ScanArray Express
Microarray Analysis System
User Manual

User Notice
Users are advised that the use of this ScanArray instrument, or the SpotArray instrument, for certain applications in analyzing microarrays may require a license from one or more third parties that have patents in this area. The following are some of the companies that have patents related to microarrays:

Affymetrix, Inc.
Incyte Genomics
Oxford Gene Technology, Ltd
Hyseq, Inc.

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Preface Summary

Contents of This Manual

The following table describes the contents of this User Manual.

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Chapter 1	Provides an overview of the ScanArray instrument hardware and software, and an overview of the ways to scan and quantitate using ScanArray Express.
Chapter 2	Helps you become familiar with ScanArray Express by running an Easy Scan and Easy Quantitation on the Geometric Test Slide.
Chapter 3	Provides instructions for scanning and quantitating using Easy Scan and Easy Quant.
Chapter 4	Provides instructions for scanning using a protocol; includes instructions for creating a Scan Protocol.
Chapter 5	Provides instructions for quantitating using a protocol; includes instructions for creating a Quantitation Protocol.
Chapter 6	Provides instructions for creating and using Protocol Groups and Batch Sets to analyze multiple experiments and to automate your scanning and quantitating.
Chapter 7	Provides instructions to set system-wide settings.
Chapter 8	Provides diagnostic and troubleshooting information.
Appendix A	Formats of files supported by ScanArray Express.
Appendix B	Specifications
Appendix C	Declaration of Conformity
Appendix D	Quality Measurement Formulas

Conventions Used in this Manual

The ScanArray Express uses the Windows® 2000 or Windows® XP Operating System. We assume that the operator is acquainted with the general use of the operating system, and therefore provide only an overview of using Windows programs.

The names of the buttons and their locations are bold. The name of windows and dialog boxes are in italics. For example:

- In the ScanArray Express *Main Window*, click **Scan** to start a scanning procedure.

All user defined file names, for example results files, must be Windows compatible (i.e.they cannot contain a slash, a backslash, a dot or any other Windows reserved character).

The **Cancel** button in all windows exits the window you are working in without saving any changes you may have entered.

Where to Get Help

If you need help installing or operating the ScanArray system, you can contact PerkinElmer in the following ways:

- Web Site - http://www.perkinelmer.com/microarray
- Customer Service by telephone
  Tel: + 617-350-9263 or (800) 551-2121
  Fax + 617-482-1380
  E-mail: PBTsupport@perkinelmer.com

Please be prepared with the following when you call:

- serial number of your instrument.
- version number of the application software, from **Help**, **About** on the ScanArray Express software, *Main Window.*
- nature of the problem
- steps you have taken to correct the problem
- access to your instrument
- your phone number, fax number, and e-mail address

For Additional Information

Additional information about the system can be found in the following:

- ScanArray Express Installation Guide
- Documentation provided with the workstation and its operating software
- Documentation provided by manufacturer of the fluorophores that are used
- The ArrayInformatics *Microarray Laboratory Integration Guide* (for ArrayInformatics users only)

Getting Help for Windows 2000/XP

The Windows 2000/XP online help provides information on learning how to use the Windows operating system.

To open Windows 2000/XP help

1. Click the **Start** button on the task bar.
2. On the Start menu, click **Help**. Navigate to a topic using the tabs.

Introduction

Chapter 1

Chapter Summary

1.1 Overview

Welcome to the ScanArray® Express from PerkinElmer Life Sciences. The ScanArray Express is an extremely sensitive microarray laser scanner with easy-to-use software for microarray scanning and quantitation.

1.1.1 The ScanArray Express System

The ScanArray Express system includes the scanning instrument with one to four lasers, and a Windows 2000 or Windows XP workstation running the ScanArray Express software.

Figure 1-1 shows the ScanArray instrument models (from left to right): the ScanArray Lite, the ScanArray Express, and the ScanArray Express HT with 20-slide autoloader.

Figure 1–1 The ScanArray Express Instrument Models

All three models run the ScanArray Express software on the workstation. The software is used to control all aspects of scanning, quantitating and saving the scanned images and quantitation results, using a network connection between the workstation and the instrument.

1.1.1.1 The Scanning Instrument

The instrument includes one to four lasers, depending on the system configuration. Usually lasers are mounted inside the scanner; however, large lasers or lasers with a significant power dissipation may be externally mounted. The laser includes appropriate beam shaping optics, an excitation filter, a shutter and beam alignment optics. Lasers turn off automatically after 120 minutes of inactivity to maximize the service life of the laser.

Optional add-ons to the instrument include a barcode reader and autoloader.

1.1.1.2 ScanArray Lite

The ScanArray Lite has two internal lasers, and can be upgraded with an optional barcode reader. This model supports:

- two Laser Excitation Wavelengths of: 543 nm and 633 nm
- two Emission Filter Wavelengths of: 570 nm and 670 nm

1.1.1.3 The ScanArray Express

The ScanArray Express can be upgraded to alternative lasers, filters, barcode reader, and autoloader. This model supports:

- up to five Laser Excitation Wavelengths of: 488 nm, 514 nm, 543 nm, 594 nm, 612 nm, and 633 nm;
- up to 11 Emission Filter Wavelengths of 508 nm, 522 nm, 530 nm, 549 nm, 570 nm, 578 nm, 592 nm, 614 nm, 660 nm, 670 nm, and 694 nm.

1.1.1.4 The ScanArray Express HT

The ScanArray Express HT includes a built-in autoloader, and can be upgraded to alternative laser, filters, and barcode reader. This model supports:

- up to five Laser Excitation Wavelengths of: 488 nm, 514 nm, 543 nm, 594 nm, 612 nm, and 633 nm;
- up to 11 Emission Filter Wavelengths of 508 nm, 522 nm, 530 nm, 549 nm, 570 nm, 578 nm, 592 nm, 614 nm, 660 nm, 670 nm, and 694 nm.

1.1.1.5 The ScanArray Express in an Integrated Microarray Laboratory

If your laboratory is integrated with an ArrayInformatics database that resides on a server or workstation on the network, you can retrieve information from and send information to the ArrayInformatics database. Selection buttons for ArrayInformatics on the ScanArray Express user interface are activated if your ScanArray Express is configured for integration, or dimmed and inactive if your ScanArray Express is not integrated.

ArrayInformatics is a microarray gene expression database and visualization software available from PerkinElmer. If you are working in an integrated laboratory, refer to the *Microarray Laboratory Integration Guide* that shipped with the ArrayInformatics software.

1.1.1.6 The User Interface

The *User Interface*, the part of the ScanArray Express software that you see displayed on the workstation monitor screen, lets you enter information or commands, and receive status information. The user interface opens to the ScanArray Express *Main Window,* described on the following page.

1.2 The ScanArray Express Main Window

When you start the ScanArray Express, the user interface displays the *Main Window* on the monitor screen, as shown in Figure 1-2 below. This is your starting point for sending commands, entering information, or receiving status information. This is also where scanning images and quantitation results display.

Note: Some ScanArray Express systems are for quantitation only; if you are working on a Quantitation system, you will not see the **Scan** button or associated windows.

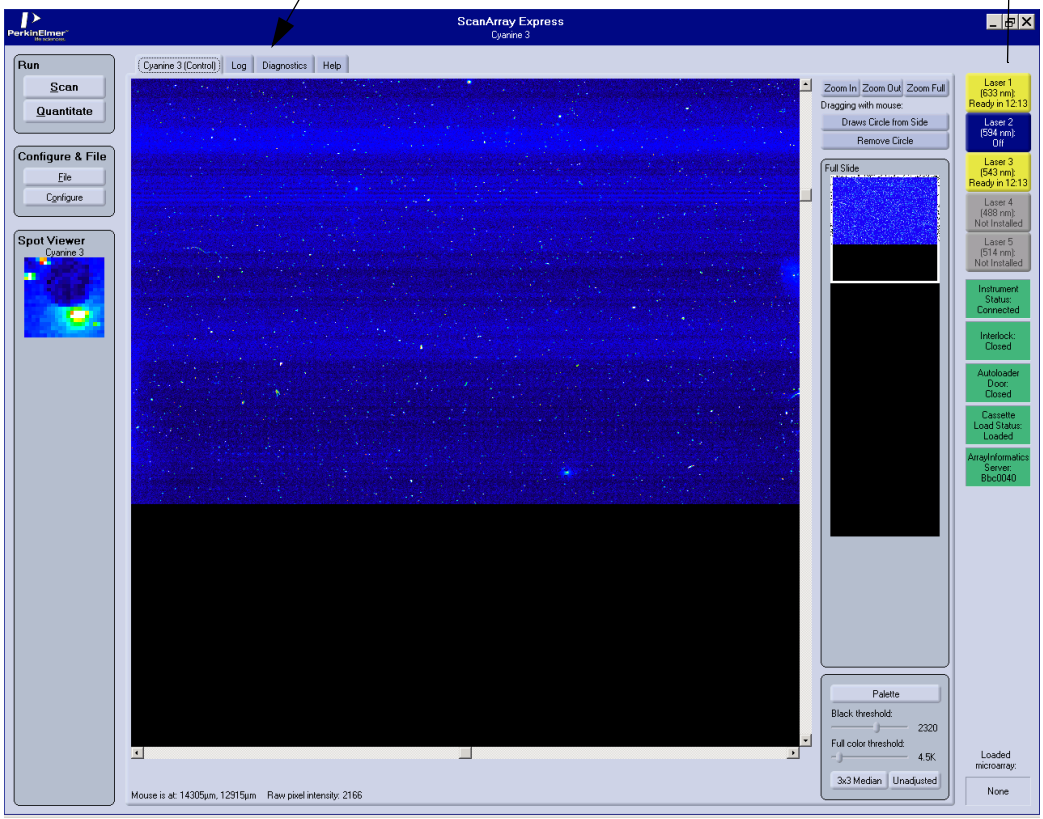
Run, Configure & File Buttons

Click Scan to select a scanning type and begin scanning.

Click *Quantitate* to select a quantitation type and begin quantitating.

Image Display Area Click a tab to view images or results.

Status buttons Display the status of the lasers and other instrument hardware. Turn lasers on or off.



Click *File* to open or save a file.

Click *Configure* to create protocols, protocol groups, and batch sets, and to configure system settings.

Figure 1–2 The ScanArray Main Window

1.3 Workflow for Scanning and Quantitation

The ScanArray Express performs two basic operations: *scanning* and *quantitation*. Depending on a workflow that you choose, scanning and quantitating can be done separately and individually, with manual or automatic saving of results, or the two operations can be partly or completely automated using *protocols* and *protocol groups*. A *Batch Set* is used for scanners with autoloaders.

The diagram below is an overview of the ways to scan and quantitate, and points you to information about each.

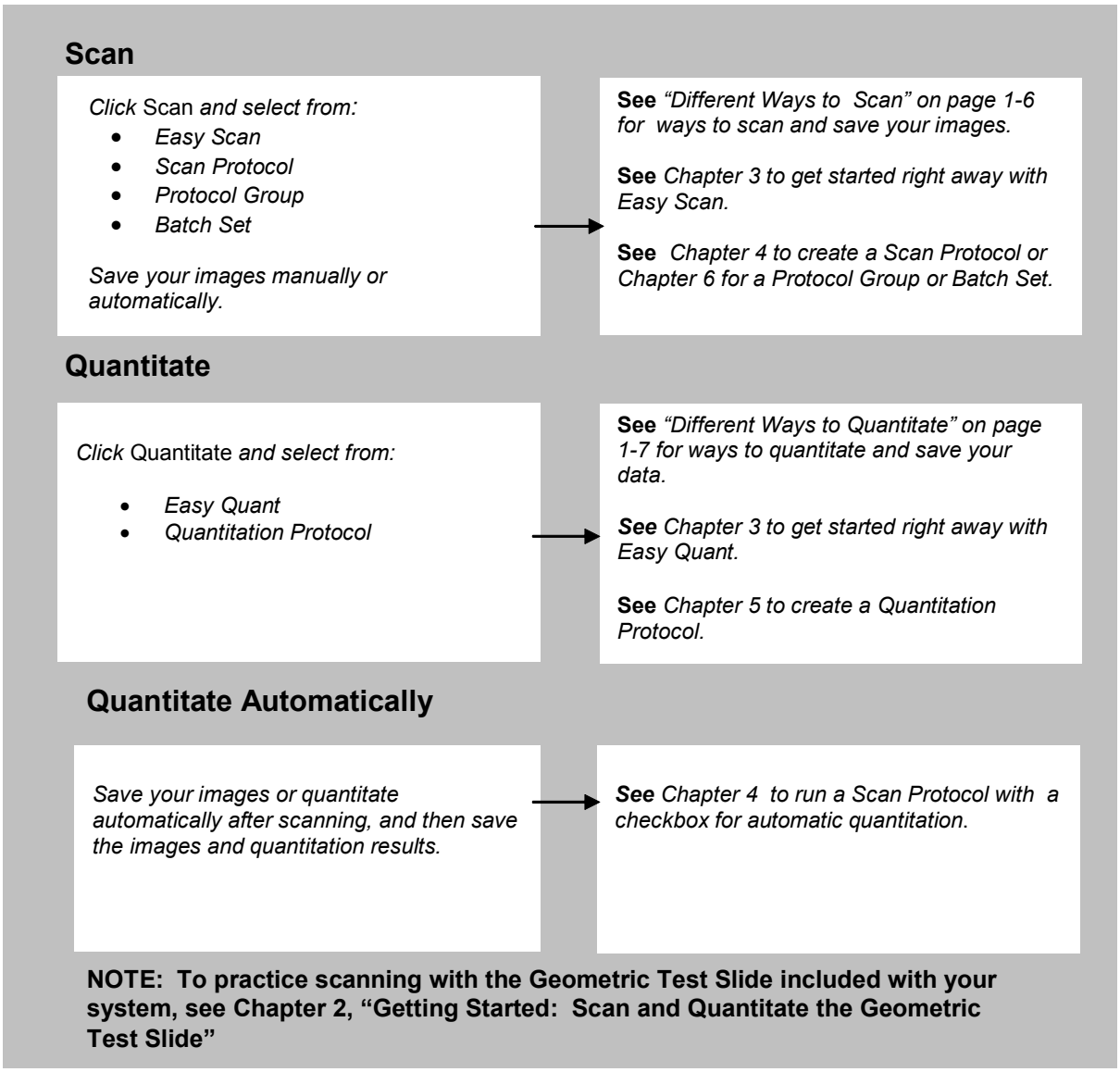


Figure 1–3 A Workflow Roadmap

1.4 The Different Ways to Scan

Before starting, review the ways you can scan your microarrays. You can automatically save the images to a local/network file system or ArrayInformatics database.

1.4.1 Easy Scan

The *Easy Scan* settings are "persistent;" that is, they remain from the last scanning session, so it's easier to scan the same type of microarrays. For the new or occasional user, Easy Scan is easy-to-use: all selectable settings are on one screen, and you can change your settings as you work.

Use Easy Scan if:

- Your lab uses one type of microarray and has one area of interest.
- You scan only one or two colors.
- You want to "walk up and use" the equipment. You can change settings as you work, including the scan resolution, the flourophores to be scanned, PMT Gain, and the area to scan.

1.4.2 Scan Protocol

A *Scan Protocol* uses pre-defined settings that can be named, saved, and recalled to use again; a protocol is useful when scanning different types of microarrays or when you want to use advanced features. Using a scan protocol is simple once it is set up: just load your microarray, select the protocol, and start.

Use a Scan Protocol if:

- You scan more than two colors, or wish to scan any fluorophore multiple times.
- Many users share the scanner and you want to automate file naming and saving for the users.
- Your lab uses more than one type of microarray: different image fields, labeling protocols, etc.
- You wish to incorporate automation features into scanning, such as Auto Sensitivity
- You want to automatically quantitate after scanning.

1.4.3 Protocol Groups and Batch Scans

Protocol Groups is a powerful new ScanArray Express feature. Using a protocol group, you can run different experiments on different areas of the same microarrays. You can run several scan protocols on a microarray, one after the other, without intervention, and automatically save the results to a specified location. In addition, you can include quantitation protocols in the protocol group, allowing you to scan, then quantitate, and save all scanned images and quantitation results to a specified location -- again, without user intervention. For more information, see Chapter 6.

Using a *Batch Set* lets you run different scan protocols or protocol groups on up to 20 slides in a cassette. To use a Batch Set requires a ScanArray Express system with the HT autoloader option. For more information, see Chapter 6.

1.5 The Different Ways to Quantitate

Before starting, review the ways you can quantitate your microarrays.

1.5.1 Easy Quant

The *Easy Quant* settings are "persistent;" that is, they remain from the last quantitation session, so it's easier to quantitate the same type of microarrays. For the new or occasional user, Easy Quant is easy-to-use: all user-selectable settings are available from one screen, and you can change your settings as you work.

Use Easy Quant if:

- Your lab uses only one type of two-color microarray.
- You want to "walk up and use" the equipment; you can change settings as you work.
- You want to test your slide; Easy Quant is a quick and easy way to do it.

1.5.2 Quantitation Protocol

A *Quantitation Protocol* uses pre-defined settings that can be named, saved, and recalled to use again. Using a quantitation protocol is simple once it is set up: just obtain your images, select the protocol, and start.

Use a Quantitation Protocol if:

- You have a variety of microarrays.
- You use more than two fluorophores.
- If the printing of the slides does not vary from microarray to microarray.
- You have complex and advanced settings that you want to use; once the settings are set up, they can be saved and easily run again.

1.5.3 Automatically Quantitating after Scanning

When running a *Scan Protocol*, you can select to automatically quantitate after the scan. While setting up to run, you must select a quantitation protocol to run, so the quantitation protocol must already be defined. For more information, see "Running a Scan Protocol" in Chapter 4.

You also can fully automate your scanning and quantitation using Protocol Groups or Batch Sets. For more information, see Chapter 6.

1.6 Preparing to Scan

To perform a scan, the instrument and the workstation must be connected, and the instrument must have completed all start-up diagnostic tests. Start the ScanArray Express and warm up the lasers for 15 minutes, and load the microarray or cassette.

Note: Lasers can vary when first turned on. After one or two minutes, they become approximately 60% stable, which is adequate for pre-scanning and investigative scanning. After five minutes, they become stable enough for all but the most sensitive applications. ScanArray Express indicates the lasers are still warming up for the full 15 minutes.

1.6.1 Turning on the ScanArray Express and Warming the Lasers

To turn on the ScanArray Express

1. Turn on the ScanArray instrument. Verify that the *Power* and *Ready* indicator lights are green.

2. Log in to Windows if required, using your assigned user name and password.

3. On the Windows workstation, double-click the *ScanArray Express* icon.

The User Interface opens to the ScanArray *Main Window*.

4. Check the status of the lasers. The laser buttons on the right side of the *Main Window* indicate the status of the lasers: blue indicates the lasers are turned off, yellow indicates the lasers are warming up; green indicates the laser are ready. To turn on a laser, click the status button for that laser.

5. Begin laser warm-up by clicking the laser status button.

1.6.1.1 Loading the Microarray or Cassette

To load a microarray

1. Hold the microarray by the thumbspace or by the edges.
2. Holding the microarray face up, and with the barcode or label toward you, slide the microarray into the slot until it touches the back. The ScanArray Express will automatically align the microarray.

To load a cassette into the autoloader

If you have a ScanArray Express system with an HT autoloader, you can load up to 20 slides in the cassette.

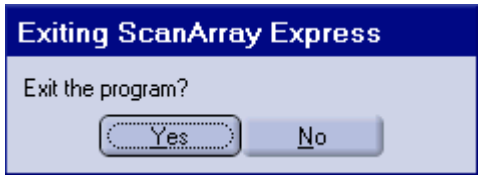
1. Pull the cassette straight up and out.
2. Inside the cassette are “fingers” that hold the slide in place. To prevent breaking these fingers, load the microarray slides from the back of the cassette.
   Insert a microarray, face up into each slot you want to use; slot 1 is on the bottom; slot 20 on the top. The barcode or label should be oriented so that it is toward the front of the instrument when the cassette is replaced in the autoloader.
3. Align the microarrays on the front of the cassette, using the metal alignment block that ships with the autoloader. The slides must be aligned to be “recognized”
4. Replace the cassette into the autoloader and close the door.

1.7 Exiting the ScanArray Express Software

You can exit the ScanArray Express user interface and the system saves the current settings.

To exit the ScanArray Express

1. Click the **X** in the top right-hand corner of the ScanArray Express *Main Window*. The following message displays:



2. Click **Yes** to exit.

3. You may leave the computer or instrument running, after exiting the ScanArray Express.

Note: If you need to shut down the instrument, use the *Service Features,* as described below.

