

# Agilent G2565CA Microarray Scanner System

with SureScan High Resolution Technology

# **User Guide**

**Eighth Edition, July 2009** 

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## Notices

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## 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 and preparation for its operation.

#### 2 Getting Started

In this chapter, learn how to safely operate the Microarray Scanner and learn how to set up and then scan slides with the Agilent Scan Control program.

#### **3 Changing Scan Settings**

Learn what the scan settings do and when and how to change them.

#### 4 Using Tools

In this chapter, learn how to set up profiles - groups of scan settings - scan regions and laser auto On/Off times.

#### 5 Maintaining and Troubleshooting Your System

This chapter describes maintenance procedures and tips, as well as error messages for troubleshooting your system.

#### 6 Reference

This chapter presents slide specifications and regulatory compliance information.

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Agilent G2565CA Microarray Scanner System (Scan Control Software 8.3) User Guide

# Introduction

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	This chapter provides a general introduction to the Agilent G2565CA Microarray Scanner System.
A powerful tool	This section describes the features that make the microarray scanner a powerful tool for Agilent's microarray analysis solution.
System description	In this section learn about the hardware and software features of the microarray scanner, the parts and system requirements, the site preparation information and the safety guidelines.
Principles of operation	This section explains how the microarray scanner works.



## A powerful tool for microarray analysis

The Agilent G2565CA Microarray Scanner is part of the microarray analysis solution from Agilent Technologies. The microarray scanner is a sophisticated laser-induced fluorescence scanner designed to read microarrays printed on standard 1 in  $\times$  3 in slides.

The microarray scanner measures the fluorescence intensity of labeled sample nucleic acid (DNA and RNA) bound to microarrays. Its ability to measure fluorescence from two dyes simultaneously facilitates all two- color microarray studies. This technology provides for rapid, high-quality, automated scanning of microarrays.



Figure 1 Agilent G2565CA Microarray Scanner

Each slide is scanned in minutes, and the files are prepared for feature extraction analysis.

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## System description

In this section you find listings of hardware and software features, parts, and computer requirements, as well as a physical description of the scanner and information on site preparation and safety.

### Hardware and software features

The microarray scanner provides the following features:

- Internal and external barcode reading
- Dynamic auto-focus
- Automatic PMT gain calibration prior to each scan
- Continuous laser power stabilization
- 2-, 3-, 5- or 10-micron pixel size
- Dynamic range of  $>10^4$  for a single scan in 16-bit scan mode, >10<sup>5</sup> for a single scan in 20-bit scan mode, and >10<sup>6</sup> for a dual scan in 16-bit scan mode (XDR)
- TIFF image file compression
- Uniformity specification of <5% CV (Coefficient of Variation)

The Scan Control software allows you to select the dye (fluorescence) channels, scan regions, resolution, dynamic range, PMT gain and output folders for each of the slides in the carousel run. These settings can be automatically loaded from saved application-specific profiles, or can be individually selected.

## **Parts list**

The Agilent G2565CA Microarray Scanner System consists of the following components:

- Microarray scanner
- Carousel and 50 slide holders
- PC workstation with recovery software on CD
- Power cables and USB cable

#### 1 Introduction

**Computer system requirements** 

- Scan Control Software 8.4
- Feature Extraction Software 10.x
- Declaration of Conformity

Agilent Scan Control Software 8.4 is designed for use with Agilent Feature Extraction Software 9.5 and higher. 5 and 10 micron, 16-bit scans are backwards compatible with FE 9.5. 2 and 3 micron scans, and all 20-bit scans, require FE 10.x.

### **Computer system requirements**

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

#### Software

- Windows XP Professional with SP2 or Windows Vista 32 with SP1
- Internet Explorer 5.5 or later

#### Hardware

- Pentium IV 3.2GHz or higher
- 2 GB RAM required (4 GB RAM required if Feature Extraction software is installed on the same PC)
- 250 GB available disk space for programs and data generation

#### Introduction 1 **Scanner physical description**

## **Scanner physical description**

### **Scanner front view**





### Scanner top view



Figure 3 Microarray Scanner, Top View

Site preparation

#### Scanner rear view





## Site preparation



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## 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.

#### 1 Introduction

Safety symbols on scanner

## **Safety guidelines**

The Agilent G2565CA 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.
WARNING	The microarray scanner must be connected to a grounded power outlet. It relies on a protective earth ground for safety.
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.
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 and 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 71 of Chapter 5 and the relative humidity specifications on page 96 of Chapter 6.

## **Principles of operation**

This section describes the operating features of the Agilent G2565CA Microarray Scanner.

### **Slide positioning**

The microarray scanner holds up to 48 1 in  $\times$  3 in slides in a removable carousel. During a carousel run, the slides are sequentially transported into scanning position, scanned, then returned 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 hybridization of the target nucleic acid to the microarray probes. Dyes similar to Cy-3 and Cy-5, such as Alexa 647, 555, and 660 dyes, can also be used.

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 bands, 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 target concentrations and for higher data confidence at lower signal levels.

### Scanning

The laser excitation is scanned rapidly back and forth across the microarray. The dynamic/tracking auto-focus ensures that the microarray is always positioned in the focal plane of the scan lens, resulting in scan uniformity.

## **Fluorescence detection**

Fluorescence from the labeled samples is converted to an electrical signal by a high-performance PMT. Very low noise amplifiers and digital integrators process the PMT signal into a digital measurement that is recorded in the TIFF file.

## **Dark offset subtraction**

At very low signal levels, noise can cause negative pixel values. Rather than setting these values to zero, which results in a biased measurement, the Agilent scanner adds a small offset to all pixel values, allowing the full pixel distribution to be reported. This constant offset is not noise, and is removed in a statistically correct manner by Agilent Feature Extraction software.



Agilent G2565CA Microarray Scanner System (Scan Control Software 8.3) **User Guide** 

# **Getting Started**

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This chapter describes how to operate the scanner, including how to set up and scan your slides quickly and easily.

If you have any problems, see Chapter 5 for troubleshooting information.



**Redeeming your high-resolution scanning license** 

## Licensing

The Agilent scanner can be ordered in either of two configurations:

- the standard high-resolution configuration, or
- with the high-resolution capability deleted

Without the high-resolution license, you can select 10 micron, 5 micron, and 5 micron double-pass, but the 2 and 3 micron selections are *not* available.

If you have purchased the high-resolution capability, you need the software license to enable this capability. In most cases, the scanner installation engineer will install the license for you. If this is not the case, or the capability is purchased later, follow the instructions below to redeem the software license.

### **Redeeming your high-resolution scanning license**

To redeem your high-resolution scanning license:

- **1** Locate the software entitlement certificate (SEC) that was shipped with the scanner.
- 2 Go to https://software.business.agilent.com/index.stm.
- 3 Select Click here to start software license redemption.
- **4** Follow the instructions to redeem your license.

### Installing your high-resolution scanning license

When you have received your license via e-mail, install the license as follows:

- **1** Close the Scan Control software window.
- 2 Locate the license file and place it in the following directory:C:\ProgramFiles\Agilent\Microarray
- **3** Restart the Scan Control software.

The 2 and 3 micron selections will now be available.

## **Non-Agilent Barcode Licensing**

If you need to scan non-Agilent barcodes in 2 or 3 micron resolution, contact Agilent for a special no-charge license for accessing this capability.

Without this license, if a slide barcode is not present, or not recognized by the scanner as an Agilent array, and the array is to be scanned in either 2 or 3 micron resolution, the scanner will skip the slide and report the following message:

"Scan settings or slide is not supported by the current scanner license. Contact Agilent via pdl-non\_ag\_barcode@agilent.com to obtain license to scan non-Agilent barcodes in high-resolution".

