Agilent G2565AA and Agilent G2565BA Microarray Scanner System

with SureScan Technology

User Guide (v 7.0)

Sixth Edition, May 2006

Research Use Only

Agilent Technologies

Notices

Manual Part Number

G2566-90017

Edition

Sixth Edition, May 2006

Printed in USA

Agilent Technologies, Inc.
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Santa Clara, CA 95051 USA

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WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide...

This user guide contains instructions to get started with, use, troubleshoot, and maintain your Agilent Microarray Scanner System.

1 Introduction

This chapter describes the Microarray Scanner, preparation for its operation, and enhancements to the software.

2 Getting Started

In this chapter, learn how to safely operate the Microarray Scanner and use the Scan Control program.

3 How Do I...?

Learn how to enter bar codes, change scan settings, and perform other useful tasks.

4 Troubleshooting

Learn how to troubleshoot problem situations.

5 Maintaining Your System

This chapter describes maintenance procedures and tips.

6 Reference

This chapter presents glass and slide specifications, as well as regulatory compliance information.

Contents

1 Introduction

This chapter provides a general introduction to the Agilent G2565AA Microarray Scanner System and Agilent G2565BA Microarray Scanner System.

A powerful tool... This section describes the features that make the Microarray Scanner a powerful tool in Agilent's gene expression analysis solution.

System description In this section learn about the external components of the Microarray Scanner, the site preparation information, and the safety guidelines.

Principles of operation This section explains how the Microarray Scanner works.

Software enhancements This section explains the differences between Version 6.3, Version 7.0 of the Scan Control software.

Hardware enhancements This section describes major improvements to the G2565BA hardware.

A powerful tool for gene expression analysis

The Microarray Scanner System is part of the Agilent Technologies gene expression analysis solution. The Microarray Scanner is a sophisticated laser-induced fluorescence scanner designed to read microarrays deposited on standard 1 in × 3 in slides.

The Microarray Scanner measures the fluorescence intensity of labeled sample nucleic acid (DNA and RNA) bound to probe arrays. The Microarray Scanner's ability to measure fluorescence from two dyes simultaneously facilitates differential gene expression studies. This technology provides for rapid, high-quality, automated, "hands-off" scanning of microarrays.

Figure 1 Microarray Scanner

Each slide is scanned in minutes, and the files are prepared for feature extraction analysis. The Microarray Scanner provides the following features:

- Internal bar code reading (and lets you enter external bar codes)
- Dynamic auto-focus
- Automatic PMT gain calibration prior to each scan
- Continuous laser power stabilization
- Pixel placement error of < 1 pixel
- 5- or 10-micron pixel size
- Single scan dynamic range > 10^4 (maximum non-saturating signal)/(detection limit)
- Dual scan extended dynamic range (XDR) > 10^5
- TIFF image file compression
- Uniformity specification of less than 5% CV global uniformity

The Scan Control software lets you set scan parameters, manage your files, and scan your microarrays. The Microarray Scanner reads each bar code, incorporating ID information from the Agilent pattern file, or you can use an external reader for non-Agilent slides.

System description

The Microarray Scanner is designed for ease of use and easy access to features. The illustrations in this section show all the customer-accessible features of the Microarray Scanner.

Parts list

The Microarray Scanner System consists of the following components:

- Microarray Scanner
- Carousel and 50 slide holders
- Crossover LAN cable
- PC workstation
- Power cables
- Software
- Diagnostic serial cable
- Norton Ghost backup & recovery software

Computer system requirements

Check that you have the following required items prior to installing and using Agilent Scan Control software (v 7.0):

Software

- Windows XP Professional or Windows 2000 with SP2 (or SP3)
- Internet Explorer 5.5 or later

Hardware

- Pentium IV 2.4 GHz or higher (Pentium IV 3.2GHz or higher recommended)
- 1 GB RAM required (2 GB RAM recommended)
- 50 GB available disk space for programs and data generation

Scanner front view

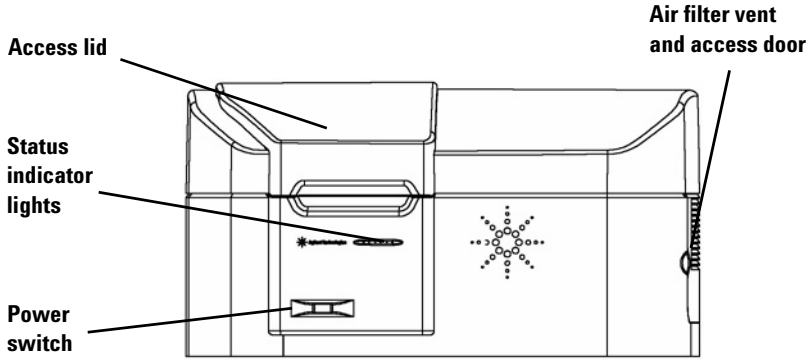


Figure 2 Microarray Scanner, Front View

Scanner top view

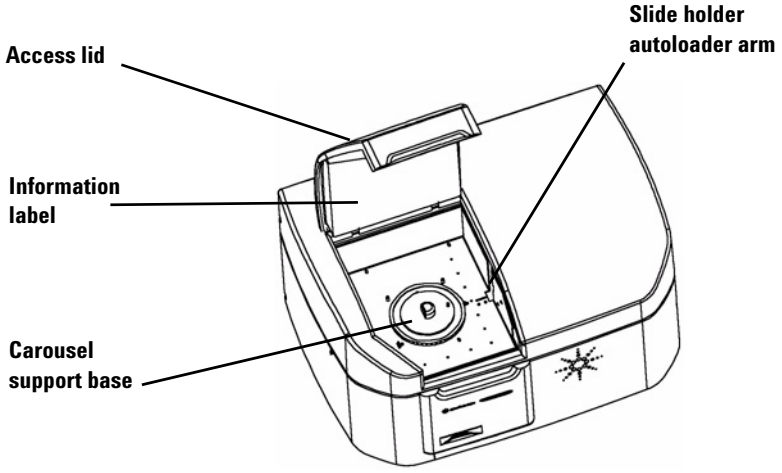


Figure 3 Microarray Scanner, Top View

Scanner rear view

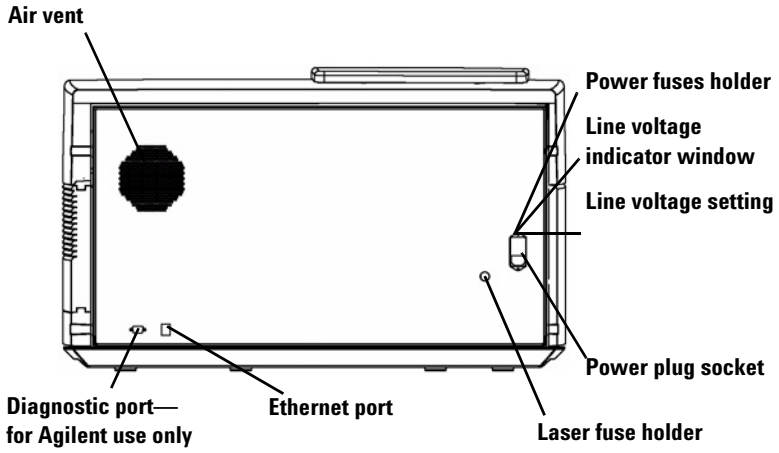
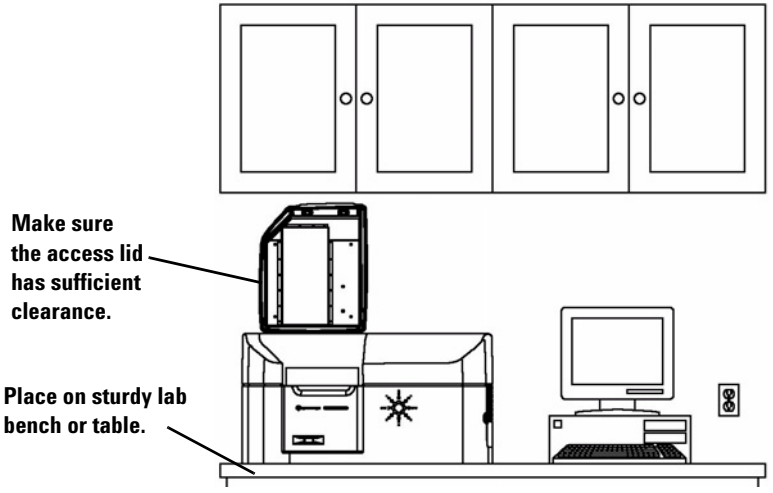


Figure 4 Microarray Scanner, Rear View

Site preparation

Make sure the environment meets the "Microarray Scanner specifications" on page 120 of Chapter 6. If you have any questions, contact your local Agilent sales and support center or www.agilent.com/chem/dnasupport.



Safety symbols on scanner

CAUTION symbol

This CAUTION symbol is placed on the product where it is necessary for you to refer to the product guide in order to understand a potential hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data.

WARNING symbol

This WARNING symbol is placed on the product within the area where hazardous voltage is present or electrical shock can occur. Only trained service persons should perform work in this area.

Safety guidelines

The Microarray Scanner has been designed for safety and ease of use. Be sure you understand and observe all the warnings and cautions before operating the Microarray Scanner.

WARNING **Do not attempt to repair or gain access to Microarray Scanner internal components. You might expose yourself to high voltage and harmful laser radiation. Removing the covers voids the warranty.**

CAUTION If you are turning on the Microarray Scanner for the first time or have moved it to a new site, verify that the voltage setting is correct and the voltage is "grounded". See "Before you start" on page 24 of Chapter 2.

CAUTION Agilent recommends that the Microarray Scanner be placed on a sturdy lab bench or table. The Microarray Scanner is a potential source of vibration. The Microarray Scanner is potentially sensitive to external vibration.

CAUTION The Microarray Scanner is potentially sensitive to condensing humidity conditions. Follow precautions stated in product documentation. See "Tips to prevent problems" on page 116 of Chapter 5.

Principles of operation

This section describes the operating features of the Microarray Scanner.

Slide positioning

The Microarray Scanner uses a circular carousel that holds up to 48 slides (1 in × 3 in). The carousel can be removed to load or remove slide holders or store for later use. When a scan run is initiated, the scanner rotates the carousel to the first scheduled slot number that contains a slide. After the carousel reaches the loading position, the Microarray Scanner transports the first slide and holder into the scanning position, scans the slide, and then returns the slide and holder to the carousel.

Laser excitation

The Microarray Scanner uses two lasers, a SHG-YAG laser (532 nm) and a helium-neon laser (633 nm). The lasers excite Cyanine-3 (Cy-3) and Cyanine-5 (Cy-5) labeled RNA or DNA to measure fluorescence after biological and chemical processes have bound the target sample to specific locations on a glass substrate. Other dyes similar to Cy-3 and Cy-5 can be used, such as Alexa 647, 555, and 660 dyes.

The Microarray Scanner is optimized for high signal-to-noise performance in the Cy-3 (550–610 nm) and Cy-5 (650–750 nm) emission channels, with a wide dynamic range (up to five orders of magnitude) and low spectral cross-talk. This allows for measurement of a very broad range of gene expression levels and for higher data confidence at lower signal levels.

Scanning

The laser excitation is scanned rapidly back and forth across the microarray. Movement of the microarray in the orthogonal coordinate is accomplished by the slow linear stage. The dynamic/tracking auto-focus assembly keeps the slide in focus with the scan lens ensuring that the microarray is always

positioned in the detection plane. These features maximize the signal-to-noise ratio by providing superior linearity, uniformity, and noise performance while scanning across the microarray surface.

Fluorescence detection

Fluorescent emissions from the labeled samples are detected using a high-performance PMT design. Very low noise amplifiers and analog-to-digital converters process the PMT signal with a high signal-to-noise ratio. Signal-to-noise performance is maintained by integrating multiple analog-to-digital samples within a single pixel.

Maximum scan region for non-Agilent slides

The maximum scan area for the 1 in × 3 in microscope slide depends on the Microarray Scanner version:

- **G2565AA:** 67.2 x 21.6 mm
- **G2565BA:** 71 x 21.6 mm

The file sizes in megabytes for the following uncompressed resolutions are:

Resolution	67.2 mm x 21.6 mm	71 mm x 21.6 mm
5 microns	232.2 MB	245.4 MB
10 microns	56.7 MB	59.9 MB

Sizes of compressed files are variable.

Dark offset subtraction

The Agilent scanner was designed to offset all signal intensity levels by a few hundred counts. This “dark offset” was introduced to ensure that no detected signal levels would ever fall below zero. Without this precaution the signal level could fall below zero either due to electronic noise or even slight fluctuations in the average level of the background. Not letting signals fall below zero ensures that an unbiased pixel distribution is reported within the data set, thus improving the accuracy of the generated data set.

Yet, the dark offset has created some confusion when users are interpreting the background level from the scanner-generated TIFF image files. An earlier software release provided a method that improves usability but does not reduce accuracy of the data set. The scanner dynamically measures the average electronic offset prior to each scan and calculates a value that can be safely subtracted from each pixel value. This calculation is designed to lower the dark offset without incorrectly skewing the data towards zero. A constant level of signal offset is not “noise” and does not negatively impact the detection of low-level signals.

Agilent G2565AA scanners that shipped with version 5.0 scanner software or earlier: An Agilent support engineer must perform an on-site calibration to enable this feature on existing scanners (prior to version 5.1) with the updated version of the software (version 5.1 or later). Until this calibration is done, the dark offset remains unchanged.

Agilent G2565AA scanners that shipped with version 5.1 scanner software or later or Agilent G2565BA scanners: The dark offset subtraction is enabled and provides scans that appear to have lower background levels than scans performed on earlier scanners that do not have this feature enabled.

Software enhancements (Version 7.0)

For hardware enhancements to the G2565BA, see "Hardware enhancements (G2565BA)" on page 22.

This edition of the user guide covers changes to the software from version 6.3 to version 7.0. With version 6.1 the hardware changed from Agilent G2565AA to Agilent G2565BA. Version 6.1, 6.3 and 7.0 software can be used on Agilent G2565AA hardware, but some of the features may be different because of differences in hardware. The differences are marked in this user guide.

Agilent Scan Control software version 7.0 is designed for use with Agilent Feature Extraction software version 9.1, but is backwards compatible to version 6.1. Although these earlier versions of Feature Extraction can display and extract uncompressed, single-scan TIF images generated by Scan Control 7.0, only versions 9.1 and later will be able to process compressed images and extract XDR data. You can use Feature Extraction software version 9.1 with any version of Scan Control software.

Extended Dynamic Range scanning

Scan Control software 7.0 provides the ability to load a slide once and automatically scan it twice at two different, user-selected sensitivity settings. By using this new feature, you can generate an eXtended Dynamic Range (XDR) scan of up to 5+ orders of magnitude.

Each XDR scan file is named according to the standard Scan Control protocol, but in addition, a distinguishing suffix is appended. The software also creates a unique ID for each pair of XDR scans and saves it to both scan image files. Feature Extraction 9.1 uses this XDR ID to link the pair of SCR scans together automatically when extracting data.

The allowable range of PMT levels for an XDR Hi scan is 100% to 10% and for an XDR Lo scan, 50% to 1%. The ratio of XDR Hi to XDR Lo is further constrained to be ≥ 2 and ≤ 20.

Reduced scanning time for 5-micron scans

Scan Control software Version 6.3 always scans each row of pixels twice when 5-micron resolution is selected. As a result, typical 5-micron scans required approximately 15 minutes to complete. Scan Control Version 7.0 offers a single pass option that reduces scan time by about half. Post-processing of the data has been implemented with this option to eliminate direction-dependent noise (DDN). The DDN information is stored into the image file and can be viewed with Feature Extraction Version 9.1 software.

Automatic image file compression option

Scan Control Version 7.0 software provides an option in the Default Settings dialog box for you to compress the scan image file at the time the image is generated. The compression algorithm used, LZW, reduces the storage space on the order of 20 to 70 percent. This feature is customizable separately for each User of the system.

The data in compressed image files can be displayed and extracted only with Feature Extraction 9.1.

More robust communication algorithms

Bootp and Connection Manager Services have been reprogrammed to be more accommodative to various networking environments. For example, it is no longer necessary to disable the house network adapter if the house connection doesn't exist or the house network is disabled.

New Carousel Report Log messages

Previous versions of Scan Control software report that a scan is "successful" simply because it scans to completion. Scan Control Version 7.0 will report "Scan completed," instead. It will also evaluate several result parameters to decide if a warning should be issued. For example, an abnormally high Autofocus Hold value will yield one of two warning messages, depending upon its magnitude.

More robust scanning algorithms

If a slide fails to load, Scan Control Version 7.0 will attempt to eject the slide and skip to the next scheduled slot in the scan run. Previous versions would simply stop the scan run.

If desired, the Laser Saver feature will now turn off the lasers at the end of a scan run and turn them back on at a scheduled time on another day.

Hardware enhancements (G2565BA)

This edition of the user guide identifies 2 major improvements to the G2565BA hardware since its introduction.

Carousel Indexing System

The Carousel Indexing System, which is a major part of the Autoloader, has been redesigned to reduce the frequency of load and eject failures. Many tolerances have been tightened and some components completely redesigned to eliminate failure modes that have been reported.

Autoloader Arm and Motor Assembly

The Autoloader Arm and Motor Assembly has been completely redesigned as well. One assembly is now compatible with both G2565AA and G2565BA scanners. Some components have been redesigned and are now manufactured out of metal instead of plastic. And most significantly, the most common source of failure in the past has been corrected by using a direct drive motor and a more robust external drive gear.

2 Getting Started

This chapter gives you instructions to set up and scan your slides quickly and easily.

Agilent slides

If you have Agilent slides, just follow the steps in this chapter and use the shipped default settings. You can change scan settings if you want.

Non-Agilent slides

If you have non-Agilent slides, you may have to change the shipped default settings. See "Step 7. Check or change the scan settings in the scan table" on page 42.

If you have any problems, see Chapter 4 for troubleshooting information.

Before you start

Before turning on the Microarray Scanner for the first time or after it has been moved to another site, verify that the input voltage setting on the rear of the instrument is set to the correct value. The input voltage setting can be set to one of four values:

- 100 VAC
- 120 VAC
- 220 VAC
- 240 VAC

1 Go to the rear of the Microarray Scanner.

2 View the input voltage setting through the small window.

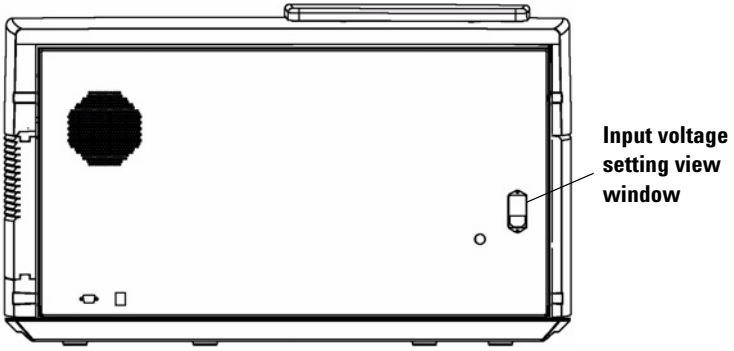


Figure 5 Input voltage setting view window

3 If the input voltage setting matches the line voltage, you can safely turn on the Microarray Scanner.

If the input voltage setting does not match the line voltage, go to step 4.

4 Disconnect the power cord.

WARNING **Always disconnect the power cord before changing the input voltage setting.**

5 Use a small flat-edge screwdriver to pry up the small plastic tab located on the bottom edge of the fuse holder until it releases.

