from one-dimensional (1D) or two-dimensional (2D) electrophoresis gels. It introduces the hardware and software and gives step-by-step instructions for utilizing the main features.

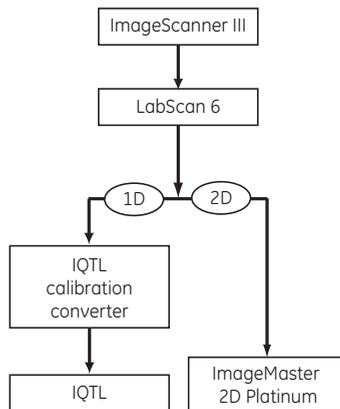


Fig 1-1. Scanning and data analysis workflow

1.1 *ImageScanner III*

ImageScanner III (Fig 1-2) is a modified version of Epson Expression™ 10000 XL.



Fig 1-2. ImageScanner III.

1.2 *LabScan*

LabScan is an easy to use software application specifically designed for acquiring images from one-dimensional (1D) or two-dimensional (2D) electrophoresis gels, using the ImageScanner III. This version of LabScan was developed by a team of researchers from the Swiss Institute of Bioinformatics

1 Introduction

1.3 IQTL calibration converter

(SIB) in collaboration with Geneva Bioinformatics (GeneBio) S.A. and GE Healthcare.

1.3 ***IQTL calibration converter***

The ImageQuant TL (IQTL) calibration converter is a software application for converting the LabScan output files to a TIFF format suitable for IQTL. It is included on the LabScan CD and is installed during the LabScan installation.

1.4 ***Software documentation***

1.4.1 **To consult the User Manual from LabScan**

- 1 Choose ***Help:User Manual*** from the menu.
- 2 LabScan opens the online document.
- 3 Click on the desired section in the ***Bookmarks***.
- 4 You immediately find the related text.

If you have trouble referring to the user manual, please make sure you have Adobe™ Reader™ installed. Adobe Reader is freely available from the following site: <http://www.adobe.com/products/acrobat/readstep2.html>.

1.4.2 **LabScan system and product information**

To quickly obtain product and system information, for your own benefit or during a technical support call, choose ***Help:System & Product Information*** from the LabScan program menu. A window shows the useful data about your LabScan version and computer system.

You can also get information about your LabScan version by looking at the release number given under ***About LabScan*** in the Help menu. Selecting ***Help>About LabScan*** displays the program splash page.

1.4.3 **Conventions used**

Before getting started with LabScan, you should have a working knowledge of your computer's operating system and its conventions, including how to use a mouse and standard menus and commands. You need to be able to adeptly open, save, and close files. If you wish to review any of these skills, please refer to the documentation that came with your computer.

Convention	Use
<i>Italic</i>	Highlights different options or parameters that are inherent to LabScan. It also draws attention to newly discussed terms.
<i>Bolditalic</i>	Highlights menu options, window names, icon names, file paths and folder names.
<i>Bolditalic:Bolditalic</i>	Indicates a menu choice. Choose the menu title and then click on the menu option.
Shift	Designates holding down the Shift key.
Ctrl	Designates holding down the Control key.
Left click	Means clicking the left mouse button.
Right click	Means clicking the right mouse button.
Double click	Means clicking the left mouse button twice.
Drag	Signifies positioning the cursor on an object and holding down the left mouse button while moving the mouse.

- 1 Introduction
- 1.4 Software documentation

2 ImageScanner III

ImageScanner III (Fig 2-1) is a modified version of Epson Expression 10000 XL. In order to be able to handle wet gels, we made some modifications to the original scanner.



Fig 2-1. ImageScanner III.

- A frame is placed around the glass plate and the body of the scanner to guide positioning of gels to be scanned.
- The scanner is sealed between the scanner body and the glass plate in order to assure moisture tightness. Nevertheless, we recommend to be careful and not to pour unnecessary liquid onto the glass plate.
- The optical distance between the bottom part and the Transparency unit (top part of the scanner) has been adjusted to afford thicker gels.
- The scanner is pre-assembled with the Transparency unit in place and tested.

2.1 Installation

Note: For complementary installation instructions, please refer to the Epson expression 10000XL Setup Guide supplied with the scanner.

Before installation of ImageScanner III the Epson Scan software must be installed, see Section 2.1.1.

2.1.1 Epson Scan software installation

Note: To control the ImageScanner III, LabScan 6.0 software uses components of the Epson Scan software. Epson Scan must be installed before starting installing LabScan 6.0.

To install Epson Scan software

- 1 Insert Epson Scan CD into the CD-ROM drive of the computer to be used. The CD contains the scanner software and the Online Reference Guide.
- 2 The installer starts automatically. If necessary, start installation by double-clicking the **EPSON CD-ROM** icon.
- 3 If a message appears to prompt you to disable the antivirus software, click **Continue**.
- 4 If a message appears to prompt you to select language, select desired language.
- 5 On the **Software Installation** screen, click **Install EPSON Scan**. Follow the on-screen instructions until the software installation is completed.
- 6 On the **Software Installation** screen, click **Install REFERENCE Guide**. Follow the on-screen instructions until the software installation is completed.
- 7 When a message appears to indicate that installation is complete, click **Exit**.

2.1.2 Unpacking.

Unpack your scanner. Make sure that all the scanner system hardware, software, cabling and documentation items are removed from the packing material.

2.1.3 Safety considerations.



WARNING! The system must be connected to a grounded power outlet.



WARNING! The power switch must always be easy to access, and the power plug must always be easy to disconnect, in an emergency situation.



WARNING! To avoid any risk of injury and damage on scanner, limited amount of liquid should be used only on the glass surface. Any liquid outside this area may cause hazard.



WARNING! If there is a risk that large volumes of spilled liquid may penetrate the casing of the equipment and come into contact with the electrical components, immediately disconnect the mains power cable of the instrument and contact authorized service personnel for further assistance.



WARNING! Never place waste containers on top of the system. If they become full and overflow, liquid may penetrate the instrument causing a hazard.

CAUTION! The system should be installed on a stable laboratory bench providing a suitable working area.

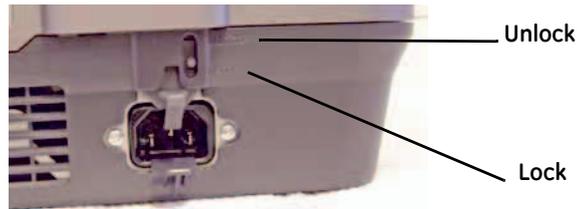
CAUTION! To maintain correct ventilation, the system requires an appropriate amount of free space. Do not block the ventilation inlets or outlets on the system!

2.1.4 Unlocking ImageScanner III

Note: Before using your scanner, ensure that it is unlocked.

To unlock the scanner

- 1 Unlock the Carriage Lock on the back of the scanner, by moving the knob from **Lock** to **Unlock** position.



- 2 Unlock the Carriage Lock on the Transparency unit (top part of the scanner) by inserting a coin in the thumbscrew and turning the arrow from **Lock** to **Unlock** position.



2.1.5 Mounting the Transparency unit

To mount Transparency unit and remove the reflective document mat



WARNING! The scanner must be turned off and the power cord unplugged when installing the Transparency unit.

- 1 The Transparency unit is supplied in a separate box.
- 2 Align the pins of the Transparency unit with the holes of the scanner.
- 3 Lower the Transparency unit until it rests securely on the scanner with the pins in the holes.
- 4 Remove the reflective documents mat from the Transparency unit by first moving it up slightly and then move it down and towards you.