#### Obtaining and installing a non-Agilent barcode license

- 1 Send an e-mail to **PDL-non\_ag\_barcode@agilent.com** with your scanner serial number in the body of the e-mail, and one will promptly be sent to you. This license will need to be renewed annually.
- **2** Once you receive your license, place the license in the following directory:

#### C:\ProgramFiles\Agilent\Microarray directory

**3** Close and re-open the Scan Control software.

The scanner will recognize the new license.

Step 1. Turn on the Agilent Microarray Scanner

## **Operating the Scanner**

### Step 1. Turn on the Agilent Microarray Scanner

- **4** Turn on the Microarray Scanner using the power switch located on the front of the instrument. You will notice:
  - **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 status indicator lights flash on and off as different functional checks are made.
  - **c** The lasers will not turn on until you start the Scan Control software.





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

 $\mathbf{Or}$ 

Select Start > All Programs > Agilent > Scan Control.



#### Figure 6 Agilent Scan Control icon

When the program starts, the lasers begin to warm up.

To learn how to set up to turn the lasers on and off automatically, see "Setting up times to turn the lasers on and off" on page 63 in Chapter 4.

Step 1. Turn on the Agilent Microarray Scanner

6 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 up	The lasers take about 30 minutes to warm up. If you have to wait for the lasers to warm up, move on to "Step 2. Insert slides into slide holders" on page 24.
Lasers are powered off	To turn on the lasers, select <b>Tools &gt; Laser Auto ON/OFF</b> <b>Settings</b> and click <b>Turn Lasers ON Now</b> .
Scanner ready	You can begin your scan.

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

		End slot:	10 💌 Profile: 📐	ilentHD_GX_2	Color	•				Operator:
Slot	Slide ID	Channels	Scan Region	Resolution	TIFF	R PMT	G PMT	XDR	Output Path	Description
1	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
2	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
3	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
4	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
5	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
6	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
7	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
8	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
9	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
10	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	



Step 1. Turn on the Agilent Microarray Scanner

## Step 2. Insert slides into slide holders

A slide must be inserted into a slide holder before loading it into the carousel. The slide holder protects the slide during scanning.



Figure 8 Loading slides into the slide holder

- **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.

For instructions on removing the slides, see "Step 5. Remove the slides" on page 33.

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 barcode.

Step 1. Turn on the Agilent Microarray Scanner





**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.



Figure 10 Proper placement of slide on ledge

Step 1. Turn on the Agilent Microarray Scanner

- **5** Align the slide with the slide holder following these two rules:
  - The end without the barcode 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 barcodes, one on each side of the glass. See Figure 11. Typically, non-Agilent slides have only one barcode. Place the active microarray side of the slide facing toward the slide holder cover.

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

An improperly inserted slide can damage the Microarray Scanner.





#### Figure 11 Slide orientation

See "Barcode and barcode label specifications" on page 99 to apply a second readable barcode.

Step 1. Turn on the Agilent Microarray Scanner

**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.





**7** To close the slide holder cover, gently press down on the cover with your thumb.



Figure 13 Close the cover

**8** To lock the cover in place, gently pull the cover back with your thumb until it locks.

Step 1. Turn on the Agilent Microarray Scanner





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



Figure 15 Properly locked cover

2

## Step 3. Load the slide holders into the carousel

When the slides are properly inserted in the slide holders, you can load the slide holders into the carousel. The carousel is designed so that you can easily load a slide holder correctly. You can load the slide holders either with the carousel in the scanner or removed from the scanner.

**1** Load 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.





CAUTION

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

**4** If the carousel is already mounted, skip to step 5. Otherwise, mount the carousel.

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.

Step 1. Turn on the Agilent Microarray Scanner

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

**a** 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.

**b** Make sure that the carousel is firmly seated.

**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.

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



Figure 17 Carousel cover

**6** Close the scanner lid.

Step 1. Turn on the Agilent Microarray Scanner

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

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



Figure 18 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.

Step 1. Turn on the Agilent Microarray Scanner

### Step 4. Scan your slides

A *scan* refers to creating an image of the probes on a single slide. A *carousel run* refers to scans of all of the specified slides in the carousel. Follow the steps below to prepare your slides for scanning and start the carousel run. See the next section, "Scanning Your Slides", for more detailed instructions.

"Step 1. Set slot locations" on page 36

"Step 2. Set a profile for changing all settings in the scan table" on page 37

"Step 3. Change individual scan settings in the scan table" on page 38

"Step 4. Start the carousel run" on page 39

"Step 5. Transfer files and archive data" on page 42

### Step 5. Remove the slides

After the carousel run is complete, you can unload the slide holders from the carousel and then remove the slides from the slide holders.

1 (Optional) Remove the carousel from the scanner.

If the slide holder autoloader arm is extended into the carousel, do not remove the carousel. Instead, follow the instructions in "Troubleshooting an autoloader arm extended into the carousel" on page 82.

- 2 Remove the slide holders from the carousel.
- **3** Remove the slides from the slide holders.
  - **a** Hold the slide holder on the sides with the Agilent logo facing up.
  - **b** Place one thumb on the center of the slide holder cover.
  - **c** Gently press down on the cover and push toward the top of the slide holder until the cover releases.



Step 1. Turn on the Agilent Microarray Scanner

- **d** Push up on the barcode end of the slide from underneath the slide holder to avoid fingerprints on the sample area.
- **e** Grasp the slide from the sides and remove from slide holder.





## Step 6. Set times to automatically turn the lasers off

You can extend laser lifetime by having the instrument automatically power off the lasers at specified times. See "Setting up times to turn the lasers on and off" on page 63 of Chapter 4, "Using Tools" for more information.

You do not need to turn off the microarray scanner.

Should you want to turn off the scanner, follow these instructions:

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** 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.

**Step 1. Set slot locations** 

## **Scanning Your Slides**

### Step 1. Set slot locations

If you want to scan slides in all 48 slots, skip to "Step 2. Set a profile for changing all settings in the scan table" on page 37.

- **1** In the **Start slot** box, select the number of the slot where the first slide that you want to scan is located.
- **2** 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 1 and 2 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 scanner scans only those slides in the slot range that you enter here.
- The **Carousel Check** command checks only the range of slots that you enter here for empty slots and for Agilent-supported barcodes. (See "Check for Agilent-supported barcodes and empty slots" on page 49 of Chapter 3.)
## Step 2. Set a profile for changing all settings in the scan table

Profile: AgilentHD\_GX\_2Color

• From the Profile list, select one of the existing profiles.

A *profile* is a collection of scan and image settings that, when selected, populate the scan table with values you entered in the Profile Editor.

iterea în the Prome Ealtor.	When you open the Scan Control main window, you see the columns populated by scan setting values. These values are set from the last profile selected for a run before closing the program.
	Agilent supplies six preloaded profiles for your selection and use with Agilent high density microarrays, Agilent G3 microarrays, and non-Agilent microarrays.
<default></default>	Default settings that you see after you install the program
AgilentHD_GX_2Color	Agilent HD 2-color gene expression microarrays
AgilentHD_GX_1Color	Agilent HD 1-color gene expression microarrays
AgilentHD_CGH	Agilent HD CGH/CNV/ChIP microarrays
AgilentHD_miRNA	Agilent HD miRNA microarrays
AgilentG3_CGH	Agilent G3 CGH/CNV/ChIP microarrays
	To change all the settings at once you can select one of the Agilent profiles or create your own from any of the existing profiles, including the <default> profile.</default>

To edit an existing profile or create your own profile, see Chapter 4, "Edit an existing profile" on page 55 or "Create a new profile" on page 56. Step 1. Set slot locations

## Step 3. Change individual scan settings in the scan table

If you do not need to change any of the settings in the scan table produced by the selected profile, wait until the **Scanner status** message says Scanner ready and move on to "Step 4. Start the carousel run" on page 39.