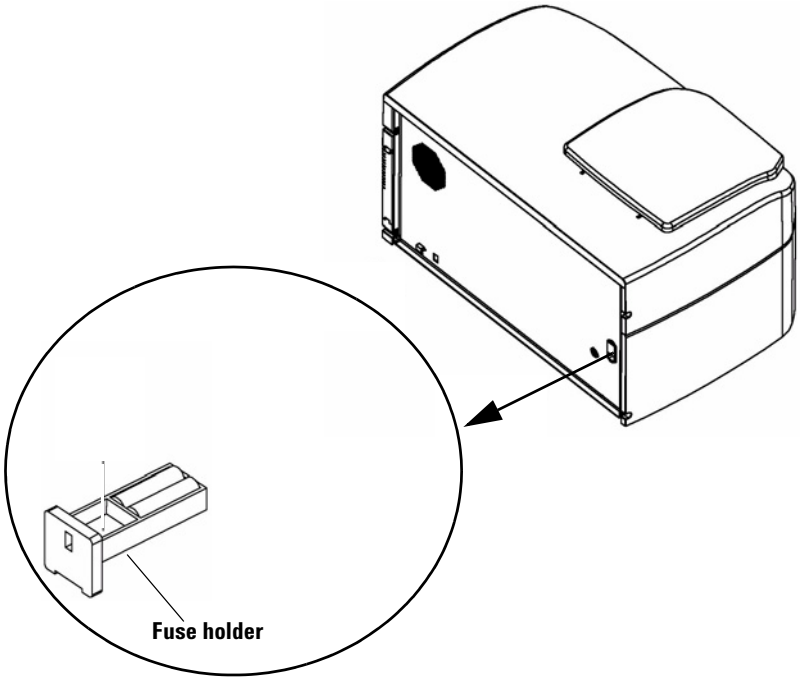


Figure 6 Removing the fuse holder for the power supply

6 Pull out the fuse holder.

7 Use needle nose pliers to remove the voltage selector.

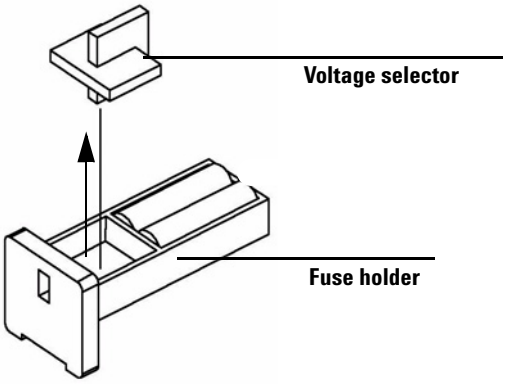


Figure 7 Removing the voltage selector from the fuse holder

8 Insert the voltage selector so that the number showing matches the line voltage.

9 Push the fuse holder back in until it clicks into place.

10 Plug in the power cord.

Step 1. Turn on the Microarray Scanner

1 Turn on the PC, but do not start the Scan Control program.

The Microsoft Windows logon prompt appears.

2 Enter your account user name and password at the Windows logon prompt.

The default logon information is the following:

- User Account: **User**
- Generic password: **none**
- Privilege: **User**

The logon information can be changed if you have Windows administrator privileges.

3 Turn on the Microarray Scanner using the power switch located on the front of the instrument. You will notice the following:

a A fan turns on, and the lid locks.

b The Microarray Scanner begins an initialization routine that takes about four minutes. During the initialization, a self-test is run during which the lights flash on and off as different functional checks are made.

c The yellow and green status indicator lights remain lit for about 20 minutes until the lasers are fully warmed up and ready to scan.

d When the Microarray Scanner is ready to scan, the yellow status indicator light turns off and the green status indicator light remains on.

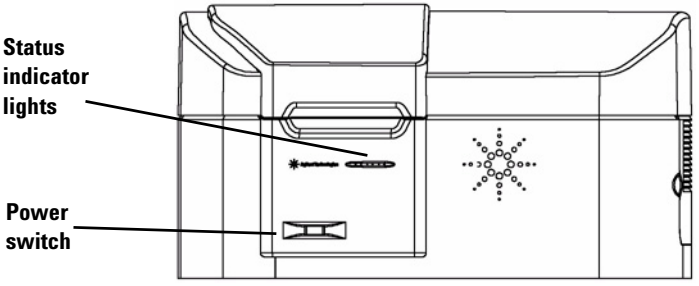


Figure 8 Location of power switch and status indicator lights

Step 2. Insert slides into slide holders

A slide must be mounted in a slide holder before inserting it into the carousel. The slide holder protects the slide during scanning.

Two types of slide holders are available:

- Version A (p/n G2505-60500) for the Agilent G2565AA
- Version B (p/n G2505-60525) for the Agilent G2565BA

CAUTION The Agilent G2565AA uses only the version A slide holder. The Agilent G2565BA uses only the version B slide holder. Using the wrong slide holder will alter scan region alignment and may cause loading failures that may damage the Microarray Scanner.

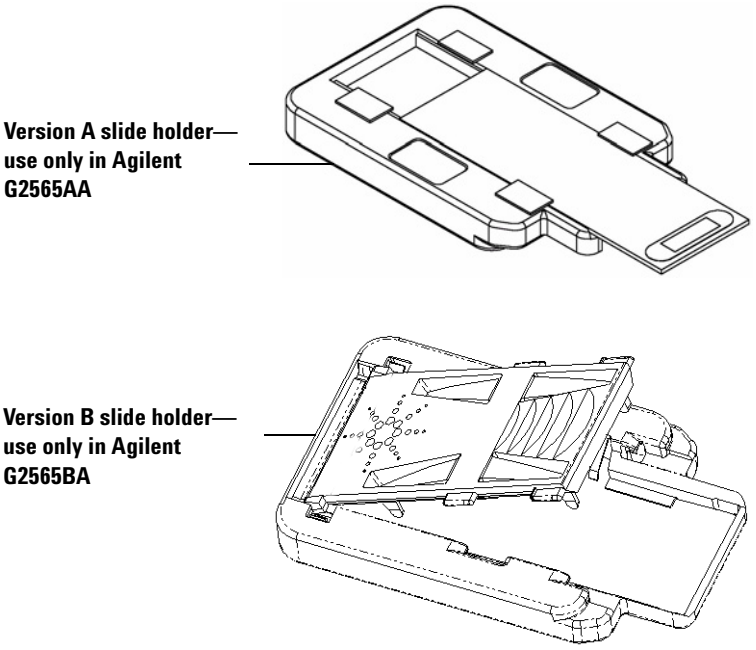


Figure 9 Loading slides into the two types of slide holders

Inserting a slide into a version B slide holder

The version B slide holder can be used only in the Agilent G2565BA.

For instructions on removing the slide, see "Remove a slide from a version B slide holder" on page 84.

Fingerprints cause errors in the fluorescence detection. For accurate readings, touch only the edges of the slide and always use gloves when handling slides.

Also, do not write on the slides with markers or place any labels on the slide other than an appropriate bar code.

1 Hold the slide holder on the sides with the Agilent logo facing up.

2 Place one thumb on the center of the slide holder cover.

3 Gently press down on the cover and push toward the top of the slide holder until the cover releases.

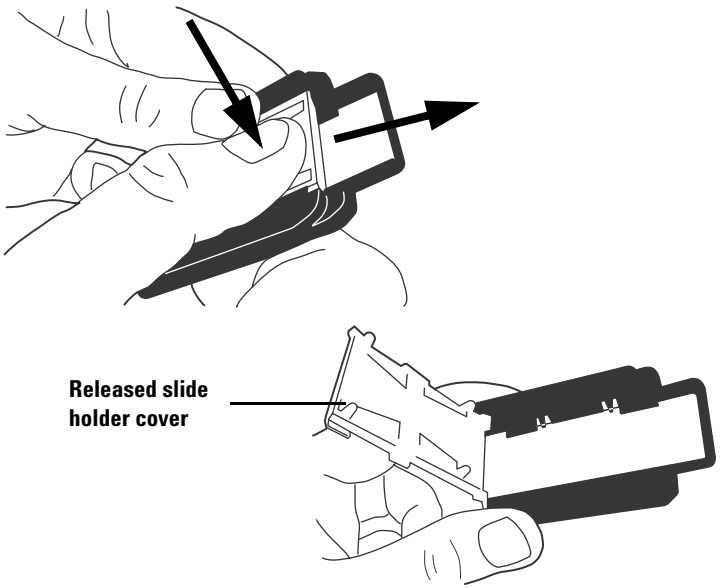


Figure 10 Release the cover

4 Place the slide into the slide holder with the end of the slide supported fully on the rear ledge.

The active microarray surface must be facing up toward the cover.

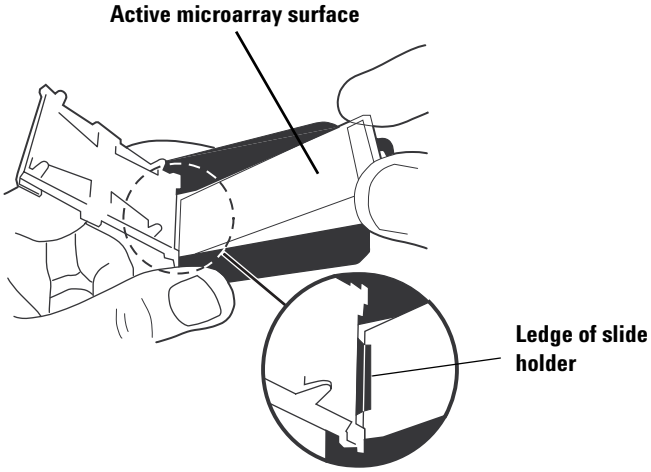


Figure 11 Proper placement of slide on ledge

5 Align the slide with the slide holder following these two rules:

- The end without the bar code label is placed on the rear ledge.
- The slide surface on which the microarray is deposited is facing the cover. The microarray is scanned through the glass.

Make sure these rules are always followed when you align a slide.

Agilent slides have two bar codes, one on each side of the glass. See Figure 12. Typically, non-Agilent slides have only one bar code. Place the active microarray side of the slide facing toward the slide holder cover.

See "Bar code specifications" on page 123 to apply a second readable bar code.

If you have a slide whose spotted array is on the opposite side of the slide as the bar code, the scanner cannot read the bar code.

CAUTION An improperly inserted slide can damage the Microarray Scanner.

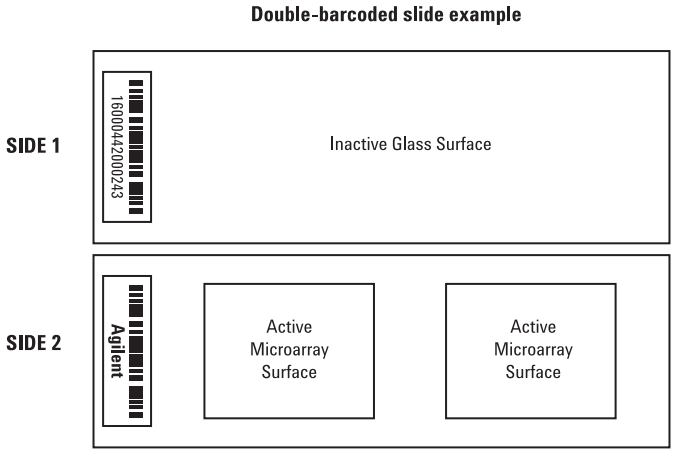


Figure 12 Slide orientation

6 Let go of other end of slide and allow it to drop into the slide holder.

It is OK if the slide is slightly tilted against the tabs on the side of the slide holder.

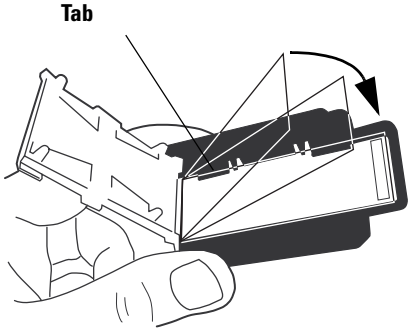


Figure 13 Slide dropping into slide holder

7 Place one thumb on the cover to gently press down on the slide holder cover and pull the cover back until it locks.

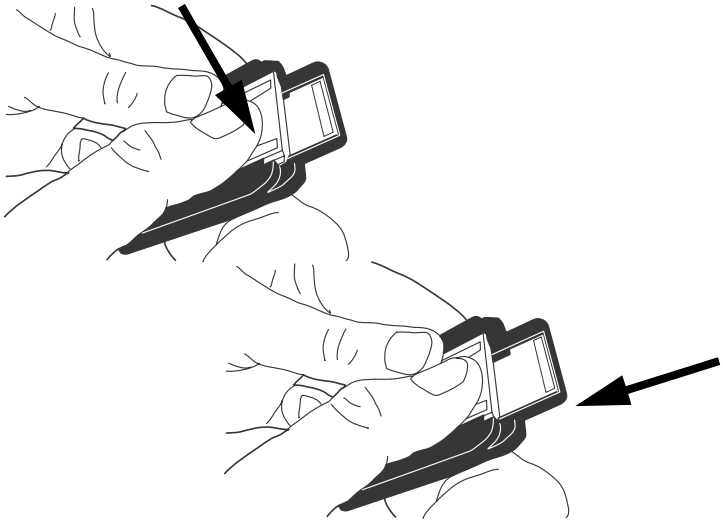


Figure 14 Lock in the cover

8 Verify that the slide holder lid is fully locked into place.

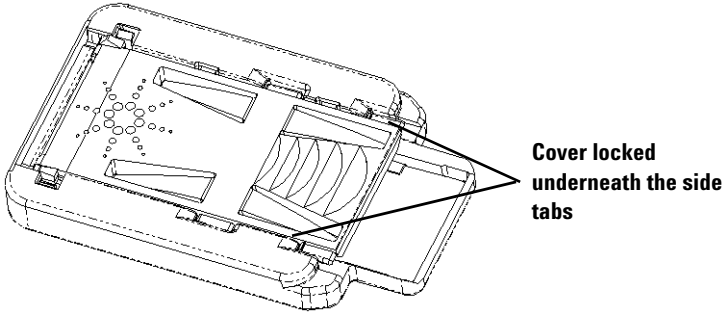


Figure 15 Properly locked cover

Inserting a slide into a version A slide holder

The version A slide holder can be used only in the Agilent G2565AA.

Fingerprints cause errors in the fluorescence detection. For accurate readings, touch only the edges of the slide and always use gloves when handling slides.

Also, do not write on the slides with markers or place any labels on the slide other than an appropriate bar code.

1 Place the slide holder on a flat surface with the inset buttons facing up.

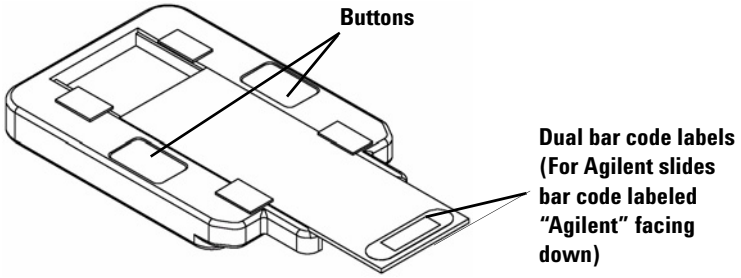


Figure 16 Slide alignment with slide holder

2 Align the slide with the slide holder following these two rules:

- The end without the bar code label is inserted into the slide holder first.
- The slide surface on which the microarray is deposited is face down in the slide holder. The microarray is scanned through the glass.

Agilent slides have two bar codes, one on each side of the glass. Non-Agilent slides have only one bar code. Place the active microarray side of the slide upside down in the holder.

See "Bar code specifications" on page 123 to apply a second readable bar code.

If you have a slide whose spotted array is on the same side of the slide as the bar code, the scanner will not be able to read the bar code.

CAUTION An improperly inserted slide can damage the Microarray Scanner.

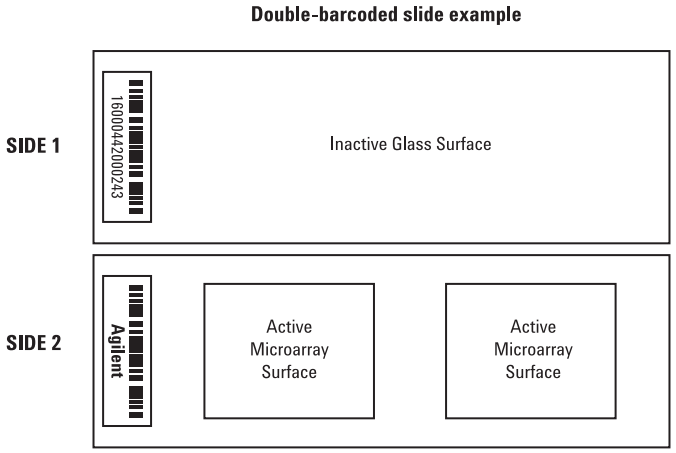


Figure 17 Slide orientation

3 While pressing the two inset buttons on the slide holder with one hand, gently push the slide into the holder with the other hand.

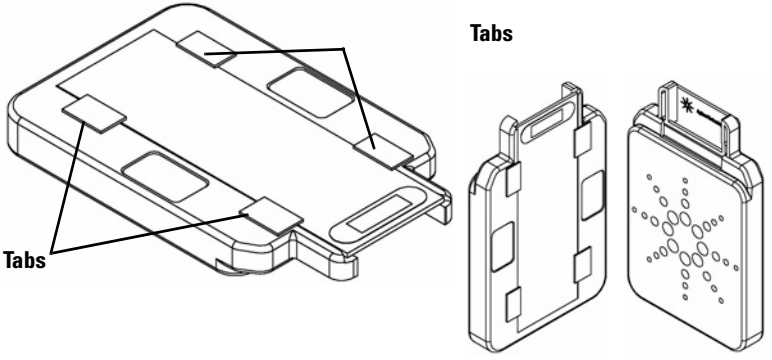


Figure 18 Slide fully inserted into slide holder

4 Verify that the slide is pushed all the way into the slide holder and the slide is underneath the four tabs.

Step 3. Insert slide holders into the carousel

When the slides are properly inserted in the slide holders, you can insert the slide holders into the carousel. The carousel is designed so that you can easily insert a slide holder correctly. This step assumes that the carousel has not been removed from the Microarray Scanner. To mount or remove the carousel, see "Mount or remove the carousel" on page 86 of Chapter 3.

CAUTION

The Agilent G2565AA uses only the version A slide holder. Using the wrong slide holder may damage the Microarray Scanner.

The Agilent G2565BA uses only the version B slide holder. Using the wrong slide holder will alter scan region alignment.

The Microarray Scanner scans slides in the order that they are detected as it rotates the carousel from lower numbered slots to higher numbered slots. The carousel run skips over any empty slots.

1. Insert a slide holder into carousel slot 1, the first slot located clockwise from the home position.

   Do not place a slide in the carousel home slot. The home slot is wider than the other slots and labeled "Home."

2. Make sure that the tapered end of the slide holder points toward the center of the carousel.

3 Make sure that the slide holder is seated in the bottom of the carousel slot.

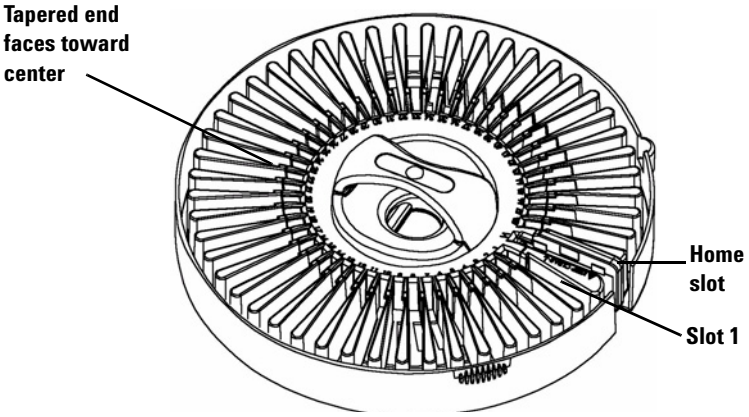


Figure 19 Carousel

CAUTION Improper placement of the slide holder in the carousel can result in severe damage to the Microarray Scanner.

4 (Optional) Place the carousel cover onto the carousel until the two side tabs snap into place.

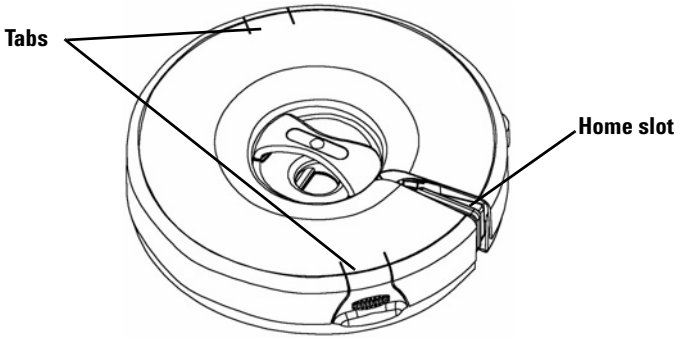


Figure 20 Carousel cover

5 Close the lid.

You cannot do any of the following tasks until you close the lid:

- Initialize the scanner
- Check the carousel for empty slots
- Read bar codes before the carousel run
- Start a carousel run

Step 4. Start the Scan Control program

The Scan Control program on the PC workstation controls the Microarray Scanner. You can set options and start and abort a scan from the user interface. You can also view a scan status messages and display a carousel run report..