Note: For complementary installation instructions, please refer to the Epson expression 10000XL Setup Guide supplied with the scanner.

2.1.6 Locking your ImageScanner III for transportation

CAUTION! To avoid damage to the scanner during transport or shipping, set the optical assembly of the bottom part and of the Transparency unit in the **Home** position and re-lock the optical assembly!

To prepare the scanner for transportation

- 1 If the scanner is powered on, turn it off.
- 2 Turn the scanner's power back on.
- 3 Wait for the scanner's **Ready** indicator to stop twinkling.
- 4 Turn the scanner off again. The optical assemblies should be in the Home position.
- 5 Re-lock the optical assembly by turning the arrow from **Unlock** to **Lock** position (see Section 2.1.4, step 2).

2.1.7 Connecting the scanner

To connect transparency unit cable

- 1 Plug the Transparency unit cable into the multi-socket connector on the back of the scanner.



- 2 Firmly tighten the screws on the end of the cable. This ensures that one end of the cable won't partially come out of the connectors.

To connect ImageScanner III to the computer

- 1 Connect ImageScanner III to the PC with a USB cable.



To connect the power



WARNING! The system must be connected to a grounded power outlet.

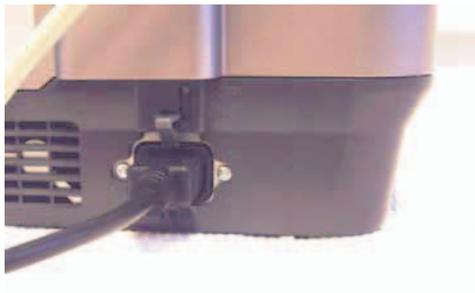


WARNING! Only use mains cables delivered or approved by GE Healthcare.



WARNING! The power switch must always be easy to access, and the power plug must always be easy to disconnect, in an emergency situation.

- 1 Plug in the scanner's power cord and turn on the power on your scanner before turning on the PC.



2.2 **Basic maintenance**

Regularly cleaning the glass plates of the bottom part and of the transparency unit will ensure that dirt or smudges will not reduce the quality of your scanned images. Before you clean the glass plates, make sure the scanner is turned off and the power cord is unplugged.

- Be sure that nothing covers the scanning area.
It is important to keep the scanning area clean since dust or smudge marks on the glass will reduce the quality of scanned gels or transparencies.
- Make sure that the gel or transparency fits in the scanning area.
- Do not leave gels on the object glass of the scanner for excessive periods of time. The warmth of the scanner and the transparency adapter lamps may cause them to shrink and deteriorate.
- Clean the glass plates with a tissue moistened with 70% Ethanol or any soft detergent.

2 ImageScanner III
2.2 Basic maintenance

3 LabScan

3.1 System requirements

The minimum system requirements for installing and running the LabScan software are:

- Windows™ XP operating systems.
- Administrative privileges to install LabScan.
- At least 128 MB RAM (256 MB is recommended).
- A high quality display, with a color resolution set to 24 bit (16.7 million colors) and a screen resolution of at least 1024 x 768 pixels.
- Internet Explorer 5 (Microsoft™ Corporation), Netscape Navigator™ 6 (Netscape Communications Corporation) or Mozilla™ 1.4 (Mozilla Foundation) installed on your computer to print reports.

3.2 Installing LabScan

Note: *Epson Scan must be installed before starting installing LabScan 6.0.*

To control the ImageScanner III, LabScan 6.0 uses components of the Epson Scan software that is delivered with the ImageScanner III. See Section 2.1.1 for installation instructions for Epson Scan software.

The LabScan software can either be installed on a PC from a CD-ROM or from a setup file downloaded from the Internet. When you insert the CD-ROM into the appropriate drive on your computer, the installation program starts automatically and guides you through a series of screens. Alternatively, you can double click on the icon of the setup file (for example LabScan_v600.exe) to launch the installer.

The LabScan installer creates a default directory tree on your hard disk, called **Program Files\GE Healthcare\LabScan**, in which the application files are placed. If this default file path is not suitable, browse for an alternative path before continuing the installation.

Once the installation is complete, it is recommended to restart your computer.

3.3 Starting and exiting LabScan

LabScan is started like any other software by selecting the **LabScan** icon (Fig 3-1) from the **Programs** folder in your Windows **Start** menu. The LabScan splash page appears while the software is loading. Click on the page to make it disappear.



Fig 3-1. LabScan icon.

Select **File:Exit** in the menu to close the program. You can also click on the **Close** button in the upper right corner of the program window. LabScan lets you choose to save unsaved gel images before exiting.

3.4 LabScan window

By reading through the following pages, you will become acquainted with the LabScan program window. It is divided into three main parts, shown in Fig 3-2, which are the **Menu bar**, the **Toolbar** and the **Image Display Zone**. The Menu bar and Toolbar are discussed in more detail below.

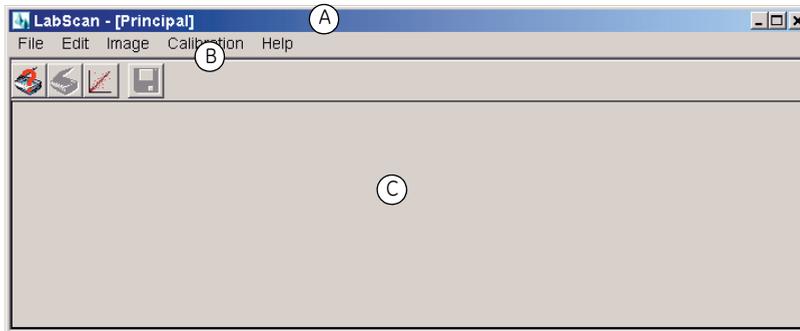


Fig 3-2. The LabScan program window. (A) Menu bar, (B) Toolbar, and (C) Image Display Zone.

3.4.1 Menu bar

Different actions may be chosen from the menu bar to be performed on the scanned image. This is done by picking the appropriate menu header and moving the cursor to the desired function while holding down the mouse button. Certain menu options have additional sub-options. You can choose one of these by moving the mouse over the small arrow found on the right hand side of the menu item.

The following menus are used to:

File	Save or print a scanned image. You can also preview a scanned image, carry out the final scan, or exit LabScan.
Edit	Undo the previous action(s), and set the scanning (mode, resolution, color filter), calibration (step tablet) and Internet settings.
Image	Obtain information about the scanned image, modify its display (flip, rotate, contrast adjustment), or display saturation.
Calibration	Create, save, load, or display a calibration.
Help	Get information about the software, access the user manual, and contact technical support.

3.4.2 Toolbar

Various options from the LabScan menu can also be accessed via the toolbar:

	The Preview tool is used to do a rapid first scan in order to define the correct settings and scan region for the final scan.
	The Scan icon is used to initiate the final scan of the object on the glass plate.
	The Calibration tool lets you create a new calibration.
	The Save As icon allows the user to save the scanned image.

3 LabScan
3.4 LabScan window

In certain modes (such as Preview, Scan, Calibration) some additional icons may appear:

	When the Hand tool is activated, you can move your image in order to show other parts of it.
	The Zoom tool lets you repeatedly zoom in (left click) or out (right click) on the whole image each time by a factor of two.
	The Region tool is useful to get a preview of the changes made during contrast adjustments.
	The Crop tool allows you to select a rectangular area on a preview of the scanned image. The crop zone can be saved, edited, and deleted.
	The Step Tablet tool is used to place the rectangles corresponding to the different gray levels on the tablet image.
	The Rotation tool is used to rotate the grid that guides you when turning an image.