- 1 Click the cell(s) whose setting(s) you intend to change.
  - Click an individual cell, or
  - · Click a column header, or
  - Drag the cursor down multiple cells in a column to select contiguous cells. An arrow appears on the right-hand side of the first cell selected.
  - Press **Ctrl** and select non- contiguous cells within a column one at a time. An arrow appears on the right-hand side of the last cell you select.
- **2** Select a new setting from the list.

The new setting appears in bold.

Scan Region		F
Agilent HD (61 x 21.6mm)	-	3
A Full Slide (71 x 21.6mm) Agilent HD (61 x 21.6mm) Agilent HD (61 x 21.6mm)		3

NOTE	An estimate of the total file size and run time is reported in the status line
NUIL	at the bottom of the Scan Control main window. Note that space is
	required on the C: drive for temporary files generated while scanning. If the
	estimated image size plus temporary file size exceed the space available,
	the scanner will not permit the carousel run to begin.

#### CAUTION

Agilent recommends that you select an Output Path for storing image files to a local drive. If you select a network drive for your output path, and the network connection fails, then files are stored on the local C: drive in the file **Program Files\Agilent\Microarray\NetDataBackup**.

## Step 4. Start the carousel run

- A carousel run scans all the selected slides in the carousel.
- **1** Verify that the **Scanner status** message in the main window says Scanner ready.

The carousel run cannot be started until the lasers have fully warmed up.

2 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.

^
~
192

Figure 20 Scan Progress dialog box

**Step 1. Set slot locations** 

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.

When the carousel run is complete, the Status box reads Carousel run completed.

The Carousel Report Log presents scan information and error messages that occur during the carousel run. At the end of a carousel run the Carousel Report Log automatically appears.

## AGILENT SCANNER -- CAROUSEL REPORT LOG

#### Fri Apr 11 11:01:38 2008

Scanner SN: US1470PPB1 Carousel run requested from Slot 1 to Slot 29 Total number of slide(s) detected: 29.

Slot 1 -----

Scanned image: D:\New Folder\US1470PPB1\_123456789012\_S01.tif Scan completed.

Slot 2 ------Scanned image: D:\New Folder\US1470PPB1\_251554817094\_S01.tif Scan completed.

Slot 3 ------Scanned image: D:\New Folder\US1470PPB1\_251554819356\_S01.tif Scan completed.

Slot 4 ------Scanned image: D:\New Folder\US1470PPB1\_251554819966\_S01.tif Scan completed.

#### Figure 21 Carousel Report Log

If you wish to re-open the final Carousel Report Log after you have closed the Scan Progress dialog box, it can be found in:

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

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

After the run is complete, you can remove the slides. See "Step 5. Remove the slides" on page 33.

#### View the Carousel Report Log during the run

You can also view the Carousel Report Log any time during the carousel run.

• Click View Report on the Scan Progress dialog box.

#### Review the data during or after the run

• After the data for one scan is transferred, display the file in the Agilent Feature Extraction software or another program.

If you installed Feature Extraction 10.x on another computer hard drive, you must transfer the image files to this drive after the run. See "Step 5. Transfer files and archive data" on page 42.

#### 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 22 Carousel Run Abort message

The Microarray Scanner can take a minute or two to finish aborting the scan even if Yes is selected. Any collected data is saved for later analysis.

Refer to the Feature Extraction Software Quick Start Guide, included with your scanner materials.

NOTE

Step 1. Set slot locations

## Step 5. Transfer files and archive data

• At the end of the run, transfer the files to another folder, if needed.

You can transfer the file or files to one of four places:

- New folder under the original folder on the local drive that you set up for the Output Path
- Another folder on the local drive
- Folder on the network

Eventually you must transfer data to the PC where the Feature Extraction program resides.

• CD or DVD

A DVD-Writer is included with every PC workstation.

**CAUTION** It is important to archive data regularly from the scanner PC to make sure enough disk space is available for future runs. When the disk is almost full, the microarray scanner stops scanning and a caution is displayed.

You can improve performance by regularly defragmenting the disk drive.



Agilent G2565CA Microarray Scanner System (Scan Control Software 8.3) User Guide

# **Changing scan settings**

3

Changing Scan Table Settings 44 Change scan table settings through profiles 45 Change individual scan table settings 46 Scan Table Settings 48

Before you scan a set of slides, you may want to modify scan or image settings.

This chapter first describes each of the scan table settings, then shows you how to change all of them at once or one at a time.



## **Changing Scan Table Settings**

You can change the scan table settings in two ways:

A *profile* is a collection of scan and image settings that, when selected, populate the scan table with values you entered in the Profile Editor.

- Change all of the settings at once through profiles
- Change individual settings through drop-down lists in the scan table

Although you can change entries in any order you want, you may want to change the entries in this order to save some time:

- **a** If you are scanning non-Agilent slides, you may want to "Create a new scan region with the Scan Region Editor." that is more relevant for your slides than the ones offered in the scan table or Profile Editor.
- **b** Whether you are scanning Agilent or non-Agilent slides, you may want to "Change scan table settings through profiles" by selecting a different profile.
- **c** After selecting a different profile (or editing or creating one), you can "Change individual scan table settings".

If you select a different profile after changing entries individually, the profile entries override the changed individual ones.

**d** Be sure to enter Slide IDs either manually or automatically after you have selected a profile. You *must* do this for non-barcoded slides. You *can* do this for Agilent slides. See "Slide ID" on page 48.

To learn about each of the scan settings, see "Scan Table Settings" on page 48.

## Change scan table settings through profiles

• From the **Profile** drop-down list, select the profile whose settings you want to use for the run.

Profile: AgilentHD\_GX\_2Color 💌



You can change the settings for the selected profile (See "Edit an existing profile" on page 55) or create a new profile from it (See "Create a new profile" on page 56).

When you open the Scan Control main window, you see the columns populated by scan setting values. These values are set from the last profile selected for a run before closing the program.

Agilent supplies six preloaded profiles for your selection and use with Agilent high density microarrays, Agilent G3 microarrays, and non-Agilent microarrays.

- AgilentHD\_GX\_2Color Agilent HD 2-color gene expression microarrays
- AgilentHD\_GX\_1Color Agilent HD 1-color gene expression microarrays
  - AgilentHD\_CGH Agilent HD CGH/CNV/ChIP microarrays
  - AgilentHD\_miRNA Agilent HD miRNA microarrays
    - AgilentG3\_CGH Agilent G3 CGH/CNV/ChIP microarrays

For Agilent slides you select the profile that applies to your type of Agilent microarray.

For non-Agilent slides you will need to make changes to individual scan table settings. Or, you can create a new profile from an existing one.

## Change individual scan table settings

With this scan table you can change entries for individual slides or groups of slides by selecting an item from the drop-down menu enabled for each column, except the Slide ID and Description columns.

- **1** Click the cell(s) whose setting(s) you intend to change.
  - Click an individual cell, or
  - · Click a column header, or
  - Drag the cursor down multiple cells in a column to select contiguous cells.

An arrow appears on the right-hand side of the first cell selected.

• Press **Ctrl** and select non-contiguous cells within a column one at a time.

An arrow appears on the right-hand side of the last cell you select.

**2** Click the arrow to show the drop-down list, and select the setting.



Figure 24 Drop-down list for scan region settings

The changed settings appear in bold letters.

See descriptions of these column settings in "Scan Table Settings" on page 48.

#### Reset scan table settings to current profile values

The profile values that appear when resetting are the original values found in the selected profile.

- 1 Click the cell or cells whose setting(s) you intend to reset.
  - Click an individual cell, or
  - Click a column header, or
  - Drag the cursor down multiple cells in a column to select contiguous cells, or
  - Press **Ctrl** and select non-contiguous cells within a column one at a time, or
  - Click the **Slot** header in the upper left-hand corner of the table to select all the cells in the scan table.
- 2 Right-click the selected area, and select **Reset to Profile** Value.