1 Double-click the **Agilent Scan Control** icon to open the Scan Control program.

Figure 21 Agilent Scan Control icon

2 Check the **Scanner status** message at the bottom of the main window.

Initializing Scanner The scanner is initializing. When the initialization is complete, the scanner lid unlocks and the lasers continue warming up.

Lasers are warming The lasers are still warming up. If you have to wait for the lasers to warm up, move on to "Step 5. Enter your name and the location of your slides" on page 40.

Lasers are powered off You need to select **Settings > Laser Power Saver** and click the **Turn Lasers ON Now** button.

Scanner ready You can begin your scan.

You cannot start a carousel run until both lasers are warmed up.

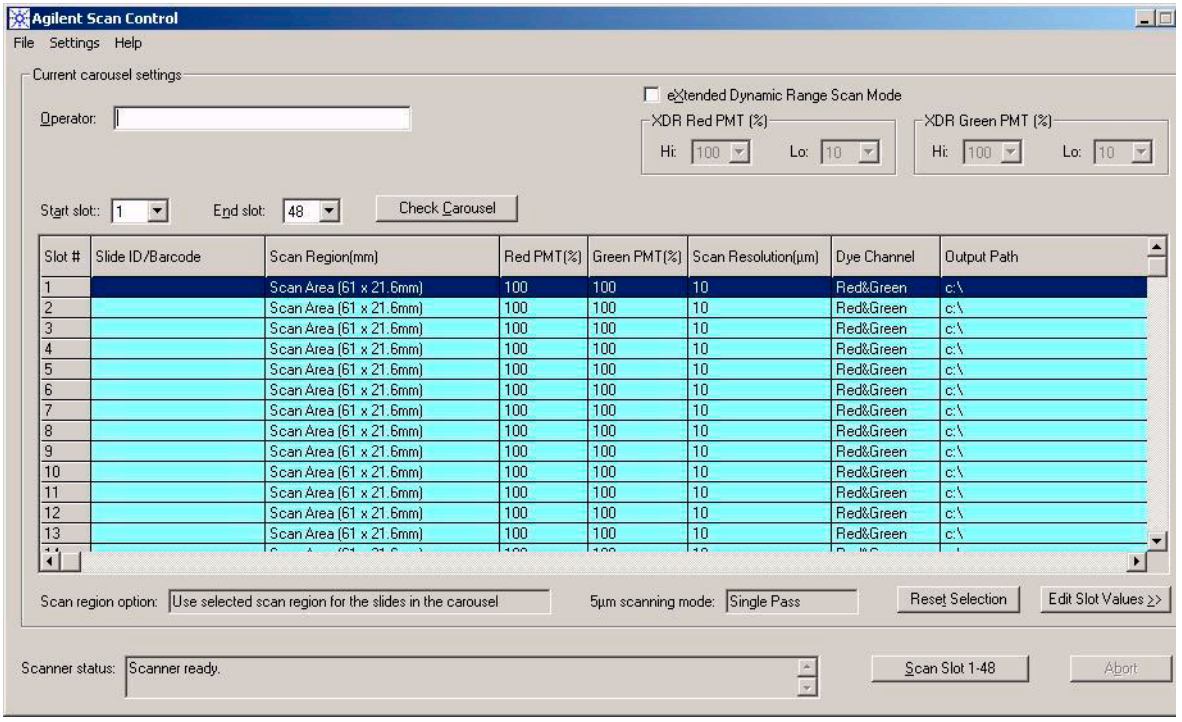


Figure 22 Scan Control main window when lasers are ready

Step 5. Enter your name and the location of your slides

A *scan* refers to the laser traversal of a single slide. A *carousel run* refers to scans of all of the specified slides in the carousel.

If you have not filled the carousel with slides in all 48 slots, you can save time by entering the location of the slots that contain your slides. When you start the carousel run, the carousel will then begin scanning the slide in the start slot and stop scanning the slide in the end slot that you enter.

1. In the Scan Control main window, enter your name in the **Operator** text box (optional).
2. If you want to scan slides in all 48 slots, skip to "Step 7. Check or change the scan settings in the scan table" on page 42.
3. In the **Start slot** box, select the number of the slot where the first slide that you want to scan is located.
4. In the **End Slot** box, select the number of the slot where the last slide that you want to scan is located.

The range of slots that you enter in steps 3 and 4 affects most of the functions in the Scan Control main window.

- Only the slot range that you enter here appears in the scan table.
- The **Check Carousel** function only checks the range of slots that you enter here.
- The scanner scans only those slides in the slot range that you enter here.

Step 6. Check the carousel for empty slots

To update the visual display of the scan table to indicate which slots have slides in them, you can run Check Carousel. You do not need to do this step because the Scan Control software automatically checks the range of slots selected for empty slots before scanning the first slide. It only attempts to scan slots that have slides in them.

1 Enter the slots that you want to check.

See steps 3 and 4 on the previous page.

2 Click **Check Carousel**.

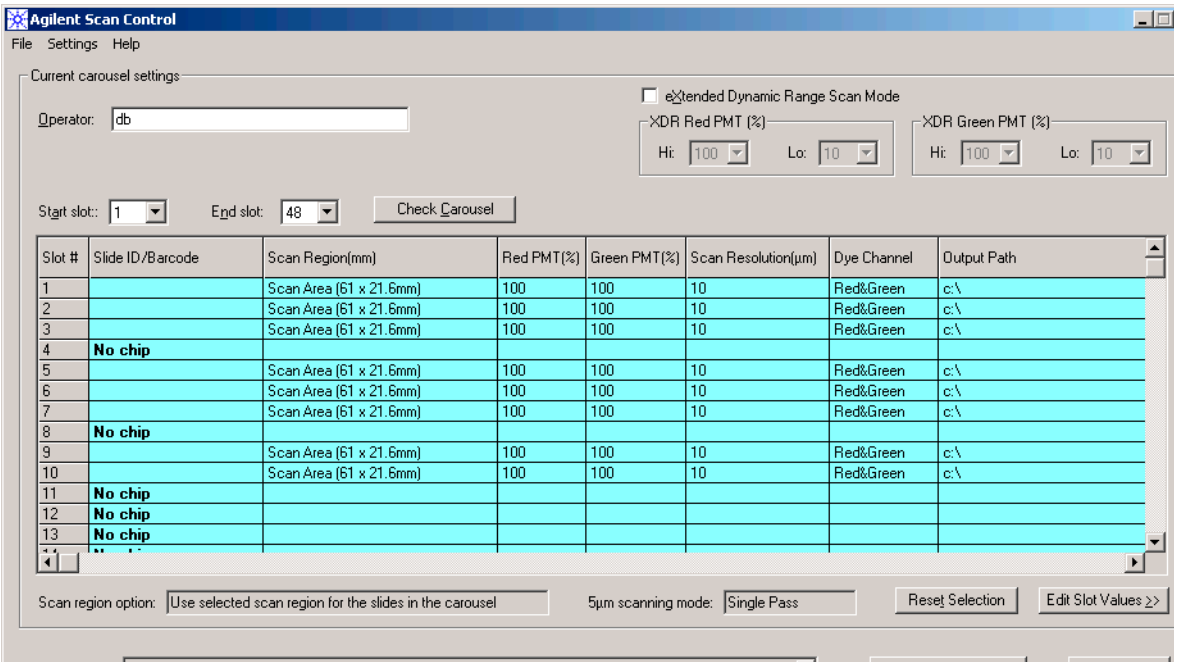


Figure 23 Example of scan table after the carousel check

See Chapter 3 for more details on changing scan table settings.

If you later place a slide into an empty slot, select the row in the scan table and then click **Reset Selection** to enter the default settings for the slide. You can then change its settings if you want.

Step 7. Check or change the scan settings in the scan table

Easy scan option for Agilent slides

When you first start the software, the Scan Control scan table in the main window contains the original default settings for Agilent slides that let you scan right away to produce usable results.

If you do not need to change any of the default settings for any slide (and you usually don't for Agilent slides), wait until the **Scanner status** message says Scanner Ready and move on to "Step 8. Start the carousel run" on page 44.

Pre-scan checklist (Agilent and non-Agilent slides)

Before you start the carousel run, review this checklist of tasks to make sure that you check or change any available scan setting that you need to. You can do the tasks in any order. You can find instructions for each of the listed tasks in Chapter 3.

Table 1 Pre-scan checklist

	Pre-scan task	Task location in Chapter 3
✔	Read bar codes for slides with Agilent-supported bar codes	page 56
	or	
	Enter bar code/slide identifier information manually	page 58
✔	Change the default scan settings	page 60
✔	Change scan table settings	page 65
✔	Select a scan region for Agilent slides,	page 67
	or	
	Select a scan region for non-Agilent slides	page 69
	or	
	Create a new scan region for non-Agilent slides	page 71
✔	Change the sensitivity, resolution, or dye channel settings	page 75

Table 1 Pre-scan checklist (continued)

	Pre-scan task	Task location in Chapter 3
✔	Change the storage directory and check drive capacity	page 76
✔	Change the prefixes for automatic file naming	page 78
✔	Rotate the scan image and split the color file	page 79
✔	Compress TIFF image	page 80

Step 8. Start the carousel run

A carousel run scans all the specified slides in the carousel.

Before you start

Make sure that the Microarray Scanner has finished warming up (maximum 20 minutes). Verify that the **Scanner status** message in the main window says Scanner Ready.

Once the carousel run is started, the Carousel Report Log from the previous carousel run is erased and a new report log is started. If you haven't reviewed the report log from the previous carousel run, we recommended that you review the report log before you begin the new carousel run. You can set a preferences option to automatically display the report log at the end of a carousel run. See "Automatically display Report Log after carousel run" on page 82 of Chapter 3.

Start the carousel run

1 Click **Scan Slot *m-n*** on the Scan Control main window.

The letter *m* represents the Start slot where your first slide is located, and the letter *n* represents the End slot where your last slide is located.

Two actions take place when you start the scan:

- The scanner lid locks.
- The Scan Progress dialog box opens showing you the status of the scan.

Agilent G2565BA only: From the Scan Progress dialog box, you can also set the software to automatically turn off the lasers after the carousel run.

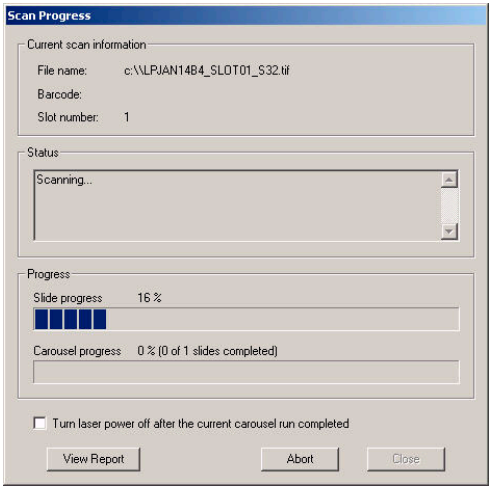


Figure 24 Scan Progress dialog box

Note the messages in the Status window of the Scan Progress dialog box that keep you informed of the progress of each scan and of the carousel run.

The *scan time* is defined as the time it takes the scanner to load, scan, and replace the slide.

The scan time for a 61 mm x 21.6 mm scan region at the 10-micron resolution setting or the single pass, 5-micron resolution setting is approximately 8 minutes. It's 15 minutes for the double pass, 5-micron resolution setting.. After each scan is complete, the data automatically transfers to the directory specified as your output directory. See "Change the storage directory and check drive capacity" on page 76 of Chapter 3.

Details of the Feature Extraction software can be found in the Feature Extraction Software User Guide, which is included with your scanner materials.

2 (Optional) When the data for one scan is fully transferred, display the file in the Agilent Feature Extraction software or another program.

CAUTION

Do NOT attempt to copy or move the file containing the scan data for the current scan while the scanner is still scanning. Data will be lost if you do this.

The *Carousel Report Log* presents scan information and error messages that occur during the carousel run.

3 (Optional) At any time during the carousel run or after the run is complete, select **View Report** to view the Carousel Report Log.

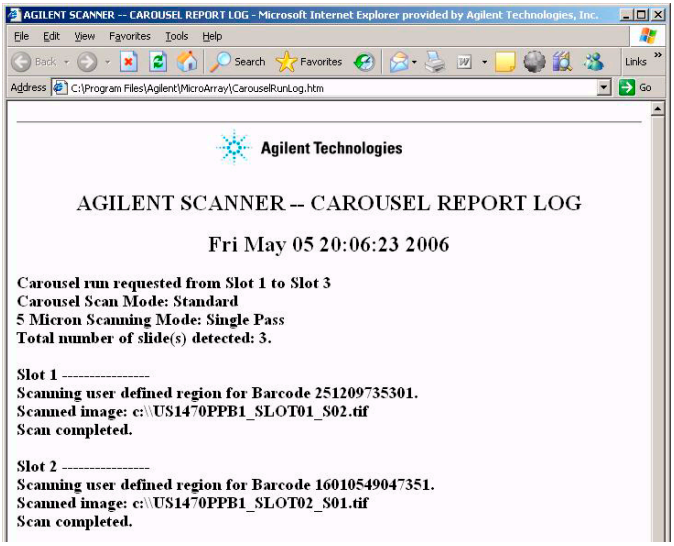


Figure 25 Carousel Report Log

Note when the carousel run is complete. The Status box reads Carousel run completed. You can now remove the slide holders or the carousel.

After the carousel run, you can find the Carousel Report Log in the following directory:

C:\Program Files\Agilent\Microarray\CarouselRunLog.htm.

This file is overwritten each time a new carousel run is started.

Abort a scan

At any time, you can stop the scan or carousel run.

- Click the **Abort** button on the Scan Progress dialog box.

  The following message appears.

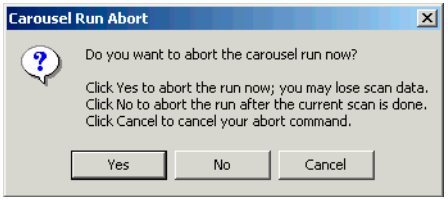


Figure 26 Carousel Run Abort message

The Microarray Scanner can take a minute or two to finish aborting the scan even if Yes is selected, depending upon the stage at which the command is issued. Any collected data is saved for later analysis.

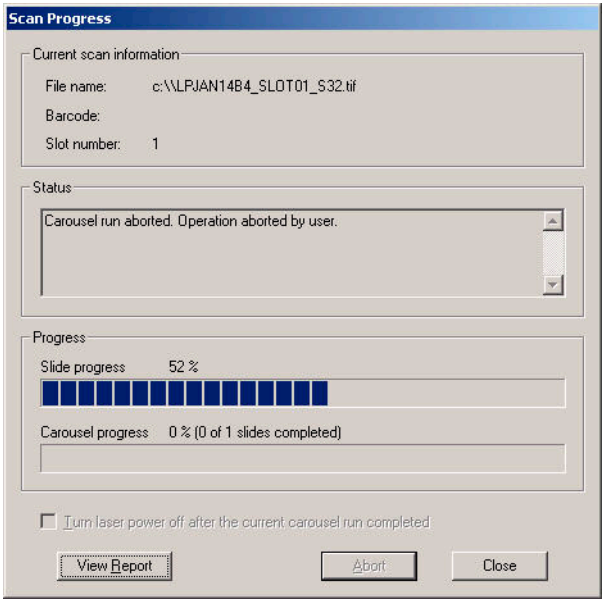


Figure 27 Scan Progress dialog box after Abort is complete

Step 9. Transfer files and archive data

- At the end of the run, transfer the files to another directory, if needed.

You can save the file or files in one of four places:

- Original directory on the local drive that you set up for the output path
- Another directory on the local drive
- Directory on the network
- CD or DVD

Agilent recommends that scanned data first be saved to the local drive so that network problems do not cause the loss of data. A DVD-Writer is included with every PC workstation.

CAUTION

Do NOT attempt to copy or move the file containing the scan data for the current scan while the scanner is still scanning. Data will be lost if you do this.

Failure to archive data regularly can result in a completely filled hard disk. When the disk is full, the Microarray Scanner stops scanning and a caution is displayed.

Use only the compression software provided in the Scan Control program to compress the image files. Other compression algorithms might cause a loss of data.

Step 10. Shut down the system

To extend laser lifetime, the Microarray Scanner should be powered off if it is not going to be used for more than four hours.

Agilent G2565BA only: You also have the option to automatically turn off the lasers after a carousel run from the Scan Progress dialog box. You can also specify laser on and off conditions from the Laser Power Saver dialog box. See "Set Laser Power Saver (Agilent G2565BA only)" on page 53 of Chapter 3 for more information.

1. Click **Exit** on the Scan Control main window.

   To avoid intermittent communication lock-ups, close the Scan Control program before you turn off the Microarray Scanner.
2. Wait a few seconds and verify that the Microarray Scanner lid is unlocked.
3. Lift the lid.
4. (Optional) Remove the slide holders (or the carousel).
5. Turn off the power switch on the front of the Microarray Scanner.

CAUTION Do not attempt to lift the lid until you hear the lid unlock. Attempting to lift the lid while the Microarray Scanner is unlocking the lid can cause a jam.

3 How Do I...?

Before you scan a set of slides, you may want to modify scan settings for one or more slides. Or you may need to perform a particular operation on the scanner only occasionally.

All of the tasks in this chapter are optional if you need to scan Agilent slides. The tasks may be necessary if you need to scan non-Agilent slides. If you have any problems, see Chapter 4 for troubleshooting information.

See Chapter 2, Getting Started, for instructions to start the software.

The tasks in this chapter assume that you have already started the Scan Control program.

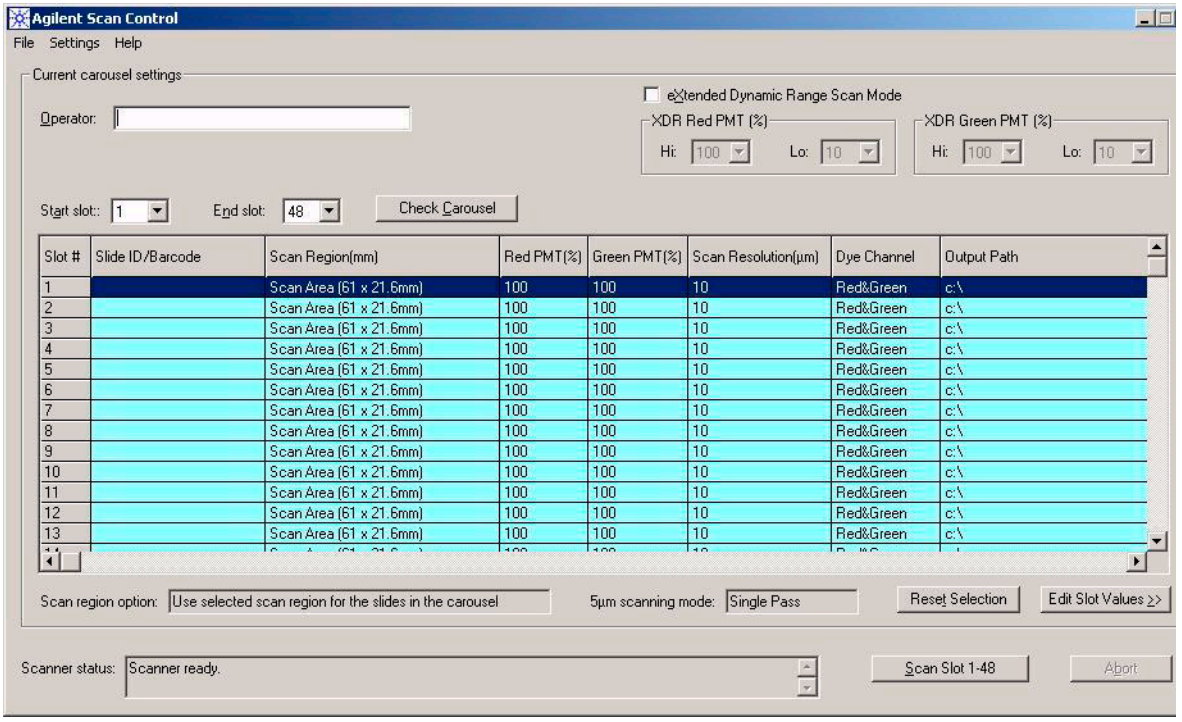


Figure 28 Scan Control main window and scan table

Set Laser Power Saver (Agilent G2565BA only)

You can activate the laser power saver, which extends the life of your lasers. This feature allows you to have the laser warmed up and ready for scanning at a specified time or turned off automatically under specified conditions.