To shut down the instrument

1. In the *Main Window*, click **Configure** in the *Configure & File* group. The *Configure* menu displays.

2. Click **Service Features**, then click **Shut down the instrument software and OS**.

3. Wait for the Ready LED on the front of the instrument to turn off; then wait 30 seconds and turn off the power switch on the right side of the instrument.

To shut down the computer

1. Your computer should automatically turn off. If not, click the **Start** menu on the task bar, then click **Shutdown**.

   A message displays on the monitor screen that it is okay to turn off the computer, and you can turn off the power.

Getting Started: Scan and Quantitate the Geometric Test Slide

Chapter 2

Chapter Summary

2.1 Overview

This Getting Started chapter leads you through a typical scanning and quantitation session, using **Easy Scan, Easy Quant** and the *Geometric Test Slide* that ships with the ScanArray Express system. The Geometric Test Slide is a test sample made of photo-resist printed on glass.

2.2 Scanning the Geometric Test Slide

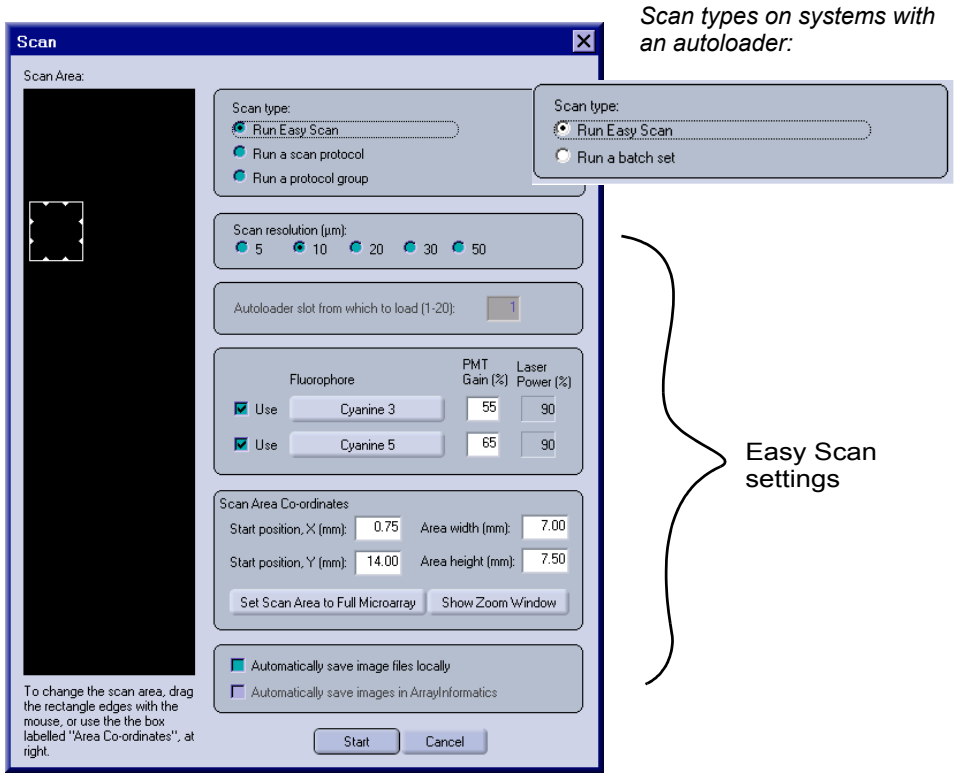
When finished, your scanned image should match the images shown in this chapter.

To run Easy Scan on the Geometric Test Slide

1. Turn on the instrument and client workstation.
2. Start the ScanArray Express software.
3. Turn on lasers 1 and 3 (click the status button for each).
4. Insert the Geometric Test Slide into the instrument. If you have an HT instrument with an autoloader, insert the slide into the cassette, align the slide using the alignment tool and insert the cassette into the instrument.

5. On the *Main Window*, click **Scan.** The *Scan* window opens.

6. Select **Run Easy Scan**. The *Scan* window displays as shown below, with the fields for Easy Scan settings.



7. Use the following settings, appropriate for the Geometric Test Slide:

   - Set the **Scan Resolution** to 10 µm.
   - If you are using an HT instrument, indicate the cassette slot into which you placed the slide.
   - Select *Cy3* as the **First Fluorophore**, with **PMT Gain** at 55; this will be the control image. To change the fluorophore, click the button labelled with the fluorophore name, select the appropriate fluorophore from the list and click **OK**.
   - Select *Cy5* as the **Second Fluorophor**e, with **PMT Gain** at 65; this will be the experimental (source) image.

- Set the **Scan Area** by entering the following values into the **Area Co-Ordinates** edit boxes:

Item	Setting for GeoSlide
Start position, X (mm)	0.75
Area width (mm)	7.0
Start position, Y (mm)	14.00
Area height (mm)	7.5

8. Click **Start.**

The ScanArray Express acquires a pair of images from the Geometric Test Slide using the *Cy3* and *Cy5* fluorophores, and automatically ejects the microarray when finished.

2.3 Viewing the Images

The scanned images display in the Main Window, each with its own tab: Cy3, Cy5, and the Composite of the two fluorophores. You can click an image tab to bring that image to the front of the display. An enlarged detail display of the image, as shown in Figure 2-1, is in the center of the window; an image of the **Full Slide** is on the right side.

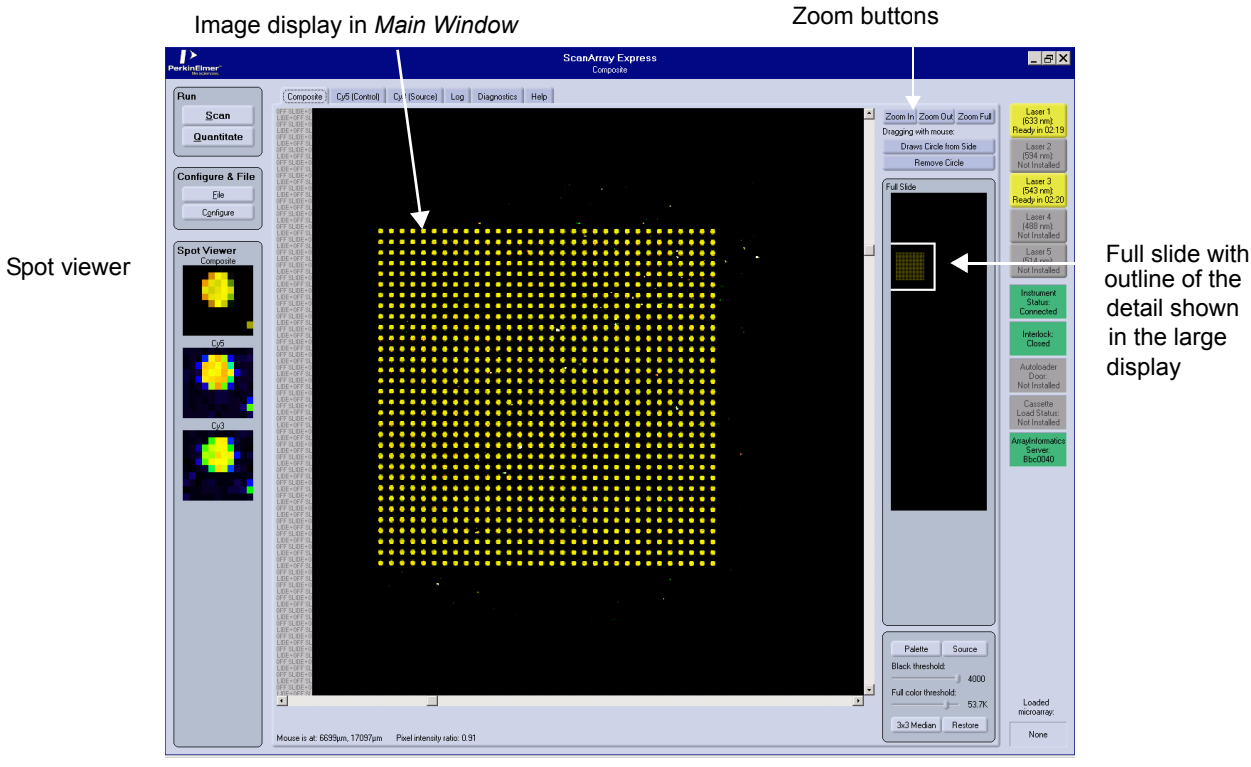


Figure 2–1 Image Display

On the left side of the window, the spot viewer shows enlarged details of the spots as the mouse cursor moves over them.

You can change the palette and adjust the display to see the spots better. Adjusting the display does not change the data for quantitation, nor the data in the file, only the appearance of the image on the screen. If there are two or more images, you can set the control image; and if three or more images, you can change the source (experiment) image.

To navigate the images

1. Click the tab of the image you want to look at, to bring it to the front.

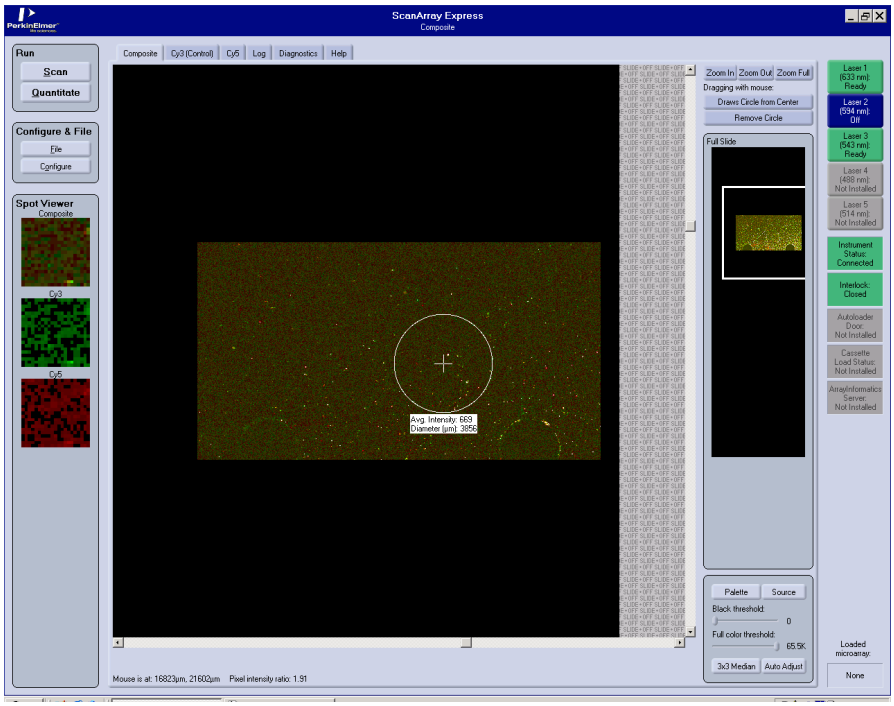
2. Use the mouse to zoom in on the details, or to shift the view to a different part of the microarray:

   - A mouse-click on either the full-slide or large image centers the field of view on the point where you click the mouse.

   - To shift the view to a completely different part of the microarray, click a point on the **Full Slide**.
     To shift the view slightly, click a point in the large image display.

   - To zoom in or zoom out on the display, click the appropriate **Zoom** button, located above the full slide view. The white rectangle in the Full Slide shows the size of the field displayed in the *Main Window,* and it changes size with the zoom level.

3. To view the average intensity of a certain area, click the **Draw Circle from the Center/Side** button. This enables you to draw a circle around the desired area using the mouse. The average intensity of spots within the circle is displayed.



2.3.1 Improving the Image Display

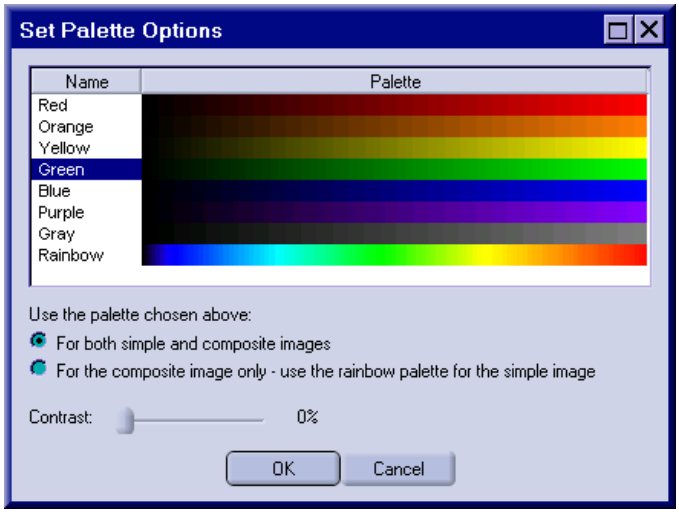
You can use the controls in the lower right corner to adjust the image display and improve the visibility. The adjustments do not affect your data, only the image on the screen.

Note: ScanArray Express auto-adjusts images during scanning or opening. The image of the Geometric Test Slide that you have just scanned using parameters in the previous section, does not need adjustments. The image is already of good quality. However, if you want to see at this point how the adjustments work, you can find more information in "Adjusting the Display," in Chapter 3.

2.3.2 Changing the Palette for the Selected Image

You can select a different palette or change the contrast. The palette used in the composite tab cannot be changed; it is always a blending of the palettes of the control and source (experiment) images or the rainbow palette. If you open a palette when the *Composite* tab is selected, the Palette window that opens allows you only to adjust the contrast.

1. Click **Palette**. The *Set Palette Options* window opens.



2. Click the palette you want to use, and select whether you want to use it for the current image and composite image, or only for the composite image (using the rainbow palette for all other images). You can adjust the contrast if needed.

Note: Using the rainbow palette for the simple images provides more visual data by providing a spread of intensities instead of limited shades of just one color. This allows you to differentiate more spots than you can with a single color.

3. Click **OK**.

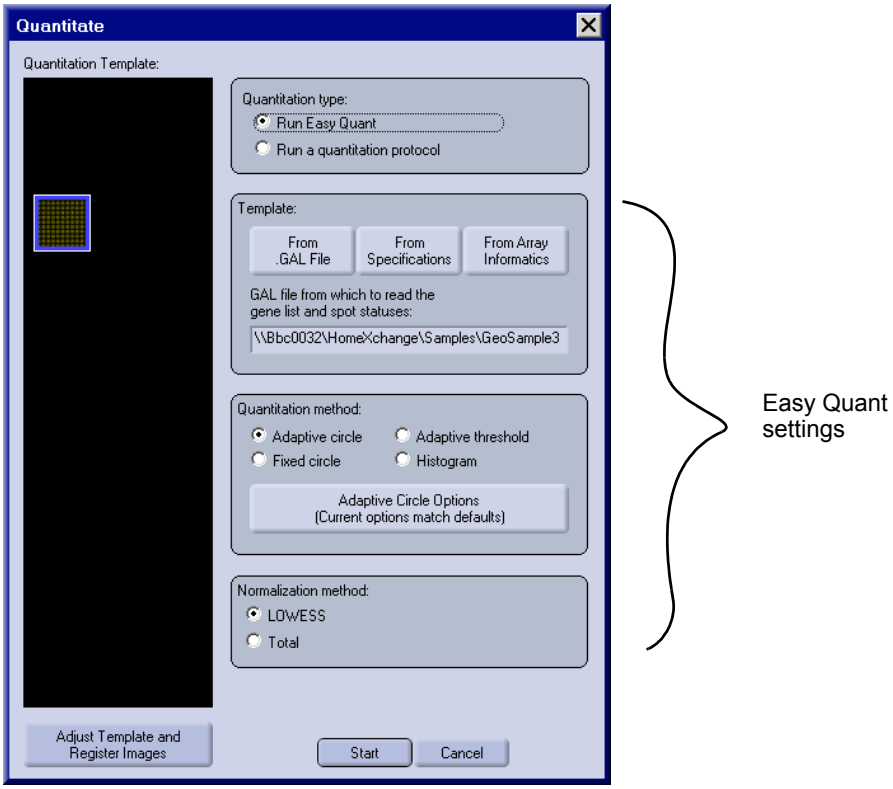
2.4 Quantitating the Geometric Test Slide

To generate numerical quantitation data from the images, quantitate the Geometric Test Slide scanned in the previous section using directions in this section.

To quantitate the Geometric Test Slide

1. With the two images generated in Section 2.2 still open, click **Quantitate** on the *Main Window*. The *Quantitate* window opens.

2. Select **Run Easy Quant**. The *Quantitate* window displays fields for easy quantitation. Use the settings in the following steps, appropriate for the Geometric Test Slide.



3. Load an array content template in the form of a .GAL file:

- click **From .GAL File**.
- In the *Open GAL File* dialog box that opens, browse to: `C:\Programs Files\PerkinElmer\ScanArray Express\Samples` and choose the file "GeoSample32x32.gal".

  (The path may be different if the ScanArray Express software was installed in a folder other than the default.)

- click **Open**.

  A template of circles appears superimposed on the microarray images.

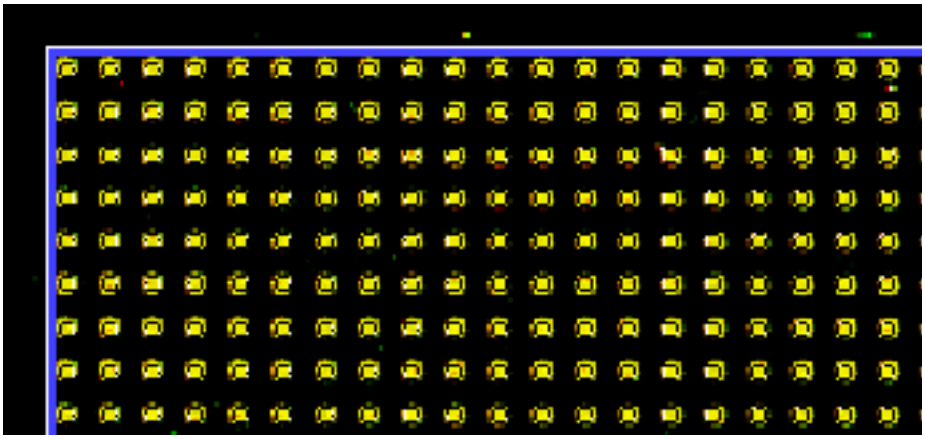
Note: When scanning the Geometric Test Slide, you don't have to adjust the template. When scanning your own slides, you need to adjust the template as described in steps 4 and 5. For more information, see "Adjusting the Template and Registering Images" in Chapter 3.

4. Click **Adjust Template and Register Images**. This opens a *Zoom* window, where you can align the template precisely, and register the images if necessary:

- Adjust the template by dragging it with the mouse until it is aligned with the spots. Leave the template in a position with the upper left-hand template circle near the upper left-hand spot in the image.

5. To register the images, click the *Composite* tab. The images are not perfectly aligned if traces of individual color such as green and red are visible around the outside of one side of each spot. Use the Zoom controls as desired to make it easier to view the registration.

- Align the images by clicking the left, right, up or down arrows located at the left side of the window. Each click on an arrow moves the image one pixel in the direction of the arrow. Move the images until they are well registered; that is, many spots appear yellow.



6. Click **OK**.

7. Under **Quantitation Method**, select **Adaptive Circle**.

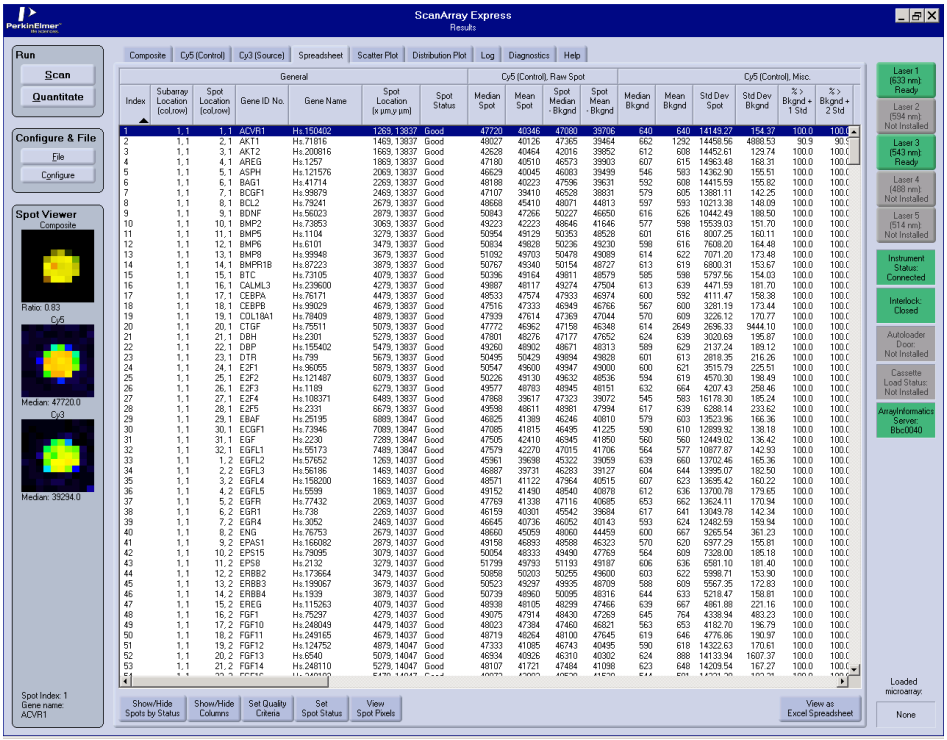
8. Under **Normalization Method**, select **LOWESS**.