4 IQTL calibration converter

The ImageQuant TL (IQTL) calibration converter makes it possible to take 1D gel images from ImageScanner III/LabScan 6 and analyze them using IQTL.

The program converts the calibration output files generated by LabScan 6 (in mel format) into a format usable in IQTL (TIFF format). The conversion performed by the IQTL calibration converter is therefore essential to run before evaluating results in IQTL. The image files contain both image data and calibration information, but the image data are not modified.

The IQTL calibration converter program is located on the LabScan 6.0 CD. The program is installed during the LabScan installation.

Convert from mel format to TIFF format

To convert scanned files to a format adapted for IQTL evaluation:

- 1 Start the IQTL calibration converter program from the Windows Start menu. Select **All programs > GE Healthcare > IQTL calibration converter > IQTL calibration converter**.
- 2 Click on **Add file(s)**.
- 3 Select the files of interest, one by one.
- 4 Click on **Convert file(s)**.

The program instantly converts the selected files into TIFF files with the letters ***IQTL*** added to the file name. The new converted files are automatically saved in the same folder as your input files. The output files are ready to use for evaluation in IQTL and contain necessary calibration data in the proper format.

4 IQTL calibration converter

5 Image scanning

5.1 General procedure

You need to capture an image with the ImageScanner III, for subsequent analysis with LabScan one-dimensional (1D) or two-dimensional (2D) image analysis software.

Note: *As gray level calibration is critical for quantitative gel analysis, make sure to use LabScan 6.0 (and not Epson Scan) for acquiring your images.*

To scan an image

- 1 Choose the settings for the scanner.
- 2 If required: carry out a scanner calibration.

Note: *The gray level calibration must be performed on a regular basis (once every month). When the previous calibration becomes obsolete, there is a reminder that lets you know that it is necessary to recalibrate the scanner using a step tablet.*

- 3 Place your gel(s) on the scanner.

Note: *Do not place it close to the left edge of the glass plate - data will be lost!*

- 4 Preview the image to be scanned.
- 5 Do the final image scan.
- 6 Save the scanned image.
- 7 Remove the gel(s) from the scanner
- 8 Close the transparency unit.

5.2 Scanner settings

First of all, you need to choose the settings for the scanner.

To change the scanner settings

- 1 Choose **Edit:Settings** from the main program menu.
- 2 Adjust the desired settings under the **Scanning (Mode, Resolution, Color)** and **Calibration** (various file paths) tabs. Further details about these settings are found in Section 5.2.1 and Section 5.2.2.
- 3 Click **OK** when finished.

5.2.1 Scanning

Mode

Choose between two different scanning modes:

- **Transparent mode**
Use Transparent mode for gels and other transparent objects.
- **Reflective mode**
Use Reflective mode to scan opaque objects.

Resolution

You must choose the scanning resolution, which is expressed in dots per inch (dpi). You have five possibilities, between 100 and 600 dpi. A resolution between 150 and 300 dpi is generally sufficient for gel analysis.

Note: *Higher resolutions (for example 600 dpi) significantly increase the file size and may slow down the analysis process.*

Color

You must choose the appropriate filter type to scan your image. Four color filters are available within LabScan:

- **Blank:** None of the colors is filtered.
- **Red:** The red channel is filtered.
- **Green:** The green channel is filtered.
- **Blue:** The blue channel is filtered.

Note: *The rule is that the scan filter color must be complementary to the sample color. You should never use the same color filter as the sample color. For example, never use a blue filter for a Coomassie stained gel or red for a silver stain.*

The following table gives some suggestions for commonly used stains. Some trial will be needed for other type of samples.

Coomassie stained gels	Red or green.
Silver stained gels	Blue or green.
Autoradiography	Blank or green.
Membranes	Blank or a color totally different from the spots or band colors.

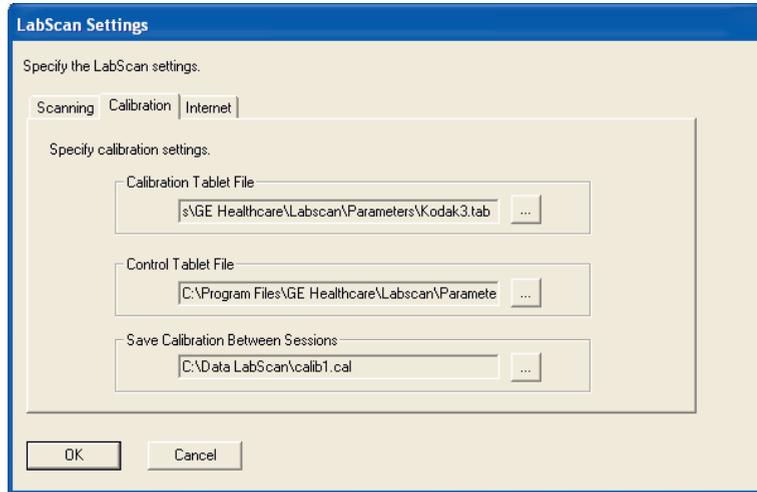
5.2.2 Calibration

The following file paths need to be entered. Please note that at least the first two should be correctly set during installation of the program.

To specify file paths

- 1 The DD values on the certificate for step tablet 3 needs to be recalculated to OD and written into the Kodak3.tab file. The DD values needs to be multiplied by 1.4 to be converted into OD values. Edit the Kodak3.tab file which is located at:
C: disk under Program files\GE Healthcare\Labscan\Parameters.
- 2 Open LabScan and go to **Edit:Settings:Calibration** and confirm that **Kodak3.tab** is selected as the Calibration Tablet File, if not change to Kodak3.tab.
- 3 Open **Edit:Settings**.
- 4 In the **LabScan Settings** window select the **Calibration** tab.

- 5 Specify calibration settings. Click the button to the right of the desired field and browse for desired files.



- **Calibration Tablet File:** Contains the step tablet definition for the calibration strip (normally the Kodak Step Tablet no. 3).
- **Control Tablet File:** Contains the step tablet definition for the control strip (normally the Kodak Step Tablet no. 3).
- **Save Calibration Between Sessions:** Contains the name of the file where the active calibration is saved when closing the current work session. This is also the file where the software reads the calibration from at the start of each new work session. Any calibration created or used during the session that should not be used as default must be saved in a separate file.

Note: *It is recommended to use a standard folder so all users easily can find and access the calibration file.*

- 6 Click **OK**.

The Calibration Tablet file and Control Tablet file should contain the appropriate information for your particular step tablets: the intensity values for the step tablet, the height and width of the tablet, the number of steps and a few other details. You can edit the files with tools such as Windows Notepad.

The Kodak Step Tablet no. 3 should be edited so that the intensity values reflect those of your calibrated tablet (see step 1).

5.2.3 Internet

Printing of tabular reports in LabScan is managed through an Internet browser. Therefore you have to set the default browser to be used for this purpose (Internet Explorer, Netscape, or Mozilla).