Regardless of how the various automatic ON/OFF settings are set, the software always allows the following:

- You can turn the scanner on or off manually anytime.
- If you open the Scan Control program, the lasers will turn on.
- If the scanner is scanning at the specified turn off time, the scanner will finish the carousel run before turning off.

1 Select **Settings > Laser Power Saver** from the menu bar.

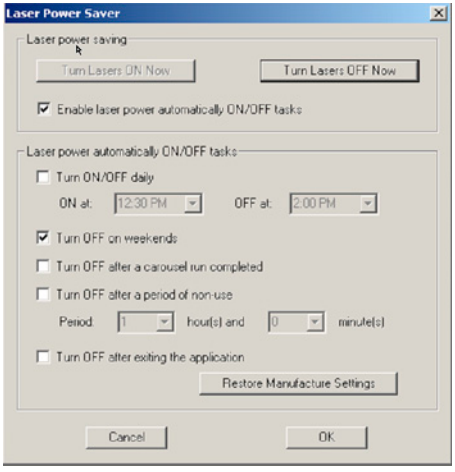


Figure 29 Laser Power Saver dialog box

2 Mark the **Enable laser power automatically ON/OFF tasks** check box.

3 Change the settings using the choices below.

Turn ON/OFF daily — Allows you to specify daily times for turning the lasers on and off.

Turn OFF on weekends — If you have set daily times for turning the lasers on and off, this option allows you to specify that those times are active only on weekdays.

Turn OFF after a carousel run completed — Turns off the lasers after every carousel run.

Turn OFF after period of non-use — Allows you to specify a specific time span of scanner inactivity to turn off the lasers.

Turn OFF after exiting the application — Turns off the lasers when you close the Scan Control program.

4 Click **OK**.

Enter bar code or slide identifier information

This section covers how to read in Agilent-supported bar codes and how to enter bar codes manually.

Agilent slides

If you have Agilent slides, you do not have to enter bar codes into the scan table before the carousel run. The scanner automatically reads Agilent bar codes into the scan table as it scans each slide. You also do not have to change any settings on the scanner after it is shipped from the factory. Just follow the instructions in Chapter 2.

You have the choice, however, of reading bar codes into the scan table before you start scanning. This section gives you instructions on how to read bar codes into the scan table either automatically or manually before you scan.

Non-Agilent slides

If you have non-Agilent slides with Agilent-supported bar codes, you can either have the scanner read the bar codes during or before the carousel run, or you can enter them manually. If you have non-Agilent slides with unsupported bar codes, you must enter the bar codes or slide identifier information manually.

See “Bar code specifications” on page 123 in Chapter 6 to learn how to place a bar code onto a non-Agilent slide to meet Agilent specifications.

Read bar codes for slides with Agilent-supported bar codes

Because Agilent-supported bar codes are directly attached to the slides, the scanner reads the bar codes as it scans. If you want the scanner to read the bar code information before the carousel run, follow these steps for Agilent-supported bar codes attached to the slides.

1 Click **Edit Slot Values** on the Scan Control main window.

The Edit slot values panel appears below the main window for you to read in the bar codes for each slide.

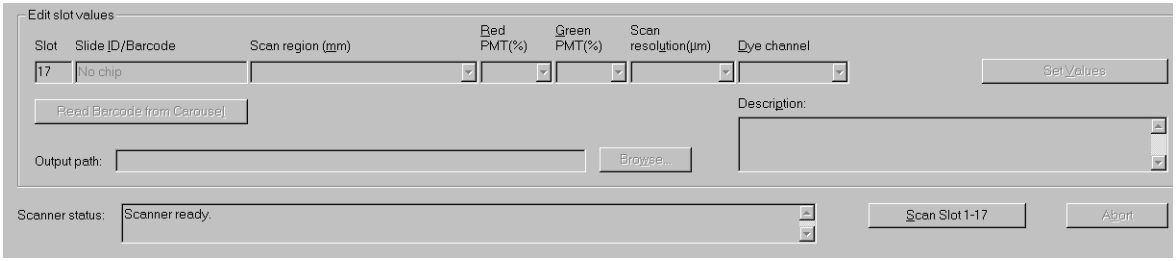


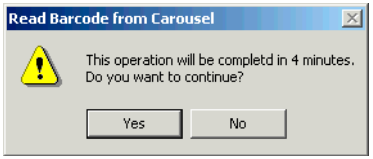
Figure 30 Edit slot values panel below the main window

2 In the scan table, select the slots or slides whose bar codes you want the scanner to read.

Drag the cursor to select more than one slide, or click the **Slide ID/Bar code** header to select all slides for entry.

3 Click **Read Bar code from Carousel** on the Edit slot values panel of the Scan Control main window.

The message similar to the one you see below appears.



4 Click **Yes**.

The main window now looks like this:

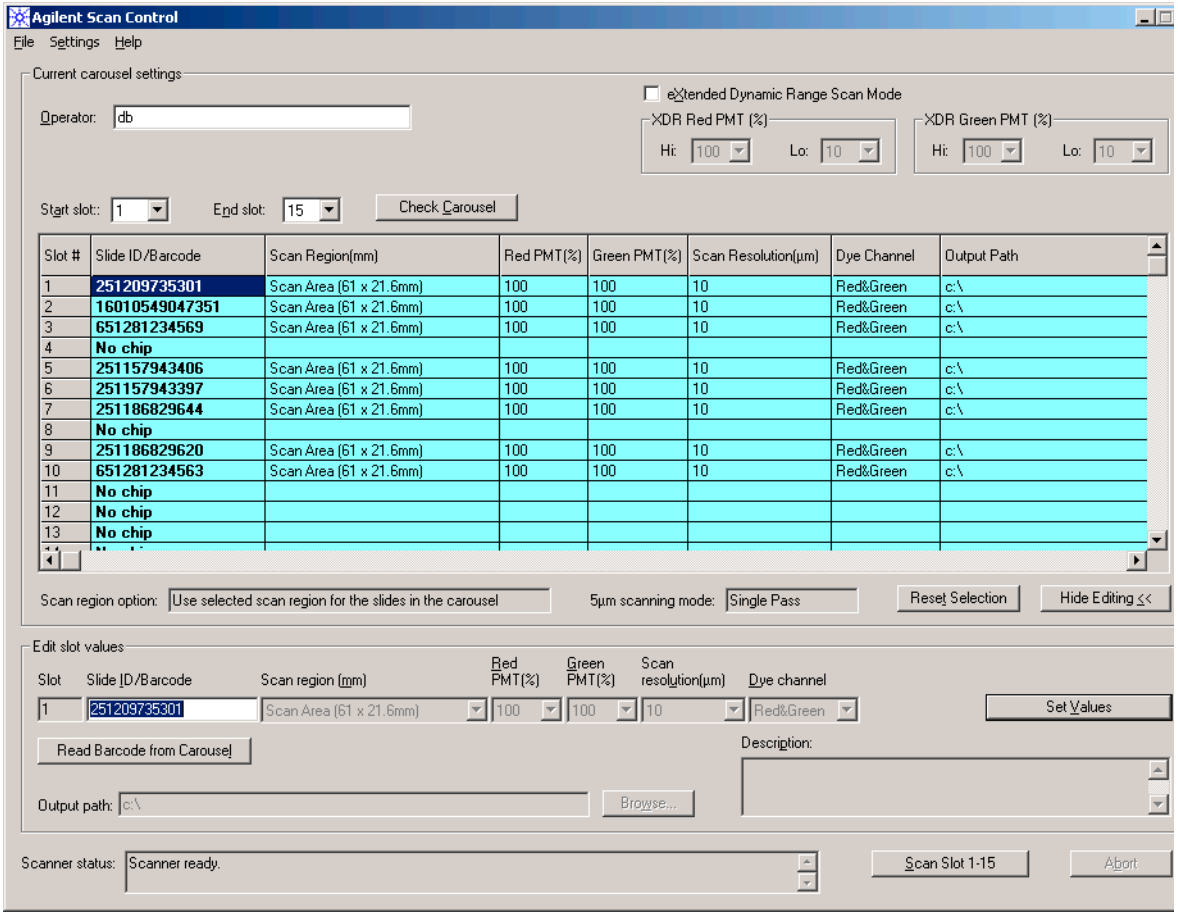


Figure 31 Main window after bar code reading

If you reset a selection to a default setting, the bar code will disappear.

Enter bar code/slide identifier information manually

You can also enter Agilent-supported bar codes or unique IDs through the keyboard or an external bar code reader. You must enter bar codes for non-Agilent slides with unsupported bar code formats this way.

1 Click **Edit Slot Values** on the Scan Control main window.

The Edit slot values panel appears below the main window for you to read in the bar codes for each slide before you start the scan.

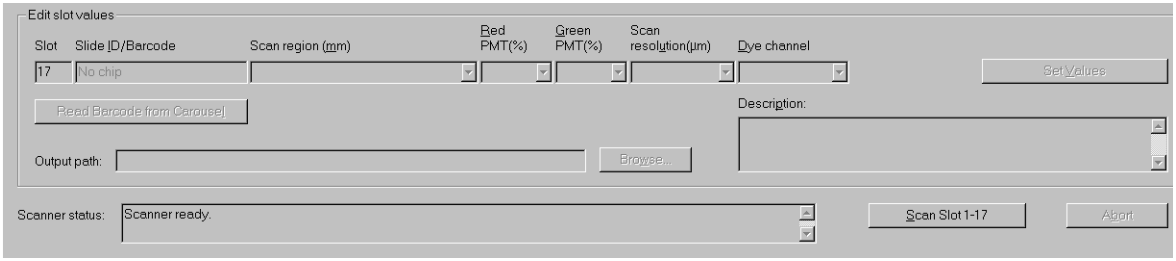


Figure 32 Edit slot values panel below the main window

2 Select the slots or slides whose bar codes you want to read.

Move the cursor to select more than one slide, or click the **Slide ID/Bar code** header to select all slides for entry.

3 Wand in a bar code for the slide with the external reader, or enter the bar code through the keyboard.

Depending on the external reader's settings, you may have to press Enter after each wand pass.

4 Repeat step 3 until you have entered bar codes for all slides.

Change scan settings

This section covers several procedures related to changing settings for Agilent and non-Agilent slides.

The scan table in the Scan Control main window can contain two kinds of scan settings:

- **Default settings:** The scan settings that you set up in the Default Settings dialog box
- **Non-default settings:** Changes that you have made in the scan table that differ from the default settings

Default settings

When you first enter the Scan Control main window, the scan table shows the shipped default settings. You cannot save changed default settings into a file, but you can have your own default settings.

When you start the Scan Control program after changing the default settings, you see the default settings that you entered or changed previously. If other users start the Scan Control program, they see their own changed default settings, not yours.

See "Change the default scan settings" on page 60.

Non-default settings

You can change a default setting in the scan table to a non-default setting with the Edit slot values panel in the main window. You can also edit the non-default settings in the same panel. See "Change scan table settings" on page 65.

You can always change a non-default setting back to a default setting. See "Change scan table settings to default settings" on page 66.

Change the default scan settings

1 Select **Settings > Modify Default Settings** from the menu bar.

When you first start the Scan Control program, the default Scan configuration settings are selected for Agilent slides.

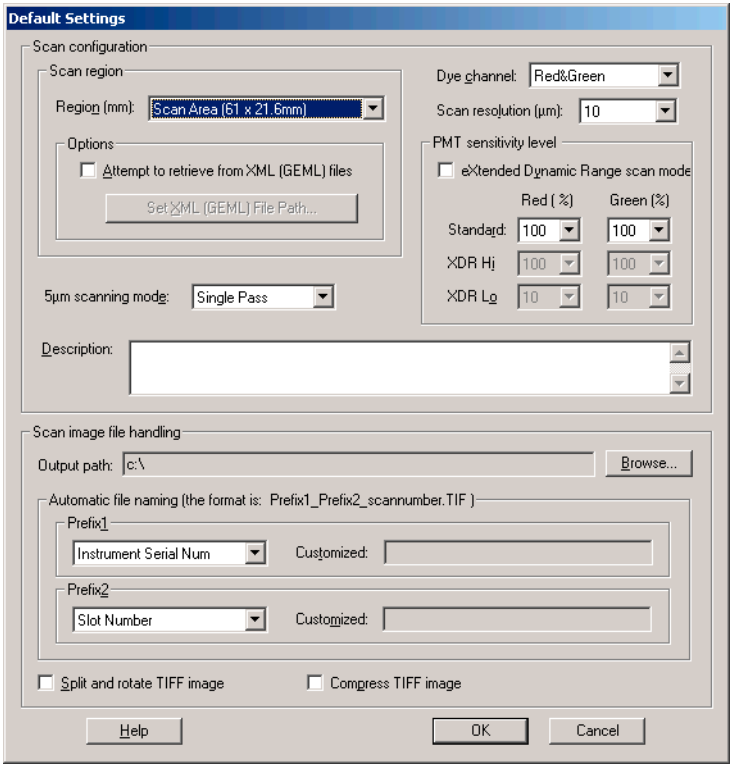


Figure 33 Default Settings dialog box

2 Change the settings using the choices below and on the next pages.

Scan region

The *scan region* determines the area of the slide that is scanned. It should be large enough to capture the entire print region of the microarray. It should be small enough to avoid scanning too close to the bar code or other non-transparent

border areas of the slide, which would affect the scanner's ability to autofocus properly. Minimizing the scan region will reduce scan time and save storage space.

For Agilent slides, the default scan region is 61 × 21.6mm. You can also select from the regions that you or others created with the Scan Region Editor. You can select from the other Agilent-provided scan regions as well, but in most cases, they will not be appropriate.

For non-Agilent slides, you can retain the Agilent default setting, or select one that better matches the expected print region from the other Agilent-provided scan regions or from regions that you or others created with the Scan Region Editor. See "Select a scan region for non-Agilent slides" on page 69, or see the Help for the Scan Region Editor.

Scan region options

For Agilent slides, you can also select **Attempt to retrieve from XML (GEML) files**. The system attempts to read the bar code and search for the scan region information in the selected XML database. If the scan region is not found, the system uses the scan region set in the scan table for each slide. You can change the XML (GEML) path.

Click **Set XML (GEML) File Path** to set or change the path to the location where the XML (GEML) files are to be found.

For non-Agilent slides, this option does not apply. Clear the check box and select from the Agilent-provided scan regions or from regions that you or others created with the Scan Region Editor.

For a combination of Agilent slides and non-Agilent slides, mark this check box if the default scan region is appropriate for the non-Agilent slides.

Dye channel

Determines whether only red (for example, Cy-5 dye), only green (for example, Cy-3 dye), or both dye channel information is gathered. The selection has no effect on the scan time, but selecting only one dye channel does reduce file size up to a factor of 2.

Scan resolution (µm)

Sets the scan resolution (pixel size) to 5 or 10 microns. Ten-micron scans and 5-micron single pass scans require about the same amount of time to complete, but a 10-micron scan consumes only one-fourth as much storage space.

Five-micron double pass scans do not require more storage space than 5-micron single pass scans, but they take twice as long to complete. Agilent recommends a 10-micron scan for features 70 microns or greater.

5 µm scanning mode

Choose between Single Pass and Double Pass scanning when 5-micron resolution is selected. The two modes differ with respect to how direction-dependent noise (DDN) is eliminated, and as a consequence, the time required to complete a scan.

Double pass mode averages signal intensities from two distinct, oppositely-directed scans of the same line. Single pass mode scans each line only once, determines the average DDN magnitude for the entire scan, and post-processes the data to compensate for it. Consequently, single pass scans take about half as long to complete.

PMT sensitivity level

Sets the sensitivity level of the photo multiplier tube (PMT). The PMT detects fluorescence emitted by the microarray. Different PMT settings are available depending on whether eXtended Dynamic Range scan mode (XDR) is marked.

With the check box cleared, the Standard sensitivity settings are active. The default output level (100%) is the recommended setting for Agilent arrays. You can reduce each color channel setting independently to as low as 1%.

If a microarray is so bright that the upper end of the output signal is saturated, the PMT sensitivity level can be lowered to a sensitivity range that allows all the information to be read. For example, an 80% setting reduces the signal intensity of the output TIFF file by 20% (above the dark level).

Alternatively, *mark* the XDR check box to automatically scan the same slide twice at two different sensitivity levels. This generates two linked images that Feature Extraction 9.1 can co-process to yield a single unified set of extracted intensity data covering up to 5+ orders of magnitude.

There are limitations to the XDR Hi and XDR Lo settings. The software automatically resets the selectable levels based on these limitations:

- Allowable sensitivity ranges
  XDR Hi: 100% to 10%
  XDR Lo: 50% to 1%
- Ratio range for XDR Hi / XDR Lo: 2 to 20

Example: If XDR Hi = 100%, the selectable range for XDR Lo is 50% to 5%. Microarrays that are too bright may eventually affect the PMT adversely.

Description

Description of each slide being scanned. This information becomes part of the file information that can be viewed. You can cut and paste within this text box.

Output path

The Browse button allows you to select a directory to store the data from each scan. The directory can be on the local hard disk or a storage area over the network, although Agilent recommends that the data be acquired to a local directory, on the secondary hard drive, i.e. Drive D. You cannot type a directory name into the Output Path text box. You must browse to an existing directory.

Automatic file naming

For *standard scans*, the Scan Control program uses three text boxes to determine the file name generated while scanning and merges them to form the file name.

Prefix1_Prefix2_scannumber.tif.

The default prefixes are the instrument serial number and the bar code. You can change Prefix 1 to a custom name, and you can change Prefix 2 to either the slot number or a custom name.

The Scan Control program automatically assigns the scan number. Other file names in the selected data directory with the same Instrument and Bar code IDs are detected and compared. If no match is found, the scan number is set to S01. If a match is found, the scan number is incremented by one until the file name is unique. This feature avoids duplicate file name conflicts.

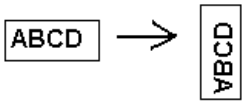
For *XDR scans*, an additional suffix (either _H or _L) is added to the filename to distinguish the XDR Hi image from the XDR Lo image:

Prefix1_Prefix2_scannumber_H.tif

Prefix1_Prefix2_scannumber_L.tif

Split and rotate TIFF image

Some analysis programs use data from only one color dye and images rotated 90 degrees. If you mark this check box, the color file is split into two color files and the images are rotated 90 degrees and flipped. An example of the image is shown below:



The split files now have the names of *FileName*_green and *FileName*_red, where *FileName* is the name that was automatically given to the file before it was split.

If you only select one dye channel for your output file, you must still mark this check box to rotate the image even if you do not need to split the file.

This option is not available for XDR scan mode.

Compress TIFF image

You can reduce the final amount of storage space occupied by scan images if this check box is marked for a carousel run. First, a temporary, uncompressed file is created, then after the scan is finished, the file is compressed and named according to the default file-naming settings. If for any reason the software fails to complete the compression step, the original uncompressed data file will be renamed according to the default file-naming settings.

The compression algorithm used, LZW, reduces the storage space on the order of 20 to 70 percent.

The data in compressed image files can be displayed and extracted only with Feature Extraction 9.1.

3 Click **OK**.

4 Change the scan table settings to the new default settings.

See "Change scan table settings to default settings" on page 66.

Change scan table settings

1 Check that the **eXtended Dynamic Range Scan mode** check box is marked or cleared appropriately to enable the scan mode you want to use. With XDR enabled, the PMT sensitivity settings are determined by the **XDR Red PMT (%)** and **XDR Green PMT (%)** values located above the scan table. The same sensitivity settings are applied to all slides in the carousel run. You can set all the other scan table parameters individually.

For the standard scan mode, i.e. when the **eXtended Dynamic Range Scan mode** check box is clear, you can change any of the scan settings for individual slides in the scan table. To return an individual setting to the setting that was entered in the Default Settings dialog box, highlight the setting and click the **Reset Selection** button.

2 Click **Edit Slot Values** to change individual scan table settings.

The window expands to show the Edit slot values panel.