9. Click **Start**.

The ScanArray Express finds the spots (a progress bar displays, showing the progress of finding spots) and immediately begins quantitation.

2.5 Viewing the Quantitation Results

The quantitation results display in the *Main Window* as a *Spreadsheet,* a *Scatter Plot*, and a *Distribution Plot*, each in a separate tab. The data is linked in all views: when you click a data point in one view, the data point is already selected if you change to a different view.



2.5.1 Spreadsheet

Each row in the spreadsheet is the data from one spot, including the gene names and ID numbers that were imported from the .GAL file. You can scroll vertically to see the data for each spot. You can scroll horizontally to view the 55 columns of data for any spot, or click **Show/Hide Columns** to hide any columns you don't want to see.

The spreadsheet is interactive: click on a row in the spreadsheet and see the spots that created that data in the spot viewer on the left side, and also see those spots highlighted and centered in the full images by selecting the *Cy3*, *Cy5*, or *Composite* image tab. Click on a column to sort using that column.

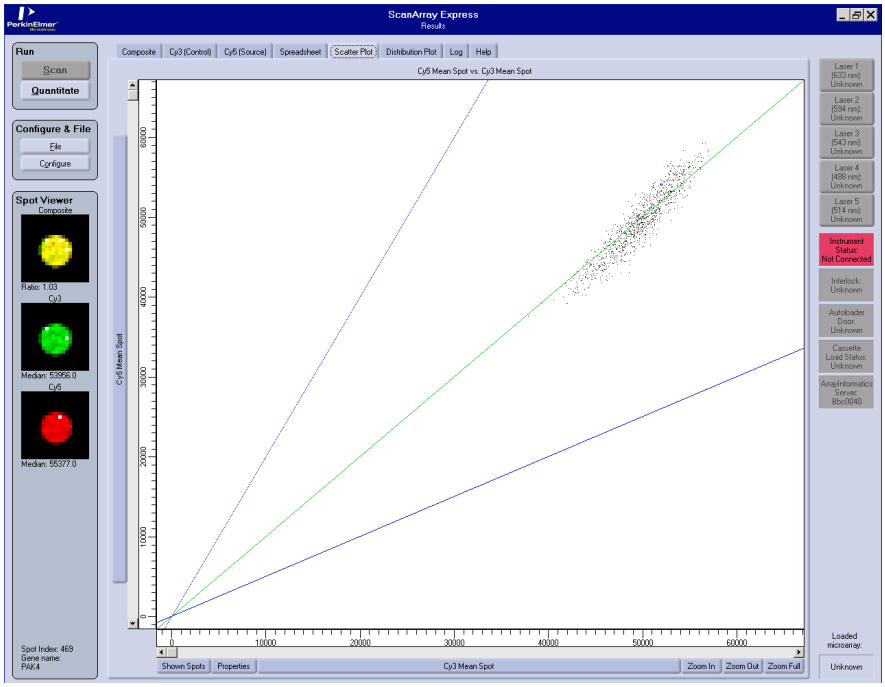
Note: If you have Microsoft Excel on your system, you can view the spreadsheet in Excel by clicking **View as Excel Spreadsheet**, in the bottom right corner of the *Spreadsheet* tab.

The following buttons on the *Spreadsheet* tab let you set the spot status and select which data to view. They are described fully in "Viewing the Spreadsheet" in Chapter 3.

Button	Description
Show/Hide Spot by Status	Lets you select spots to hide or display, depending on their status.
Show/Hide Columns	Let you select which columns of information to show or hide.
Set Quality Criteria	You don't need to set this for the Geometric Test Slide. For more information, see "Viewing the Quantitation Results" in Chapter 3.
Set Spot Status	Lets you change the status for a spot: Good, Bad, Found, Not Found, Absent.
View Spot Pixels	Shows which pixels were treated as part of the spot during quantitation.

2.5.2 Scatter Plot

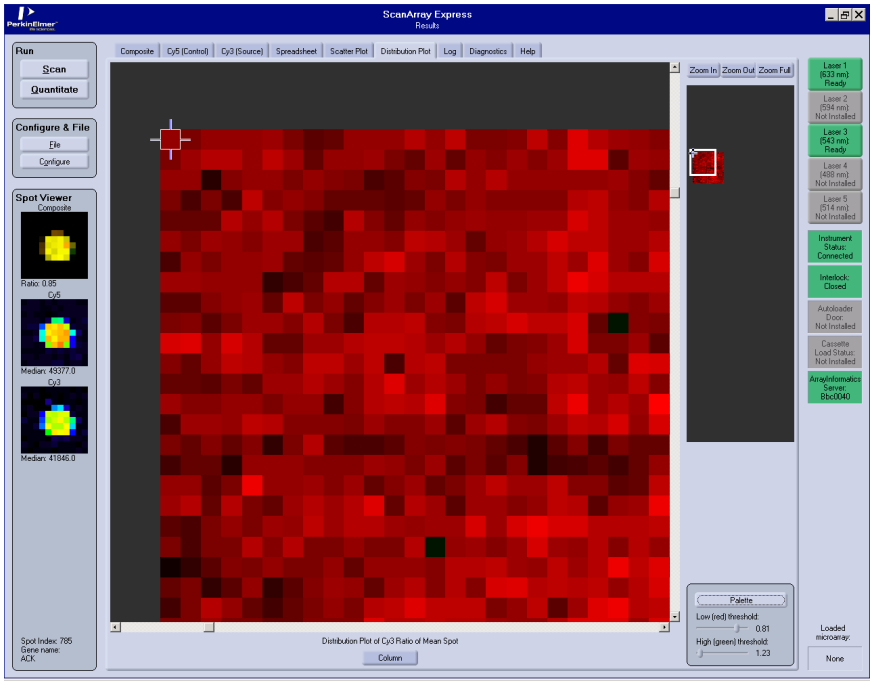
The *Scatter Plot* allows you to see any column of data plotted against any other column.



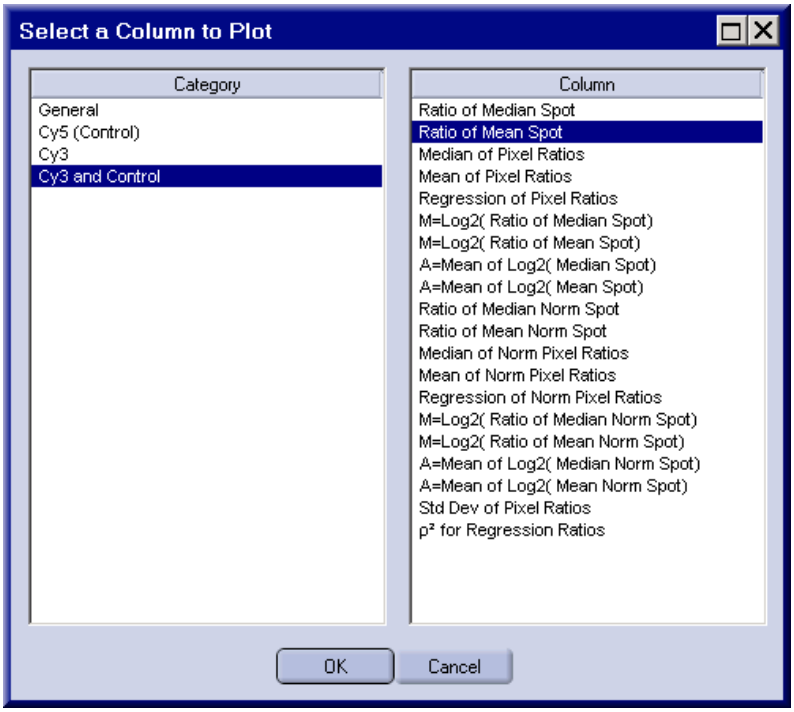
When you click a data point on the scatter plot, the selected data point displays in the spot viewer and is highlighted in both the Spreadsheet and Image views when you switch back to those tabs. *The data is linked in all views.*

2.5.3 Distribution Plot

The *Distribution Plot* allows you to see trends that are area-sensitive on the slide; for example, if one side of the slide is being over- or under-washed, or if a pin is partially clogged.



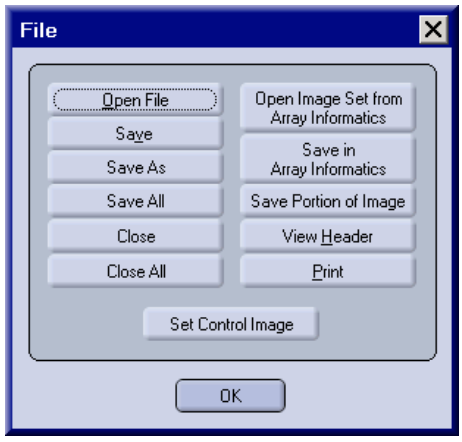
You can select a column of spots to plot from any one of the images, or any images with the control image.



2.6 Saving the Images

To save the images

1. On the *Main Window,* click **File** in the *Configure & File* group, then click **Save** or **Save as** to save the selected image.
   Click **Save All** to save all of the images.



2. In the *Save Image* dialog box that opens, enter a filename and pathname for the image.

2.7 Saving the Results

You can save the results as a .GPR file or as a .CSV file. The format of these files is described in Appendix A.

To save the results

1. Click the *Spreadsheet*, *Scatter Plot*, or *Distribution Plot* tab.

2. On the *Main Window*, click **File** in the *Configure & File* box, then click **Save** and provide a filename and file type.

2.8 Scan and Quantitate Your Microarrays

You have completed scanning and quantitating the Geometric Test Slide and saved the results! Use the same process for your own microarrays, specifying at each step along the process the settings that are appropriate for your microarray. See Chapter 3 for more information on selecting settings for Easy Scan and Easy Quant.

Using Easy Scan and Easy Quant

Chapter 3

Chapter Summary

3.1 Overview

The Easy Scan and Easy Quant settings, once set, remain set until you change them. This makes it easy to scan microarrays with the same spot configuration and quantitate the resulting images using the same settings.

This chapter describes how to scan, including how to adjust the display of the images; and how to quantitate, including reviewing the results, using Easy Scan and Easy Quant.

Tip: *To become familiar with how scanning and quantitation work, first use the Geometric Test Slide that ships with the ScanArray Express. Run an Easy Scan and Easy Quant on the Geometric Test Slide, using recommended settings. See Chapter 2, "Getting Started."*

3.2 Using Easy Scan

Note: You can stop the scan at any time by clicking **Stop** on the *Main Window*. The image of what was scanned to that point can be saved just as a fully scanned image can be saved. See ***Saving or Printing Images*** on page 3-7.

When scanning with *Easy Scan*, you can specify the PMT settings in real-time, when you are ready to scan. If you don't change the settings, the settings remain the same as the last time a scan was performed.

To run an Easy Scan

1. Load your microarray.
2. In the *Main Window,* click **Scan**. The *Scan* window opens; select **Run Easy Scan**.

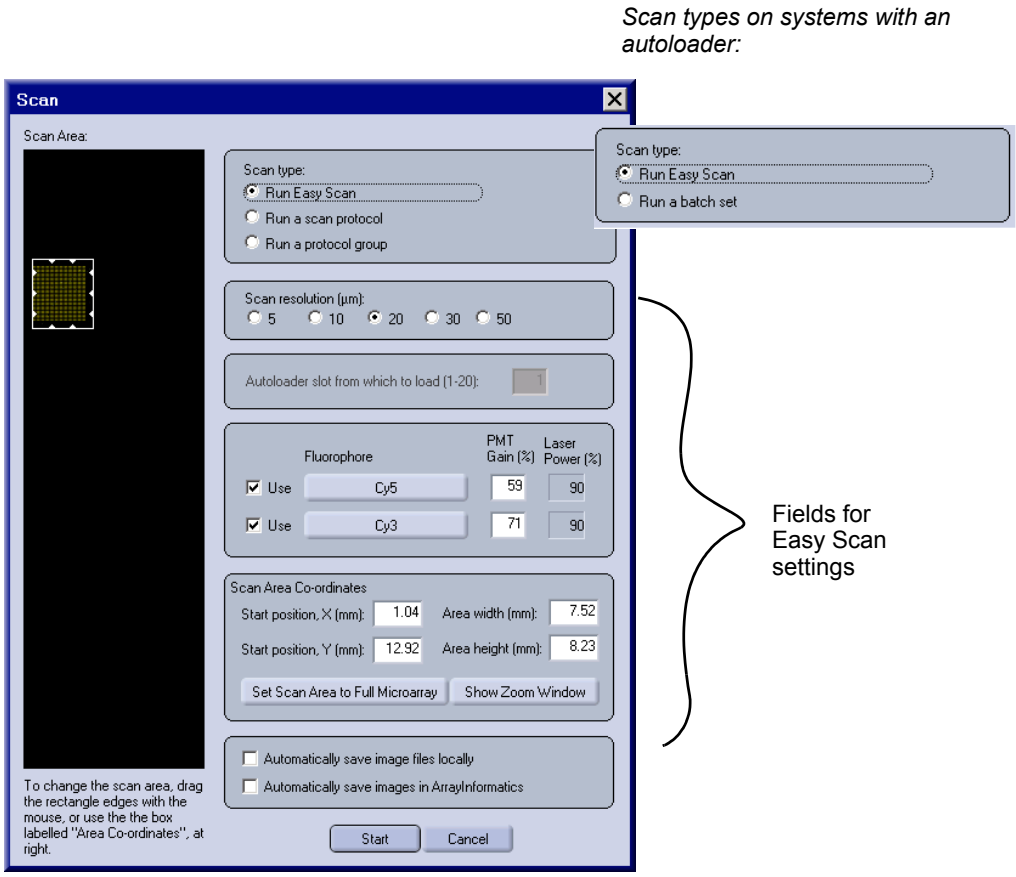


Figure 3–1 Scan Window - Run Easy Scan

3. Use the settings from the last session if you are using the same type of microarray, or see Section 3.2.1 to change the settings.
4. Click **Start**.

You may or may not see a message asking if you want to skip warming up the lasers. Skipping the warmup does not cause any damage, but the sensitivity of the resulting scans will vary slightly. If you are concerned with precise repeatability or uniformity of scanning, wait for the lasers to warm up.

3.2.1 Changing the Settings

To change the Easy Scan settings, refer to the table below for information. To make it easier to select a scan area using the mouse, you may want to obtain an image first by scanning the full slide at 50 microns.

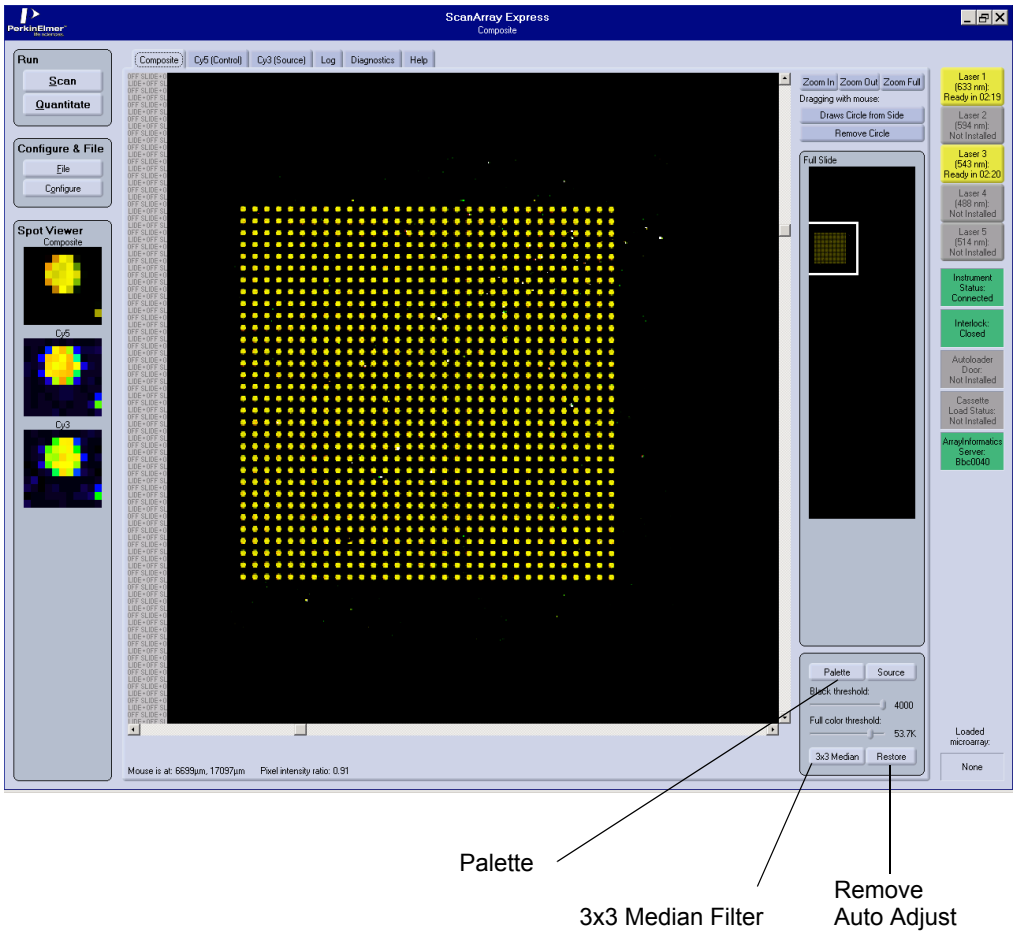
Item	Description
Scan Type	Select **Run Easy Scan**. The scan types that display depend on your system. You may see Run Easy Scan, Run a scan protocol, Run a protocol group, or on HT autoloader systems, you will see Run Easy Scan and Run a batch set.
Scan resolution	Select a scan resolution. Scan resolution represents the individual pixel size in the scanned image, and can be set to 50, 30, 20, 10, or 5 microns. Higher settings provide a quicker scan with reduced detail. The default resolution is 10 microns.
Autoloader slot from which to load	For instruments with an autoloader, specify the slot number of the microarray to be scanned. The default is 1. If your system does not have an autoloader this field is dimmed and unavailable.
Fluorophores	Select one (first checkbox) or two fluorophores to be scanned and set the PMT Gain for each:
Use	Check the box for a fluorophore, and click the button below it to open the *Select a Fluorophore* window. Find and select your fluorophore in the list, and click **OK**. The window closes, and the button fills with the fluorophore name.
PMT Gain (%)	Set a value for the PMT Gain for each selected fluorophore. The value defaults to the value used in the last scan session. ***NOTE:*** Increasing the PMT Gain percentage improves sensitivity, but also increases background noise and causes saturation. A PMT Gain of approximately 50 percent lowers the background. The laser power is set in the ScanArray Express application settings and cannot be changed here.
Scan Area	The diagram on the left side of the window is the full slide area, showing the selected scan area outlined in a white box. Select or change a scan area by using the mouse cursor to outline the desired area on the diagram. Alternatively, you can enter specific X and Y coordinates, or select the full microarray, as described under *Area Co-ordinates*, below.
Area Co-ordinates	You can enter precise coordinates here for the scan area, instead of selecting an area with the mouse. Start position, X (mm); Area Width (mm) Start position, Y (mm); Area Height (mm)
Set Scan Area to Full Microarray	Click here to set or reset the scan area to the full microarray.
Show Zoom window	Click here to open the *Scan Area for Zoom* window where you can zoom in on the display, and get help with selecting an area.

Item	Description
Automatically save images	Check this box to automatically save the scanned images locally. When you click **Start**, a dialog box opens where you specify a filename and path for saving the image(s).
Automatically save in ArrayInformatics	Check this box to automatically save the scanned images in the ArrayInformatics database. If your scanner is not integrated with ArrayInformatics, this field is dimmed and unavailable to select.

3.2.2 Adjusting the Display

The scanned image(s) display on the *Main Window*: a tab for each fluorophore, and a *Composite* tab of both fluorophores if two were selected. You can change the palette and adjust the display to see the spots better. Adjusting the display does not change the data for quantitation, or the data in the file, only the appearance of the image on the screen.

You can change the control image, and if there are more than two images, you can set or change the source (experiment) image.



To adjust the display

1. The ScanArray Express scans the microarray using the **Auto Adjust** settings that provide the best contrast between the spots and the background to make the spots more visible. Click **Unadjusted** to remove the auto adjustment and display the raw data.