Figure 25 Shortcut menu for returning all selected settings to their original values in the current profile

The settings in the current profile appear in the selected area.

#### 3 Changing scan settings

## **Scan Table Settings**

This section describes the settings in the scan table that you can change. The next section tells you how to change them.

🖁 Agilen	t Scan Contro	a l								
ile <u>T</u> ools	Help									
St <u>a</u> rt slo	t: 1 💌	E <u>n</u> d slot.	10 💌 Profile: 🗛	jilentHD_GX_2	Color	•				Operator:
Slot	Slide ID	Channels	Scan Region	Resolution	TIFF	R PMT	G PMT	XDR	Output Path	Description
1	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
2	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
3	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
4	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
5	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
6	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
7	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
8	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
9	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	
10	<auto detect=""></auto>	R+G	Agilent HD (61 x 21.6mm)	5 um	20 bit	100%	100%	<no xdr=""></no>	D:\ScanData	

Figure 26 Scan Control main window and scan table with preloaded profile settings

**Slide ID** An identifier for the slide. The Slide ID entries will be used as part of the image file name if specified as part of the file name in the current profile. See "Naming prefix 1 and Naming prefix 2" on page 56.

**Automatic entry** If your slides have barcodes conforming to the specifications in "Barcode and barcode label specifications" on page 99, you do not have to enter Slide ID into the scan table before the carousel run. The "Auto detect" function in the Slide ID column means the scanner automatically reads the Agilent-supported barcode into the scan table as it scans each slide.

You also have the choice of entering Slide ID manually.

**Manual entry** You must enter IDs for non-barcoded slides, or slides with unsupported barcode formats, using the keyboard or an appropriate external barcode reader.

If you enter the ID through the keyboard, double-click the Slide ID cell first before typing in the barcode or a unique text identifier.

**Check for Agilent-supported barcodes and empty slots** To view the Agilent-supported barcodes and check for empty slots in the Slide ID column, you click Tools > Carousel Check.

The program first checks the carousel for empty slots and places "No chip" in each Slide ID cell with no slide. Then the program reads the barcodes for all the slides and places them in the Slide ID cells.

**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 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 barcode or other non-transparent border areas of the slide and affecting the scanner's ability to autofocus properly. Minimizing the scan region will also reduce scan time and save storage space.

Agilent provides a scan region suitable for all Agilent High Density and G3 slides, and another suitable for scanning full 1 by 3 slides without barcode labels.

For non-Agilent slides, you may need to use a custom scan region that better matches the printed region. See "Create a new scan region with the Scan Region Editor." on page 60.

Scan resolution (μm)
Sets the scan resolution (pixel size) to 2, 3, 5 or 10 microns.
For 10 micron scans, each row of pixels in the TIFF image represents the average of two scan lines, one acquired in each direction. For 5, 3 and 2 micron scans, you can select double-pass scanning to perform this averaging, or acquire only a single scan line for each image row.

# TIFF fileSets the dynamic range to 16-bit or 20-bit. The G2565CAdynamic rangescanner has extended the dynamic range of the PMT and<br/>signal processing electronics. By selecting the 20-bit TIFFfile option, you can access this extended range, to enable<br/>quantitation of high and low signal features in a single scan.

The Scan Control software still enables conventional XDR 16-bit scanning, as described below.

Below is a table showing the storage space and scan time for a single and double pass scans for each resolution selected for either a 16-bit TIFF dynamic range or a 20-bit one. The Scan Region is 61 X 21.6 mm.

The Status bar at the bottom of the Scan Control main window shows an estimate of the storage space and run time required for the complete carousel of slides.

Resolution	Storage Space, Mb, 16-bit	Storage Space, Mb, 20-bit	Scan Time, min.
2 micron single pass	1300	1600	20
3 micron single pass	620	760	15
5 micron single pass	200	300	8
10 micron single pass	52	115	8
2 micron double pass	1300	1600	40
3 micron double pass	620	760	30
5 micron double pass	200	300	16

Table 1Storage Space and Scan Time for Single and Double Pass<br/>Scans for Agilent HD Scan Region 61 x 21.6 mm

Double pass scans do not require more storage space than single pass scans, but they take twice as long to complete.

Red PMT Gain and	Sets the sensitivity level of the red channel and green channel PMTs. The PMT detects fluorescence emitted by the microarray.
Green PMT Gain	The default output level (100%) sets the gain to the factory-calibrated level; this is the recommended setting for Agilent microarrays. 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.
XDR Ratio	Before the availability of the 20-bit TIFF file dynamic range, the eXtended Dynamic Range (function) had to be used on both the scanner and Feature Extraction software to capture all the data scanned between very low signal features and very high signal features. Now you can select the 20-bit TIFF file dynamic range to capture the wide dynamic range of data as an alternative to using XDR.
	With the XDR function turned on, the scanner automatically scans the same slide twice at two different PMT sensitivity levels. This generates two linked images that Feature Extraction can co-process to yield a single unified set of extracted intensity data covering up to 6+ orders of magnitude.
	The high sensitivity level is set by the Red or Green PMT Gain. The XDR function is automatically turned off (once on) at values lower than 10%.
	Setting the XDR ratio column instructs the scanner to perform a second scan at lower PMT settings. For example, if the Red and Green PMTs are set to 100%, and the XDR ratio is set to 0.1, then the second scan will be performed with the PMTs set to 10%. The lowest available PMT setting is 1%.
Output path	The Browse button lets you select a directory to store the data from each scan. Agilent recommends that the data be acquired to a local directory on a secondary hard drive. You may also select a network directory. If a network access problem is experienced during the scan, data will be saved to a temporary local directory, and a warning will be included in the carousel run report.

#### **3** Changing scan settings

SlideDescription of each slide being scanned. This informationDescriptionbecomes part of the file information that can be viewed in the<br/>Feature Extraction software.



Agilent G2565CA Microarray Scanner System (Scan Control Software 8.3) User Guide

# **Using Tools**

4

Creating/Editing profiles using the Profile Editor 55 Creating/Editing scan regions using the Scan Region Editor 59 Setting up times to turn the lasers on and off 63 Additional Tools 65

If you want to create or modify a profile or a scan region or if you intend to set up times for the lasers to turn on and off automatically, you use the tools in the Tools menu. This chapter shows you how to use these tools:



Figure 27 Tools menu



#### 4 Using Tools

Profile Editor	With the Profile Editor you can modify the settings for any of the profiles that come with the software:
<default></default>	The profile whose settings you see loaded upon installation
AgilentHD_GX_ 2Color	Agilent HD 2-color gene expression microarrays
AgilentHD_GX_1Color	Agilent HD 1-color gene expression microarrays
AgilentHD_CGH	Agilent HD CGH/CNV/ChIP microarrays
AgilentHD_miRNA	Agilent HD miRNA microarrays
AgilentG3_CGH	Agilent G3 CGH/CNV/ChIP microarrays
	You can also create new profiles from existing ones.
Scan Region Editor	You may need to scan microarray regions not installed by default. To create new ones, you use the Scan Region Editor.
Lasers Auto On/Off Settings	To extend the lifetime of the lasers, you set up times for the system to automatically turn the lasers on and off with this dialog box.

# Creating/Editing profiles using the Profile Editor

## Edit an existing profile

You can edit and save an existing profile. Or you can edit an existing profile and save the edits to a new profile name. (See "Create a new profile" on page 56).