5.3 Intensity calibration

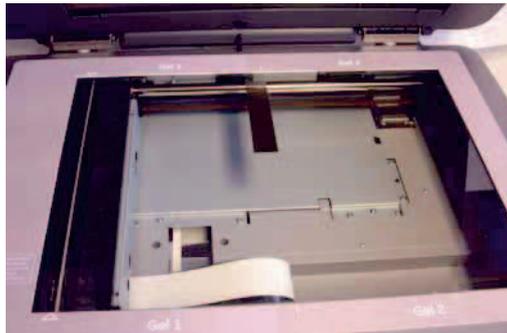
The first time you use your scanner you must do a calibration using the step tablet that was provided. Afterwards, you only need to perform this intensity calibration every month. Before first calibration, check setup (see section 5.2.2).

Note: *If you do not calibrate, darker material may not be measured in the correct proportions to the lighter material.*

To perform a calibration

- 1 Choose **Calibration:Calibrate** from the menu or click on the **Calibration** icon in the program toolbar.
- 2 You are asked to position the step tablet (no. 3). Place it as indicated in the middle of the scanner with the light end against the edge and the glossy side against the glass.

Note: *Do not place it close to the left edge of the glass plate - data will be lost!*



- 3 Click **OK** to continue the calibration process.

5 Image scanning
5.3 Intensity calibration

- 4 The image of the scanned step tablet appears in the main window, together with the red calibration step tablet tool overlay. The **Create Calibration** window is also displayed (see Fig 5-1).

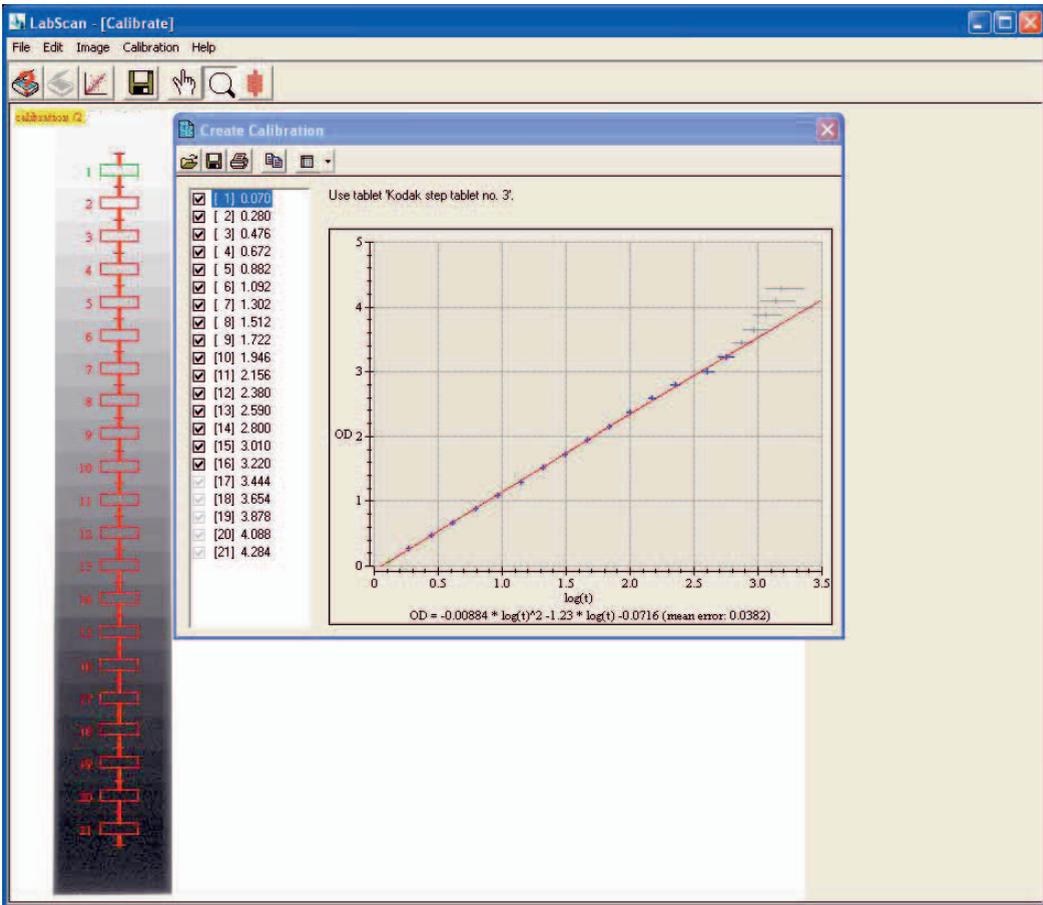


Fig 5-1. Image of the step tablet with the red calibration step tablet tool overlay. The Create Calibration window shows the calibration curve and the OD values for the different steps (on the left).

- 5 Select the **Step tablet** tool in the main program toolbar and adjust the position of the steps by dragging the overlay while holding the left mouse button. To help you differentiate the darkest steps, you can adjust the contrast (see below).

- 6 At the left of the **Create Calibration** window, you see the theoretical optical density (OD) values of the different steps in the tablet. LabScan may automatically deselect some of the steps (grayed out) because of their non reliable values, and you can deselect additional ones if you estimate that they should be excluded from the calibration process. At the right of the **Create Calibration** window you see the calibration curve between the logarithmic transmittance values on the X-axis, and the OD intensities on the Y-axis. Note that the measured intensity values for each step are calculated as median intensities over all the pixels in the small rectangle area for each step (on the step overlay). The horizontal dispersion intervals in blue (or gray for deselected spots) represent the intensity ranges when 10% of the less intense and 10% of the most intense pixel values are removed. The calibration formula and error are given below the graph.
- 7 You can additionally display some reports to judge the quality of your scanner calibration (see below for more details).
- 8 Once you are satisfied with the calibration, close the **Create Calibration** window. The software asks whether you want to validate the calibration. If you answer **Yes**, LabScan automatically saves the calibration in the default folder indicated in the LabScan **Settings** (see Section 5.2).

Note: *It is strongly recommended to use a standard folder as destination for Calibration files. Doing that enables all users access to the calibration file.*

Note: *When you select a point in the step tablet list (in the **Create Calibration** window), the corresponding step becomes automatically highlighted in green on the step tablet overlay and in the calibration graph. This makes it very easy to correlate the three sources of information (point in tablet list, point in graph, and step rectangle).*

5 Image scanning
 5.3 Intensity calibration

Please note the various icons in the toolbar of the **Create Calibration** window. These are used to:

	Open another tablet definition.
	Save the calibration under a different name (Save As).
	Print the calibration graph.
	Copy the calibration graph to the clipboard.
	Display related Reports .

Two reports are available from the **Reports** icon in the **Create Calibration** window:

- The **Fitting Report** displays the coefficients of the regression function, the error, and the number of correlated points.
- The **Calibration Report** displays for each step: the step number, the experimental averaged gray level, the theoretical intensity value, and the fitting error (difference between the curve and the point).

The Fitting and Calibration reports can be saved, printed, and copied to the clipboard:

	Save a report. Enter the File name in the displayed window and choose the desired format in the Save as type field. The reports can be saved in the LabScan report format (XML file carrying the extension .rpt), in text format with tabulations separating the columns (.txt), or as a Microsoft Excel™ Workbook (.xls).
	Print a report. The report table to be printed is first displayed in the default browser. This is because the XSL stylesheet located in the Template\Reports folder of the LabScan installation directory is used to transform the XML report into an attractive table. You can subsequently use the print option in your browser to get a paper copy.