2. Click the **3x3 Median** filter to make the image appear smoother and less "noisy" - cleaning up miscellaneous pixels. The button changes to **Remove Filter** so you can return to the previous appearance.

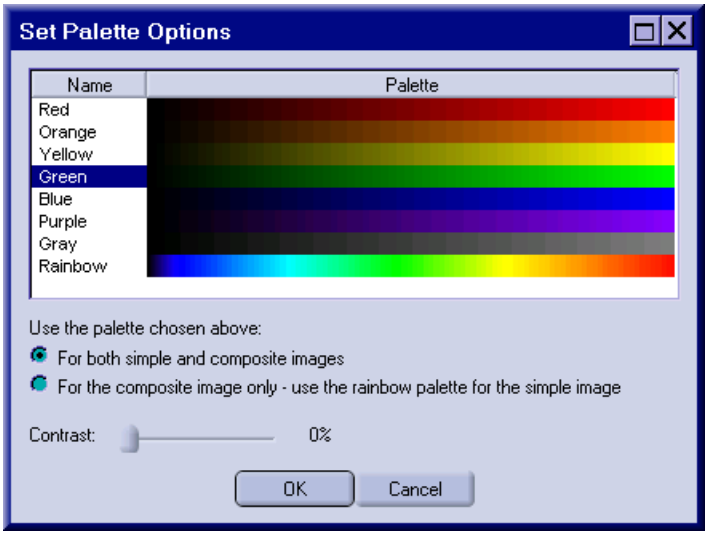
3. Black threshold and full-color threshold; use these two slide controls together to adjust the image to minimize background noise and to increase the color for better visibility.

4. Repeat for each tab you want to adjust.

To set palette options

You can select a different palette or change the contrast. The palette used in the composite tab cannot be changed; it is always a blending of the palettes of the control and source (experiment) images or the rainbow palette. If you open a palette when the *Composite* tab is selected, the *Composite Palette* window that opens allows you only to adjust the contrast.

1. Click **Palette**. The *Set Palette Options* window opens.



2. Click the color you want to use, and select whether you want to use it for the current image, or only for the composite image (using the rainbow palette for all other images). Adjust the contrast if needed.

Note: Using the rainbow palette for the simple images provides more visual data by providing a spread of intensities instead of limited shades of just one color. This allows you to differentiate more spots than you can with a single color.

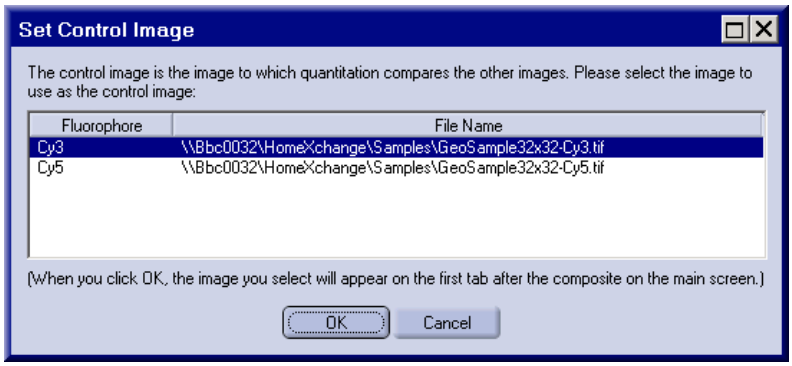
3. Click **OK**.

3.2.2.1 Changing the Control Image or Source (Experiment) Image

The first image scanned becomes the *Control Image*, the image to which quantitation compares the other images. The *Source* image is the one that is combined to form the Composite. You can change which image is used for the Control or, if you have three or more images, which is used for the Source (experiment).

To change the control image

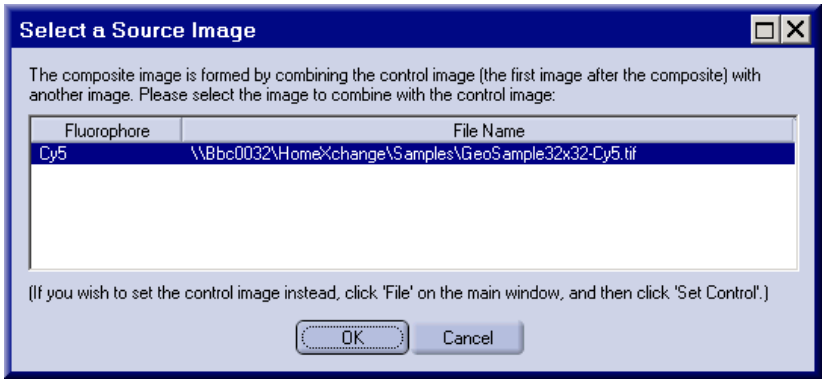
1. On the *Main Window,* click **File,** then **Set Control**. The *Set Control Image* window opens.



2. Select the image to use as the Control Image and click **OK**. The selected image appears in the first tab after the *Composite* tab in the *Main Window.*

To change the source image

1. On the display, click **Source** in the lower right corner. The *Select a Source Image* window opens.



2. Select the image to use as the Source image; the selected image appears in the first tab after the *Control* image tab.

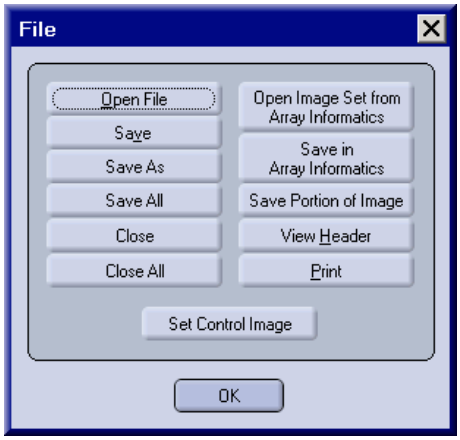
Note: With the images still displayed, you can immediately quantitate. See ***Using Easy Quant*** on page 3-8.

3.2.3 Saving or Printing Images

If you did not select to save images automatically by checking the *Autosave* checkbox(es) before scanning, you can save the images after scanning. You can also save a portion of an image to make a smaller file.

To save images that were not automatically saved

1. On the *Main Window,* click **File** in the *Configure & File* group, then click **Save** or **Save as** to save the selected image.
   Click **Save All** to save all of the images.



2. In the *Save Image* dialog box that opens, enter a filename and pathname for the image.

To save a portion of an image

1. Select an image tab.
2. On the *Main Window*, click **File** in the *Configure & File* group, then click **Save Portion of Image**.
3. In the *Save Portion of Image* window that opens, use the mouse to select the portion of the image that you want to save.
4. Click **OK**. In the *Save Portion of Image* dialog box that opens, enter a filename and click **Save**.

To print an image

1. Click the tab of the image you want to print.
2. On the *Main Window*, click **File** in the *Configure & File* group, then click **Print**.

3.3 Using Easy Quant

The ScanArray Express remembers your settings from the last quantitation session, or you can change them as you are ready to quantitate. Scan the images to quantitate, or open previously scanned images, by clicking **File** in the *Configure & File* group on the *Main Window*, then clicking **Open File.**

To run Easy Quant

1. In the *Main Window,* click **Quantitate**. The *Quantitate* window opens; select **Run Easy Quant**.

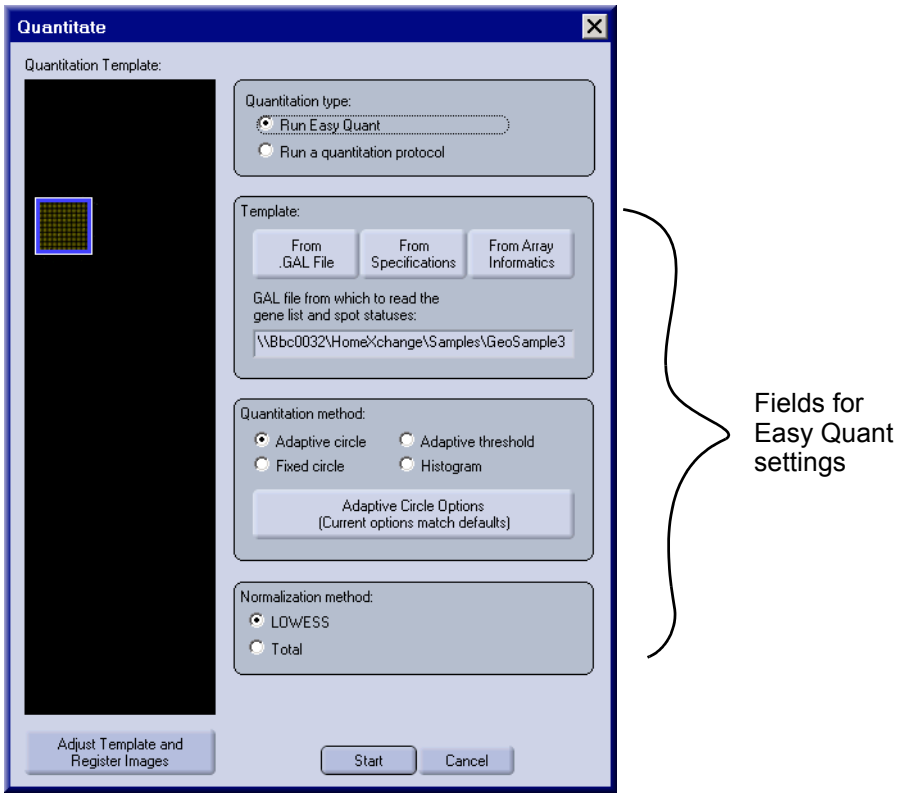


Figure 3–2 Quantitate Window - Run Easy Quant

2. Use the settings from the last session if you are using the same type of microarray, or see Section 3.3.1 to change the settings. If you make any changes, be sure to adjust the template and register the images. See ***Adjusting the Template and Registering Images*** on page 3-12.

3. Click **Start**.

   The ScanArray Express finds the spots (a message box displays, showing the progress of finding spots) and then quantitates immediately.

3.3.1 Changing the Settings

To change the Easy Quant settings, refer to the table below for information.

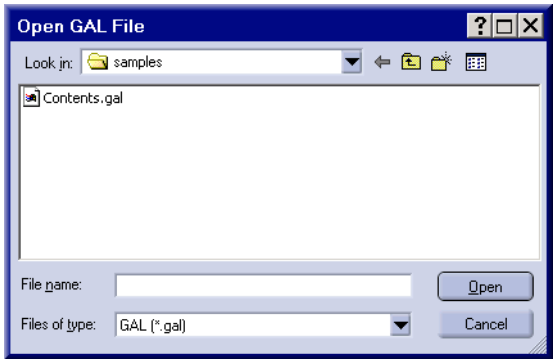
Item	Description
Quantitation Type	Select **From Easy Quant**. This selection lets you enter or change settings, or use the default or previous settings.
Template	A spot template must be defined that matches the layout of the arrays on the selected scan. Load a template from one of the methods listed below. A template of circles appears superimposed on the microarray image; you can then adjust and align the template.
From .GAL file	Click this button to open the *Open GAL File* window, where you can browse for and select a .GAL file to use for your template. For a description of .GAL files, see Appendix A. The template field fills with the name of the selected .GAL file. See ***To define a template using a .GAL file*** on page 3-10.
From specifications	Click this button to manually define the array pattern. The *Template Specification* window opens, where you enter the values. See ***To define a template from specifications*** on page 3-11.
From ArrayInformatics	Click this button to import template information from the ArrayInformatics database, if your ScanArray Express system is integrated with ArrayInformatics. Refer to the *Microarray Laboratory Integration Manual* that ships with your ArrayInformatics software. If your system is not integrated with ArrayInformatics, this button appears dimmed and is unavailable to select.
Quantitation Method	Select **Adaptive Circle**. This is the default method. For more information on quantitation methods, see “Choosing a Quantitation Method” in Chapter 5.
Normalization Method	Select **LOWESS**. This is the default method. For more information on normalization methods, see Chapter 5.
Adjust Template and Register Images	Click this button to open the *Adjust Template* window to adjust the template, and register the images if there are two images. When you start the quantitation, ScanArray Express finds the spots using the template, and then starts quantitation. For fastest spot finding, adjust the template within a row or column or two of the microarray spots. See ***Adjusting the Template and Registering Images*** on page 3-12.

3.3.1.1 Defining a Template

Specify a template to be used for quantitation. You can import a .GAL file, which also assigns gene names to each spot on the microarray, or you can manually define the array pattern by entering values to describe the subarray and spots. For a detailed description of a .GAL file, see Appendix A.

To define a template using a .GAL file

1. In the *Quantitation* window under Template, click **From .GAL File**. The *Open GAL File* dialog box opens.



2. Highlight the desired file and click **Open**. The name and location of the selected .GAL file displays in the *Quantitate* window under the **From .GAL File** button.

To define a template from specifications

1. In the *Quantitation* window under Template, click **From Specifications.** The *Template Specifications* window opens.

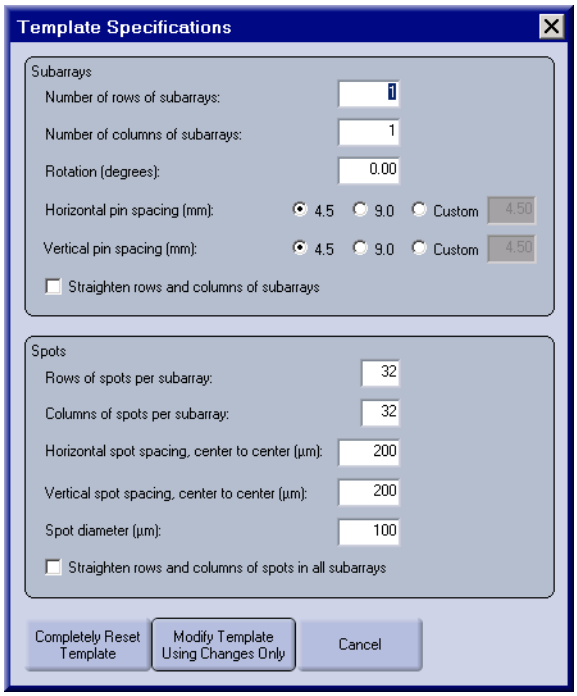


Figure 3–3 Define Quantitation Template Dialog Box

2. Provide the subarray and spot information as described in the following table.

Item	Description
Subarrays	Describe the geometry of the microarray:
Number of rows and number of columns of subarrays	Enter the number of subarray rows and the number of subarray columns.
Rotation	If the microarray is skewed on the substrate, you can rotate the template to align the template to the microarray.
Pin spacing, horizontal; Pin spacing, vertical	Enter the horizontal and vertical pin spacing that was used to create the microarray: 4.5 - if 384-well plates were used to print the microarray 9.0 - if 96-well plates were used to print the microarray Custom - to enable the edit boxes where you can enter values for custom pin spacing.
Straighten rows and columns of subarrays	This forces the subarrays into straight rows and columns for quantitation. The straightening does not display on the image, but affects the quantitation results.

Item	Description
Spots	This describes the position and size of each spot in a subarray.
Rows of spots and Columns of spots per subarray	Enter the number of rows and the number of columns of spots that are in each subarray.
Horizontal spot spacing (center to center (µm)	Enter the horizontal and vertical spot spacing, measuring from the center of a spot to the center of the spot in the next row or column.
Spot diameter	Enter the spot diameter in µm.
Straighten rows and columns of spots in all subarrays	This forces the spots into alignment for quantitation. The straightening does not show on the displayed image but affects the quantitation results.
Completely Reset Template	This resets the template.
Modify Template Using Changes Only	Modifies the template using only the changes just entered.

3.3.1.2 Adjusting the Template and Registering Images

After loading the template, adjust it to precisely fit the subarrays, and register the images. The images are not perfectly aligned if traces of individual color such as green and red are visible around the outside of each spot. Use the Zoom controls as needed to make it easier to adjust the template and view the registration.

To adjust the template

1. Click **Adjust Template and Register Images**. The *Adjust Template and Register Image* window opens (see Figure 3-4 on the next page).

Adjust Template controls

Register Image controls

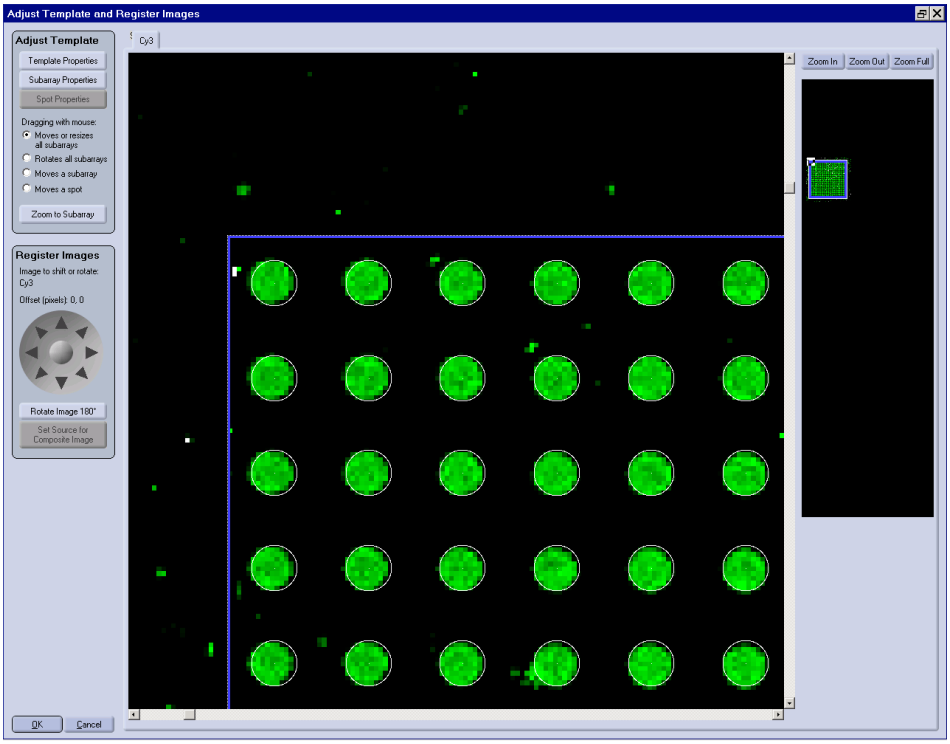


Figure 3–4 Adjust Template and Register Images Window (with Zoomed Display)

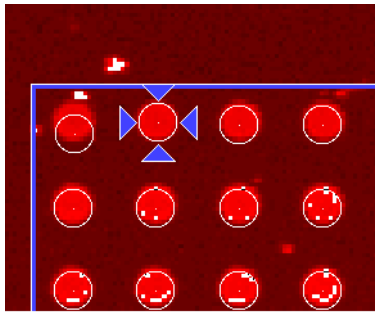
2. Click on a subarray of the template. (The example above shows just one subarray.) The selected subarray turns blue and the *Adjust Template* group is activated. You can move the highlighted subarray template by clicking **Move a subarray** under the *Dragging with Mouse:* options, then dragging the subarray along the substrate.

Or,

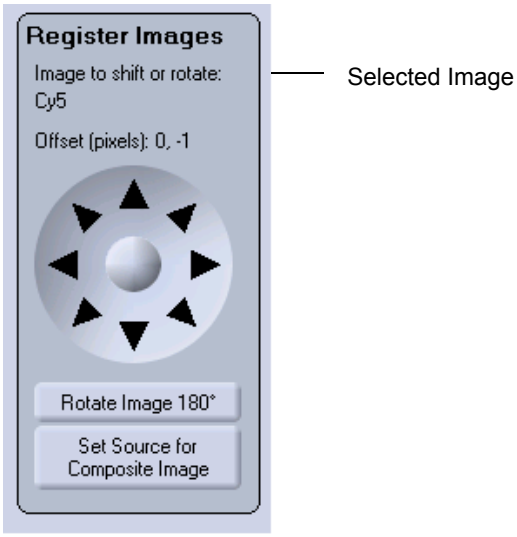
you can click **Subarray Properties** to enter specific information about the location of the subarray template and spots in the subarray.

Figure 3–5 Subarray Template Properties Dialog Box

3. To move a spot, click **Move a Spot** under "Dragging with Mouse." Four blue triangles appear around the spot; you can drag the spot in any direction to align it perfectly to the circle of the template.



4. To register the images, use the buttons and arrows in the *Register Images* box to move the selected image, in the direction of the arrow you click, until the images are aligned.



5. When the template is aligned and the images are registered, click **OK**. This returns you to the *Quantitate* window (page 3-8), where you can make any additional changes or start your quantitation.

3.4 Viewing the Quantitation Results

Quantitation results display in the *Main Window*. They include:

- *Spreadsheet* tab, with information about each spot and subarray on the scanned microarray, including each gene name, the accession number, subarray location, spot location, and spot intensity values listed by fluorophore, and normalized spot intensity values.
- *Scatter Plot* tab, lets you view spot intensity two channels at a time.
- *Distribution* Plot, lets you see "geographic" trends for your microarrays - areas of the slide that are being under or overwashed, for example.