1 Select Tools > Profile Editor from the menu bar (Figure 27).

3 General		
Description	Default configuration settings	Save
Scan Settings		1.17
Dye channel	R+G	Save As
Scan region	Agilent HD (61 × 21.6mm)	
Scan resolution	5 um	
Tiff file dynamic range	16 bit	Remove
Red PMT gain	100%	- 12 <u></u>
Green PMT gain	100%	
XDR Ratio	<no xdr=""></no>	
🖃 Image File Settings		
Output path	c:\	
Slide description		
Naming prefix 1	<instrument #="" serial=""></instrument>	
Naming prefix 2	<slot #=""></slot>	
Compress Tiff	No	
Solit/votate image	No	



Note that you can never remove the <default> profile from the list. You can remove all others, including any profiles you create from the <default> profile.

- **2** Select a profile to edit.
- **3** Change the scan and image settings using the choices described in "Changing Scan Table Settings" on page 44 and "Profile settings not in the scan table" on page 56.
- 4 Click Save.

## Create a new profile

You can create a new profile from any existing profile by saving the existing profile as a new name.

- 1 Select Tools > Profile Editor.
- 2 Select an existing profile to save as a new one.
- **3** Click **Save As** to save the existing file to a new name.
- 4 Enter the name, then click Save.
- **5** Change the scan and image settings using the choices described in "Changing Scan Table Settings" on page 44 and "Profile settings not in the scan table" on page 56.
- 6 Click Save.

## Profile settings not in the scan table

The following settings do not appear in the scan table. These parameters apply to all scans in the carousel run.

Naming prefix 1For standard scans, the Scan Control program uses three nameandsegments to compose the filename.

Naming prefix 2 Prefix1\_Prefix2\_ScanNumber.tif.

For *XDR scans*, an additional segment (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

The default *Prefix1* is **Instrument Serial #**, but you can select either **Slide ID** or your own custom prefix.

The default *Prefix2* is Slide ID, but you can select Slot#, Scan Date Time or your own custom prefix.

#### Using Tools 4

Profile settings not in the scan table

🖻 Image File Settings		
Output path	c:\	
Slide description		
Naming prefix 1	<instrument #="" serial=""></instrument>	
🖃 Naming prefix 2	<pre><customize></customize></pre>	-
Customized	<slide id=""></slide>	
Compress Tiff	<scan datetime=""></scan>	
Split/rotate image	<slot #=""></slot>	2
	<customize></customize>	

- **Slide ID** If selected, the entry in the Slide ID cell appears in the filename. If <Auto detect> is in the Slide ID cell, the barcode will appear in the filename.
- **Slot#** The number of the slot in which the scanned slide resides
- **Scan DateTime** If you select this option, the date and time, in format YYYYMMDDHHMM, will be used.
- **Customize** Enter a custom prefix if you select Customize (36 character maximum).

The Scan Control program automatically assigns the *Scan Number*.

The program compares the filename of a new scan with those in the selected data directory.

If Prefix1\_Prefix2 is unique, the scan number is set to S01.

If a match is found, the scan number is increased until the filename is unique.

#### Example

US4510PP02\_251485023883\_S03.tif

- Instrument Serial # = US4510PP02
- Slide ID = 251485023883
- ScanNumber = S03. This is the third scan file in the directory with the same Instrument Serial # and Slide ID.
- **Compress TIFF** You can reduce the final amount of storage space occupied by scan images by compressing the TIFF files.

The compression algorithm used, LZW, reduces the storage space by 20 to 70 percent.

#### 4 Using Tools

Profile settings not in the scan table

Split/rotateSome analysis programs require data from one-color images<br/>rotated 90 degrees. If you set the Split/Rotate option to Yes, the<br/>color file is split into two color files, and the images are rotated<br/>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 select only one dye channel for your output file, you must still make this selection to rotate the image even if you do not need to split the file.

This option is available only for 16-bit TIFF dynamic range scans.

# Creating/Editing scan regions using the Scan Region Editor

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 barcode label placement and size, and areas with frosted glass. 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. The new region appears as a selection in the scan table. and in the Profile Editor.

## **Start the Scan Region Editor**

#### From the Scan Control main window

• Select **Tools > Scan Region Editor** from the menu bar (Figure 27).



Figure 29 Scan Region Editor

#### 4 Using Tools

Create a new scan region with the Scan Region Editor.

#### From the Start menu

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

You cannot access the Scan Region Editor 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.

### **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 barcode label and does not overlap any other opaque or translucent areas of the slide.

1 Select File > New Region from the menu bar in the Scan Region Editor.

🖲 Add	New Region	
Name:	-	
		Ranges(mm):
X:	mm	3.10 - 69.10
Y:	mm	1.90 - 23.40
Width:	mm	5.00 - 71.00
Height	mm	0.10 - 21.60
	Save	Exit



- **2** Enter the name or size of the region.
- **3** Enter the measurements in mm for the region, using the criteria listed below.

Invalid values will be 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.

Change an existing scan region for non-Agilent slides

- Y Y-axis measurement for the upper left-hand corner.
- WidthWidth of the scan region measured from the end of the X-axis<br/>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.

Name	X Pos (mm)	Y Pos (mm)	Width (mm)	Height (mm)
Custom Region 1	3.15	1.95	70.90	21.50

Figure 31Scan regions list

Show Custom<br/>regions onlyWhen this check box is marked, only the custom scan regions<br/>appear in the list in the Scan Region Editor. When the check<br/>box is clear, you can also see the regions provided by Agilent<br/>in the list. However, you cannot change these regions with<br/>the Scan Region Editor.

**5** Close the Scan Region Editor.

The newly created regions now appear in the Scan Region lists, both in the Profile Editor and the scan table.

## 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.

#### 4 Using Tools

Change an existing scan region for non-Agilent slides

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

💐 Modi	fy Region	
Name:		
	,	
		Ranges(mm):
X:	4.00 mm	3.10 = 69.10
Y:	2.00 mm	1.90 - 23.40
Width:	6.00 mm	5.00 - 70.10
Height	1.00 mm	0.10 - 21.50
	Save	Exit

Figure 32 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 60.
- 5 Click Save.

4

## Setting up times to turn the lasers on and off

To extend the life of the scanner lasers, you can enable the Scan Control program to turn the lasers on or off automatically. This feature allows you to have the laser warmed up and ready for scanning at a specified time or turned off automatically at a specified time or when idle.

Regardless of Laser Auto ON/OFF settings:

- You can turn the lasers on or off manually anytime.
- When you start the Scan Control program, the lasers turn on.
- If the scanner is scanning at the specified turn-off time, the lasers will remain on.

## Enable the lasers to turn on or off automatically

- 1 Select Tools > Laser Auto ON/OFF Settings from the menu bar (Figure 27).
- 2 Double-click No to set Enable laser auto ON/OFF to Yes.

Laser Auto ON/OFF Settings			
🗖 General			
Enable laser auto ON/OFF	Yes		
Auto ON Settings			
Turn ON daily	Every day		
Turn ON at	7:00 AM		
Auto OFF Settings			
Turn OFF daily	Yes		
Turn OFF at	8:00 PM		
Turn OFF when idle	Yes		
Idle for(hr)	1		
- · ·			
<u>R</u> eset <u>I</u> urn Lasers on I	Now OK Cancel		
	11.		

Figure 33 Laser Auto ON/OFF Settings properties page

#### 4 Using Tools

Turn the lasers on or off manually

NOTE	If the selection is NO, you must turn the lasers on and off manually, as described in the next section.
Turn ON daily	Select <b>Weekdays only</b> or <b>Weekends</b> , and specify the time for turning the lasers ON on weekdays or weekends.
Turn OFF daily	Select <b>Yes</b> and specify the time for turning the lasers OFF on weekdays and weekends.
Turn OFF when idle	Select <b>Yes</b> and specify a time span of scanner inactivity beyond which the scanner turns off the lasers. If <b>No</b> , the scanner will not turn off under this condition.
	3 Click OK.

## Turn the lasers on or off manually

- 1 Open the Laser Auto ON/OFF Settings properties page.
- 2 Click Turn Lasers on Now or Turn Lasers off Now.
- 3 Click OK.