	<p>Copy to Clipboard. You can export your table directly into another program such as spreadsheet software (Excel). To do so, select the desired lines in your table using the Shift or Ctrl keys. Then copy the selection to the clipboard by choosing the Copy to clipboard button in the toolbar or the Copy to clipboard option in the contextual menu (available by clicking the right mouse button). Directly paste into the desired software.</p>
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5.3.1 Display calibration

Normally, once you do a calibration, it remains valid for one month and you need not worry about it during that time. Occasionally, it may be useful to display the current calibration. For example, in order to verify that you are using the correct one. You can do this with the **Display Calibration** mode, which cannot be used for editing a calibration, only for viewing existing calibrations.

To open a Display Calibration window

- 1 Choose **Calibration:Display** from the menu.
- 2 A **Display Calibration** window opens, showing your current calibration.

The **Display Calibration** window is very similar to the **Create Calibration** window. It uses the same type of graphics and toolbar icons. The only differences lie in the fact that the calibration cannot be edited (no possibility to deselect any calibration steps) and that the **Open** icon allows you to open other calibration files. A step tablet definition cannot be selected as is the case in the **Create Calibration** mode.

5.3.2 Control calibration

The **Control Calibration** mode allows you to verify whether you are using a correct calibration. It requires a different step tablet (Kodak Step Tablet no. 3, for instance), which you compare to your previous calibration results.

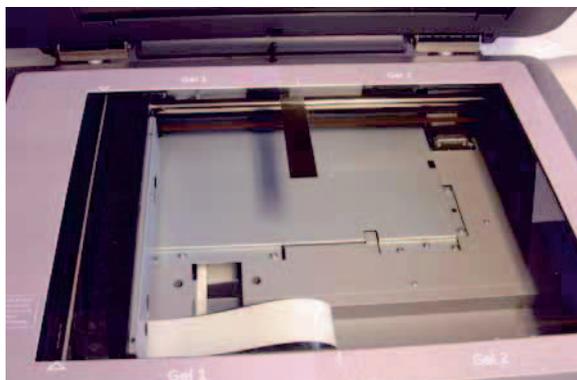
To perform a control

- 1 Choose **Calibration:Control** from the menu.
- 2 You are asked to position the appropriate step tablet (for example no. 3). Remember that the path to this tablet definition file is set in the LabScan **Settings**.

5 Image scanning

5.3 Intensity calibration

Place it as indicated in the middle of the scanner with the light end against the edge and the glossy side against the glass.

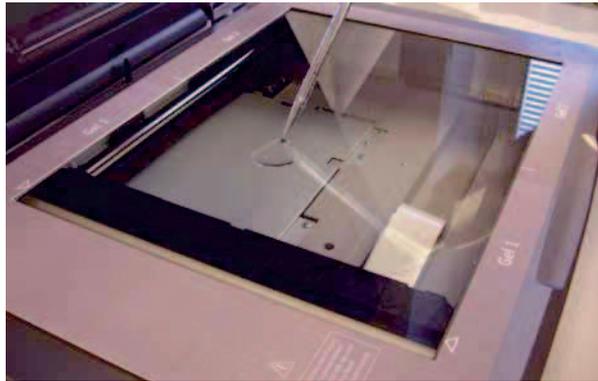


- 3 Click **OK** to continue the calibration process.
- 4 The image of the scanned step tablet appears in the main window, together with the red calibration step overlay. The **Control Calibration** window is also displayed.
- 5 Select the **Step tablet** tool and adjust the position of the steps.
- 6 You should now verify that the calibration curve is passing through the data points correctly and with minimum dispersion intervals. If this is not the case, try to find out why your current calibration does not seem to work properly.

5.4 Gel positioning

To position gell(s) in the scanner

- 1 Add 5-7 ml liquid on the scanner's glass document table, to avoid air bubbles under the gel. Use the same liquid as the gel(s) is stored in (deionized water or 7.5% Acetic acid).



WARNING! To avoid any risk of injury and damage on scanner, only a limited amount of liquid should be used on the glass surface. Any liquid outside this area may cause hazard.

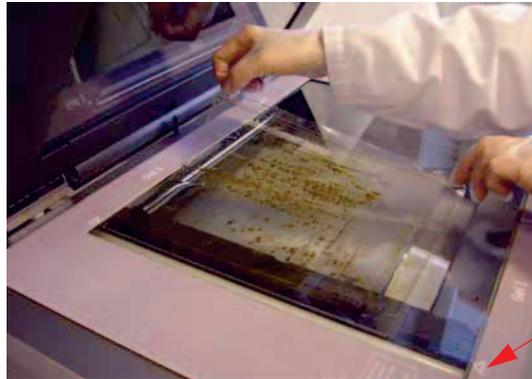


WARNING! If there is a risk that large volumes of spilled liquid may penetrate the casing of the equipment and come into contact with the electrical components, immediately disconnect the mains power cable of the instrument and contact authorized service personnel for further assistance.

5 Image scanning

5.5 Preview

- 2 Position the gel(s) directly on the scanner's glass document table, as indicated by **Gel 1** and **Gel 2**. Borders are labeled with triangles and dashes



Left scan area
marker

Note: *White balance calibration is performed on the area left of the triangles on the left end of the frame. It is critical not to cover this area with any material.*

- 3 Remove excess liquid with a tissue.

5.5 Preview

Preview allows you to rapidly inspect the object to be scanned and to delimit the zone you really want to capture. Please note that a preview image cannot be saved.

To display a preview of your image, just choose **File:Preview** from the menu or click on the **Preview** icon in the toolbar. The software displays a preview of the image to be scanned. In the preview mode, various tools are available. Most of these also function in scan mode.

5.5.1 Image information

To obtain information on the image size and resolution, choose **Image:Info** in the menu. This opens a window (Fig 5-2) containing data such as the size of the full image and the crop zone (final size of the scanned image), both in terms of pixel dimensions and memory (KB). The resolution for both the full image and the crop zone is expressed in dots per inch (dpi).

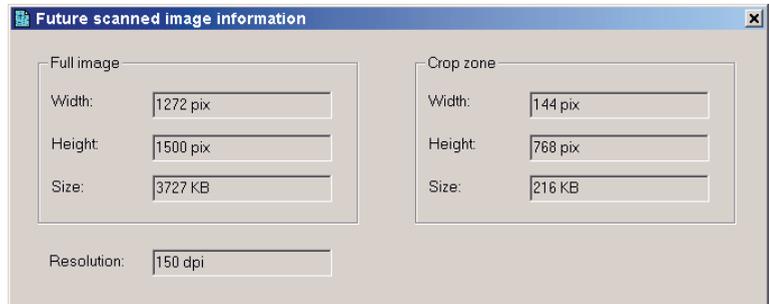


Figure 5-2. Future scanned image information window displaying the sizes (in terms of pixels and memory) of the full image and the crop zone, as well as image resolution.

5.5.2 Hand tool

The **Hand** tool is a straightforward means to move your image in the Image Display Zone, enabling you to look at a specific part of the image that may have been hidden before.

To move an image

- 1 Pick the **Hand** tool in the LabScan toolbar.
- 2 Put the cursor inside the image and hold down the left mouse button while moving the mouse. The image follows your movement.
- 3 Release the mouse when you reach the desired position.

5.5.3 Zoom

The **Zoom** tool lets you repeatedly zoom in or out on the whole image.