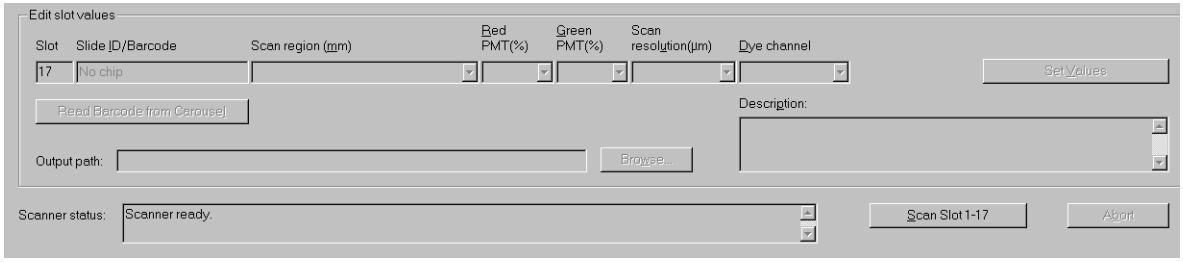


Figure 34 Edit slot values panel

3 Select the slide or slides whose setting you intend to change.

- Click the **Slot** header in the upper left corner of the table to select all the slides.
- Select an individual slide.
- Drag the cursor down multiple slides to select more than one slide.

4 Change the setting.

See “Change the default scan settings” on page 60 for information on the selections in the Edit slot values panel, or see the individual task in this section that relates to the setting that you intend to change.

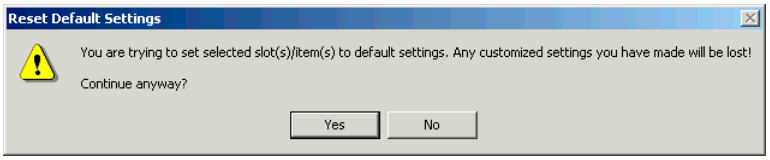
Change scan table settings to default settings

1 Select the slot or slots to contain the new default settings.

- Select one slot in the scan table
- Select more than one contiguous slot by dragging the cursor over the slots
- Select the **Slot** header in the upper left-hand corner of the table to select all the slots in the scan table.

2 Click **Reset Selection**.

The Reset Selection message appears.



3 Click **Yes**.

After applying the default settings to one or more slots, the remaining non-default settings appear in bold.

Select a scan region for Agilent slides

The *scan region* determines the area of the slide that is scanned. The larger the region, the longer the scan time. Scan regions vary because of factors such as bar code label placement and size, and areas with frosted glass.

When you first start the Scan Control program, the default settings are selected for Agilent slides. The scan region is 61 mm x 21.6 mm, and the **Attempt to retrieve from XML (GEML) files** check box is *not* marked.

You can select a different scan region for one or more slides if you want, but normally you do not have to. See "Change scan table settings" on page 65.

Set the XML(GEML) path

The *XML database* contains the microarray layout files that have critical information about the microarray. The information can include scan region, feature extraction settings, location of each microarray feature, and name and sequence of each microarray probe.

If the **Attempt to retrieve from XML (GEML) files** check box is marked, the system attempts to read the bar code and search for the scan region information in the selected XML database. If the scan region is not found, the system uses the scan region set in the scan table. You can modify the XML (GEML) path.

1 Select **Settings > Modify Default Settings** on the menu bar.

The Default Settings dialog box appears.

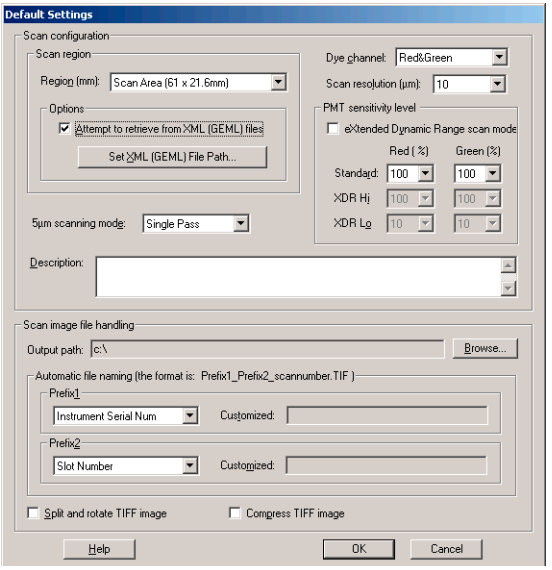


Figure 35 Default Settings dialog box

2 Make sure that the **Attempt to retrieve from XML (GEML) files** check box is marked.

3 Click **Set XML (GEML) file path**.

4 Click **Browse** to find a directory to add, and click **OK**.

5 Click **Add**, and click **OK**.

To remove a directory, select the directory and click **Remove**.

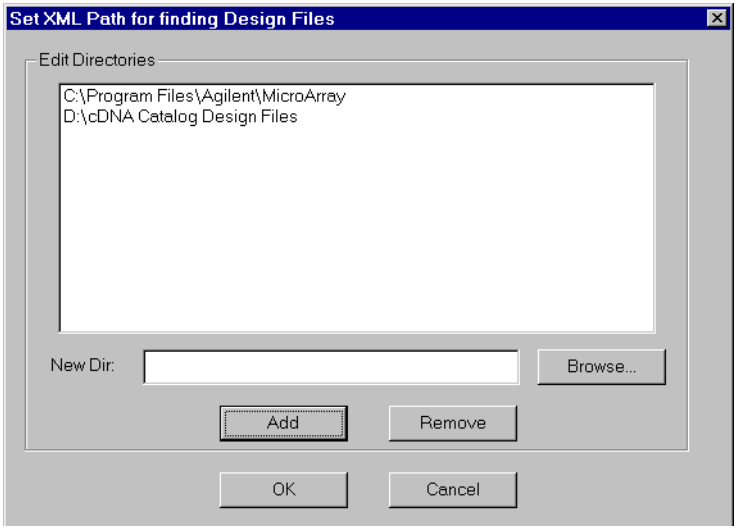


Figure 36 Set the XML (GEML) path

6 Click **OK** in the Default Settings dialog box.

Select a scan region for non-Agilent slides

If you use non-Agilent slides, you can select from the already existing scan regions, either the Agilent-supplied scan regions for non-Agilent slides or the scan regions that you or others created.

Select a default scan region setting

1 Select **Settings > Modify Default Settings** from the menu bar.

The Default Settings dialog box appears. See Figure 35.

2 Under **Scan Region**, clear the check box **Attempt to retrieve from XML (GEML) files**.

3 In the **Region (mm)** drop-down list box, select an Agilent-supplied scan region or an existing one that you or someone else created.

Agilent-Supplied Scan Regions
Scan Area (71 x 21.6 mm) - Agilent G2565BA only
Scan Area (67.2 x 21.6 mm)
Scan Area (61 x 21.6 mm)
Scan Area (55 x 21.6 mm)
Scan Area (50 x 21.6 mm)
Scan Area (45 x 21.6 mm)
Scan Area (40 x 21.6 mm)

4 Click **OK**.

5 Change the scan table scan region to the new default settings.

(See "Change scan table settings to default settings" on page 66.)

Select an existing scan region for slides in the scan table

1. Click **Edit Slot Values** on the Scan Control main window.

   The Edit slot values panel appears below the main window for you to change the scan region for individual slides. See Figure 30.

2. Select the slide whose region you want to change.
3. Select the scan region from the list and click **Set Values**.

   You can also press Enter to place the new setting in the scan table.

4. Repeat steps 2 and 3 for all the slides whose scan region you intend to change.
5. Click **Hide Editing**.

To change a setting back to its default setting, select the slide or slides in the scan table, and click **Reset Selection**. You apply the default settings to all slides when you click **Slot** in the upper left of the scan table and click **Reset Selection**.

See "Change scan table settings to default settings" on page 66.

Create a new scan region for non-Agilent slides

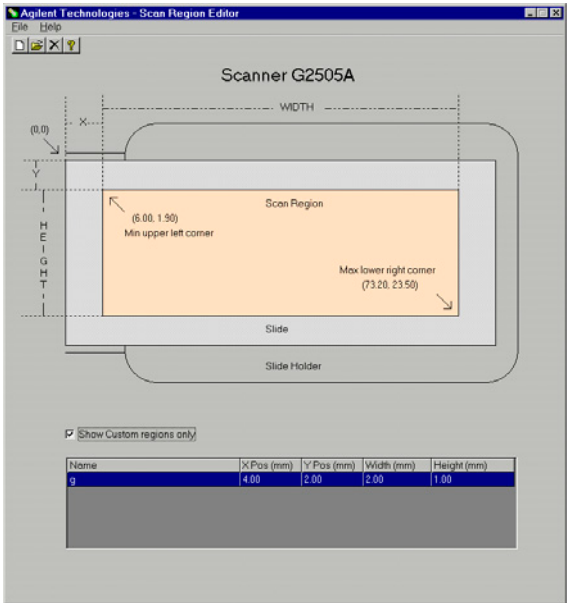
If you use non-Agilent slides, you can create your own scan region or change a scan region up to the maximum scan region of 71 mm x 21.6 mm for the Agilent G2565BA and 67.2 mm x 21.6 mm for the Agilent G2565AA. Follow the instructions below.

Start the Scan Region Editor from the Scan Control main window

The Scan Region Editor is not available from the Scan Control main window when the scanner is scanning. See "Start the Scan Region Editor from the Start menu" on page 74.

- Select **Settings > Customize Scan Region** from the menu bar.

Agilent G2565AA



Agilent G2565BA

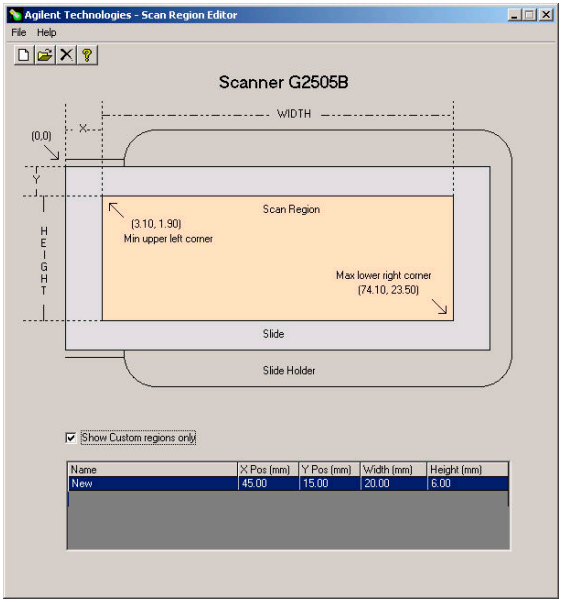


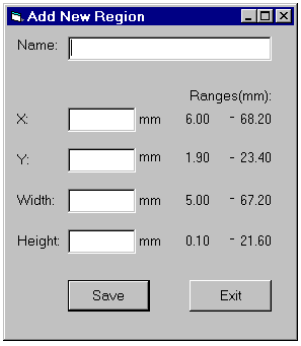
Figure 37 Scan Region Editor

Create a new scan region with the Scan Region Editor.

When creating a new scan region or using existing scan regions, make sure the scan region is at least 4 mm away from the bar code label and does not overlap any other opaque or translucent areas of the slide.

1 Select **File > New Region** from the menu bar.

Agilent G2565AA



Agilent G2565BA

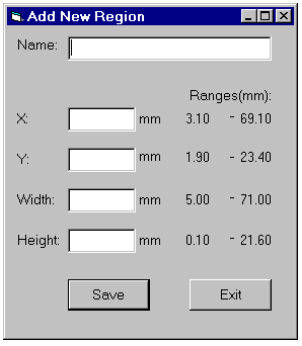


Figure 38 Add New Region dialog box

2 Enter the name or size of the region.

3 Enter the measurements in mm for the region, using the criteria listed below.

Invalid ranges are marked in red.

X X-axis measurement for the upper left-hand corner. You enter this measurement and that of the Y-axis in mm to position the region on the slide.

Y Y-axis measurement for the upper left-hand corner.

Width Width of the scan region measured from the end of the X-axis measurement in the upper left-hand corner.

Height Height of the scan region measured from the end of the Y-axis measurement in the upper left-hand corner.

Ranges Range of values that you can enter for each measurement.

4 Click **Save**.

If no errors are found, the Scan Region Editor appears with the new region listed in the Scan Region Editor.

5 Close the Scan Region Editor.

6 Select **Settings > Refresh Scan Region** from the menu bar.

The newly created regions now appear in the Scan Region lists.

Red entries in the table are invalid entries that are not available to the Scan Control program. This can occur when you specify that you have a Agilent G2565BA when you really have a Agilent G2565AA.

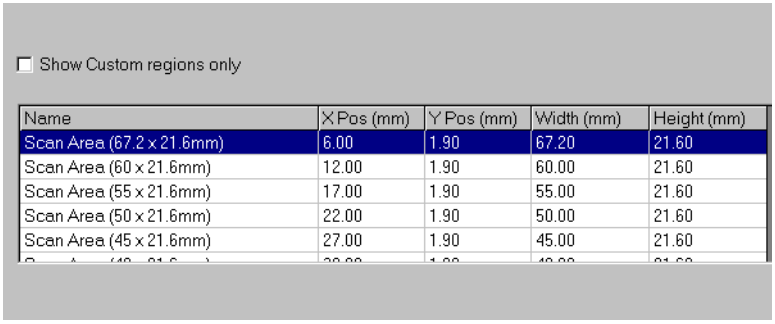


Figure 39 Scan regions list

Show Custom regions only

When this check box is marked, only the created scan regions appear in the list in the Scan Region Editor. When the check box is clear, you can also see the regions provided by Agilent in the list. However, you cannot change these regions with the Scan Region Editor.

Change an existing scan region for non-Agilent slides

You can only change the scan regions that you created. You cannot change or remove the regions provided by Agilent.

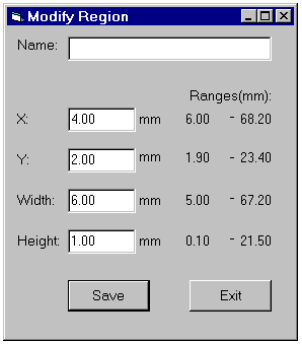
When creating a new scan region or using existing scan regions, make sure the scan region is at least 4 mm away from the bar code label.

1 In the Scan Region Editor, select the region to change.

2 Select **File > Open Region** from the menu bar.

The Modify Region dialog box appears.

Agilent G2565AA



Agilent G2565BA

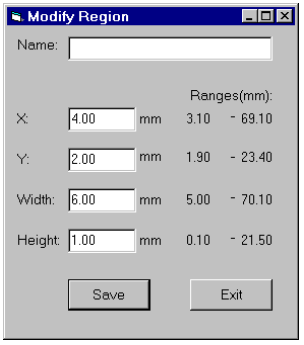


Figure 40 Modify Region dialog box

3 Change the name or size of the region.

4 Change the measurements for the region, using the criteria listed in "Create a new scan region with the Scan Region Editor." on page 72.

5 Click **Save**.

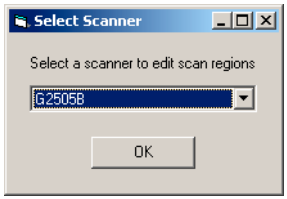
Start the Scan Region Editor from the Start menu

You cannot access the Customize Scan Region menu item in the main window when the scanner is in operation. You can only create and change new scan regions through the Start menu.

If you attempt to start the Scan Region Editor when a second one is already running, a message appears that says that another Scan Region Editor is running and you cannot open another one.

1 Select **Start > Programs > Agilent Life Sciences > Scan Region Editor** on the taskbar.

2 Select your microarray scanner model and click **OK**.



Change the sensitivity, resolution, or dye channel settings

This section covers how to change the default settings and scan table settings.

Change the default settings

1 Select **Settings > Modify Default Settings** from the menu bar.

The Default Settings dialog box appears. See Figure 35.

2 Select the sensitivity, scan resolution, or dye channel setting that you need.

Use the definitions in "Change the default scan settings" on page 60 to help make your selections.

3 Click **OK**.

4 Change the scan table settings to the new default settings.

See "Change scan table settings to default settings" on page 66.

Change the scan table settings

1 Click **Edit Slots Values** on the Scan Control main window.

The Edit slot values panel appears below the main window. See Figure 30.

2 Select the slot whose settings you want to change.

You can select more than one slot at a time by dragging the cursor up and down the table.

3 Select the new settings for this slide.

See the definitions of the settings in the topic "Change the default scan settings" on page 60 to help you select the ones to change.

4 Click **Set Values**.

You can also press Enter to place a setting in the scan table.

5 Click **Hide Editing**.

To change a setting back to its default setting, see "Change scan table settings to default settings" on page 66.

Change the storage directory and check drive capacity

This section covers how to change settings in the storage directory and how to check the drive capacity.

Check the drive capacity

- Determine the free space on the hard disk:

  a In Windows Explorer, right-click on the data drive and select Properties from the shortcut menu.

  b Click the **General** tab.

  The total drive capacity as well as remaining free space are displayed on the General tab.

  A 10-micron scan is 50 MB, and a 5-micron scan is 200 MB.

Agilent recommends that you do not use the system partition (usually drive C:) to store data and that you scan to the local hard drive first and then move or archive the scan files to a network or CD drive later.

Change the default settings for the storage directory

1 Select **Settings > Modify Default Settings** from the menu bar.

The Default Settings dialog box appears. See Figure 35.

2 Change the path and enter a description for the slides that you want to change.

You can use the cut and paste functions for the description.

3 Click **OK**.

Change the scan table settings for the storage directory

1 Click **Edit Slot Values**.

The Edit slot values panel appears under the Scan Control main window. See Figure 30.

2 Select the slide or slides whose output path you want to change.

You can select more than one slide at a time. Just drag the cursor up and down the list of slides. You cannot select non-contiguous slides.

3 Change the path and enter a description for the slides that you want to change.

See the topic "Change the default scan settings" on page 60 for more information.

4 Click **Hide Editing**.

To change a setting back to its default setting, select the slide or slides in the scan table, and click **Reset Selection**. You apply the default settings to all slides when you click the **Slot** header in the upper left of the scan table and click **Reset Selection**.

See "Change scan table settings to default settings" on page 66.

Change the prefixes for automatic file naming

The Scan Control program uses three text boxes to determine the file name generated while scanning and merges them to form the file name.

Prefix1_Prefix2_scannumber.tif.

The default prefixes are the instrument serial number and the bar code. If you want to change these prefixes, do the following:

1 Select **Settings > Modify Default Settings**.

The Default Settings dialog box appears.

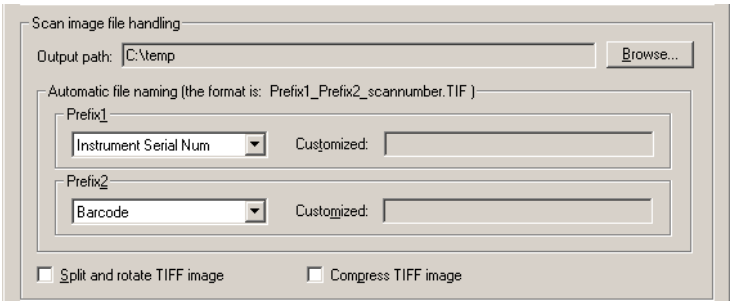


Figure 41 "Scan image file handling" section of the Default Settings dialog box

2 Under **Prefix1**, select from the following choices:

- Instrument Serial Number
- Customize

3 Enter your own custom prefix in the **Customized** box, if you selected Customize (Maximum characters = 36).

4 Under **Prefix2**, select from the following choices:

- Bar code
- Slot Number
- Customize

5 Enter your own custom prefix in the **Customized** box, if you selected Customized (Maximum characters = 36).

6 Click **OK**.

Example 2583_02506190000015_S03.tif

- Instrument serial number = 2583
- Bar code = 02506190000015
- Scan Number = S03. This is the third scan file in the directory with the same instrument and bar code.

The Scan Control program automatically assigns the Scan Number. Other file names with the same Instrument and Bar code IDs are detected and compared. If no match is found, the scan number is set to S01.

If a match is found, the scan number is incremented by one until the file name is unique. This feature avoids duplicate file name conflicts.

Rotate the scan image and split the color file

Some post-scan analysis programs need to have the scan image rotated 90 degrees and can only analyze data of one color. So the Agilent Scan Control program lets you tell the system to rotate the image and split the two-color composite file into two separate one-color files automatically.