## **Additional Tools**

The following additional tools are available on the Tools menu.

## **Unlock Door**

The unlock door tool (**Tools > Unlock Door**) can be used to attempt to manually unlock the door if the door lock did not operate correctly. This can happen, for example, if the lid is being lifted as the lock operates automatically at the end of a carousel run.

## **Reset Calibration Warnings**

During normal operation, the G2505C scanner calibrates the PMTs before every scan, and calibrates the lasers during system initialization and after every carousel run. If the calibration is unsuccessful, or if the calibration changes significantly since the previous time it was performed, the scanner software will record that fact, and generate warnings in the Carousel Run Log.

The PMT calibration warning is set when the PMT voltage changes by more than 20% from the previous calibration value.

The laser calibration warnings are set when the lasers cannot achieve their specified power within the warm up period. If this occurs, the system sets the warning, and recalibrates the lasers at 80% of their specified power. The Scan Control program scales the TIFF file to compensate for the lower laser power.

You can reset these warnings by selecting **Tools > Reset Calibration Warnings**.

If the next calibration is again unsuccessful, and the warnings reappear, you should contact your local Agilent sales and support center.



## **Extended Laser Calibration**

Normal calibration of the lasers does not measure the maximum power output. The extended laser calibration tool performs a more comprehensive check of the laser subsystem. If you are receiving repeated laser calibration warnings, Agilent support may request that you use the extended laser calibration tool, then e-mail the log files containing the results.



Agilent G2565CA Microarray Scanner System (Scan Control Software 8.3) User Guide

# 5 Maintaining and Troubleshooting Your System

Maintaining your system 68 Scheduled software maintenance 68 Scheduled hardware maintenance 69 Tips to prevent problems 71 Troubleshooting your system 73 Technical Support 74 Frequently Asked Questions (FAQs) 76 Hardware Troubleshooting 78 Using HyperTerminal to Capture Diagnostic Data 83 Software Troubleshooting 87 Microarray Scanner Error Messages 88

This chapter provides maintenance and troubleshooting information for the Agilent G2565CA Microarray Scanner System.



5 Maintaining and Troubleshooting Your System

Scheduled software maintenance

## Maintaining your system

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	These sections describe the software and hardware
maintenance	maintenance tasks that you need to perform on a regular basis
tasks	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 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 34 Opening and removing the air filter access door

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

#### 5 Maintaining and Troubleshooting Your System

Scheduled software maintenance





#### **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.

#### 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 96.)

• 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 96.) Always allow 10 hours thermal equilibration time on site before opening the shipping box.

#### 5 Maintaining and Troubleshooting Your System

**Scheduled software maintenance** 

#### 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.
- To extend the life of the lasers, set up to automatically power your lasers on and off. See "Setting up times to turn the lasers on and off" on page 63 of Chapter 4.
- After turn- on, allow time for laser warm- up and stabilization. The maximum warm- up time is 30 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.
## Troubleshooting your system

	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.

#### Use the Web to reach technical support

The software arrives with a default URL that lets you access Agilent technical support for the microarray scanner.

• Click Help > Technical Support.

## **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 click **Help > Technical Support**.

#### 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 83 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 10-character serial number. Please make a note of the serial number of your Agilent Microarray Scanner, the software version # 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:

## Find the version information for scanner software

- 1 Select **Help > About** from the menu bar to find version information.
- 2 To close the program, click OK.

**Technical Support** 

## Frequently Asked Questions (FAQs)

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

FAQ	Answer		
I want to move the microarray scanner to another area.	Scanner performance could be adversely affected by the move. Call your local Agilent sales and support center for assistance in moving the scanner and assuring proper operation afterwards. Agilent recommends that you save your data files directly to the local hard disk. Data files may also be saved to a network directory. If a network access problem is experienced during the scan, data will be saved to a temporary local directory, and a warning will be included in the carousel run report.		
Can I save files over the network while scanning?			
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.		
l cannot open the top lid to access the carousel.	<ul> <li>Do not attempt to force the lid open. Forcing the lid could damage the microarray scanner.</li> <li>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.</li> <li>2 Close and then restart the Scan Control program.</li> <li>3 Try opening the lid.</li> <li>4 If the lid still does not open, close the Scan Control program and turn the microarray scanner off, then back on.</li> <li>5 Try opening the lid.</li> <li>6 If the lid still does not open, contact your local Agilent sales and support center.</li> </ul>		
The Scan Control program does not respond.	<ul> <li>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:</li> <li>1 Open the Windows Task Manager (Ctrl-Alt-Del) and close the Scan Control program. Then restart the Scan Control program.</li> <li>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.</li> </ul>		

## Table 2 FAQs

FAQ	Answer
What do I do if a slide is only halfway inserted in the carousel by the autoloader arm?	<ol> <li>Gently push or pull the autoloader arm, whichever resists least, to the end of its range. Don't force it.</li> <li>Close and restart the Scan Control program.</li> <li>If the problem persists, call your local Agilent sales and support center.</li> </ol>
The microarray scanner is turned on, but the yellow light does not go off after 20 minutes.	<ol> <li>Verify that the PC workstation is turned on.</li> <li>Verify that the USB cable is connected from the PC workstation to the microarray scanner.</li> <li>Contact your local Agilent sales and support center.</li> </ol>
The microarray scanner is turned on and the Scan Control program is open, but the scanner won't scan.	<ol> <li>Close and then restart the Scan Control program. The microarray scanner must be turned on before the Scan Control program is started. If you started the Scan Control program first, the connection is not made when the scanner is turned on.</li> </ol>
	2 Contact your local Agilent sales and support center.

## Table 2FAQs (continued)

## 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 74 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 36 Location of the status indicator lights

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

LED Status			Possible Causes	Suggested Action
Green	Yellow	Red		
Off	Off	Off	No power to the scanner.	<ul> <li>Check the following:</li> <li>power switch is on</li> <li>power cord is plugged in and there is power at the source</li> <li>power fuses are not blown</li> </ul>
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. Use HyperTerminal to capture diagnostic information. See "Using HyperTerminal to Capture Diagnostic Data" on page 83.
Off	Flashing (20 sec. off/ 20 sec. on)	Off	Attempting to connect to PC	Install Scan Control Software 8.0 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 30 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.

## Table 3 Indicator Light Status

**Technical Support** 

#### **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.
- **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 (Part# 2110-1309).
- **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.



Figure 37 Removing the fuse holder for the power supply

**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.

#### 5 **Maintaining and Troubleshooting Your System Technical Support**



Figure 38 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 (Part # 2110-0458).
- **5** Push the fuse holder back into place and screw on the cap.

## Troubleshooting an autoloader arm extended into the carousel

- **1** Turn off the scanner.
- 2 Rotate the carousel and push the arm back into position.

If this does not work, call your local Agilent sales and support center for instructions.

## CAUTION

Removing the carousel when the slide holder autoloader arm is extended into the carousel will cause serious damage to the microarray scanner.

## **Using HyperTerminal to Capture Diagnostic Data**

Agilent service personnel and users can capture information for troubleshooting the scanner by setting up a HyperTerminal session. These instructions apply to Windows XP systems. For instructions on capturing diagnostic data for scanners operating on Windows Vista 32 systems, please see the release notes associated with Scan Control Software version 8.4

## Set up HyperTerminal

- **1** Turn on the scanner to start initialization.
- 2 Start the Scan Control program to turn on the lasers.
- **3** Make sure the scanner is on for at least 30 seconds before starting HyperTerminal (step 4).
- 4 Click Start > Programs > Accessories > Communications > HyperTerminal.
- **5** In the Connection Description dialog box:
  - a Type Scanner in the Name text box.



**b** Click **OK**.

**Technical Support** 

## CAUTION

When the scanner is turned on and HyperTerminal is running, the scanner cannot initialize. Wait at least 30 seconds after you turn on the HyperTerminal must be started no earlier than 30 seconds after the scanner has been turned on.