To zoom

- 1 Pick the **Zoom** tool in the LabScan toolbar.
- 2 To zoom in, click on the image in the desired region.
- 3 To zoom out, right click on the image.

5.5.4 Contrast mapping

Because typical computer screens are only able to display 256 gray levels, mapping has to be undertaken between the calibrated pixel gray levels in the image (65536 for a 16-bit image) and the 256 screen gray levels. By default, LabScan uses a linear mapping function for this, where the lightest point in the image is mapped to 0 (white) and the darkest point is mapped to 255 (black).

This is illustrated with the gray level histogram in Fig 5-3. The histogram displays the frequency with which each gray level (from 10247 to 65327) occurs in the image. The red line on your screen indicates that the minimum and maximum gray levels are remapped by the default linear mapping. The vertical axis for the red re-mapping graph corresponds to the 256 screen gray levels.

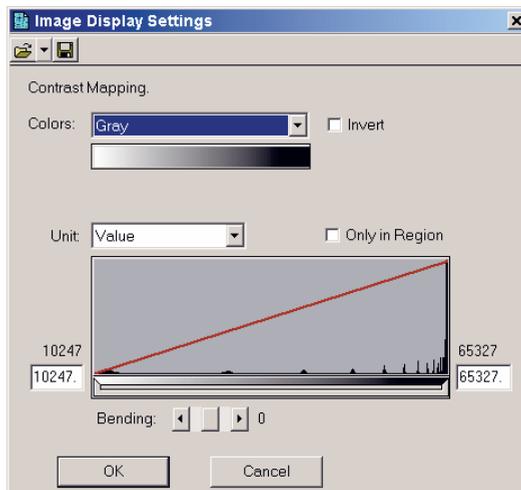


Figure 5-3. Adjusting the contrast of displayed colors.

However, you can choose other mapping functions to highlight small light spots. LabScan offers two ways to change the default gray level mapping and thus improve the visual display of the images.

You can define the minimum and maximum gray levels. That is, you can look at only the light or the dark regions of the images. Do this by decreasing the size of the gray level range to be linearly remapped to the screen gray levels. To accomplish this, move the left or right borders of the scrollbar that is found below the histogram function. Once the size (interval) of the scrollbar is decreased, you can also displace the interval by moving your cursor to the middle of the scrollbar, holding down the mouse button, and shifting to the left or right (Fig 5-4).

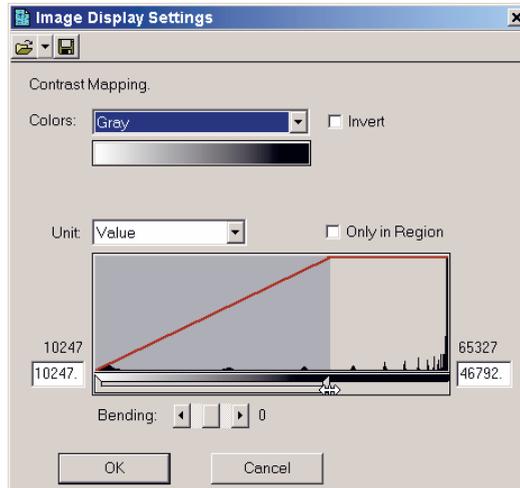


Fig 5-4. Remapping of the gray levels.

Another way to change the default gray level mapping is to use a non-linear mapping function. The **Bending** parameter expands or compresses the contrast range at the dark or light ends of the range. When bending is positive, the image is lighter. When the bending parameter is negative, the image is darker (Fig 5-5).

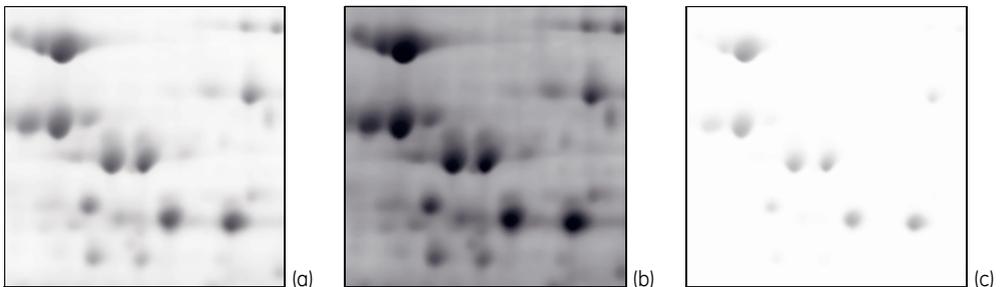


Fig 5-5. Region of a gel showing a contrast adjustment. (a) Original image with bending parameter 0, (b) same area with bending parameter set to -2, (c) same area with bending parameter set to 2.

To adjust the image contrast

- 1 Choose **Image:Adjust Contrast** from the menu.
- 2 Select the **Region** tool in the toolbar and draw a region in the image to get a preview of the contrast mapping modifications.
- 3 Select the units you would like to use from the Unit list (see below) and indicate whether you want to modify the contrast mapping as a function of the complete image range or only that of the selected region (see below).

- 4 In the **Image Display Settings** window, change the minimum and maximum gray levels by displacing the scrollbar borders, or typing in the desired numbers in the boxes at the lower left and right corners of the histogram. You can also use the Bending slider.
- 5 If you are happy with your choice, click **OK** to apply the changes to the whole image. This does not modify the raw pixel data. The change is only visual.

In some cases, you may want to save a specific mapping function so that it can be later applied to other images with similar gray level properties. To do this, click on the **Save** icon in the **Image Display Settings** window and enter a file name as well as the desired destination folder. LabScan saves the mapping function with the extension .glt (Gray Levels Transformation).

Units

Two different units are used for displaying the gray level minimum and maximum:

- **Value** uses the raw pixel counts as displayed in all the reports. When a calibration has been done, these correspond to the calibrated pixel values.
- **Percentage (%)** allows you to choose the range as a percentage of the total gray level range in the histogram.

Only in region

By checking the Only in Region box in the **Image Display Settings** window, LabScan only considers the gray levels that are present in the selected region.

The **Only in Region** option is very useful in combination with the % Units and the choice of a relatively small region. Under these conditions, you enter an adaptive mode that allows you to adjust to the local gray levels. The result is a local increase in contrast that effectively enables you to look for very faint spots. This mode can also be combined with the Gray+Saturation palette in the Colors list.

5.5.5 Pseudo colors

You may want to use a particular color palette instead of the standard gray levels in your gel. LabScan offers color palettes such as Coomassie Blue, Silver Stain, Hot Iron, Spectrum, etc. A palette that is particularly useful is the Gray+Saturation palette. It corresponds to the standard Gray option, except for the maximum value that is displayed in red and the minimum value that appears in blue. It is helpful when you would like to visualize saturated spots (red) or the background (blue). Of course, you can modify the minimum and maximum gray levels, as explained above, to decrease the stringency of what is

considered as saturation or background. Finally, you can just inverse the gray levels by checking the Invert box.

To display (part of) a gel with pseudo colors

- 1 Choose **Image:Adjust Contrast** from the menu.
- 2 Select the **Region** tool in the toolbar and draw a region in the image.
- 3 Select one of the color palettes in the **Colors** list and/or invert the gray levels by checking the **Invert** box. You can also adjust the contrast as indicated above.
- 4 To go back to the default gray levels, choose the **Gray** option from the **Colors** list.