1 Select **Settings > Modify Default Settings** from the menu bar.

The Default Settings dialog box appears. See Figure 35.

2 Under scan image file handling mark the check box **Split and Rotate TIFF Image**.

3 Click **OK**.

The image will be flipped as below:

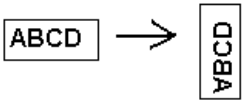


Figure 42 Result of the flip rotation

The split files now have the names of *FileName*_green and *FileName*_red, where *FileName* is the name that was automatically given to the file before it was split.

If you only select one dye channel for your output file, you must still mark this check box to rotate the image even if you do not need to split the file.

This option is not available for XDR scan mode.

Compress TIFF image

You can reduce the final amount of storage space occupied by scan images if this check box is marked for a carousel run. First, a temporary, uncompressed file is created, then after the scan is finished, the file is compressed and named according to the default file-naming settings. If for any reason the software fails to complete the compression step, the original uncompressed data file will be renamed according to the default file-naming settings.

1. Select **Settings > Modify Default Settings** from the menu bar.

   The Default Settings dialog box appears. See Figure 35.

2. Under scan image file handling, mark the check box **Compress TIFF Image**.
3. Click OK.

The compression algorithm used, LZW, reduces the storage space on the order of 20 to 70 percent.

The data in compressed image files can be displayed and extracted only with Feature Extraction 9.1.

Do other useful tasks

This section covers the following useful tasks you will need to know:

- Setting the technical support URL
- Automatically displaying the Carousel Report Log
- Finding version information
- Removing a slide from a version B slide holder
- Mounting or removing the carousel

Set the URL to reach technical support

The software arrives with a default URL that lets you access Agilent technical support for the Microarray Scanner. You can change this support URL if you want to have users go through your own support or another support organization first.

1 Select **Settings > Preferences** from the menu bar.

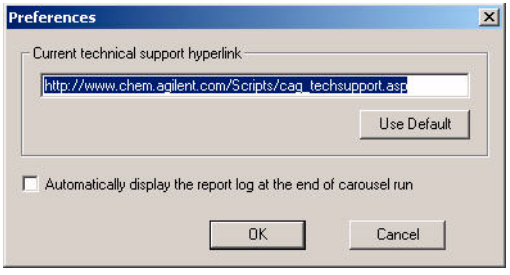


Figure 43 Preferences dialog box

2 Enter the URL of your choice, and click **OK**.

When you select **Help > Technical Support** from the menu bar, you access the Web site with the URL you entered.

To return to the original Agilent URL, click **Use Default**.

Automatically display Report Log after carousel run

You can set a preference option to automatically display the Carousel Report Log after each carousel run. We recommend that you check the log for errors before beginning the next carousel run because the previous log is erased once you start the new carousel run.

1 Select **Settings > Preferences** from the menu bar.

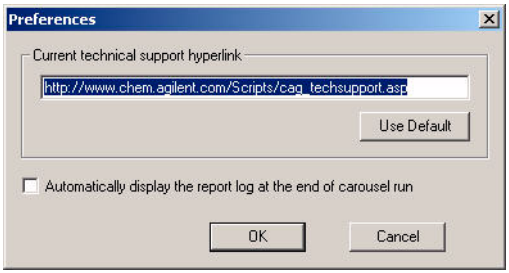


Figure 44 Preferences dialog box

2 Select the **Automatically display the report log at the end of carousel run** check box.

3 Click **OK**.

Find the version information for scanner software components

1 Select **Help > About** from the menu bar to find version information.

2 To close the program, click **OK**.

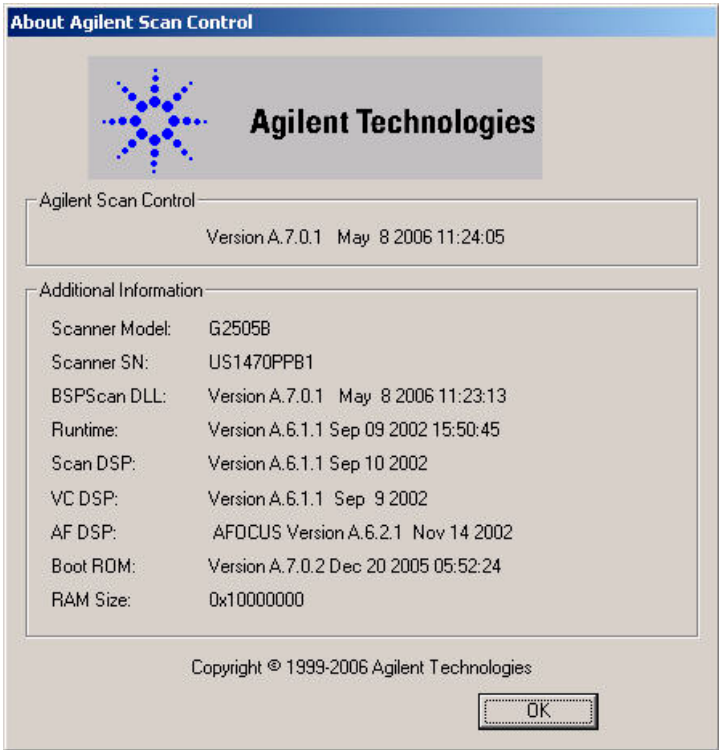


Figure 45 Example of version information for software components

Remove a slide from a version B slide holder

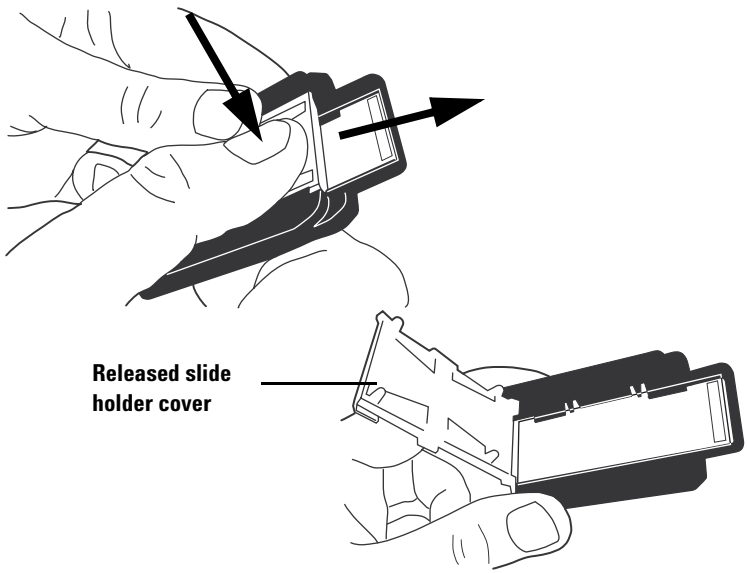
The version B slide holder can be used only in the Agilent G2565BA. For instructions on inserting the slide, see "Inserting a slide into a version B slide holder" on page 29 of Chapter 2.

Fingerprints cause errors in the fluorescence detection. For accurate readings, touch only the edges of the slide, and always use gloves when handling slides.

1 Hold the slide holder on the sides with the Agilent logo facing up.

2 Place one thumb on the center of the slide holder cover.

3 Gently press down on the cover and push toward the top of the slide holder until the cover releases.



4 Release the cover.

5 Push up on the bar code end of the slide from underneath the slide holder to avoid fingerprints on the sample area.

6 Grasp the slide from the sides and remove from slide holder.

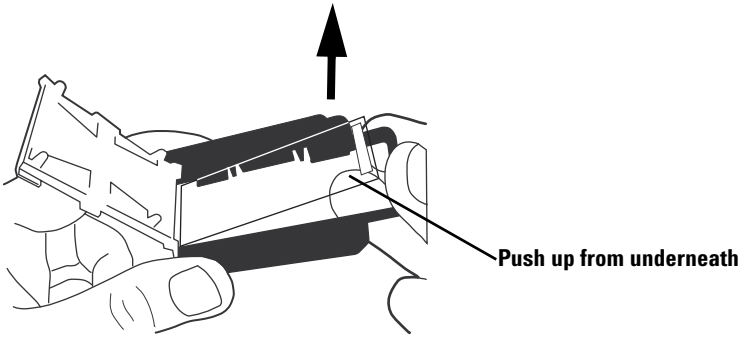


Figure 46 Proper removal of slide

Mount or remove the carousel

If you have placed the slide holders into a carousel that was removed from the Microarray Scanner, follow these steps to remount the carousel.

CAUTION When mounting the carousel, be careful not to knock the carousel against the autoloader arm. Knocking the autoloader arm can cause misalignment.

CAUTION If you need to rotate the carousel manually, do so only when the instrument is turned off or you may damage the instrument.

1. Mount the carousel so that the carousel alignment pin holes fit in with the alignment pins on the Microarray Scanner carousel mounting area.

   Align the D-shaped hole in the bottom of the carousel with the D-shaped center post of the scanner tabletop (where the carousel fits) and gently place the carousel into the scanner straight down.
2. Make sure that the carousel is firmly seated.
3. Close the Microarray Scanner access lid.

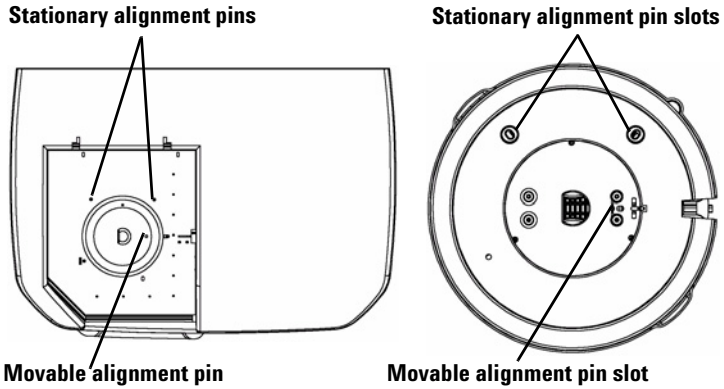


Figure 47 Alignment pins and slots on scanner and carousel

If an error message appears in the Carousel Log Report that says there is a motor time-out, this time-out may be due to an improperly mounted carousel. Check the carousel alignment and remount, if necessary.

```
AGILENT SCANNER -- CAROUSEL REPORT LOG
Fri May 10 15:44:53 2002
Carousel run requested from Slot 1 to Slot 48.
Checking carousel failed. Description:Motor timed out.
```

CAUTION Removing the carousel when the slide holder autoloader arm is extended into the carousel will cause serious damage to the Microarray Scanner. Call your local Agilent sales and support center for instructions if the autoloader arm is extended.

4 Troubleshooting

The Agilent Microarray Scanner was designed for low-maintenance requirements and high user-friendliness. If you cannot resolve the problem you are experiencing with the system, please read over this chapter before calling your local Agilent sales and support center.

Technical support This topic gives important information about technical support.

Frequently asked questions Refer to this section to learn about solutions to frequently asked questions related to support and maintenance.

Hardware troubleshooting This topic describes the indicator lights and how to replace the fuses.

Software troubleshooting This topic covers the precautions you must take when you use the Recovery CD to recover the software and data on your hard drive.

Scanner error messages Refer to this topic to learn about important error messages and solutions.

Technical Support

Technical support is available for the Agilent Microarray Scanner System. Please read the rest of this chapter prior to calling your local Agilent sales and support center.

PC workstation support

If you have a problem with your PC workstation, see the documentation that came with the PC. If you are still unable to resolve the problem, contact your local Agilent sales and support center or see Agilent support at www.agilent.com/chem/dnasupport.

Microarray Scanner support

If you have a problem with your Microarray Scanner that requires assistance from your local Agilent sales and support center, you may be asked to provide the latest diagnostic log files that the Scan Control program created. These files are located in the folder **C:\Program Files\Agilent\Microarray\Logs**.

Additional diagnostic information including results of the Power-on Self Test can be captured using the HyperTerminal program. See “Using HyperTerminal to Capture Diagnostic Data” on page 99 for more information on setting up and using this communication protocol.

Each Agilent Microarray Scanner is identified by a unique 10-character serial number. The serial number is located on a label in two places on the Microarray Scanner: one in the carousel bay and one behind the filter door.

When corresponding with your local Agilent sales and support center about your Microarray Scanner, be sure to include the model number and the full 10-character serial number.

Please make a note of the serial number of your Agilent Microarray Scanner and the installation date in the spaces below (if you print this page) or on a sheet of paper that you keep close to your scanner.

Scanner information

Model #: _____

Serial #: _____

Installation Date:_____

Software Version#: _____

Software Update Version#/Date: _____

Software Update Version#/Date: _____

Frequently Asked Questions (FAQs)

The following are frequently asked questions (FAQs) and the corresponding answers that can help you operate and maintain the Microarray Scanner System and troubleshoot issues that occur.

Table 2 FAQs

FAQ	Answer
I want to move the Microarray Scanner to another area.	If you need to move the scanner, there is a chance that its performance could be adversely affected. Call your local Agilent sales and support center for assistance in moving the scanner and assuring proper operation afterwards.
Can I save files over the network while scanning?	Save your data files directly to the local hard disk. Saving to a storage device over the network can cause data loss or other problems if network traffic becomes congested.
How do I obtain the Scan Control program revision information?	When communicating with Agilent Technical Support, you might be asked to obtain the Scan Control program revision information. This is done by selecting Help > About in the main menu.
Where do I find support information, such as drivers, guides, and troubleshooting solutions, for my PC workstation?	If you have a problem with your PC workstation, see the documentation that came with the PC. If you are still unable to resolve the problem, contact your local Agilent sales and support center.
I cannot open the top lid to access the carousel.	Do not attempt to force the lid open. Forcing the lid could damage the Microarray Scanner. **1** Push down on the lid to reduce any existing spring tension, and then attempt to open the lid. If this fails, then go to step 2. **2** Close and then restart the Scan Control program. **3** Try opening the lid. **4** If the lid still does not open, close the Scan Control program and turn the Microarray Scanner off, then back on. **5** Try opening the lid. **6** If the lid still does not open, contact your local Agilent sales and support center.

Table 2 FAQs (continued)

FAQ	Answer
The Scan Control program does not respond.	The Scan Control program has stopped responding—no changes in the status message for more than 15 minutes and you are unable to close the program. Do the following: **1** Open the Windows Task Manager (Ctrl-Alt-Del) and close the Scan Control program. Then restart the Scan Control program. **2** If the previous step fails, reboot the PC workstation, turn the Microarray Scanner off and then back on, wait for the yellow status indicator light to go off, and then restart the Scan Control program.
What do I do if a slide is only halfway inserted in the carousel by the autoloader arm?	**1** Gently push or pull the autoloader arm, whichever resists least, to the end of its range. Don't force it. **2** Close and restart the Scan Control program. **3** Call your local Agilent sales and support center if the problem persists.
The Microarray Scanner is turned on, but the yellow light does not go off after 20 minutes.	**1** Verify that the PC workstation is turned on. **2** Verify that the Ethernet cable is connected from the PC workstation to the Microarray Scanner. **3** Contact your local Agilent sales and support center.
Can I use both slide holders (version A and version B) on all Microarray Scanners?	**Agilent G2565AA**: Use only version A slide holders. Version B slide holders may fail to load or eject reliably and could damage the Microarray Scanner. **Agilent G2565BA**: Use only version B slide holders. Version A slide holders may fail to load or eject reliably. The largest scan region would not be usable.
I created a custom scan region and it doesn't show up as one of the choices for the scan regions.	After creating a custom scan region, you must synchronize scan regions. From the main Scan Control menu, select **Settings > Refresh Scan Region** from the top menu.

Hardware Troubleshooting

The Microarray Scanner has very few parts that you can service yourself. You can take actions based on the errors shown by the status indicator lights on the front. You can also replace the fuses that protect the system. Any other problems, including jams, should be handled by contacting your local Agilent sales and support center.

If you are having a problem with your PC workstation, see "PC workstation support" on page 90 for support information.

Troubleshooting with the status indicator lights

The front panel holds three status indicator lights indicating the current status of the Microarray Scanner.

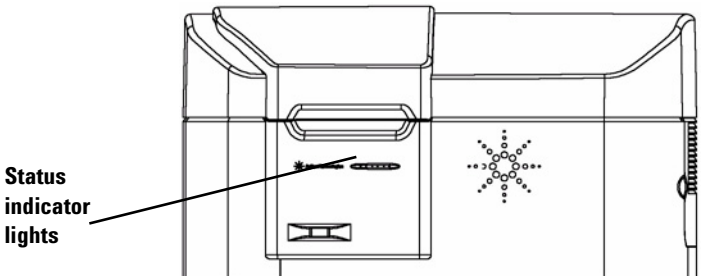


Figure 48 Location of the status indicator lights

Table 3 describes the actions that you take given the status of the indicator lights.

Table 3 Indicator Light Status

LED Status			Possible Causes	Suggested Action
Green	**Yellow**	**Red**		
Off	Off	Off	No power to the scanner.	Check the following: • power switch is on • power cord is plugged in and there is power at the source • power fuses are not blown
Flash once	Flash once	Flash once	LED test at power-on (duration < 1 sec.)	No action required.
Flashing	Flashing	Off	Power-on Self Test in operation	No action required. User HyperTerminal to capture diagnostic information. See "Using HyperTerminal to Capture Diagnostic Data" on page 99.
Off	Flashing (20 sec. off/ 20 sec. on)	Off	Attempting to connect to PC	Install Scan Control software version 7.0 if not already installed.
Off or On	Flashing (period = 1.3 or 1.0 sec.)	Off	Firmware downloading and DSP initializing	No action required.
On	On	Off	Lasers are warming up and are not ready to scan.	Wait. Maximum warm-up time is 20 minutes.
On	Off	Off	Instrument is ready to scan.	No action required.
Flashing	Off	Off	Instrument scanning	No action required.
Any condition	Any condition	On	Error detected.	Contract your local Agilent sales and support center.

Checking and replacing scanner fuses

The Microarray Scanner has three fuses, two fuses for the power supply and one fuse for the red laser. The fuses can be found on the rear of the Microarray Scanner. The power supply fuses are directly above the power cord plug. The laser fuse is located in a circular fuse holder to the left of the power cord plug.

The fuses can be ordered directly from Agilent Technologies.

WARNING **Always disconnect the power cord before checking or replacing the fuses.**

Checking and replacing the power supply fuses

If the Microarray Scanner cannot be powered on even though the power outlet is active when tested, you should check and replace the fuses if needed. You do this from the rear of the unit.

1 Disconnect the power cord.

2 Use a small flat-edge screwdriver to pry up the small plastic tab located on the bottom edge of the fuse holder until it releases.

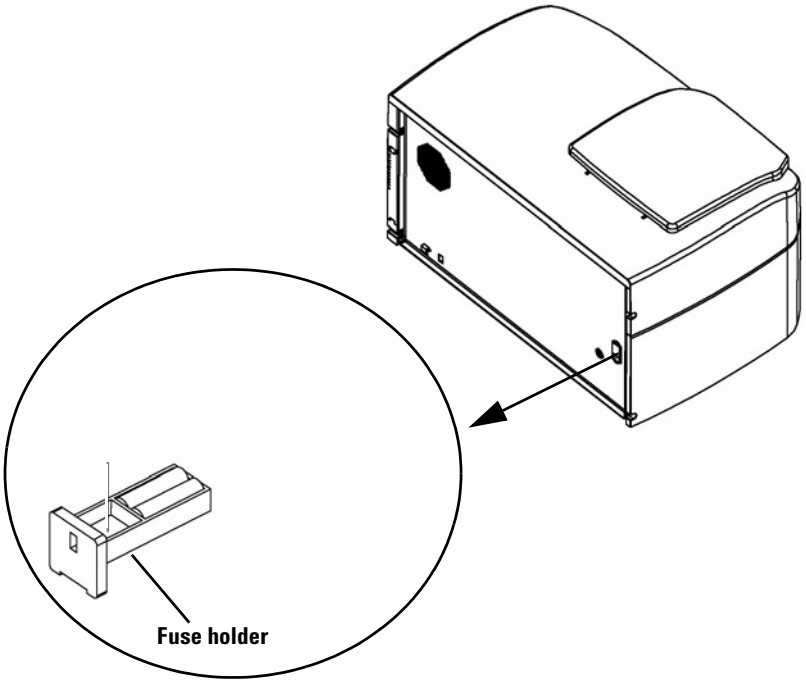


Figure 49 Removing the fuse holder for the power supply

3 Pull out the fuse holder, and check the fuse integrity.

4 If a fuse is blown, replace the fuse with a T4A, 250 VAC rated fuse.

5 Push the fuse holder back in until it clicks into place.

6 Plug in the power cord.

CAUTION Replace the fuses with only the same or equivalent rated fuses. If you are unsure about the fuses, contact your local Agilent sales and support center before installing.