- 6 In the Connect To dialog box:
  - **a** From the **Connect using** drop-down list, select the highest COMX number.

This will usually be COM3, but you can verify this through Windows Device Manager. The scanner COM port is listed as "USB Serial Port (COMX)".

Connect To	? 🛛
🇞 Scanner	8
Enter details for	the phone number that you want to dial:
Country/region:	United States (1)
Area code:	510
Phone number:	
Connect using:	СОМЗ
	OK Cancel

**b** Click **OK**.

- 7 From the COMX Properties dialog box:
  - a Select 19200 as Bits per second.
  - **b** Select None as Flow Control.

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OM3 Properties		?
Port Settings		
Bits per second:	19200	
Data bits:	8	
Parity:	None	
Stop bits:	1	
Flow control:	None	
	Restore Default	5
0	K Cancel Ap	ply

### c Click OK.

The Scanner - HyperTerminal window appears.

- 8 Click File > Save to save the file to Scanner.ht, and close the window.
- 9 Click Yes when asked if you want to sever the connection.
- 10 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.

**Technical Support** 

**11** 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 filename Capture *yymmdd-#* in the **filename** 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.

Folder:	C:\Documents and Settings\da	wid_baker\M	y
<u>F</u> ile:	inal Capture Files\Capture 060	105-1.TXT	Browse
	_	Ci uni	Cancel

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.

### Start a HyperTerminal capture session after initial set-up

- If the lasers are warmed up, skip to step 3.
- **1** Turn on the scanner to start initialization.
- 2 Start the Scan Control program to warm up the lasers.
- **3** Double-click on the Desktop **Scanner.ht** icon.
- 4 From the HyperTerminal **Transfer** drop-down menu, click **Capture Text.**

- 5 In the Capture Text dialog,
  - **a** Change the filename in the **File** text box to reflect today's date and capture session.
  - **b** Click Start.

older:	L:\Documents and Settings\david_baker\M	y .
File:	inal Capture Files\Capture 060105-1 TXT	Browse

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

## **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.

To use this CD successfully you must disconnect the USB cable that attaches to the back of the PC before you use the CD.

## CAUTION

If you do not disconnect this cable, the Agilent default settings for the USB devices may be changed, and the software will not recognize the scanner when you restart the software.

5 Maintaining and Troubleshooting Your System Software Troubleshooting

## **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 Scan Control 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.

The title bar of the error message indicates that this message is related to the carousel hardware.

Browsing	carousel	×
8	Description: The chip	carousel is not present. Please place the carousel in the scanner and try again.
		ОК

Figure 39 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 74 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. Scan your slides" on page 32 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 "View the Carousel Report Log during the run" on page 41 of Chapter 2.

We recommend that you check the Report Log at least after the first scan and when the carousel run is completed. The report is automatically generated at the end of a carousel run.

#### Troubleshooting with error messages

The Agilent 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.

Error Message	<b>Description or Solution</b> You cannot run two copies of the Scan Control program at the same time.	
Application Is Already Running		
Autofocus error: Did not detect two reflective surfaces during home.	<ol> <li>Do the following:         <ol> <li>Verify that the slide is inserted properly in the slide holder. See "Step 2. Insert slides into slide holders" on page 24, or try another slide holder.</li> <li>Verify that the slide meets the Agilent specifications. See "Slide specifications" on page 98.</li> <li>If the problem persists with one slide, then something might be wrong with that slide.</li> <li>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 "Create a new scan region with the Scan Region Editor." on page 60.</li> <li>Contact your local Agilent sales and support center.</li> </ol> </li> </ol>	

Table 4	Error N	lessages
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Software Troubleshooting

Error Message	Description or Solution	
Autofocus error. No PSD signal is detected. Make sure there is a slide in the chip holder.	<ul> <li>Do the following:</li> <li>1 Verify that the slide is inserted properly in the slide holder. See "Step 2. Insert slides into slide holders" on page 24,</li> <li>2 Verify that the slide meets the Agilent specifications. See "Slide specifications" on page 98.</li> <li>3 If the problem persists with one slide, then something might be wrong with that slide.</li> <li>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 "Create a new scan region with the Scan Region Editor." on page 60.</li> <li>5 Contact your local Agilent sales and support center.</li> </ul>	
Autofocus was unable to retain focus as it travelled to the scan start position.	<ul> <li>Do the following:</li> <li>1 Verify that a barcode has not obstructed the scan start position.</li> <li>2 Choose a smaller scan region.</li> <li>3 If the problem persists with one slide, then there might be something wrong with that slide.</li> <li>4 Contact your local Agilent sales and support center.</li> </ul>	
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.	<ul> <li>Do the following:</li> <li>1 Open the scanner lid.</li> <li>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.</li> <li>3 If the problem persists, contact your local Agilent sales and support center.</li> </ul>	
Command timed out.	<ul> <li>Do the following:</li> <li>1 Verify that the USB cable from the microarray scanner to the PC workstation is connected.</li> <li>2 Turn the microarray scanner off, then on again.</li> <li>3 Restart the Scan Control program.</li> <li>4 If the problem persists, contact your local Agilent sales and support center.</li> </ul>	

## Table 4 Error Messages (continued)

Error Message	<ul> <li>Description or Solution</li> <li>Possible causes for this error include: <ul> <li>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.</li> <li>The microarray scanner might not be connected to the PC workstation. Verify the USB cable is connected to both the microarray scanner and PC workstation. If the USB cable is disconnected, reconnect the cable, reboot the PC computer, and if needed turn the microarray scanner off then on.</li> <li>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.</li> <li>Windows XP firewall may be preventing communication. Disable the firewall.</li> <li>If you rebooted the PC workstation, you may need to turn the microarray scanner off and then back on after Windows has restarted</li> </ul> </li> </ul>	
Connection manager reports that there are no instruments.		
Error ejecting chip. The chip was ejected into the home slot.	<ol> <li>Do the following:</li> <li>1 Turn off power to the scanner.</li> <li>2 Open the scanner lid.</li> <li>3 Rotate the carousel by hand until access to the home slot is not blocked by the Autoloader Arm.</li> <li>4 Remove slide from home slot.</li> <li>5 Gently push the Autoloader Arm back into the scanner until it comes to a stop.</li> <li>6 If the problem persists, contact your local Agilent sales and support center.</li> </ol>	
Failed to communicate with connection manager.	<ul> <li>Do the following:</li> <li>1 Reboot the PC workstation.</li> <li>2 Turn the microarray scanner off and then back on.</li> <li>3 After several minutes, start the Scan Control program.</li> </ul>	
Lasers never became ready.	<ol> <li>Do the following:</li> <li>Verify that the number showing in the voltage selector on the back of the scanner matches the input power supplied.</li> <li>If you rebooted the PC workstation, you may need to turn the microarray scanner off and then back on, after Windows has restarted.</li> <li>If the problem persists, contact your local Agilent sales and support center.</li> </ol>	