The views in all three tabs are linked -- a data point selected in one tab is automatically selected when you change to another tab.

3.4.1 Viewing the Spreadsheet

An example of the spreadsheet is shown below. Each row in the spreadsheet is the data for one spot. If you use a .GAL file for your template, the gene name and ID are imported for each spot.

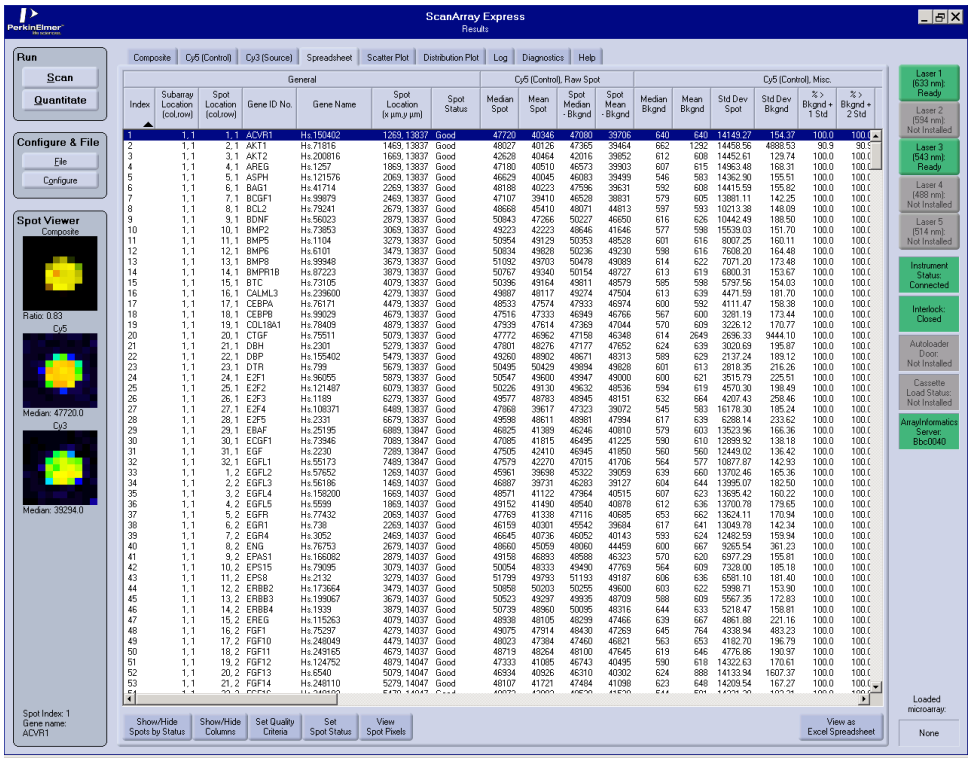


Figure 3–6 Quantitation Results Tab

You can scroll down to see all of the listed spots or scroll across to see all columns of data; click **View as Excel Spreadsheet** to view and save as a Microsoft Excel spreadsheet.

Several spot intensity values and ratios between multiple fluorophores are listed in the spreadsheet. Spot intensity values are automatically normalized to correct the intensity of each spot for variations in the overall intensity of the image, with respect to a control image. Values include:

Value	Description
Median	This column normalizes the ratio of the median value of all spots in each fluorophore to the ratio of the median value of the spots in the control fluorophore.
Mean	This column normalizes the ratio of the mean value of all spots in each fluorophore to the ratio of the mean value of the spots in the control fluorophore.

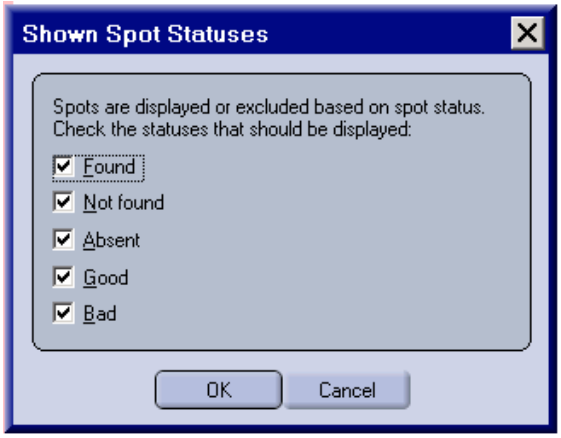
Value	Description
Total	Each spot's data is normalized to the data of all spots.
Background Subtracted	Reports the intensity of each spot in the array minus the background for that spot.

3.4.1.1 Filtering the Spreadsheet Data

You can use the buttons at the bottom of the *Spreadsheet* tab to filter the data, as described in this section.

To show or hide spots by their status

1. Click **Show/Hide Spots by Status**.



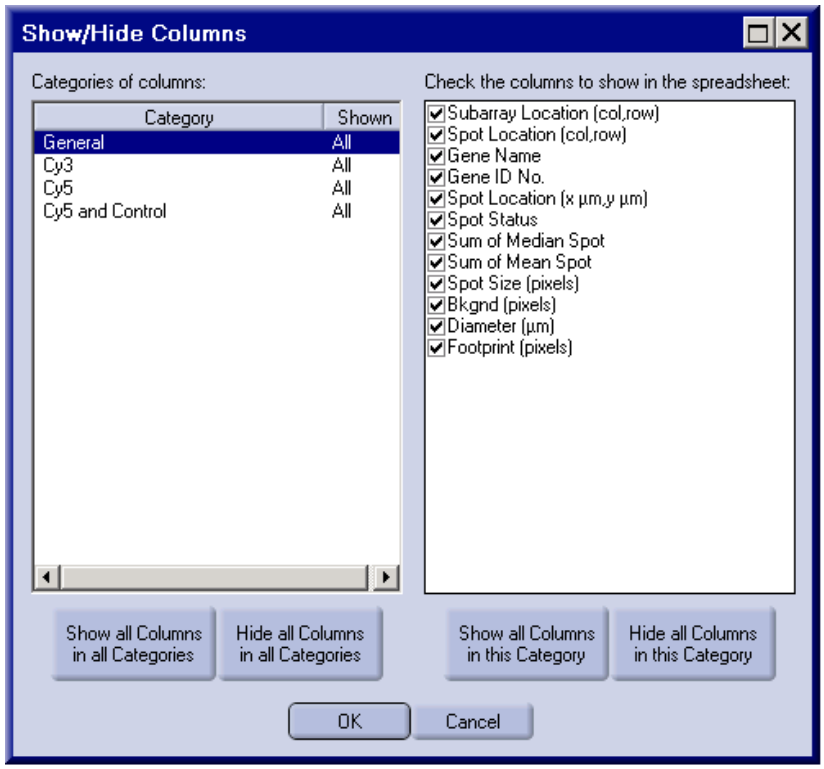
The spot status definitions are as follows:

Status	Definition
Found	The spot was found within that position on the spot template.
Not found	No spot was found within that position on the spot template.
Absent	The spot was reported as missing within the .GAL file.
Good	The spot met the quality criteria.
Bad	The spot did not meet the quality criteria.

2. Check each status for which you want spots to display; uncheck each status for which you want spots to be hidden.

To show or hide columns of information

1. Click **Show/Hide Columns**. The *Show/Hide Columns* dialog box displays.

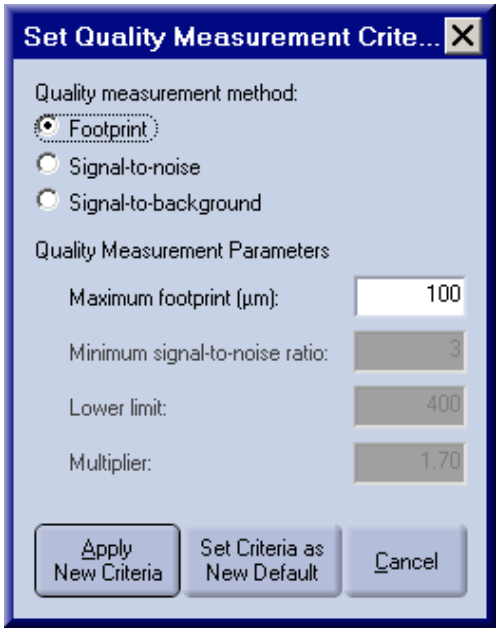


2. Check the corresponding boxes for the columns you wish to show or hide. To quickly select entire groups, click on the appropriate **Category**.

3. Click **OK** to implement the changes.

To set or change quality measurements

1. Click **Set Quality Criteria** to set or change quality measurement criteria.The following window opens.



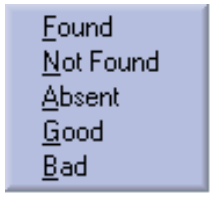
2. Set the criteria you want to use. The default method is Footprint. Refer to the following table for information.

Item	Description
Quality measurement method	Select one of the methods below. *Footprint* is the default method. The formulas used by the different methods use the settings in the ScanArray Express Application Settings window. The formulas are provided in Appendix D.
Footprint	This is the distance between the expected position of a spot and its actual position. Choosing this method enables the *Maximum footprint* field below.
Signal-to-noise	Uses the ratio of the spot intensity to the standard deviation of the local background of all spots on the microarray. Choosing this method enables the *Minimum signal-to-noise ratio* field below. Spots with a low signal-to-noise ratio can be quickly identified for later review.
Signal-to-background	Uses the comparison of the mean of spot intensity to the mean of local background. Choosing this method enables the *Lower Limit* and *Multiplier* fields below.
Quality Measurement Parameters	The following fields will be enabled, or dimmed and unavailable for selection, depending on which method you select:

Item	Description
Maximum footprint (µm)	Enter the maximum size of the footprint in microns. This measurement helps to distinguish spots from artifacts on the slide. The default setting is 0. The minimum/maximum settings are 100 through 72,000.
Minimum signal-to-noise ratio:	This field is enabled when you choose the Signal-to-noise method. The default value is 3. Valid values are 1 through 10,000.
Lower limit:	For Signal-to-background method. Valid values are 0 through 64,000 . More accurate spot intensities can be produced with a low intensity, uniform background around all spots.
Multiplier	For Signal-to-background method. The valid values are 1 through 65000.

To set the status of a spot

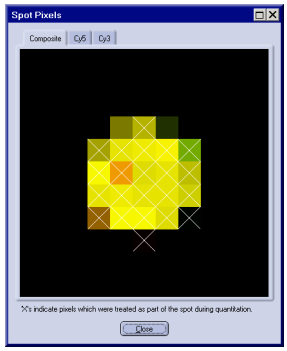
1. Highlight the individual spot, and click **Set Spot Status**. The options are: Found, Not Found, Absent, Good or Bad.



2. Select the status you want to assign.

To view spot pixels

1. Click **View Spot Pixels**.



The image shows which pixels were treated as part of the spot during quantitation.

3.4.2 Viewing the Scatter Plot

The *Scatter Plot* tab displays a scatter plot of spot brightnesses from both fluorophores. Any data that appears on the spreadsheet can be selected and viewed in the scatter plot. The axis measurements and scales can be changed by clicking on the axis label button.

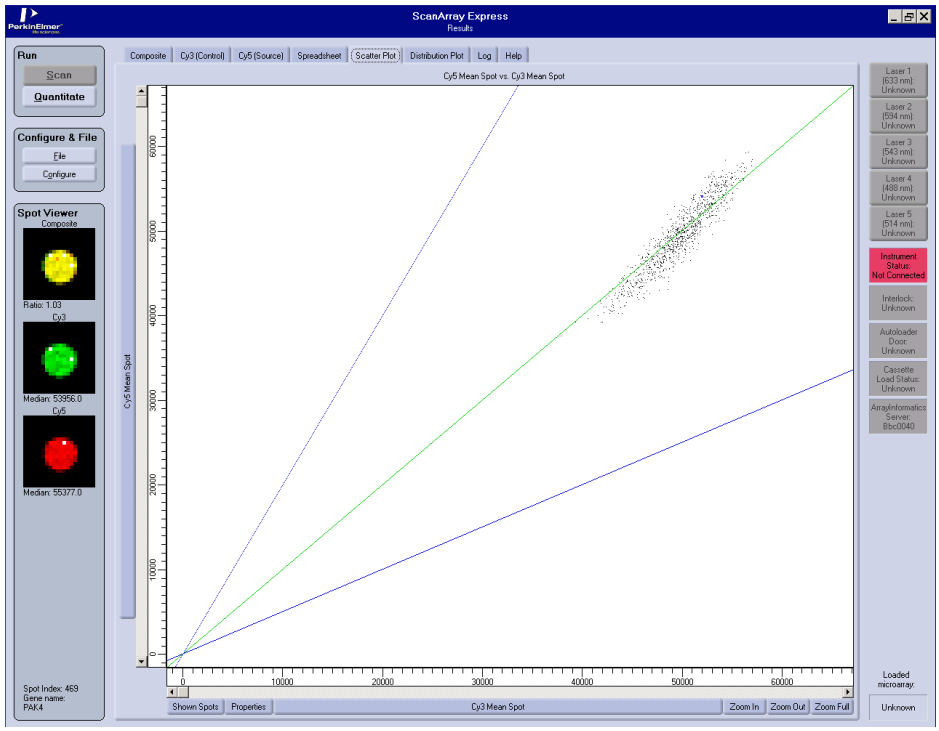


Figure 3–7 Scatter Plot Tab

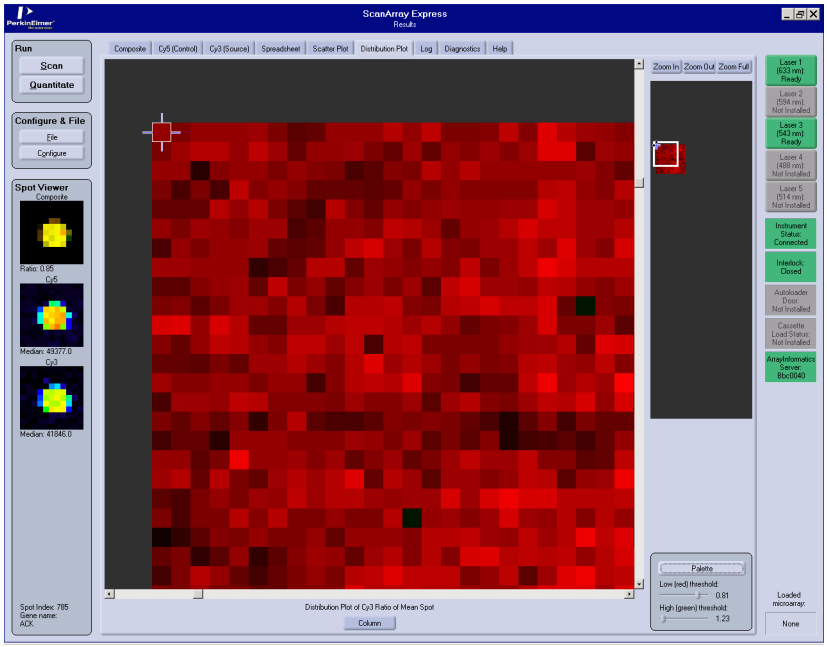
Data can be filtered and displayed based on status, by clicking the **Shown Spots** button at the bottom of the plot. Set axis boundaries by clicking the **Properties** button.

3.4.3 Viewing the Distribution Plot

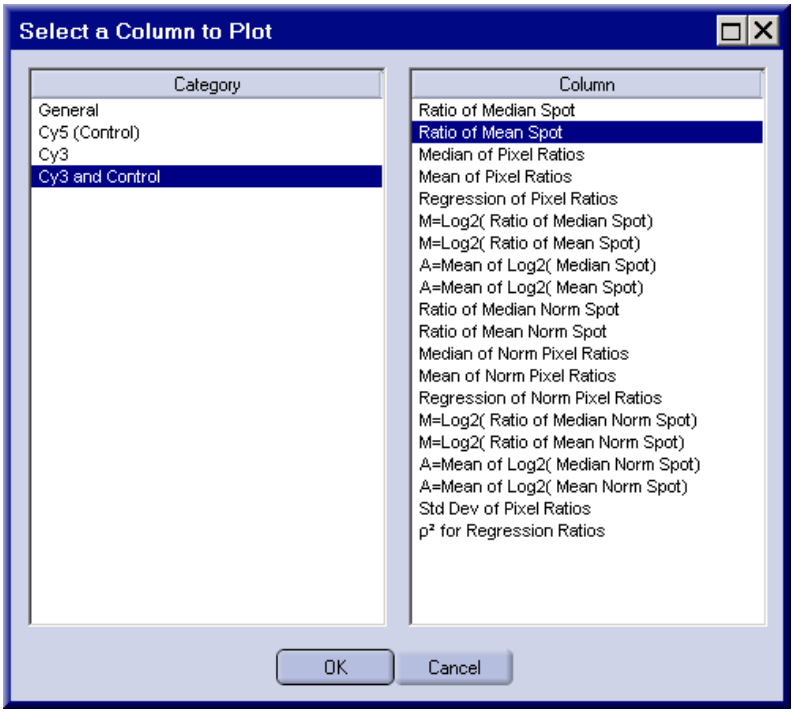
The *Distribution Plot* is a simple yet powerful data visualization tool. This feature allows you to select any type of quantitated data displayed in the spreadsheet and map it back to the spatial arrangement of the microarray, using colors to indicate the value of the data.

The Distribution Plot is of particular value for diagnosing or troubleshooting microarray problems based on the idea that in a well-processed array with a large number of spots, there should be no significant spatial correlation of any property.

For example, if ratio data were biased toward the green at the top of each subarray and biased toward red at the bottom of each subarray, that would suggest a problem with attachment or hybridization. Similarly, if the spots in one subarray had diameters significantly smaller than the others, that would suggest a problem with a pin. The *Distribution Plot* allows you to see all of these phenomena and more with ease.



You can select a column of spots to plot from any one of the images, or any images with the control image.

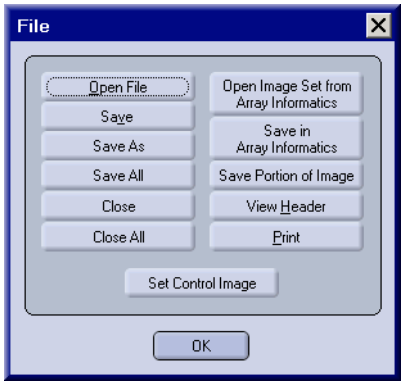


3.4.4 Viewing Information About the Images

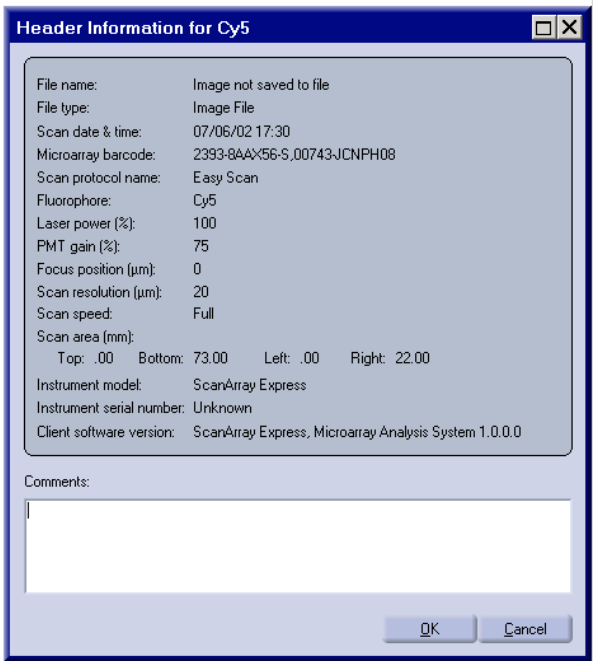
You can view information about one of the displayed (non-composite) images, including whether or not it has been saved, the filename if saved, and the settings that were used.

To view image information

1. Click the tab for which you want to view information.
2. On the *Main Window*, click **File** in the *Configure & File* group.



3. On the *File* menu, click **View Header**. A *Header Information* window opens; the example below is for the current image.



4. Add comments if you wish, and click **OK**.

3.4.5 Saving the Quantitation Results

You can save quantitation results as a .GPR or .CSV file or as an Excel spreadsheet. The .GPR file does not save all of the columns; if you want to save all of the data, be sure to save it as a .CSV file. See Appendix A for a description of .GPR and .CSV files.

To save the results as a GPR or CSV file

1. On the *Main Window*, click **File** in the *Configure & File* group, then click **Save** or **Save As**.

2. In the dialog box that opens, enter a file name and select the file type, CSV or GPR.

Note: To save the file in ArrayInformatics, click **File** in the *Configure & File* group, and then click **Save in ArrayInformatics**. If your system is not integrated with ArrayInformatics, this button is dimmed and unavailable to use.