Checking and replacing the red laser fuse

If the Microarray Scanner can be powered on but the red laser is not on, you can easily check and replace the laser fuse from the rear of the unit.

1 Disconnect the power cord.

2 Use a small flat-edge screwdriver to unscrew the small plastic circular cap until it releases.

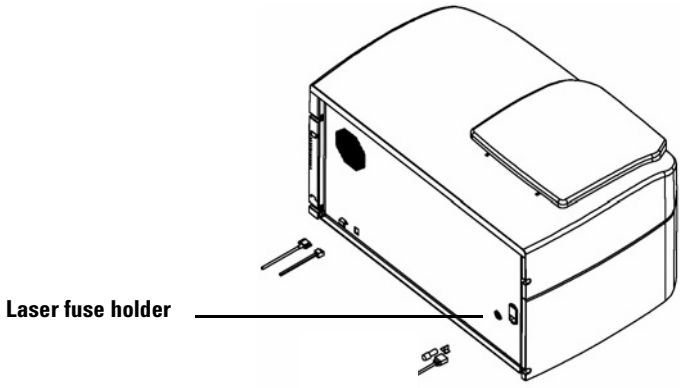


Figure 50 Laser fuse holder location

3 Pull out the fuse holder, and check the fuse integrity.

4 If a fuse is blown, replace the fuse with a T500mA, 250 VAC rated fuse.

5 Push the fuse holder back into place and screw on the cap.

Using HyperTerminal to Capture Diagnostic Data

The Agilent Scanner comes with a diagnostic cable that allows Agilent service personnel to do troubleshooting. To capture information that comes through the cable, you will need to set up a HyperTerminal session. These instructions apply to Windows 2000 systems.

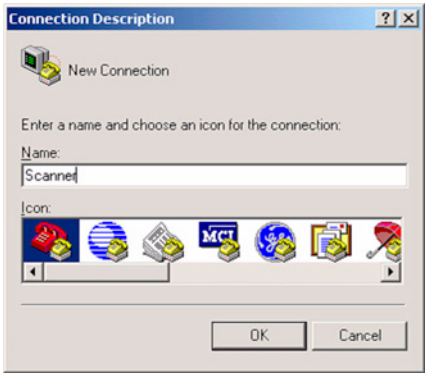
To set up HyperTerminal

1 Click **Start > Programs > Accessories > Communications > HyperTerminal.**

2 In the Connection Description dialog box:

   a Type `Scanner` in the **Name** text box.

   b Click **OK**.



3 In the Connect To dialog box:

   a From the **Connect using** drop-down list, select **COM1**.

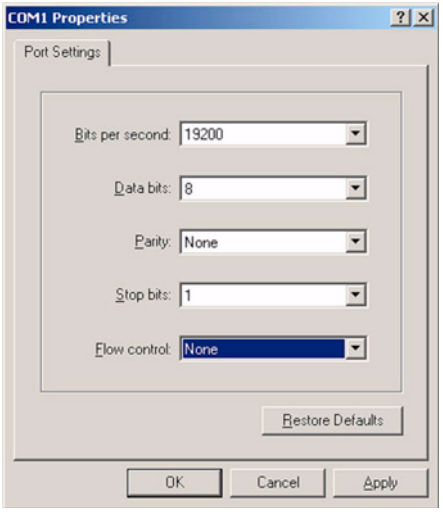
   b Click **OK**.

4 From the COM1 Properties dialog box:

a Select **19200** as **Bits per second**.

b Select **None** as **Flow Control**.

c Click **OK**.



The Scanner - HyperTerminal window appears.

5 Click **File > Save** to save the file to **Scanner.ht**.

6 Create a **Scanner.ht** shortcut icon on the Desktop:

   a Click **Start > Programs > Accessories > Communications.**

   b From the HyperTerminal submenu, right-click **Scanner.ht.**

   Note that the HyperTerminal submenu has been added to the bottom of the Communications menu. Do not right-click the HyperTerminal program icon towards the top of the menu.

   c From the shortcut menu, click **Create Shortcut.**

   d Click and drag **Shortcut to Scanner.ht** to the Desktop.

7 Place a **Scanner.ht** shortcut icon in **All Users Startup** folder:

   a Right-click **Shortcut to Scanner.ht** on the Desktop.

   b Select **Copy.**

   c In Windows Explorer, paste the shortcut to **C:\Documents and Settings\All Users\Start Menu\Programs\Startup**.

8 Start a HyperTerminal text capture session:

   a Double-click the **Scanner.ht** icon on the Desktop.

   b Click **Transfer > Capture Text**.

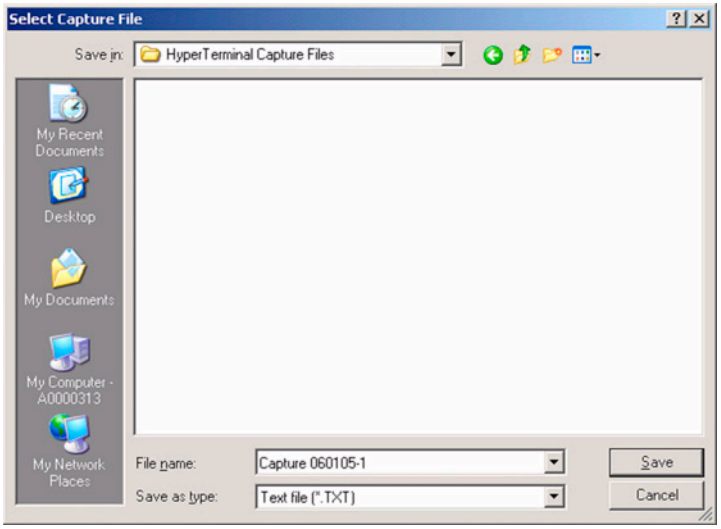
   c Click **Browse**.

   d In the **Select Capture File** dialog box, find the Archive (E:) drive.

   e Create a new folder named **Support**.

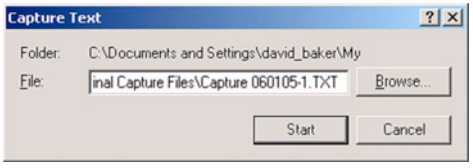
   f In the **Support** folder, create a new folder and call it **HyperTerminal Capture Files**.

   g In the **HyperTerminal Capture Files** folder, type the file name `Capture` *`yymmdd`*`-#` in the **File name** text box, where *yymmdd* represents today's date, and # represents the number of the capture session for that day.



h Click **Save**.

i Click **Start** in the Capture Text dialog box.



HyperTerminal data will continue to append to this file until HyperTerminal is closed or the **Capture Text...** session is stopped from the **Transfer** drop-down menu.

HyperTerminal will close when you log off.

To start a HyperTerminal capture session after initial set-up

1 Log onto the Scanner PC.

If the shortcut to **Scanner.ht** is in the All Users Startup folder, HyperTerminal will start automatically.

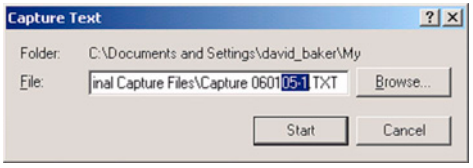
2 If HyperTerminal does not start, double-click on the Desktop **Scanner.ht** icon.

3 From the HyperTerminal **Transfer** drop-down menu, click **Capture Text.**

4 In the **Capture Text** dialog,

a Change the file name in the **File** text box to reflect today's date and capture session.

b Click **Start**.



You can also click Browse to open the Select Capture File dialog box. From the Select Capture File dialog box, you can create new folders or go to a different folder, and create a new file. Make sure you choose a new folder or a new file name, or else your original capture session file will be overwritten. When you are done, click **Save** to continue.

5 Turn on the scanner, if it's not already on.

6 When the scanner is warmed up (about 5 minutes after you turn it on), start the Scan Control software.

Software Troubleshooting

In case your computer crashes or you want to reload the hard drive image that Agilent ships with the PC, Agilent supplies a recovery CD that lets you re-image the hard drive. In order to use this CD successfully you must disconnect the two LAN cables that attach to the LAN cards in the back of the PC before you use the CD. One cable is from the scanner and the other is to the company/corporate network (if the company has one).

CAUTION If you do not disconnect these cables, the Agilent default LAN settings for these cards may be changed, and the software will not recognize the scanner when you restart the software.

Microarray Scanner Error Messages

This section explains how to use error messages you may encounter.

Sources of error messages

The computer monitor shows error messages for the Microarray Scanner hardware, the scanning and feature extraction software, Microsoft Windows, and the PC workstation. Check the title bar of the error message to determine the source of the error message.

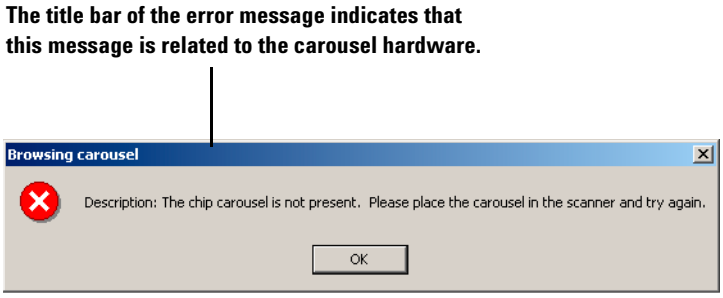


Figure 51 Title bar of a Scan Control error message

This topic presents the error messages for the Microarray Scanner hardware and the Scan Control program.

If the title bar indicates that you are having a problem with your PC workstation or Windows, see "PC workstation support" on page 90 for support information.

Where scanner error messages can appear

Error messages can appear in the following places:

- Errors during the Microarray Scanner initialization appear in the **Scanner status** message in the Scan Control window. See "Step 4. Start the Scan Control program" on page 38 of Chapter 2.
- Errors before the scan starts are shown in message boxes.

- Some errors during the scan are shown in the **Status** message in the Scan Progress dialog box.
- Error messages are logged in the Carousel Report Log as the carousel run progresses. See "Step 8. Start the carousel run" on page 44 of Chapter 2. We recommend that you check the Report Log at least after the first scan and when the carousel run is completed. You can set an option in the Preferences menu to automatically show the report log after a run is completed. See "Automatically display Report Log after carousel run" on page 82 of Chapter 3.

Troubleshooting with error messages

The Microarray Scanner System creates error messages to help you solve issues that may arise. Many of the error messages include a solution within the text box; follow those instructions.

The following table contains some of the error messages that appear without instructions. These descriptions contain information that can help you resolve your problem.

Table 4 Error Messages

Error Message	Description or Solution
Application Is Already Running	You cannot run two copies of the Scan Control program at the same time.
Autofocus error: Did not detect two reflective surfaces during home.	Do the following: **1** Verify that the slide is inserted properly in the slide holder. See "Step 2. Insert slides into slide holders" on page 28, or try another slide holder. **2** Verify that the slide meets the Agilent specifications. See "Slide specifications" on page 123. **3** If the problem persists with one slide, then there might be something wrong with that slide. **4** If needed, select or create an appropriate scan region. For some non-Agilent slides with translucent or opaque borders, the scan region selected may be located too near to the non-transparent areas of the glass. See "Select a scan region for non-Agilent slides" on page 69. **5** Contact your local Agilent sales and support center.

Table 4 Error Messages (continued)

Error Message	Description or Solution
Autofocus error. No PSD signal is detected. Make sure there is a slide in the chip holder.	Do the following: **1** Verify that the slide is inserted properly in the slide holder. See "Step 2. Insert slides into slide holders" on page 28. **2** Verify that the slide meets the Agilent specifications. See "Slide specifications" on page 123. **3** If the problem persists with one slide, then there might be something wrong with that slide. **4** For some non-Agilent slides with translucent or opaque borders, the scan region selected may be located too near to the non-transparent areas of the glass. See "Select a scan region for non-Agilent slides" on page 69. **5** Contact your local Agilent sales and support center.
Autofocus was unable to retain focus as it travelled to the scan start position.	Do the following: **1** Verify that a bar code has not obstructed the scan start position. **2** Choose a smaller scan region. **3** If the problem persists with one slide, then there might be something wrong with that slide. **4** Contact your local Agilent sales and support center.
Cannot eject chip. There is already a chip in the slot.	Do the following: **1** Open the scanner lid. **2** Remove the slide holder from the home slot.
Cannot load chip, moving the arm may be unsafe.	Do the following: **1** Open the scanner lid. **2** If the AL Arm is partially extended into the carousel compartment, gently push it back into the scanner until it comes to a stop. **3** If the problem persists, contact your local Agilent sales and support center.
Command timed out.	Do the following: **1** Verify that the Ethernet cable from the Microarray Scanner to the PC workstation is connected. **2** Turn the Microarray Scanner off then on again. **3** Verify that the LEDs on the PC network adapter that is connected to the scanner are lit and/or flashing. **4** Restart the Scan Control program. **5** If the problem persists, contact your local Agilent sales and support center.

Table 4 Error Messages (continued)

Error Message	Description or Solution
Connection manager reports that there are no instruments.	Possible causes for this error include: • The Microarray Scanner might not be on, or you turned on the Microarray Scanner but did not wait long enough for the Microarray Scanner to complete its self-test and initialization. Select OK to close the Scan Control message, wait a few minutes, and try again. • The Microarray Scanner might not be connected to the PC workstation. Verify the Ethernet connector is connected to both the Microarray Scanner and PC workstation. If the Ethernet connector is disconnected, reconnect the cable, reboot the PC computer, and if needed turn the Microarray Scanner off then on. • The Ethernet cable connected to the scanner may be a regular LAN cable and not a crossover cable. If the PC Ethernet cables have been disconnected recently, the House and Scanner cables may have been switched. They are not interchangeable; the scanner must be connected to the PC with a crossover cable. • The PC network adapter that is connected to the scanner may have failed. Verify that the LEDs on the PC adapter are lit or flashing. • The Internet Protocol (TCP/IP) networking properties for the scanner may have been altered. Verify that the IP address for the scanner's Local Area Connection is 10.0.0.1 and its subnet mask is 255.255.255.0. • Windows XP firewall may be preventing communication. Disable the firewall. • If you rebooted the PC workstation, you may need to turn the Microarray Scanner off, and then back on, after Windows has restarted. • If you are using a version of Scan Control software prior to 7.0: ◦ The scanner PC is not connected to the House network, but the House network adapter card is enabled. Disable the House network adapter card with the Windows operating system. ◦ The scanner PC is connected to the House network, and the network goes down or becomes inaccessible. As a temporary fix, disconnect the House LAN cable and disable the House network adapter using the Windows operating system. ◦ The Connection Manager service is stopped. To correct this situation, set the Connection Manager service recovery options for the First Failure, Second Failure, and Subsequent Failures, to “Restart the Service.”

Table 4 Error Messages (continued)

Error Message	Description or Solution
Error ejecting chip. The chip was ejected into the home slot.	Do the following: **1** Turn off power to the scanner. **2** Open the scanner lid. **3** Rotate the carousel by hand until access to the home slot is not blocked by the Autoloader Arm. **4** Remove slide from home slot. **5** Gently push the Autoloader Arm back into the scanner until it comes to a stop. **6** If the problem persists, contact your local Agilent sales and support center.
Failed to communicate with connection manager.	Do the following: **1** Reboot the PC workstation. **2** Turn the Microarray Scanner off and then back on. **3** After several minutes, start the Scan Control program.
Lasers never became ready.	Do the following: **1** Verify that the number showing in the voltage selector on the back of the scanner matches the input power supplied. **2** If you rebooted the PC workstation, you may need to turn the Microarray Scanner off and then back on, after Windows has restarted. **3** If the problem persists, contact your local Agilent sales and support center.
Missing or corrupt ChipRegions.ini file.	Do the following: **1** If you have a backup copy of your customized ChipRegions.ini file, copy it to **C:\Program Files\Agilent\Microarray**. Having a backup copy of **ChipRegions.ini** will avoid loss and re-creation of custom scan regions. **2** If you don't have a backup copy, search the folder for ChipRegions.bak, and rename it to ChipRegions.ini. **3** If neither file exists, re-install the Scan Control program. **4** Contact your local Agilent sales and support center.
Not enough disk space to create file.	The hard drive does not have enough disk space to create a file to store scan data. You can do one of the following: • Go to the Scan Control main menu and set the output directory to another directory on another hard drive. • Delete or transfer files from the current hard drive. See "Step 9. Transfer files and archive data" on page 48.
PMT calibration may be unstable. Using last known PMT setting.	Contact your local Agilent sales and support center.

Table 4 Error Messages (continued)

Error Message	Description or Solution
PMT calibration lost! Using factory default settings.	Contact your local Agilent sales and support center.
The autoloader loading arm is in an unknown location. It is unclear if it is safe to move the carousel.	Do the following: **1** Open the scanner lid. **2** If the Autoloader Arm is partially extended into the carousel compartment, gently push it back into the scanner until it comes to a stop. **3** Close and restart the Scan Control program. **4** If the problem persists, contact your local Agilent sales and support center.
The chip carousel is not present. Please place the carousel in the scanner and try again.	Put the carousel in the Microarray Scanner and reinitiate the carousel run.
The carousel may be misaligned. Please remove the carousel, make sure that its opening is aligned with home position and reinsert the carousel.	See "Mount or remove the carousel" on page 86.
Slide skipped because it is outside the allowable thickness range or the selected scan region contains an obstruction to the initial focus positions.	Do the following: **1** Check the skipped slide for a bar code, hybridization residue, or other non-transparent obstruction that has interfered with the initial focus position. The primary initial focus position is located 1 to 2 mm to the left of the scan region on the top row of the scan region. **2** Measure the glass thickness and verify that it meets with the specifications in "Slide specifications" on page 123.
The slide failed to eject. Pressing OK will re-initialize the instrument.	When you press OK, the Microarray Scanner re-initializes. If this fails, do the following: **1** Open the scanner lid. **2** If the Autoloader Arm is partially extended into the carousel compartment, gently push it back into the scanner until it comes to a stop. Do not force it if it does not move easily. **3** Close and restart the Scan Control program. **4** If the problem persists, contact your local Agilent sales and support center.
The software has detected a serious sensor error that may have existed prior to the installation of this version of software.	Contact your local Agilent sales and support center.

Table 4 Error Messages (continued)

Error Message	Description or Solution
Unable to lock instrument specified by connection manager.	Do the following: **5** Reboot the PC workstation. **6** Turn the Microarray Scanner off and then back on. **7** Start the Scan Control program.
You must select a region to scan first.	To enter a non-default scan region into the scan table: **1** Select the slot or slots whose region you intend to enter. **2** Click **Edit Slot Values** in the Scan Control main window. **3** Select a scan region from the Scan region list. See "Select a scan region for non-Agilent slides" on page 69. **4** Select **Set Values**. **5** Start the scan or carousel run. To enter a default scan region into the scan table: **1** Select the slot or slots whose region you intend to enter. **2** Click **Reset Selection**. Enter the scan region set in the Default Settings dialog box.
You must select at least one dye channel.	**1** Select the slot or slots whose dye channel you intend to change. **2** Click **Edit Slot Values** in the Scan Control main window. **3** Select a dye channel setting from the list. See "Change the sensitivity, resolution, or dye channel settings" on page 75. **4** Select **Set Values**.
You must close the door first.	Microarray Scanner initialization and scan operations cannot be performed with the Microarray Scanner lid open. Close the lid.
Warning: The laser power does not fall within expected thresholds. Please contact an Agilent representative.	Wait the required warm-up time (about 20 minutes) when the yellow indicator light turns off. If this message still appears, contact your local Agilent sales and support center. In most cases, this error indicates that the maximum laser power achieved is below a threshold value required to maintain an internal set point. In these cases, treat the error as an early warning message. The PMT voltage will be elevated precisely to compensate for lower laser power levels that are as much as 20% below the set point.

If an error message does not appear in the table

This table does not list all the possible error messages. If you have an error message that is not listed and you are unable to resolve the problem, do the following:

1. Write down the error message.
2. Restart the Scan Control program.
3. If step 2 does not solve the problem, do the following:
   a. Reboot the PC workstation.
   b. Turn the Microarray Scanner off, then back on.
   c. Restart the Scan Control program.
4. If step 3 does not solve the problem, contact your local Agilent sales and support center.