## Table 4 Error Messages (continued)

Software Troubleshooting

Error Message	Description or Solution
Missing or corrupt ChipRegions.ini file.	<ul> <li>Do the following:</li> <li>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.</li> <li>2 If you don't have a backup copy, search the folder for ChipRegions.bak, and rename it to ChipRegions.ini.</li> <li>3 If neither file exists, re-install the Scan Control program.</li> <li>4 Contact your local Agilent sales and support center.</li> </ul>
Not enough disk space to create file.	<ul> <li>The hard drive does not have enough disk space to create a file to store scan data. You can do one of the following:</li> <li>Go to the Scan Control main menu and set the output directory to another directory on another hard drive.</li> <li>Delete or transfer files from the current hard drive. See "Step 5. Transfer files and archive data" on page 42.</li> </ul>
PMT calibration may be unstable. Using last known PMT setting.	You can reset this warning using the <b>Reset Calibration Warnings</b> tool. See "Additional Tools" on page 65. If this warning persists or reoccurs, contact your local Agilent sales and support center.
PMT calibration lost! Using factory default settings.	You can reset this warning using the <b>Reset Calibration Warnings</b> tool. See "Additional Tools" on page 65. If this warning persists or reoccurs, contact your local Agilent sales and support center.
Slide skipped because it is outside the allowable thickness range or the selected scan region contains an obstruction to the initial focus positions.	<ul> <li>Do the following:</li> <li>1 Check the skipped slide for a barcode, 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.</li> <li>2 Measure the glass thickness and verify that it meets with the specifications in "Slide specifications" on page 98.</li> </ul>
The autoloader loading arm is in an unknown location. It is unclear if it is safe to move the carousel.	<ul> <li>Do the following:</li> <li>1 Open the scanner lid.</li> <li>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.</li> <li>3 Close and restart the Scan Control program.</li> <li>4 If the problem persists, contact your local Agilent sales and support center.</li> </ul>
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. See "Step 2. Insert slides into slide holders" on page 24.

## Table 4 Error Messages (continued)

Table 4	Error Messages	(continued)
---------	----------------	-------------

Error Message	Description or Solution
The carousel may be misaligned. Please remove the carousel, make sure that its opening is aligned with home position and reinsert the carousel.	See "Step 2. Insert slides into slide holders" on page 24.
The slide failed to eject. Pressing OK will re-initialize the instrument.	<ul> <li>When you press OK, the microarray scanner re-initializes. If this fails, do the following:</li> <li>1 Open the scanner lid.</li> <li>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.</li> <li>3 Close and restart the Scan Control program.</li> </ul>
	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.
Unable to lock instrument specified by	Do the following:
connection manager.	<ol> <li>Reboot the PC workstation.</li> <li>Turn the microarray scanner off and then back on.</li> <li>Start the Scan Control program.</li> </ol>
You must select a region to scan first.	<ul> <li>To enter a scan region not specified in the selected profile into the scan table:</li> <li>Select the slot or slots whose region you intend to enter.</li> <li>Click the down arrow next to the selection(s).</li> <li>Select a scan region from the Scan region list. See "Change individual scan table settings" on page 46.</li> <li>Start the scan or carousel run.</li> </ul>
You must select at least one dye channel.	<ol> <li>Select the slot or slots whose dye channel you intend to change.</li> <li>Click the down arrow next to the selection(s).</li> <li>Select a dye channel setting from the list. See "Dye channel" on page 49.</li> </ol>

**Software Troubleshooting** 

Error Message	Description or Solution
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 30 minutes) when the status message in the Scan Control program says Status Ready. You can reset this warning using the Reset Calibration Warnings tool. See "Additional Tools" on page 65. If this warning persists or reoccurs, contact your local Agilent sales and support center.
	The Scan Control program scales the TIFF file to compensate for the lower laser power.

#### Table 4 Error Messages (continued)

### 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.



Agilent G2565CA Microarray Scanner System (Scan Control Software 8.3) User Guide

## Reference

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Microarray scanner specifications 96 Slide specifications 98 Regulatory information 102 Input voltage check 105

	This chapter includes specifications, regulatory information and instructions for checking the voltage if you move the instrument.
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 barcode 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.
Input voltage check	In this section you can make sure the input voltage is correct after a move.



## **Microarray scanner specifications**

	The microarray scanner operates within the following specifications:
Approximate	Height: 97 cm (38 in); with lid closed, 55 cm (21.5 in)
dimensions	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 (Part# 2110-1309)
	One laser fuse: T500mA, 250 VAC (Part# 2110-0458)
Temperature	Operating: 15° to 30°C
range	Storage: $-20^{\circ}$ to $+50^{\circ}C$
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	Excitation wavelengths:
information	• SHG-YAG laser: 532 nm
	• Helium-Neon (HeNe) laser: 633 nm
	Power: 20 mW at 532 nm and 23 mW at 633 nm, both controlled to 13 mW

Maximum scan region	Agilent G2565CA: 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)	2, 3, 5 or 10 microns	
Pixel placement error	< 1 pixel at 5-micron resolution	
Uniformity	5% CV global non-uniformity, average local non-uniformity is typically 1% based upon 100um features.	
Scan time	Table 5Scan Time for Single and Double Pass Scans for Agilent HDScan Region 61 x 21.6 mm	

Resolution	Scan Time, min.
2 micron single pass	20
3 micron single pass	15
5 micron single pass	8
10 micron single pass	8
2 micron double pass	40
3 micron double pass	30
5 micron double pass	16

Dynamic range	Single scan 16-bit dynamic range > $10^4$
	Single scan 20-bit dynamic range $>10^5$
	Dual scan extended dynamic range (XDR) $>10^6$

## **Slide specifications**

## Scan dimensions

The scan region is specified in Figure 40. All dimensions are in millimeters and the reference point is the lower right side of the glass.





## **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  $\times$  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)
- 1 mm thick (+/-0.1 mm)
- No mirrored slides
- High quality with low intrinsic fluorescence
- Index of refraction from 1.510 to 1.515

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## **Barcode and barcode label specifications**

## **Barcode specifications for Agilent slides**

The G2565CA scanner reads barcodes placed on the active side of the slide.

For backwards compatibility with the G2565AA model scanners, Agilent microarrays continue to have barcodes on both sides. The label with the text "Agilent" denotes the active side; the label with the numeric value is the inactive side.



#### Double-barcoded slide example



## **Barcode specifications for non-Agilent slides**

- The barcode 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 barcode labels on slides whose scan region exceeds 61 mm. You must enter the slide identifier or barcode manually for slides whose scan regions exceed 61 mm.

**Barcode and barcode label specifications** 

• For a barcoded slide, the distance between the edge of the microarray scan region and the edge of the barcode label can be no less than 4 mm.



Figure 42 Barcode location on non-Agilent slide

- The barcode label width should be about 6 mm; the recommended barcode label width is for use with the 61 mm scan region. Larger or longer barcode labels can be used by decreasing the scan region proportionally.
- Barcode labels should be narrow enough so as not to interfere with the slide holder tabs.
- Maximum allowable tilt in the placement of the barcode label on the slide is ±3°.

The barcode must meet the following requirements:

- The minimum quiet zone (the region between the edge of the label and the start or end of the barcode) is 10 times the X-dimension on each side of the barcode.
- Barcode lines must be parallel to the long edge of the slide within tilt tolerance of ±3°.
- Agilent supports the following barcode formats:
  - Code 128: Non-Agilent barcodes with 12 digits must not start with 2
  - CODABAR: Maximum of 8 digits; minimum line thickness
     6 mils

**Barcode and barcode label specifications** 

- 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 barcode must be
  - Code 128: 15% of barcode length for Code 128
  - Code 39, Code 93, CODABAR: 5 mm or 15% of barcode length, whichever is greater

**Barcode and barcode label specifications** 

## **Regulatory information**

This section lists regulatory information for the microarray scanner.

#### For research use only

The Agilent G2565CA 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 IEC 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.

Reference Barcode and barcode label specifications 6

**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)

Barcode and barcode label specifications

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

## Input voltage check

## Verify input voltage

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 43 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, change the voltage setting.

**Change the voltage setting** 

## Change the voltage setting

**1** Disconnect the power cord.

## WARNING

## Always disconnect the power cord before changing the input voltage setting.

**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.





**3** Pull out the fuse holder.

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**4** Use needle nose pliers to remove the voltage selector.



- **5** Insert the voltage selector so that the number showing matches the line voltage.
- 6 Push the fuse holder back in until it clicks into place.
- 7 Plug in the power cord.

Change the voltage setting
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