Scanning with Protocols

Chapter 4

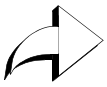
Chapter Summary

4.1 Overview

A *Scan Protocol* lets you set up parameters for scanning -- including scan speed, scan area, fluorophores, PMT Gain, laser power, and AutoSensitivity -- and save the parameters to be used again. An *Image Autosave Protocol* is used to save the scanned images; it specifies the folder, file naming convention and file type to use when saving the images.

Protocols can also be defined in a *Protocol Group*, allowing you to use multiple protocols to scan a single microarray. A *Batch Set* allows you to run multiple protocols or protocol groups on 1 to 20 slides in an autoloader. See Chapter 6, "Automating Scans and Quantitation" for more information on protocol groups and batch sets.

This chapter provides instructions for running a Scan Protocol and for creating Scan Protocols and Image Autosave Protocols.



Tip: *If you are a new or infrequent user, you can use Easy Scan to become familiar with the scanning process before creating and using Scan Protocols. See Chapter 3 for information on using Easy Scan.*

For a tutorial that leads you through scanning and quantitation with a sample slide, see Chapter 2, "Getting Started: Scan and Quantitate the Geometric Test Slide."

4.2 Running a Scan Protocol

A scan protocol is the most efficient and reliable method of scanning microarrays requiring the same scanning parameters. With a user programmed protocol, each user can scan microarray slides using the same parameters, thus eliminating user-to-user variability from slide to slide.

To run a Scan Protocol

1. Click **Scan** on the *Main Window*. The *Scan* window opens, where you can choose a scan type.

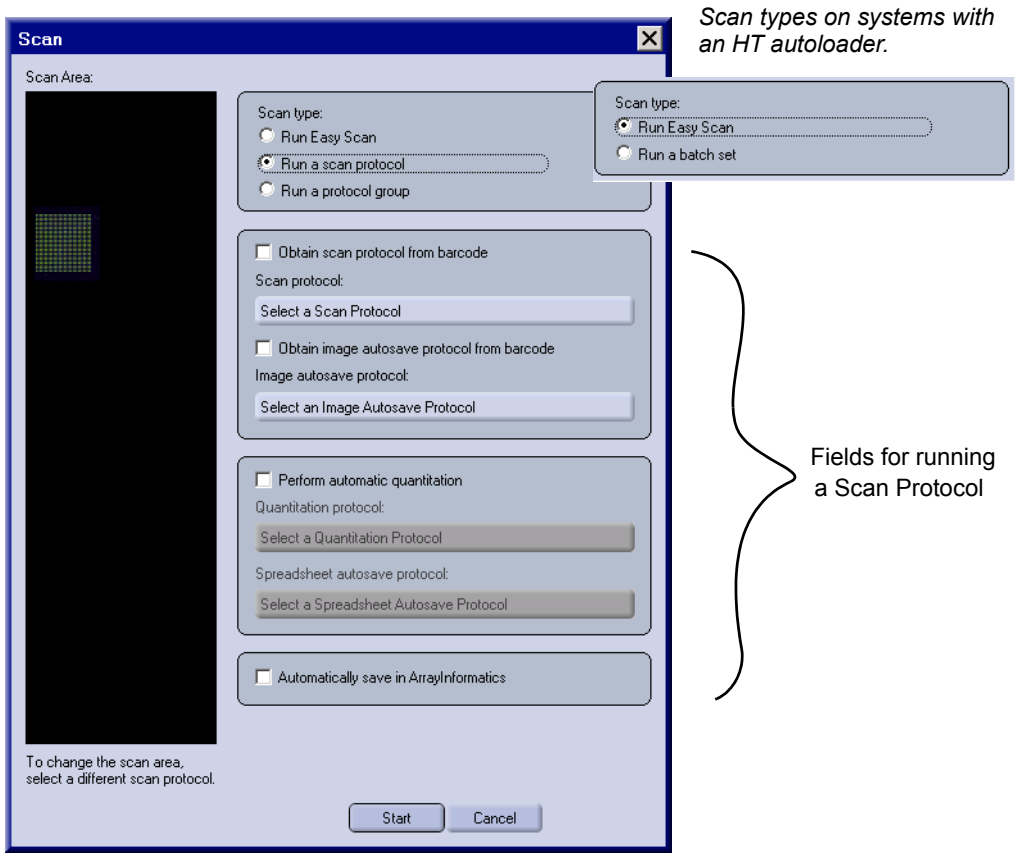


Figure 4–1 Scan window

2. Select **Run a scan protocol.**

   The fields for a scan protocol display, as shown in Figure 4-1 above. The settings for performing the scan, including the scan area, are included in the scan protocol itself. Unlike when using Easy Scan, you cannot change the scan area or settings in this window.

   You can set or change settings when *running* the scan, as described in section 4.2.1.

3. Select your scan protocol and image autosave protocol. You can obtain this information from barcodes on the microarrays. See ***Other Scan Settings*** on page 4-3.

4. Click **Start**. The scan protocol scans the microarray and saves the images using the Image autosave protocol. The images are displayed in the *Main Window*.

4.2.1 Other Scan Settings

You cannot change the protocol settings from this window, but you can change some of the settings for how the Scan is to run, including getting the protocol information from barcodes.

To change the scan protocol settings, refer to the table below for information.

Item	Description
Scan type	The scan types that display depend on your system. Without an autoloader the types are: Run Easy Scan, Run a scan protocol, Run a protocol group, On an HT autoloader system the types are: Run Easy Scan and Run a batch set.
Scan area	The scan area has been defined in the scan protocol. If you want to change the scan area, you must select a different protocol.
Obtain scan protocol from barcode	Check this box to read the barcode of the microarray for the appropriate scan protocol. If a barcode reader is not installed, or if the barcodes are not configured, this box is dimmed and unavailable to select. See “Configuring Barcodes” in Chapter 7.
Scan Protocol	To select a scan protocol, click the **Select a Scan Protocol** button, and select a protocol from the *List of Scan Protocols* window that opens. The button on the Scan window fills with the name of the protocol.
Obtain image autosave protocol from barcode	Check this box to read the barcode of the microarray for the appropriate image autosave protocol. If a barcode reader is not installed, or if the barcodes are not configured, this box is dimmed and unavailable to select. See “Configuring Barcodes” in Chapter 7.
Image autosave protocol	To select an image autosave protocol, click the **Select an Image Autosave Protocol** button, and select a protocol from the *List of Image Autosave Protocols* window that opens. The button on the Scan window fills with the name of the protocol.
Perform automatic quantitation	Make sure this box is *not* checked, unless you want to automatically quantitate after scanning. See ***Automatic Quantitation after Scanning*** on page 4-4. Checking this box enables the following fields for selecting a quantitation protocol and spreadsheet autosave protocol.
Quantitation Protocol	You must select an already defined Quantitation Protocol. Click the **Select a Quantitation Protocol** button to select a protocol from the *List of Quantitation Protocols* window that opens.
Spreadsheet Autosave Protocol	You must select an already defined Spreadsheet Autosave Protocol for saving the quantitation results. Click the **Select a Spreadsheet Autosave Protocol** button to select a protocol from the *List of Spreadsheet Autosave Protocols* window that opens.
Automatically Save in ArrayInformatics	Check this box to automatically save the scanned image files to the specified location. If your system is not integrated with ArrayInformatics, this box is dimmed and unavailable to select.

4.2.1.1 Automatic Quantitation after Scanning

To automatically quantitate the images after scanning

1. In the *Scan* window, select your scanning settings, then check the **Perform automatic quantitation** box.
2. Select a quantitation protocol and spreadsheet autosave protocol.
3. Click **Start**.

The ScanArray Express scans the microarray and saves the images. As the image is completed, ScanArray Express immediately starts spot finding, using the template called for in the quantitation protocol, and starts quantitating. When quantitation is finished, ScanArray Express saves the results.

4.3 Creating a Scan Protocol

To create a scan protocol

1. On the *Main Window*, click **Configure** in the *Configure & File* group. In the Configure menu, click **Scan Protocols.**

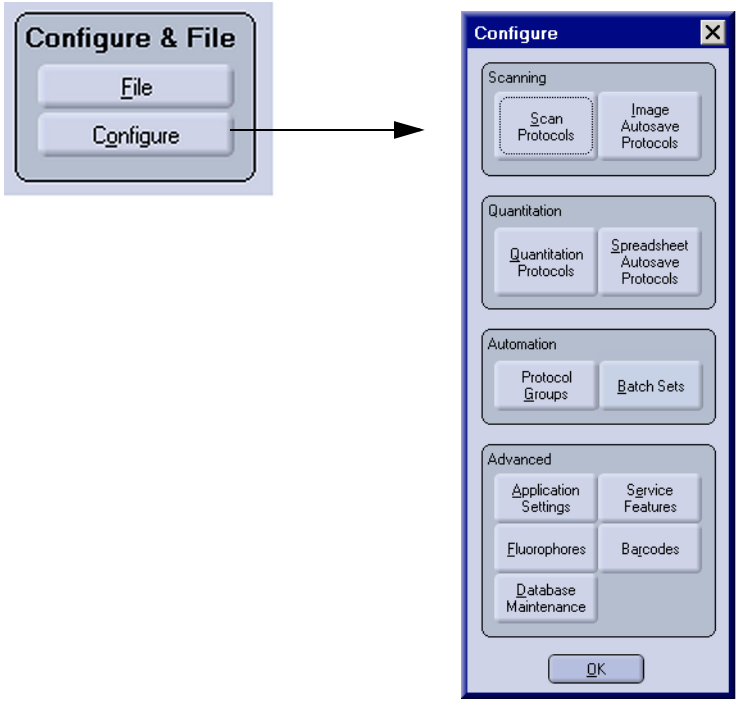


Figure 4–2 Configure & File group and Configure menu

2. The *List of Scan Protocols* window opens. The ScanArray Express includes several default protocols that display in this window, along with any protocols that have been created.

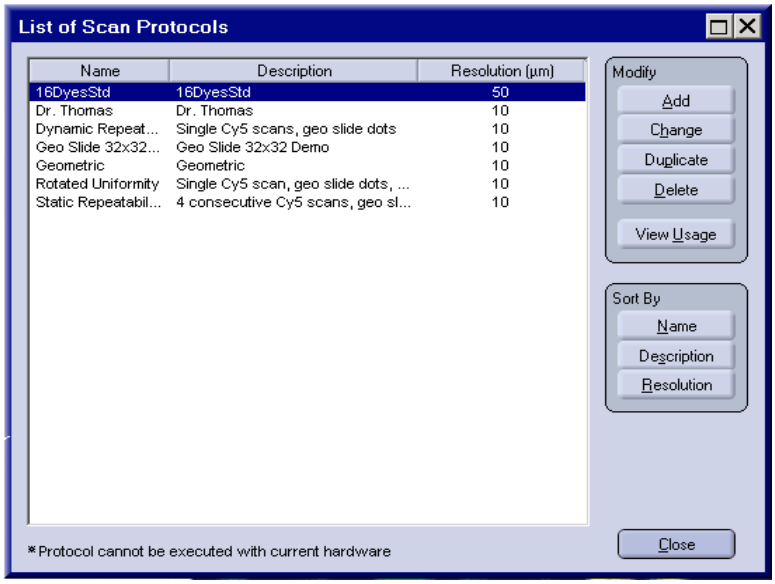


Figure 4–3 List of Scan Protocols Window

3. Sort the list (optional) by **Name**, **Description**, or **Resolution**.

4. Click one of the following in the *List of Scan Protocols* window to open the Scan Protocol wizard:

- **Add** to create a new protocol using the Scan Protocol wizard.
- **Change**, after selecting a protocol, to modify an existing protocol.
- **Duplicate**, makes a duplicate of the highlighted protocol to help you create a new protocol from one that is already close to what you want.

Or, click:

- **Delete**, after selecting a protocol, to delete a protocol; you cannot delete a protocol that is part of a batch set or protocol group.
- **View Usage,** after selecting a protocol, to display a list of batch sets and protocol groups which include the selected protocol.

4.4 The Scan Protocol Wizard

The *Scan Protocol* wizard opens with the *Basic Information* window displayed, as shown in Figure 4-4. From this window, using the wizard, you can display and set all of the scanning parameters.

The only required steps in the wizard are **1, Basic Information** and **3, Fluorophores**. At least one fluorophore must be added to a new protocol for it to be created and saved.

Tip: *You may want to run an Easy Scan to acquire an image before configuring your scan protocol, to help in setting up the protocol.*

You can move through the windows by clicking the numbers on the left, or by using the buttons on the bottom of the window:

Click the number to move from one window display to another.

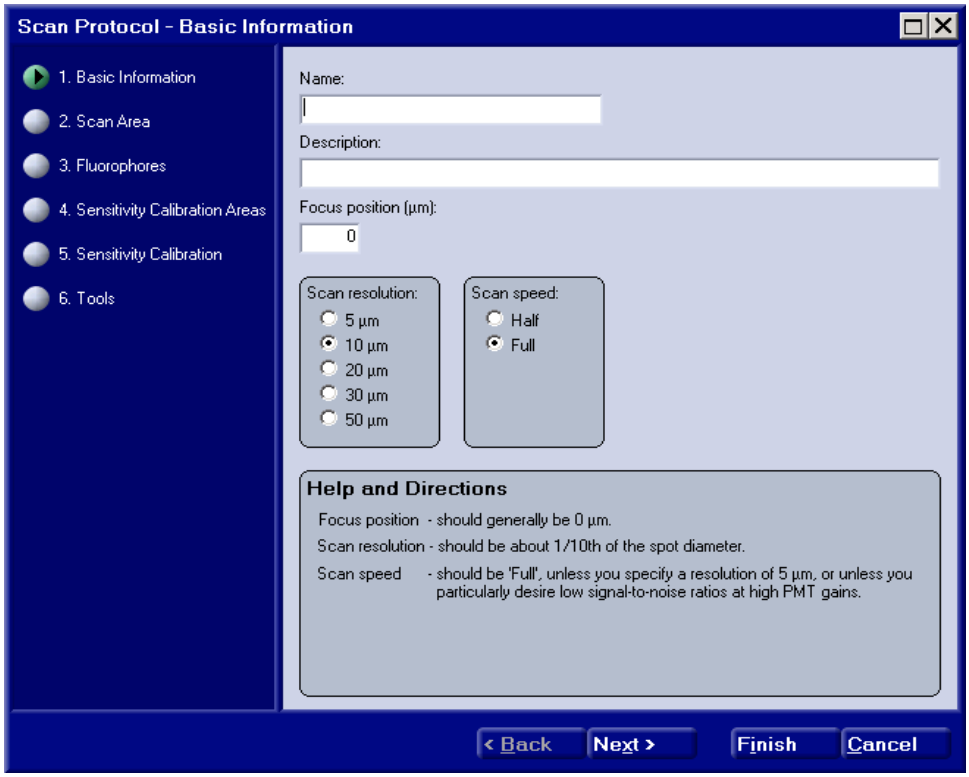


Figure 4–4 Scan Protocol - Basic Information Window

- Click **Next** to move to the next window and **Back** to move to the previous window.
- Click **Finish** to save all of your changes and close the wizard.
- Click **Cancel** to close the wizard without saving any of your changes.

4.4.1 Basic Information

In the *Scan Protocol - Basic Information* window, provide a name and description for the protocol, and choose the scanning resolution and speed.

To name a scan protocol

1. Fill in the information as listed in the following table.

Item	Description
Name	Enter a name for the scan protocol so you can recall it easily. You must enter a name in order to proceed.
Description	Enter a description for the protocol.
Focus Position	You do not need to adjust this for each scan. Leave the default value at 0 for conventional flat slide substrates. To determine the focus position for unconventional slides, use the Scan Protocol Tools.
Scan Resolution	Select a resolution that is appropriate for your microarrays: 5 µm for spots that are less than 90 µm diameter 10 µm for spots that are 100 to 200 µm diameter 20 µms for spots that are greater than 200 µm diameter Higher settings result in a quicker image with reduced detail. The default is 10 microns.
Scan Speed	Select a scan speed, usually **Full** (the default setting). You may want to use **Half** speed for better signal to noise ratio on dim arrays that require PMT settings greater than 75.

2. Click **Next** to display the *Scan Protocol - Scan Area* window.

4.4.2 Selecting the Scan Area

In the *Scan Protocol - Scan Area* window, select the area of the slide to be scanned. To set the scan area to include only that part of the slide with the image, obtain an image to help you see the area. There are two ways you can get an image:

- The first is to run an Easy Scan on your slide before you start to configure the scan protocol.
- The second, if you've already started configuring the protocol, is to run a Quick Scan from the Tools section of this protocol.
  See ***Running the Quick Scan*** on page 4-15.

To select the Scan Area

1. Open the *Scan Protocol - Scan Area* window, shown in Figure 4-5.

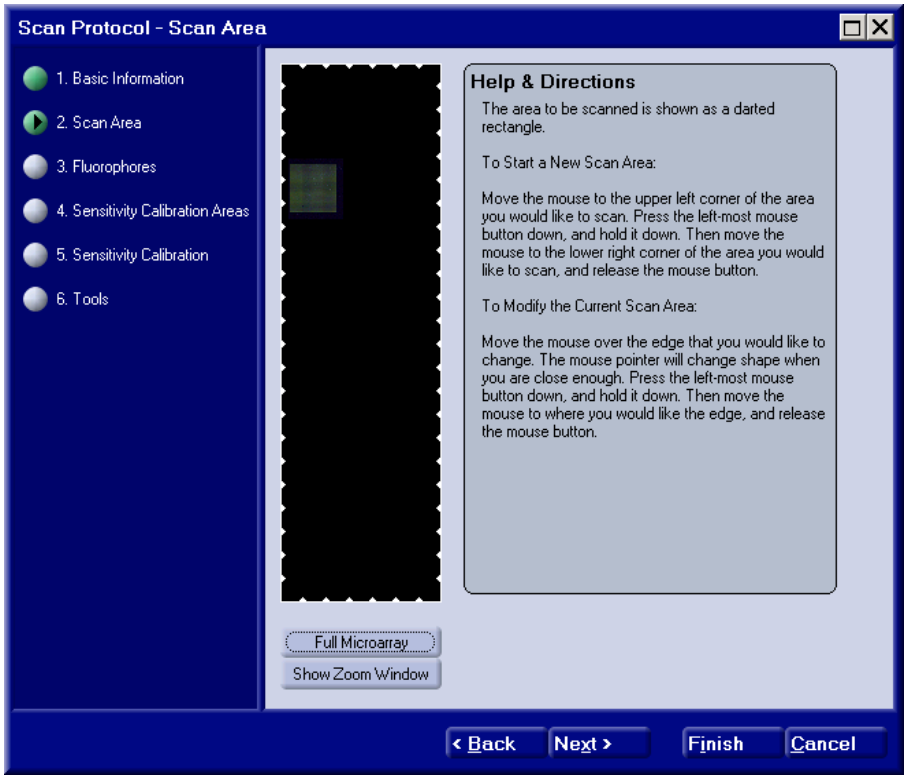


Figure 4–5 Scan Protocol - Scan Area Window

2. Use the mouse to set the area to be scanned by drawing a rectangle of the scan area on the slide image or click **Show Zoom Window.** A larger image allows you to select an area with greater accuracy.

Refer to the following table:

Item	Description
Microarray Diagram	Displays the last scanned image, or the resulting image if you ran a Quick Scan, and lets you use the mouse to select an area to scan.
Full Microarray	Automatically selects the entire microarray. Use this button for 50 µm scans to “find” the array.
Show Zoom window	Opens a window with a larger diagram, for easier selection of an area with the mouse, and edit boxes where you can enter numerical scan area parameters instead. Enter the coordinates for the Start position, Area width and Area Height values in millimeters for X and Y. The default scan area is the entire slide, or 22 mm for width and 73 mm for height. The default start X and Y positions are 0.00 mm. The minimum width and height for the scan area are 0.1 mm and 0.05 mm respectively.

3. Click **Next** to display the *Scan Protocol - Fluorophores* window.

4.4.3 Fluorophores

In the *Scan Protocol - Fluorophores* window, you can select or change fluorophores to be included in the scan protocol and set a percentage for the **PMT Gain** and **Laser Power**. You can also change the order of the listed fluorophores. The first fluorophore listed is the control fluorophore.