5 Maintaining Your System

This chapter provides maintenance information for the Agilent G2565AA and Agilent G2565BA Microarray Scanner System.

Scheduled maintenance tasks This section describes the software and hardware maintenance tasks that you need to perform on a regular basis to keep the Microarray Scanner operating efficiently.

Tips to prevent problems This section presents hints on the operation and care of the Microarray Scanner to help you avoid problems.

Scheduled maintenance tasks

With proper care and maintenance, the Microarray Scanner will provide you with trouble-free use. When in doubt about the use or care of the Microarray Scanner, contact your local Agilent sales and support center to answer your questions.

Scheduled software maintenance

Perform these tasks to help maintain the performance of your PC workstation and MS Windows operating system:

- At least once a week, check disk space and archive data as needed.
- Once a week delete any temporary files (*.mp, *.tmp files) from the c:\Temp folder.
- If sluggish performance is observed, defragment the hard disk using defragmentation software.

Scheduled hardware maintenance

Perform these tasks to help maintain the performance of your Microarray Scanner.

Inspecting the vents and air filter

Good air flow through the Microarray Scanner is essential to proper operation. Blocked vents and dirty air filters can adversely affect performance.

Vents Check all vents weekly to ensure that they are not blocked by dust, debris, furniture, or other instrumentation.

Air filter The air filter in the Microarray Scanner should be checked once a month for excessive dirt, using the following procedure:

1 Turn the Microarray Scanner off.

2 Open and remove the air filter access door, which is located on the right side of the Microarray Scanner.

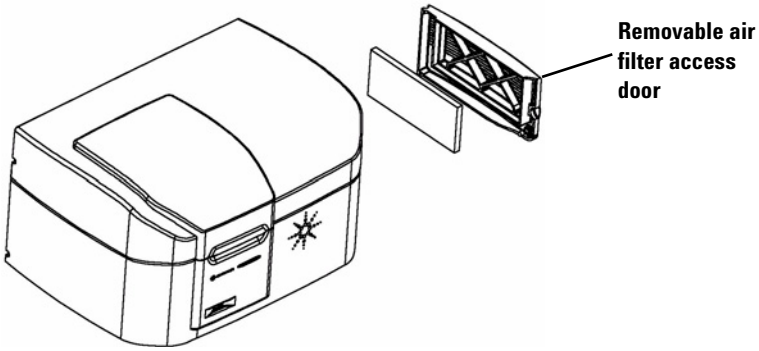


Figure 52 Opening and removing the air filter access door

3 Remove the air filter from the door housing, and inspect the air filter visually.

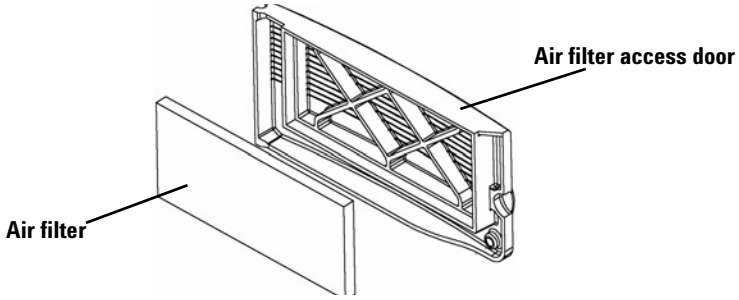


Figure 53 Removing the air filter

Cleaning the air filter

If excessive dirt has built up, or six months have passed since the air filter was cleaned, clean the air filter.

1 Wash the air filter in warm water.

2 Squeeze the air filter to wring out the water.

All excess water should be wrung out of the air filter.

3 Allow the air filter to dry completely before installing. You can use paper towels to blot the filter dry.

4 Re-install the air filter in the air filter access door housing and close the door.

Verify that the air filter was replaced after drying. Failure to replace the air filter can cause dust buildup inside the instrument that could adversely affect performance.

Air filters (p/n 3150-0956) can be ordered directly from your local Agilent sales and support center.

Tips to prevent problems

Perform the scheduled maintenance tasks described on the previous pages, and follow these tips to help you maintain the Microarray Scanner and its performance.

Tips to avoid data loss

- Avoid loading software programs that cause high CPU workload, as this might affect the acquisition of data during scanning.
- During scanning, save your data files directly to the local hard disk. Saving to a storage device over the network can cause data loss or other problems if network traffic becomes congested. Transfer the files when the scanning is complete.

Tips to avoid environmental damage to the scanner

- Keep liquids and vapors away from the Microarray Scanner.
- Minimize and control temperature fluctuations.

  Do not place the Microarray Scanner in direct sunlight. Do not locate the Microarray Scanner near windows even if they have blinds or window coverings. The hot sun can heat up the Microarray Scanner's housing in a non-uniform fashion, which may cause problems with the alignment of the optics.

  Scan only when the laboratory temperature is consistent with the operating temperature specifications for the Microarray Scanner. To assure optimal Microarray Scanner performance, operate the scanner only in the specified temperature ranges (see "Microarray Scanner specifications" on page 120).
- Control the humidity.

  The Microarray Scanner is potentially sensitive to condensing humidity conditions. To ensure optimal performance, operate the Microarray Scanner only in the specified humidity ranges (see"Microarray Scanner specifications" on page 120.) Always allow 10 hours thermal equilibration time on site before opening the shipping box.

Tips to maintain hardware performance

- Avoid moving the Microarray Scanner.

  If you need to move the Microarray Scanner, there is a chance that its performance could be adversely affected. Call your local Agilent sales and support center for assistance in moving the Microarray Scanner.
- Place the Microarray Scanner on a sturdy lab bench or table.
- Avoid leaning on the Microarray Scanner.
- Leave the instrument powered off when not in use for over 4 hours to extend the life of the lasers. Use the laser power-save feature to automatically power on and off your lasers. See "Set Laser Power Saver (Agilent G2565BA only)" on page 53 of Chapter 3.

- After turn-on, allow time for laser warm-up and stabilization. The maximum warm-up time is 20 minutes.
- Keep carousel floor clean using a wipe and no solvent.
- Do not use acetone or other solvents for cleaning.
- Do not touch gearing in the carousel area.

WARNING **Do not remove the covers. Do not attempt to repair or gain access to internal components. You might expose yourself to high voltage and harmful laser radiation.**

Tip to prevent scanner/software miscommunication when using the Recovery CD on a PC running Windows 2000

Agilent supplies a recovery CD that lets you reload the hard drive image that Agilent ships with the PC. In order to re-establish the LAN connection with the scanner after you reload the image, you must restart the connection manager program.

Keep the recovery CD in a safe place. You may need the CD to re-image the hard drive later.

1. Select **Administrative Tools** in the Windows 2000 Control Panel.
2. Select **Services** > **Connection Manager**.
3. Click on the **Start** button.

   The connection between the scanner and the PC restarts.

6 Reference

The Microarray Scanner System includes the following:

- Microarray Scanner
- 48-position carousel
- 50 slide holders
- Crossover LAN cable
- Scan Control software
- Feature Extraction software
- PC workstation with recovery software on CD
- Declaration of Conformity

Microarray scanner specifications This section describes the specifications for the Microarray Scanner instrument. For specifications for the PC workstation, see the user guide that came with the PC.

Slide specifications This section presents the glass and bar code specifications that the scanner is designed to accept.

Regulatory information This section lets you know which regulations for acoustic noise, recycling and disposal, electromagnetic interference, and safety that the Microarray Scanner complies with.

Microarray Scanner specifications

The Microarray Scanner operates within the following specifications:

Approximate dimensions	Height: 97 cm (38 in); with lid closed, 55 cm (21.5 in) Width: 91 cm (36 in) Depth: 71 cm (28 in) These dimensions account for maximum height and width when access doors are open and for an 80 mm (3 in) clearance in depth to provide adequate venting.
Weight	99 Kg (218 lbs)
Power input	100, 120, 220, or 240 VAC at 4 A 50/60 Hz
Fuses	Two power supply fuses: T4A, 250 VAC One laser fuse: T500mA, 250 VAC
Temperature range	Operating: 15° to 30°C Storage: -20° to +50°C
Humidity	Operating: 15% to 95% RH at 30°C Potentially sensitive to condensing humidity conditions. Follow precautions stated in Chapter 5.
Altitude	Operating maximum: 2,300 m (7,500 ft) Storage maximum: 4,600 m (15,000 ft)
Usage	Indoor use
Laser information	Excitation wavelengths: • SHG-YAG laser: 532 nm • Helium-Neon (HeNe) laser: 633 nm Power: 23/20 mW at laser orifices (nominal); 20 mW at 532 nm and 23 mW at 633 nm

Maximum scan region	Agilent G2565AA: 67.2 mm x 21.6 mm Agilent G2565BA: 71 mm x 21.6 mm
Suggested microarray print region	1 mm smaller than scan region on the right, 2 mm on the left, and 0.6 mm on the top and bottom. For non-Agilent slides, the print region is smaller than this by an amount equal to the array printing tolerance.
Dyes supported	Cyanine-3 (Cy-3) and cyanine-5 (Cy-5) and dyes similar to Cy-3 and Cy-5 and Alexa 647, 555, and 660 dyes
Resolution (pixel size)	5 or 10 microns
Pixel placement error	< 1 pixel at 10-micron resolution
Uniformity	5% CV global non-uniformity, average local non-uniformity is typically 1% based upon 100um features.
Scan time	For two colors simultaneous data acquisition, about 8 minutes per scan at 10-micron resolution (scan region of 61 mm x 21.6 mm) and 5-micron resolution/single-pass mode, and about 15 minutes for 5-micron resolution/double-pass mode per scan. Includes slide loading and ejection.
Dynamic range	Single scan dynamic range > 10^4 (16-bit data format) Dual scan extended dynamic range (XDR) > 10^5
Data transfer rate	The data transfer rate between the scanner and the PC is 10 MB/sec.

Scan and print dimensions

The scan and print regions for the Agilent G2565BA are described in Figure 54 and Figure 55. In the figures, all dimensions are in millimeters and the reference point is the lower right side of the glass.

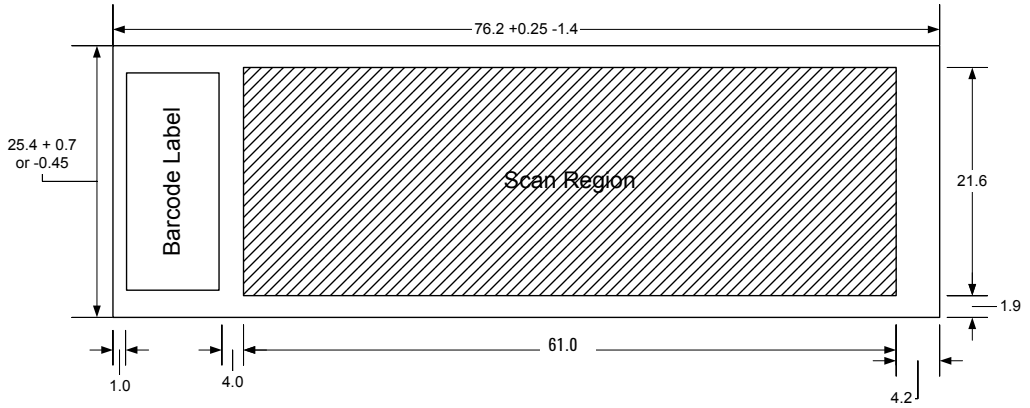


Figure 54 Scan region for Agilent G2565BA

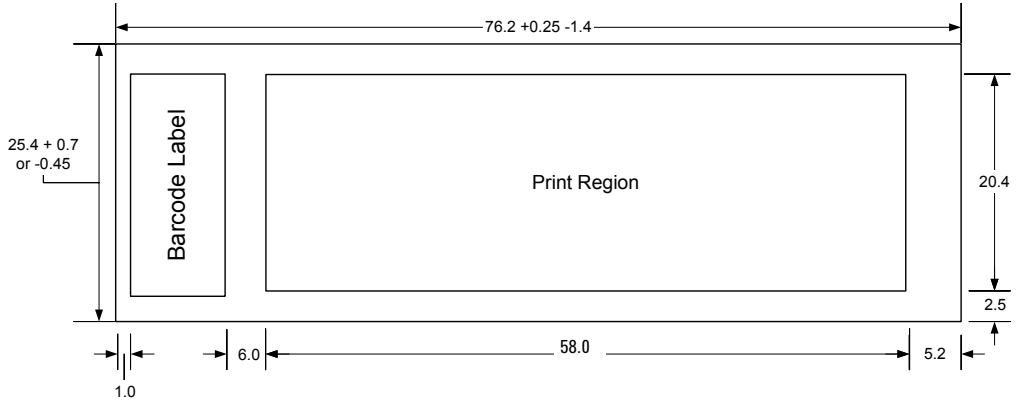


Figure 55 Print region for Agilent G2565BA

Slide specifications

Slide specifications include glass thickness and bar code information.

Glass specifications

The Microarray Scanner uses slide holders to move the microarrays in and out of the carousel. These slide holders are designed to accept a 1 in × 3 in nominal piece of glass.

The detailed specifications of the glass are as follows:

- 1 inch wide (25.4 mm, -0.45 mm, or +0.7 mm).
- 3 inches long (76.2 mm, +0.25 mm, or -1.4 mm).
- Version A slide holder: 1 mm thick (1 mm ±0.025 mm).
  Version B slide holder: 1 mm thick (+0.2 mm or -0.1 mm).
- No mirrored slides.
- Glass should be of high quality with low intrinsic fluorescence.
- Index of refraction from 1.510 to 1.515.

Bar code specifications

The bar code specifications vary for Agilent or non-Agilent slides.

Agilent slide specifications

Agilent slides have had the bar code on the side opposite the microarray. From mid-2002, Agilent slides contain two bar codes, one on each side of the slide.

The microarrays are printed on the active side of the slide containing the bar code labelled “Agilent.” The numeric bar code alone is on the inactive side of the slide.

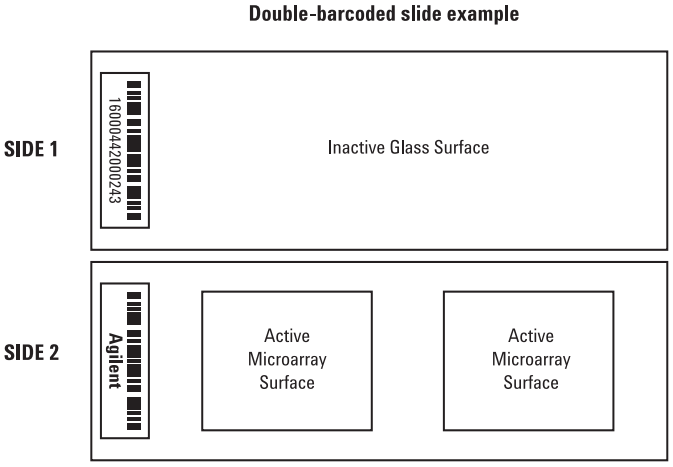


Figure 56 Agilent slide bar code orientation vs. microarray surface

Non-Agilent slide specifications

Non-Agilent slides may have the microarray deposited on the same side as the bar code.

To place a bar code on the side opposite the microarray surface for a non-Agilent slide, you must consider the following requirements:

- Bar code placement tolerance is 1.0 mm.
- The bar code label maximum thickness is 0.2 mm. Make sure there are no bubbles in the label. Do not add labels on top of old labels or replace labels that are beginning to peel off.
- Do not place bar codes on slides whose scan region exceeds 61 mm. You must enter the slide identifier or bar code manually for slides whose scan regions exceed 61 mm.

If you are using non-Agilent slides for your microarrays, the bar code is not read by the G2565AA Microarray Scanner unless you place a bar code on the side opposite the microarray surface.

- For a bar coded slide, the distance between the edge of the microarray scan region and the edge of the bar code can be no less than 4 mm.

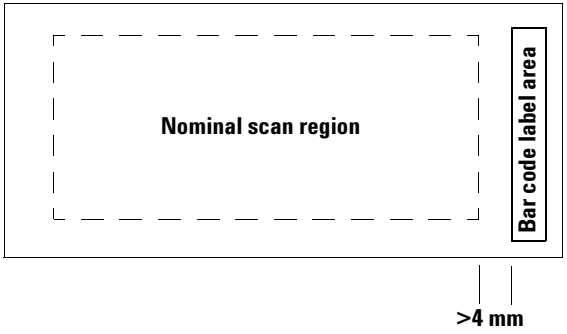


Figure 57 Bar code location on non-Agilent slide

- The bar code width should be about 6 mm; the recommended bar code width is for use with the 61 mm scan region. Larger or longer bar codes can be used by decreasing the scan region proportionally.
- 21.6 mm scan region will be centered for a 25.4 mm glass width (1.9 mm from the top and bottom), or above-center by 0.2 mm for a 25.0 mm glass width.
- Bar codes should be narrow enough so as not to interfere with the slide holder tabs.
- Maximum allowable tilt in the placement of the bar code label on the slide is ±3°.

The bar code must meet the following requirements:

- The minimum quiet zone (the region between the edge of the label and the start or end of the bar code) is 10 times the X-dimension on each side of the bar code.
- Bar code lines must be parallel to the long edge of the slide within tilt tolerance of ±3°.

- Agilent supports the following bar code formats:
  - Code 128: Non-Agilent bar codes with 12 digits must not start with 2
  - CODABAR: Maximum of 8 digits; minimum line thickness = 6 mils
  - Code 39: Maximum of 8 digits; minimum line thickness = 6 mils
  - Code 93: Maximum of 8 digits; minimum line thickness = 6 mils
- Minimum height of bar code must be
  - **Code 128:** 15% of bar code length for Code 128
  - **Code 39, Code 93, CODABAR:** 5 mm or 15% of bar code length, whichever is greater

Regulatory information

This section lists regulatory information for the Micorarray Scanner.

For research use only

The Agilent Microarray Scanner is intended for RESEARCH USE ONLY.

Acoustic noise information

Manufacturer's Declaration:

English This statement is provided to comply with the requirements of the German Sound Emission Directive, from 18 January 1991. Sound Pressure Lp < 70 dB(A), at operator's position, normal operation, according to EN 27779 (Type Test).

Deutsch Die folgende Information wird in Übereinstimmung mit den Anforderungen der Maschinenlärminformationsverordnung vom 18. Januar 1991 erteilt. Schalldruckpegel am Arbeisplatz bei normalem Betrieb, Lp < 70 dB(A), nach EN 27779 (Typprüfung).

Recycling and disposal

Contact Agilent Technologies for more information on recycling and disposal.

This device is designed to accommodate recycling at the end of its useful life. Please dispose of this device in accordance with local regulations.

Electromagnetic interference

The scanner is intended for use with shielded cables only.

Emissions Complies with the emissions levels for Class A equipment as required in lEC 61326-1. This equipment is not intended for use in residential areas.

Immunity This device complies with the immunity levels required in IEC 61326-1 for a non-controlled, non-industrial environment. See accompanying Declaration of Conformity for specific levels.

Canada This ISM (Industrial-Scientific-Medical) device complies with Canadian ICES-001.

Cet appareil ISM est conforme a la norme NMB-001 du Canada.

Safety information

This scanner complies with the following safety standards:

CAN/CSA No. C22.2 No. 1010.1 - 92	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements (Includes Amendment 1)
CAN/CSA-C22.2 No. 1010.1B-97	Amendment 2 to CAN/CSA-C22.2 No.1010.1-92, Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
UL Std No. 3101.1	Electrical Equipment for Laboratory Use: Part 1: General Requirements
UL Std No. 3111-1	Equipment for Measurement Use; Part I: General Requirements
ISA S82.01-1994	Safety Standards for Electrical and Electronic Test, Measuring, Controlling, and Related Equipment - General Requirements
IEC 61010-1	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
IEC 825-1	Safety of laser products Part 1: Equipment classification, requirements and user's guide.
EN60825-1	
(Including Amendment 1:1992 and Amendment 2:1995)	

- Pollution Degree: 2
- Installation Category: II
- Class 1 Equipment; requires a grounding system
- Class 1 Laser Product
- UL/CSA Approved Product

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Printed in USA
Sixth Edition, May 2006

Part Number: G2566-90017

Agilent Technologies