Please note that the color palettes cannot be permanently applied to the image. When you click **OK** to close the **Image Display Settings** window, only the contrast adjustments are applied. The color settings are no longer visible.

5.5.6 Cropping

The **Crop** tool (Fig 5-6) allows you to define the zone you are to scan.

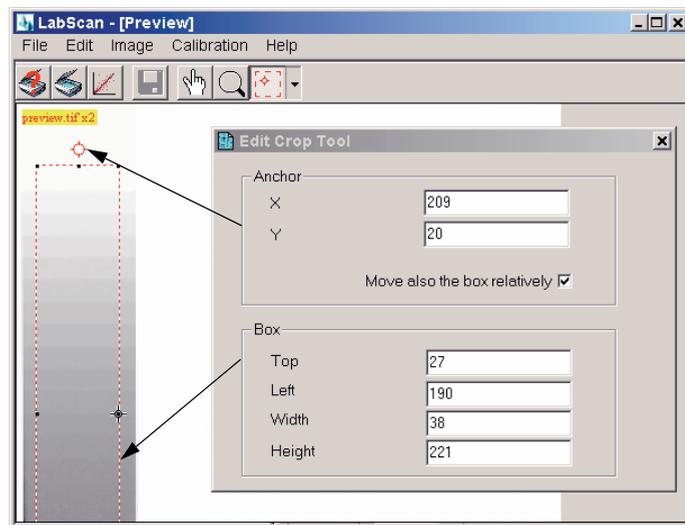


Figure 5-6. Crop tool.

To use the Crop tool

- 1 Select the **Crop** tool and draw a rectangle on the image corresponding to the region you want to scan. To modify the rectangle size, click on one of the rectangle handles (small black squares) and drag it while holding down the left mouse button.

5 Image scanning

5.6 Scanning

- 2 If you have a clear visual landmark (spot or marker) on your image, you can double click on its position to define an anchor (red circle) that can be used to reposition the crop zone in a similar way on the next gel to be scanned.
- 3 Move the crop zone by clicking in it and dragging it to another position.
- 4 You can also use the **Edit** option from the **Crop** tool drop down menu in the toolbar. This opens an **Edit Crop Tool** window as shown in Fig 5-7. Directly modify the values for the anchor and the box dimensions in this window. You can also check the option called **Move also the box relatively** that allows you to change the box position by moving the anchor, while maintaining the same separation distance.
- 5 To define a new crop zone, the old one should be removed. This is automatically the case if you reduce the size of your box so that it is less than 5 pixels large. You can also use the **Delete** option from the **Crop** tool drop down menu in the toolbar.

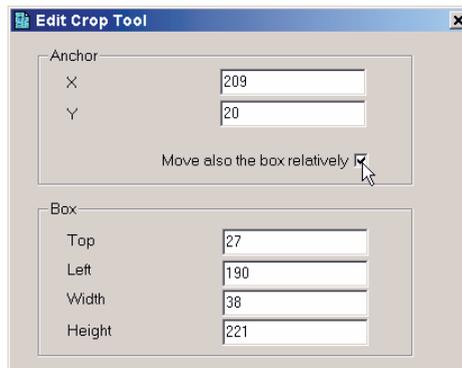


Figure 5-7. Edit Crop Tool.

Once you have set the crop area, you can save it to reload later, by selecting **Save** from the **Crop** tool drop down menu in the toolbar. In any event, the crop box and anchor are passed on (same position) to the next gel preview. You can then move the anchor to the same visual landmark. Since the box moves with the anchor, you easily scan the corresponding region.

5.6 Scanning

Once you have set the crop area in the preview, scan the selected part of the image by choosing **File:Scan** or by clicking on the **Scan** icon in the toolbar. The resulting scanned image is displayed in its entirety with the maximum possible zoom. The scanned image is then manipulated and modified with the tools mentioned above (in the Preview section) and those described hereafter.

5.6.1 Crop tool

As with Preview, the **Crop** tool is used to define a new area in the acquired image for final saving. When you use **File:Save As**, you are asked whether you want to save only the selected area. If you answer **Yes**, the image is reduced to the crop zone and saved with the new file name.

Note: *This new crop area is independent of the one defined in **Preview** mode. However, it is similarly maintained for the next scanned image.*

5.6.2 Displaying saturation

You can visualize the saturated pixels in your image. Saturated pixels have OD values higher than a threshold that is defined as the OD of the step in the calibration curve that is just above the last activated one.

To view saturated pixels (spots)

- 1 Choose **Image:Display Saturation** from the menu.
- 2 The saturated pixels are displayed in orange on the image.
- 3 Choose **Image:Display Saturation** again to deactivate the function.

5.6.3 Flipping images

Images are sometimes scanned in the wrong direction, and you may have to flip them to create their correct mirror image.

To flip images horizontally and vertically

- 1 Choose **Image:Flip** from the menu and select the desired option (**Horizontally** or **Vertically**).
- 2 The image flips.

5.6.4 Rotating images

When images are scanned in the wrong orientation, they can be rotated to correct for this. You can typically rotate your gels by 90° clockwise (90 CW), 180° and 90° counter clockwise (90 CCW). Free rotation can also be applied.

To rotate an image

- 1 Choose **Image:Rotate** from the menu and select the desired rotation angle.
- 2 Alternatively you can choose **Image:Free Rotate**.
- 3 The image appears with a grid on it. The bold horizontal grid line plays the role of landmark to help you visualize the rotation. It becomes the new horizontal in your rotated image.
- 4 Click on any position in the scanned gel and rotate the grid while holding down the left mouse button. Release the button when you obtain the desired rotation angle. You can also manually enter a rotation angle in the **Rotation Tool** box.
- 5 Click **OK** to confirm your rotation or **Cancel** to return to the initial orientation of the gel.

5.7 Undo

LabScan allows you to cancel any unwanted modifications. You can return to an earlier state of settings simply by using the **Undo** option.

Each Action Descriptor in the Undo list is preceded by the time at which the action was carried out. This information can help you to identify the particular sequence of actions that you would like to cancel.

To undo the last action performed

- 1 Choose **Edit:Undo** from the menu.
- 2 The last action is selected by default.
- 3 Click **OK**.
- 4 To cancel several successive actions:
- 5 Choose **Edit:Undo** from the menu.
- 6 Select a prior action to be undone.
- 7 When you answer **OK**, the selected action and all following actions are undone automatically.

It is not possible to undo operations such as saving and printing files.

5.8 Saving and printing

To save a scanned image

- 1 Choose **File:Save As** from the menu or click on the **Save As** icon in the toolbar.
- 2 Choose the destination folder and name for your image. Also select the desired output format (TIFF™ or ImageMaster™ format).
- 3 If a crop area was selected in the **Scan** mode, LabScan asks whether you want to save only the selected area. Answer **Yes** or **No**.
- 4 Click **OK** when finished.

To print a scanned image

- 1 Choose **File:Print** from the menu.
- 2 If a crop area was selected in the **Scan** mode, LabScan asks whether you want to print only the selected area. Answer **Yes** or **No**.
- 3 The image prints in full size.

Please note that the image prints as it is displayed on the screen, retaining properties such as contrast mapping, pseudo colors, etc.

5.9 Removing gels

To remove gel(s) after scanning

- 1 Remove gel(s) from the scanner.
- 2 Wash the scanner's glass document table with water.
- 3 Wash the scanner's glass document table with 70% Ethanol.
- 4 Dry the scanner's glass document table with a tissue.