To select Fluorophores

1. Open the *Scan Protocol - Fluorophores* window.

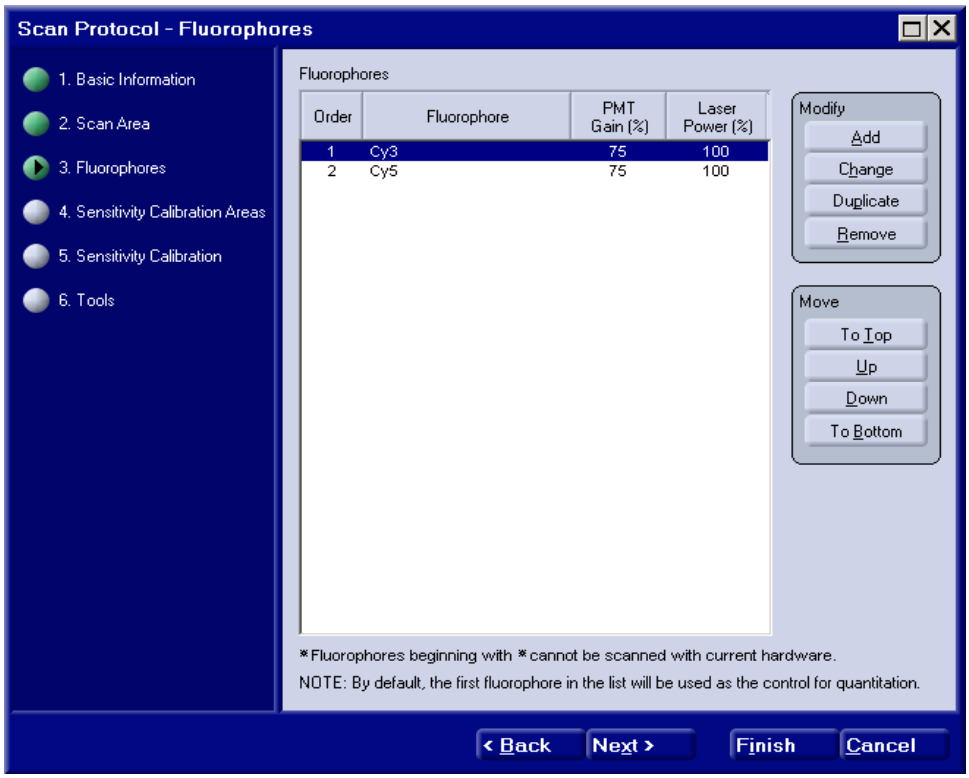


Figure 4–6 Scan Protocol - Fluorophores Window

The window displays the current order of scanning and the name, PMT Gain, and Laser Power for each fluorophore in the list. The first fluorophore scanned is the control image, the second one is the source image (experiment).

2. Double-click a fluorophore to change it, or click one of the following:

- **Add** to add a new fluorophore.
- **Change**, after selecting a fluorophore, to modify an existing fluorophore.
- **Duplicate**, makes a duplicate of the highlighted fluorophore to help you create a new fluorophore from one that is already close to what you want.
- **Remove**, after selecting a fluorophore, to remove it from the protocol.

3. When you click **Add**, the *Fluorophore* window opens.

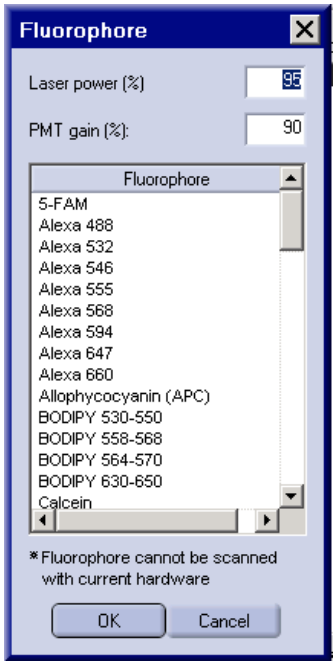


Figure 4–7 Fluorophore Window

4. Select a fluorophore and specify the **Laser Power** and **PMT gain.**

   - Increasing PMT gain improves sensitivity, but also increases background noise and causes saturation. The optimal signal-to-noise is obtained using 70-80% for the PMT Gain.
   - **Leave the laser at 90% unless scanning under special conditions.**

5. Click **OK** to accept the changes and return to the protocol wizard**.**

6. Click **Finish** if the PMT Gain and laser settings are known for this scan and will be kept constant for all scans performed with this Scan Protocol. No further configuration is required for the protocol.

   Click **Next** if the maximum fluorescence values are significantly different from microarray to microarray. While it will significantly increase individual scan time, ScanArray Express can automatically calibrate each microarray for maximum fluorescence values with minimal saturation before scanning takes place.

4.4.4 Sensitivity Calibration Areas

In the *Scan Protocol - Sensitivity Calibration Areas* window, you can select an area of the microarray to be used to adjust the sensitivity of each fluorophore. In this window, you select the area; in the next window, *Automatic Sensitivity Calibration*, you specify the settings for the calibration.

The larger the area that you specify as the calibration area, the more time it will take to perform the calibration.

To set the Sensitivity Calibration Area

1. Open the *Scan Protocol - Sensitivity Calibration Areas* window.

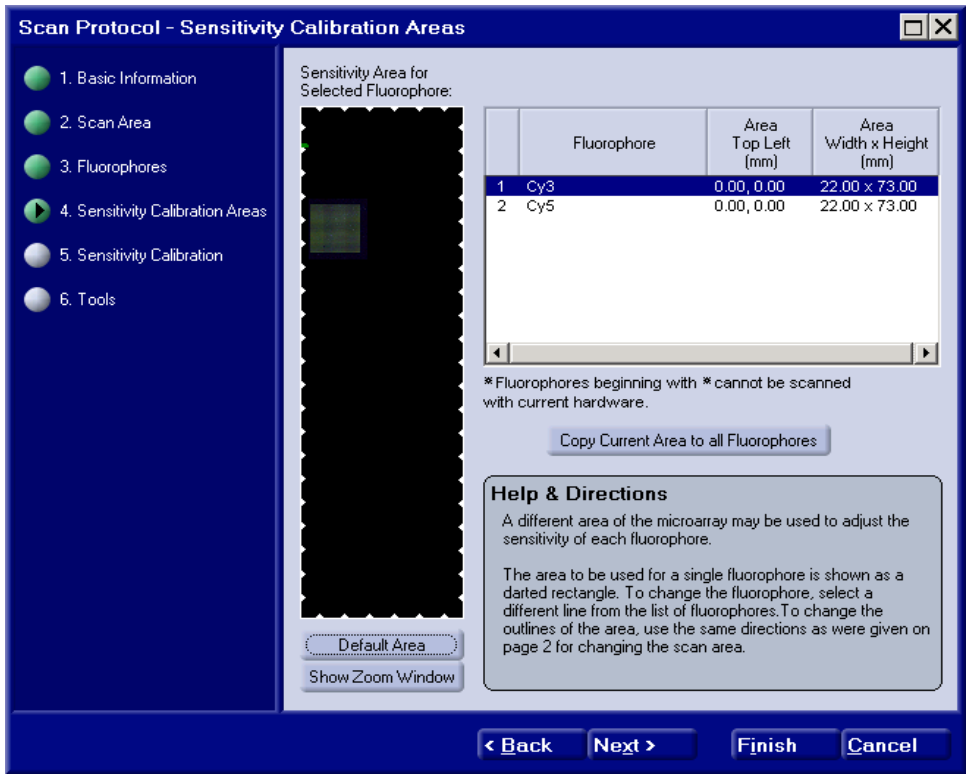


Figure 4–8 Scan Protocol - Sensitivity Calibration Areas Window

2. To set the area to be used for automatically calibrating the signal intensity, select a fluorophore and click **Show Zoom Window.** The *Zoom* window allows greater accuracy when defining an area.

   Setting as small an area as possible is recommended. In the *Zoom* window**,** draw a rectangle on the slide image using the mouse, or enter the **Area Co-ordinates**.

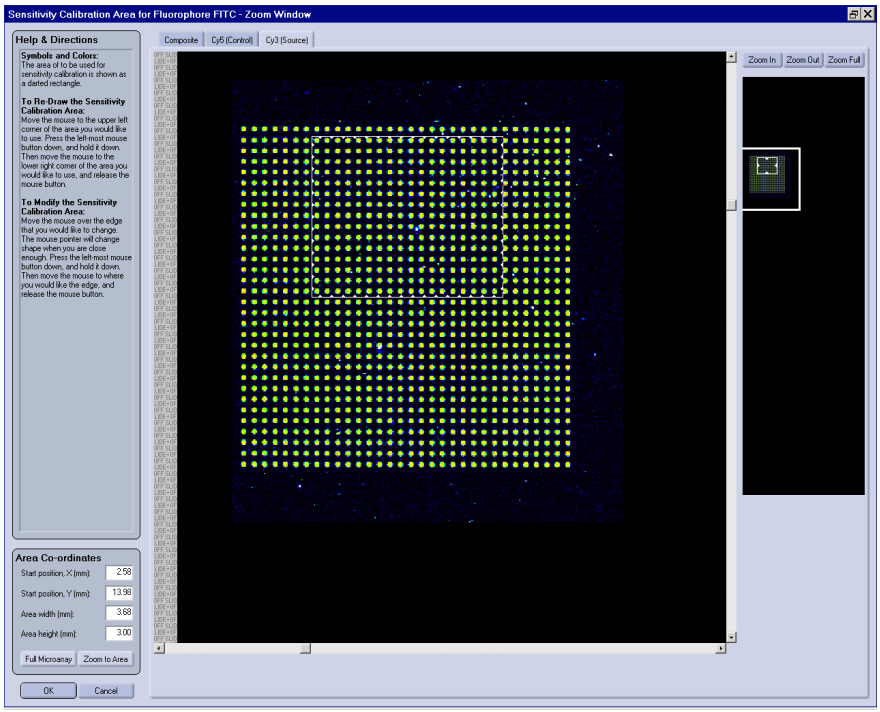


Figure 4–9 Drawing a Rectangle on the Slide Image - Zoom Window

The default scan area is the full substrate, for each fluorophore.

A different area of the microarray may be used to adjust the sensitivity of each fluorophore, or the same area may be used for all fluorophores in the protocol by clicking **Copy Current Area to all Fluorophores**.

Note: Be sure to set the area for all fluorophores. If you select an area for one fluorophore, but neglect to set the area for all fluorophores, any fluorophores not set will scan the whole slide (the default), which could take a long time, possibly hours, for dense arrays.

3. Click **Next** to display the *Scan Protocol - Automatic Sensitivity Calibration* window.

4.4.5 Automatic Sensitivity Calibration

In the *Scan Protocol - Automatic Sensitivity Calibration* window, you can adjust the scanner so that the brightest features in a defined area of an array will have a chosen signal level. The area was defined in the previous window, the *Sensitivity Calibration Area* window.

To run Automatic Sensitivity Calibration

1. Open the *Scan Protocol - Automatic Sensitivity Calibration* window.

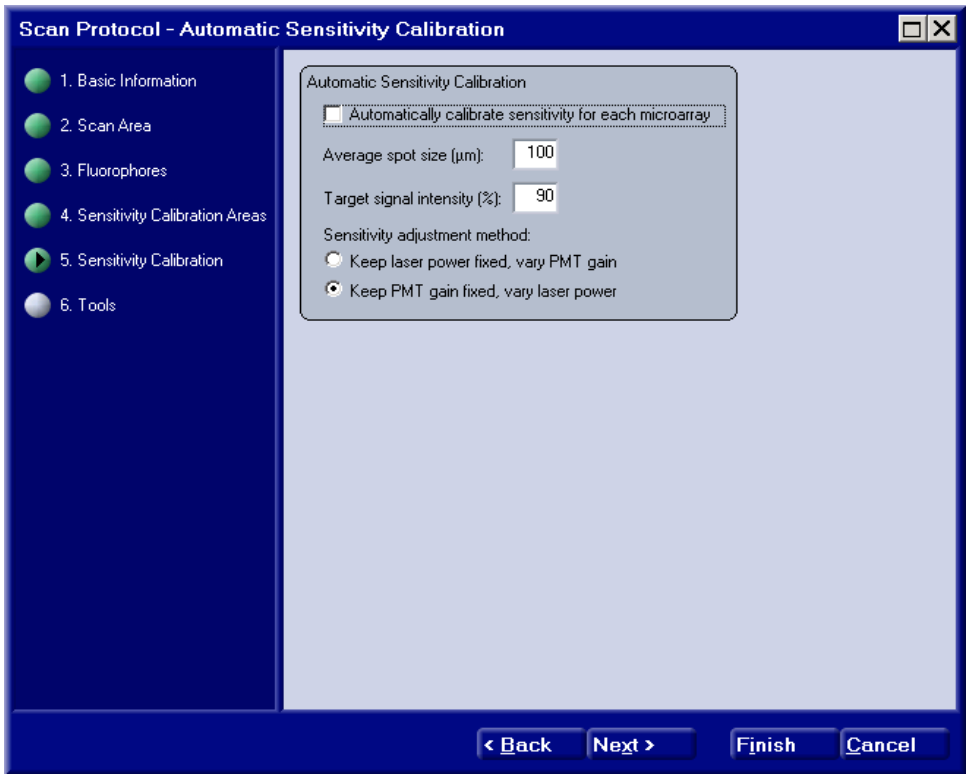


Figure 4–10 Scan Protocol - Automatic Sensitivity Calibration Window

2. Use the following table for information on settings.

Item	Description
Automatically calibrate sensitivity for each microarray	Check this box to have AutoSensitivity run automatically on every scan with this protocol.
Average spot size (µm)	Enter the average diameter of your microarray spots for the greatest particle/dust suppression. If the spots are poorly formed, set the average spot size to a smaller value for better results. The minimum and maximum spot sizes are 75 and 500 µm. The default spot size is 100 µm.

Item	Description
Target signal intensity (%)	AutoSensitivity automatically adjusts the laser or PMT so the brightest feature in the Sensitivity Calibration area is at this percentage of full-scale. The Target signal intensity for poorly formed or mottled spots should be lowered to avoid saturation within the brightest spots. The minimum and maximum target intensities are 50 and 100%. The default target intensity is 90%.
Sensitivity adjustment method	For the best signal to noise ratio, go to Fluorophores and set the laser power to 95 for all fluorophores, then choose “Keep laser power fixed, vary PMT gain.”

3. Click **Finish** to save the Scan Protocol.

4.4.6 Scan Protocol -Tools

The *Scan Protocol - Tools* window provides tools you can use to determine or refine correct settings for this protocol. After you run a tool and accept the results, the settings are automatically saved back to the protocol. When you save the protocol, all settings are saved.

Note: The *Quick Scan* can be used to test protocol parameters to see the resulting scanned image. Only experienced users should use the other scan protocol tools.

All of the tools perform specialized scans, and you must have a microarray in the scanner before you run them. Tools include:

- *Quick Scan,* which allows you to adjust the settings during a scan and save them;
- *Line Scan* to help you determine the appropriate focus position, laser power, and PMT Gain for various fluorophores;
- *Automatic Sensitivity Calibration*, for determining appropriate laser power and PMT Gain settings; and
- *Automatic Focus Calibration* for determining the appropriate focus setting.

When a tool has finished running, click **Accept the Changes** to save the information to the protocol.

To access the Tools

1. Open the *Scan Protocol - Tools* window, shown in Figure 4-11.

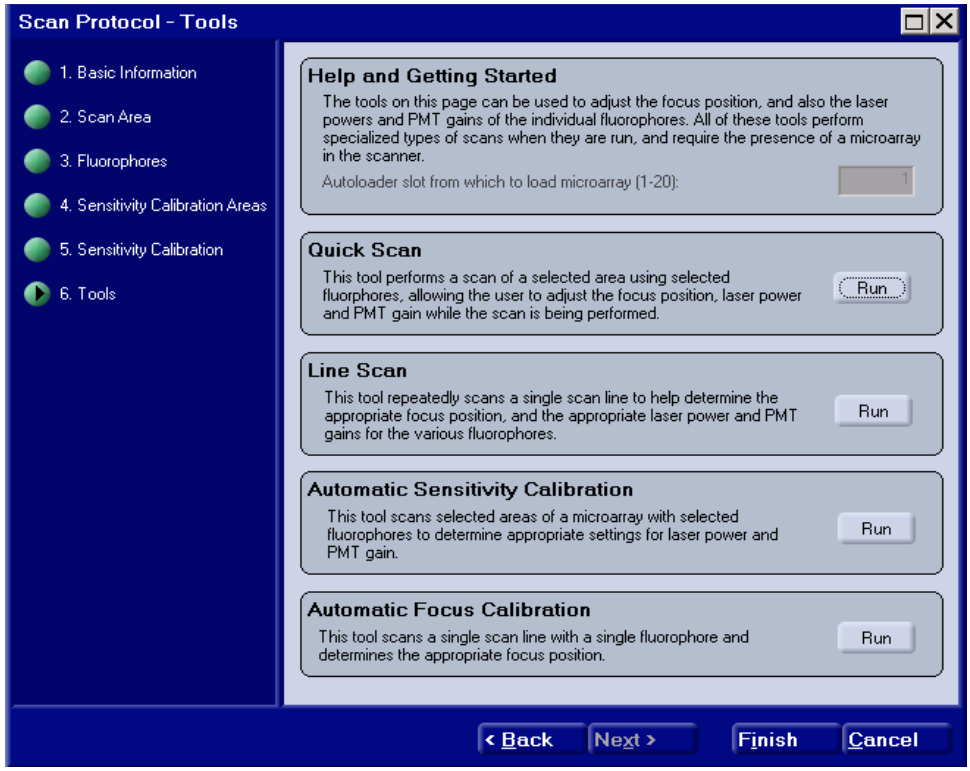


Figure 4–11 Scan Protocol - Tools Window

Each of the tools is described in a section below. To use these tools, the instrument must be connected and a microarray inserted.

Note: For instruments with HT capability, specify the slot number of the microarray to be scanned, before selecting a tool to run. The default slot value is 1.

2. After using the Tools to refine settings, click **Finish** to save the protocol.

4.5 Using Scan Protocol Tools

To use the tools, run a Quick Scan first; only experienced users should run the other tools.

4.5.1 Running the Quick Scan

The **Quick Scan** allows you to check the setup of the protocol. Any changes made during a Quick Scan do not persist unless you save them to the protocol. The Quick Scan performs a scan of a selected area using selected fluorophores, allowing you to adjust the laser power and PMT gain while the scan is being performed.

To perform a Quick Scan

1. Click **Run** in the **Quick Scan** area of the *Scan Protocol - Tools* window. The *Quick Scan* dialog box displays.

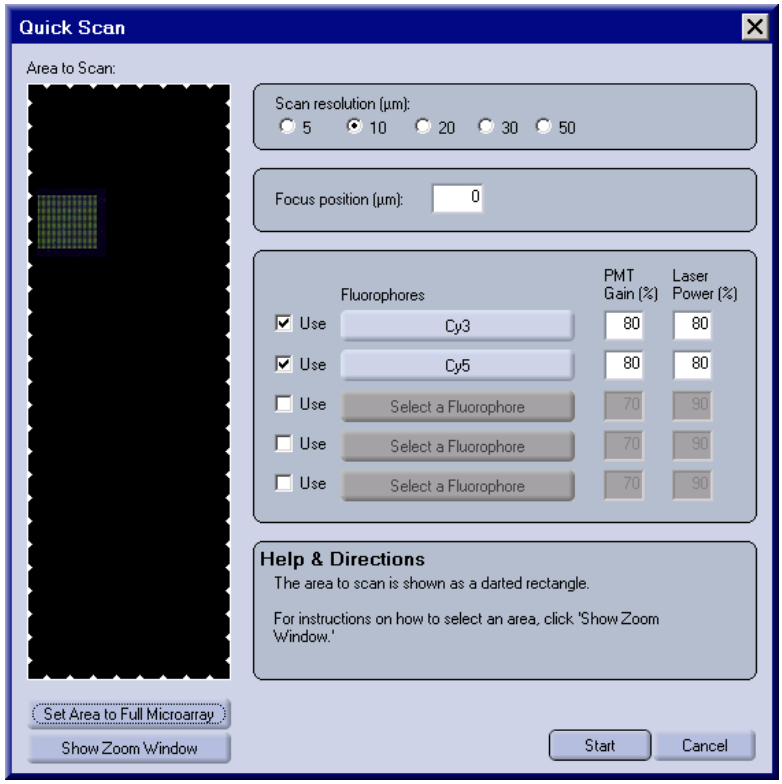


Figure 4–12 Quick Scan Window

2. Change settings only if needed. Refer to the following table for information. The default value of each item is the value specified in the protocol's parameters.

Item	Description
Scan resolution (µm)	The minimum and maximum resolutions are 5 and 50 microns.
Focus position (µm)	This is the distance from the lens to the calibrated nominal focus.
Fluorophores	Check the fluorophores you wish to use for the scan.
PMT Gain	This value can be changed at any time during the scan. Enter the PMT Gain for each selected fluorophore.
Laser Power	This value can be changed at any time during a scan. Enter the Laser Power for each selected fluorophore.

3. Set the area to be scanned by clicking **Show Zoom Area** and drawing a rectangle, using the mouse, on the image of the slide in the *Quick Scan* window. Alternatively, enter the X and Y coordinates if preferred. Click **OK** to return to the *Quick Scan* window.