5 Image scanning
5.9 Removing gels

6 FAQ

Workflow using ImageScanner III, LabScan 6 and IQTL Calibration Converter.

Protein concentrations may be estimated from the intensity of bands on 1-D gels. To date, however, no automated technology has been shown to deliver precise, quantitative measurements, partly due to the difficulty in converting band intensities to discrete numeric values across a sufficiently wide linear pixel intensity range. The generation of such a linear standard curve is essential for confident concentration measurements from bands in a gel and may well be a regulatory demand in settings requiring validated methods of analysis. Recent software updates to the flatbed scanner ImageScanner III™, fulfill this requirement via accurate calibration, making the instrument an excellent choice for the quantitative analyses of proteins in 1-D gels.

These FAQ's are designed to guide you through the most common questions encountered:

Q How is the ImageScanner III calibrated using step tablet 3?

A

- 1 Check your kodak3.tab file, if the values are still in DD values. The DD values are the values written at the certificate delivered together with step tablet 3. Calculate the OD values from these DD values by multiplying the DD values with 1.4. Edit the kodak3.tab-file manually to get OD values in the tab-file before starting the calibration.
- 2 Put the step tablet in the scanner, as described in the manual, with the glossy side down and towards the upper middle part of the glass plate, see figure 1.
- 3 Click on Calibration/Calibrate to start the calibration.
- 4 Move the step tablet tool so that the first step of the ladder is placed in the first step of the image of the step tablet (the biggest and lightest step). The graph should now show a straight line going through the crosses, see figure 2.
- 5 Close the window and the calibration is automatically saved.



Fig 1. Placing step tablet on the glass plate with the glossy side down.

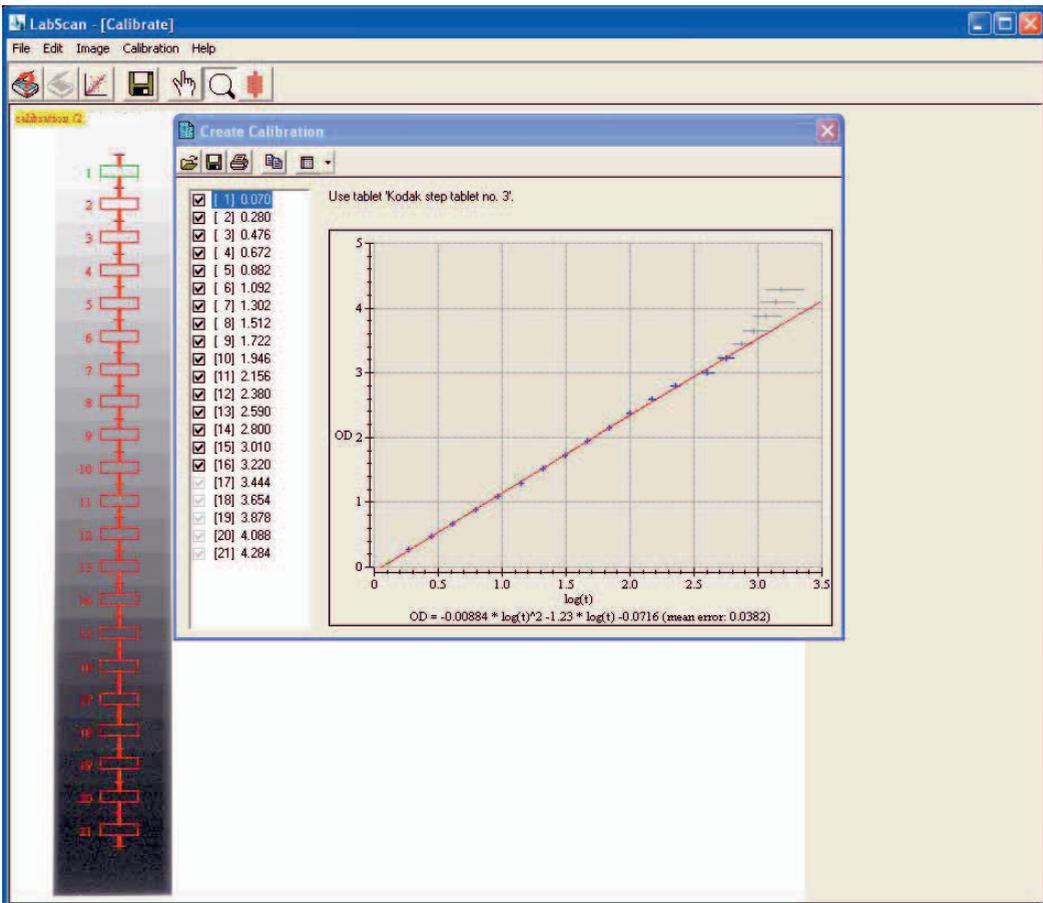


Fig 2. Showing how to place the step tablet tool at the first step in the first big step of the step tablet.

**Q Why does the line not pass through 0 when calibrating?
Why are weak bands cut off?**

A This might be caused by an earlier manual calculation error when changing DD values into OD values. By dividing instead of multiplying, the weak bands may get a negative value and thereby are cut off. The reason might also be that during the calibration procedure the wrong step has been selected as step number one on the step tablet.

Q Why is the image of a cropped area so poor? Is it not possible to save a cropped area?

A There is a bug in the LabScan 6 version so that information gets lost if a cropped area is chosen. Therefore, always answer NO and instead save the total area to ensure that you save all the information you need.

Q Can an output-file from LabScan 6 be directly used for quantification in IQTL?

A No, first the file needs to be converted by the IQTL Calibration Converter. This is because the format of the calibration data from LabScan 6 isn't readable in IQTL and needs to be converted to a format accepted by IQTL. Without the calibration data, the quantification gets unreliable. The IQTL Calibration Converter is free of charge.

Q How does one know that the points are passing through the curve correctly?

A The recommendation is:

- 1 Calibration should reach 3 OD.
- 2 The fitting error in the calibration report should be close to 0.02 in the range 1 to 2.5 OD. Below 1 OD the error is lower and above 2.5 OD the error is larger.

Q Why do I get a calibration OD lower than 3.0 or higher than 3.5?

A If the scanner does not manage to calibrate above 3.0, it may be because the scanner needs service or that something is wrong with the calibration. Check if the OD values are correctly calculated.

If the scanner is calibrated to above 3.5 OD, it may depend on an incorrectly edited Kodak3.tab file. Check if the OD is correctly calculated.

Q What is the time limit for expiry date of step tablet 3 from the day it is delivered?

A There will be no shipping regulation depending on expiry date, but the customer will be able to know the expiry date when placing the order.

Q Why are stripes visible when using reflective mode (for membranes)?

A The scanner is not developed for western blots. Western blots have a very narrow dynamic range and therefore quantification methods are not recommended. The western blots can be scanned for documentation without stripes if the filter is dry.

Q Is it possible to review a previous scan in LabScan 6?

A No, this is not possible to review directly in LabScan 6. The saved file has to be read into another program.

Q There are white bands over the image, what is it?

A Several small bands when scanning gels can be caused by a number of reasons: the lamps need to be changed, a cropped area has been saved (save the total area instead), the gel has been placed on the left edge of the glass plate, or the contrast is set high due to low intensities of the gel.

One big stripe over the image can be caused by dirt or a wet spot on the very left side of the scanning glass plate. Be sure to keep the glass plate clean around the edges.

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