   The scan area will default to that specified in the protocol's parameters.

4. Click **Start**.

5. The *Quick Scan* image displays.

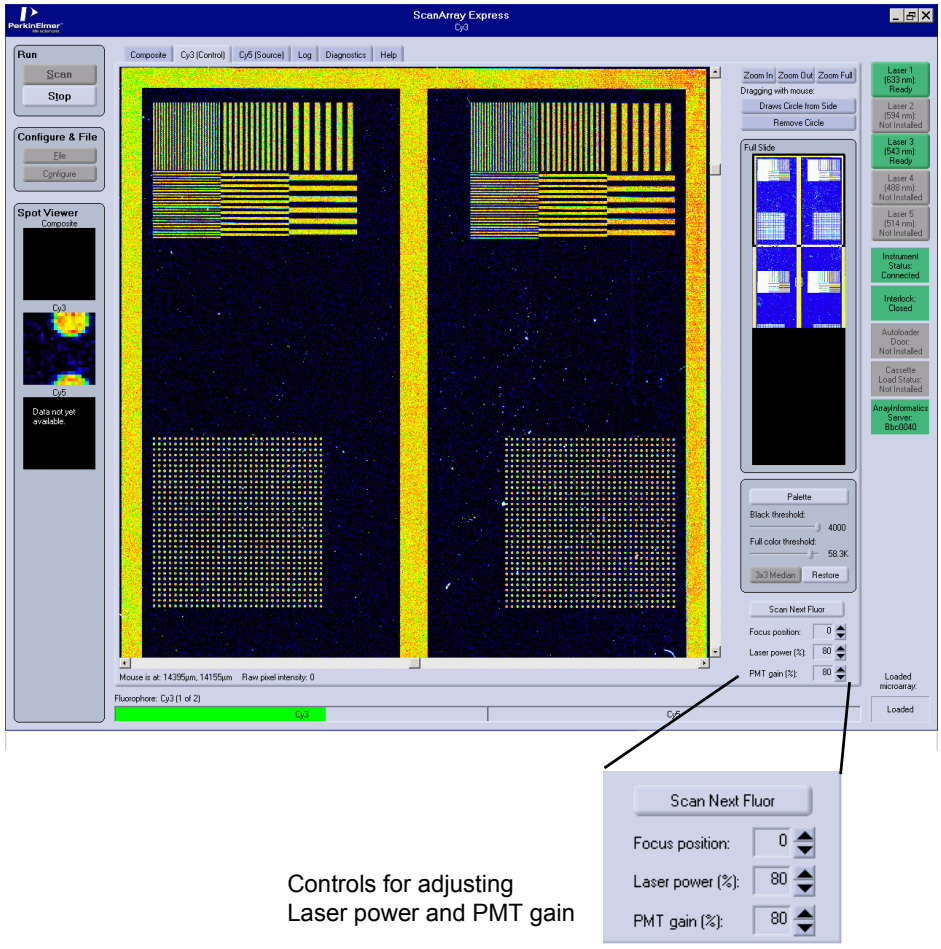


Figure 4–13 Quick Scan Image

6. The scanned image displays on the workstation. The name of the fluorophore is identified on the tab of each image.

   You can change the focus position, laser power, and PMT gain during the scan, using the up arrow and down arrows in the bottom-right corner of the window.

7. Click **Scan Next Fluor** to scan the next fluorophore.

8. To view the average intensity of a certain area, click the **Draw Circle from the Center/Side** button. This enables you to draw a circle around the desired area using the mouse. The average intensity of spots within the circle is displayed.

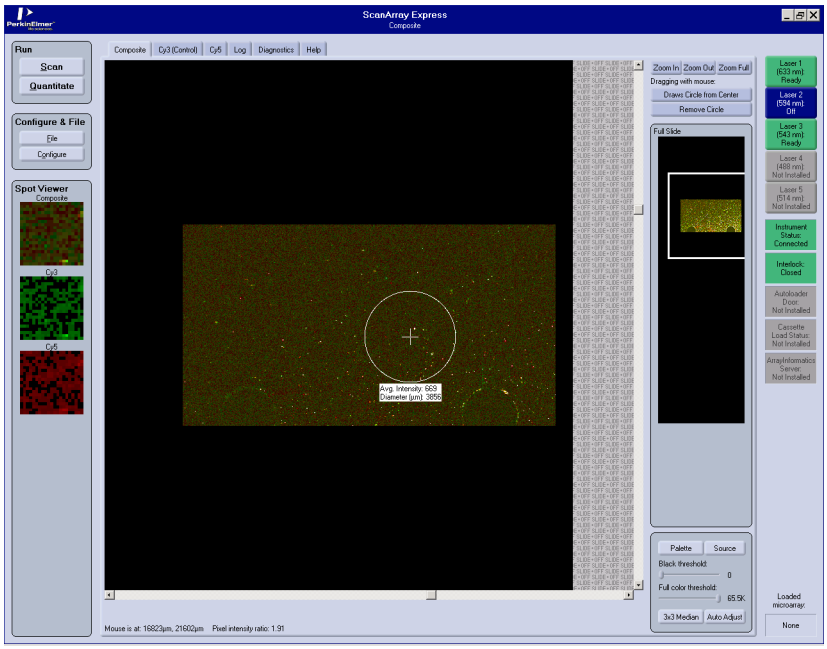
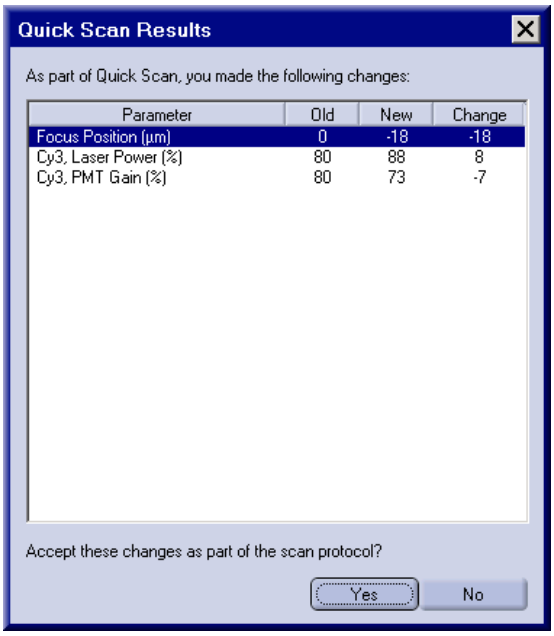


Figure 4–14 Viewing Average Intensity of an Area

9. When the Quick Scan is done, a *Quick Scan Results* window appears.



10. Click **Yes** to accept the changes as part of the scan protocol.

11. To cancel a Quick Scan at any time during the scan, click **Stop** on the *Main Window*.

4.5.2 Running a Line Scan

Line Scan should be used only by advanced users. The **Line Scan** repeatedly scans a single scan line to help determine the laser power and PMT gains for the various fluorophores, and displays the signal intensity for the specified line scan area on an oscilloscope-like screen. You can adjust the focus position, Y start position, laser power and/or PMT gain while reviewing real-time changes in signal intensity.

To perform a Line Scan

1. Click **Run** in the **Line Scan** area of the *Scan Protocol - Tools* window. The *Line Scan* dialog box displays.

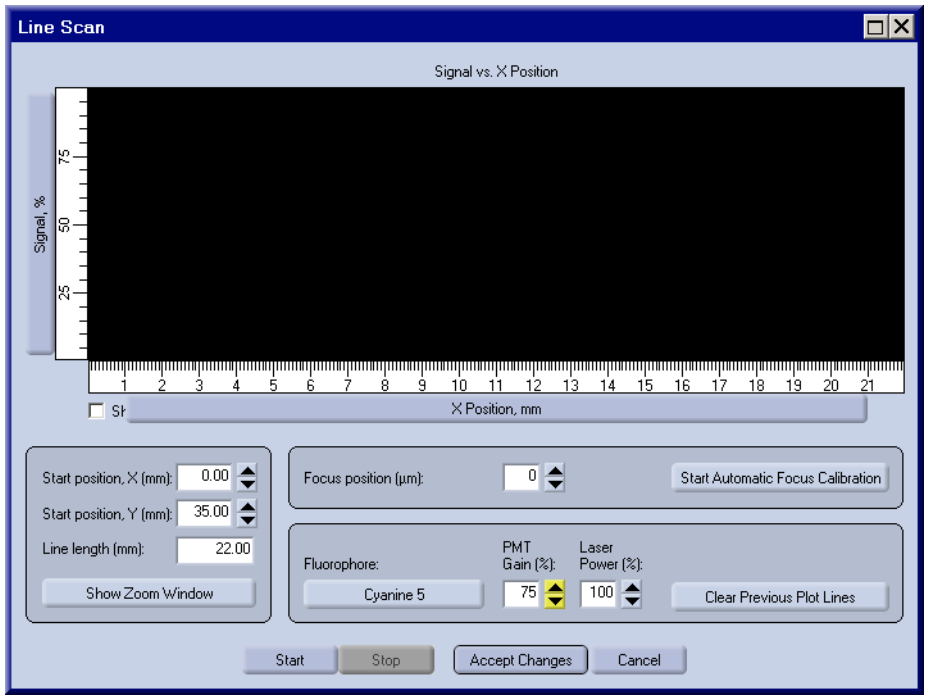


Figure 4–15 Line Scan Window

2. Set the scan area and describe where the line is:

Item	Description
Start position, X, Y	Enter the coordinates of the area to be scanned.
Line length	Enter the length of the scan line.
Show Zoom Window	Click to open the Zoom window where you can select an area with greater precision. Using the mouse, draw a line over the area of interest on the slide image. The line should be through the middle of the selected dots.

3. Select the fluorophore. Change the **PMT Gain** and **Laser Power** while the scanning is in progress to find the optimal settings.

Item	Description
Fluorophore	Select the desired Fluorophore.
PMT Gain, Laser Power (%)	Change the instrument sensitivity by adjusting the Laser Power and PMT Gain settings so that the signal is within 90% of maximum to prevent saturation. The Line Scan changes dynamically with each adjustment.
Clear Previous Plot Lines	Click this to clear the display.

4. Focus position rarely needs adjustment when using conventional genomic and proteomic microarrays printed on flat substrates. Focus position may need to be adjusted for less conventional applications such as tissue arrays and three-dimensional substrates.

Item	Description
Focus position	Do not change the focus position for conventional microarrays.
Start Automatic Focus Calibration	If you have selected a spot on the sample that has measurable signals for the selected fluorophore in the protocol, click **Start Automatic Focus Calibration** to automatically determine the optimum lens position. A progress bar displays indicating automatic focus calibration. Upon completion click **Accept Changes** to accept the adjustment or click **Cancel** to cancel the adjustment. If the signal trace approaches 100%, reduce the Laser Power and/or PMT Gain or re-run the automatic focus.

5. Click **Start**, review the results, and click **Accept Changes** to save the results to the protocol.

4.5.3 Automatic Sensitivity Calibration

Only experienced users should use this tool. The **Automatic Sensitivity Calibration** tool scans selected areas of a microarray with the selected fluorophores to determine appropriate settings for laser power and PMT gain.

To perform Automatic Sensitivity Calibration

1. Click **Run** in the **Automatic Sensitivity Calibration** area of the *Scan Protocol - Tools* window. The *Automatic Sensitivity Calibration* dialog box displays.

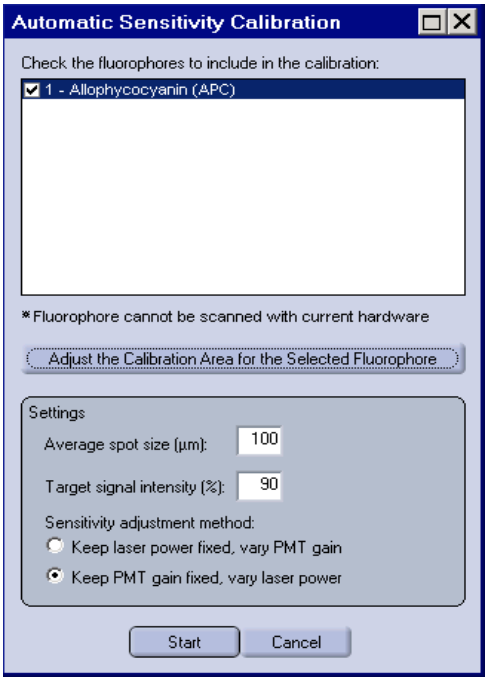


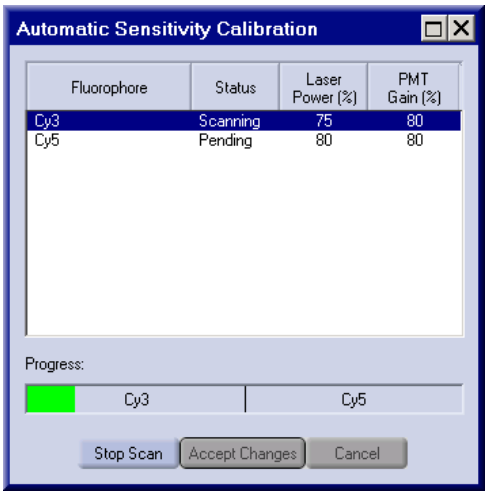
Figure 4–16 Automatic Sensitivity Calibration Window

2. Select the fluorophores to include in the calibration, and specify the settings. Refer to the following table:

Item	Description
Check the fluorophores to include in the calibration	Check each fluorophore to be included, and adjust the calibration area for each fluorophore, if necessary.
Adjust the Calibration Area for the Selected Fluorophore	Select a fluorophore and click this button to adjust the area in the window that opens.
Average spot size (µm)	Enter a value. The default is 100. Valid values are 75 (µm) to 500 (µm).
Target signal intensity (%)	Enter a value. The default is 90%. Valid values are 50% to 100%.
Sensitivity Adjustment Method	Select a method to use: **Keep laser power fixed, vary PMT gain**. This is the default. **Keep PMT Gain fixed, vary laser power**.

3. Click **Start** to run Automatic Sensitivity Calibration. It can be cancelled at any time by clicking **Stop,** then **Cancel**.

4. A progress bar displays 100% when complete. Upon completion, review the results, and click **Accept Changes** to accept the results.

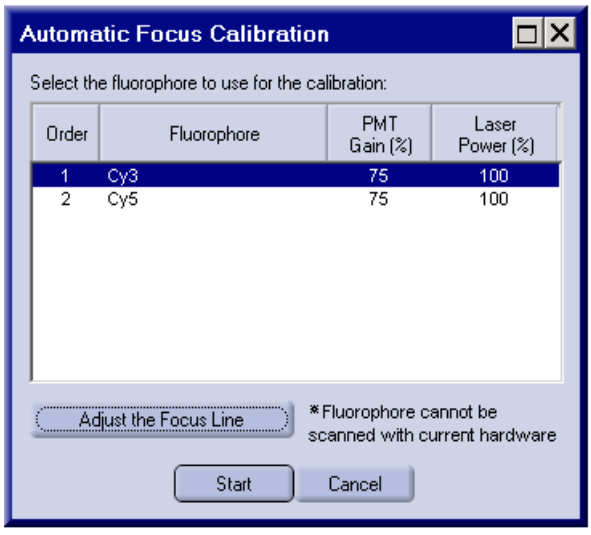


4.5.4 Automatic Focus Calibration

Only experienced users should use this tool. The **Automatic Focus Calibration** tool scans a selected area of a microarray with a single fluorophore and determines the appropriate focus position.

To perform Automatic Focus Calibration

1. Click **Run** in the **Automatic Focus Calibration** area of the *Scan Protocol - Tools* window. The *Automatic Focus Calibration* dialog box displays.



2. Select the fluorophore to use for the calibration. To change the area to be used for focus calibration, click **Adjust the Focus Line** to move to an area with a lot of features. The *Focus Line Definition* window opens, where you can move the focus line.

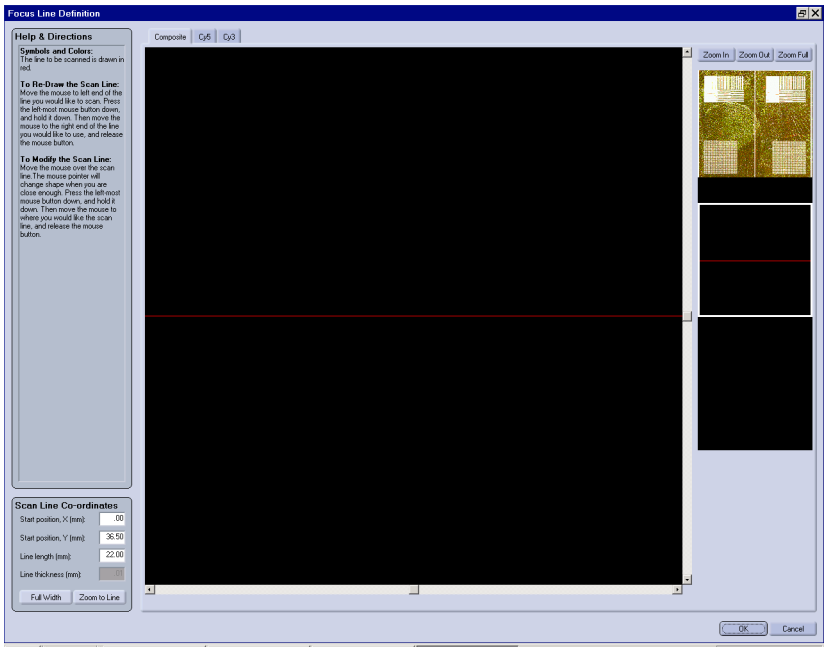


Figure 4–17 Focus Line Definition Window

3. Click **OK** to close and return to the *Automatic Focus Calibration* window.

4. Click **Start**. A progress bar displays while the focus is being calibrated. You can cancel at any time by clicking **Stop**.

5. Upon completion of the calibration, review the results, and click **Accept Changes** to accept the results.

Note: If you have used the Scan Protocol Tools, and accepted changes made by the tools, be sure to save your Scan Protocol to save the changes.

4.6 Creating an Image Autosave Protocol

Image Autosave Protocols specify the folder, file naming convention and file type to use when automatically saving image files during a scan.

To create an Image Autosave Protocol

1. On the *Main Window*, click **Configure** in the *Configure & File* group. In the *Configure* menu that displays, click **Image Autosave Protocols.** The *List of Image Autosave Protocols* window opens.

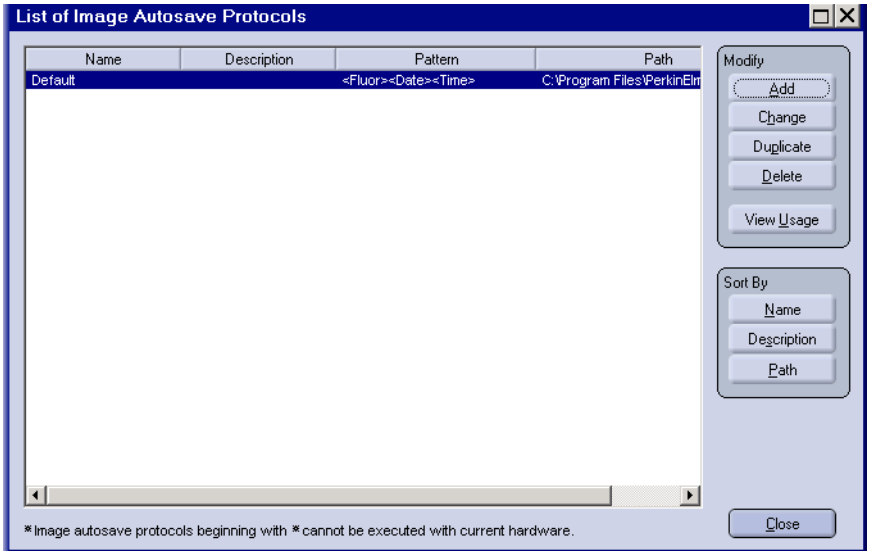


Figure 4–18 List of Image Autosave Protocols Window

2. Sort the list (optional) by **Name**, **Description**, or **Path**.

3. Click one of the following in the *List of Image Autosave Protocols* window:

   - **Add** to create a new protocol.
   - **Change**, after selecting a protocol, to modify an existing protocol.
   - **Duplicate**, makes a duplicate of the highlighted protocol to help you create a new protocol from one that is already close to what you want.
   - **Delete**, after selecting a protocol, to delete it.
   - **View Usage,** after selecting a protocol, to display a list of protocol groups and batch sets which include the selected protocol.

4. Click **Add**. The *Image Autosave Protocol* dialog box displays. In this window, you can check each item that you want to include in the filename. To create a unique filename, you must include the fluorophore, the date, and the time. You can also check *Custom Text* and add a version number (e.g., v1, v2, etc.)

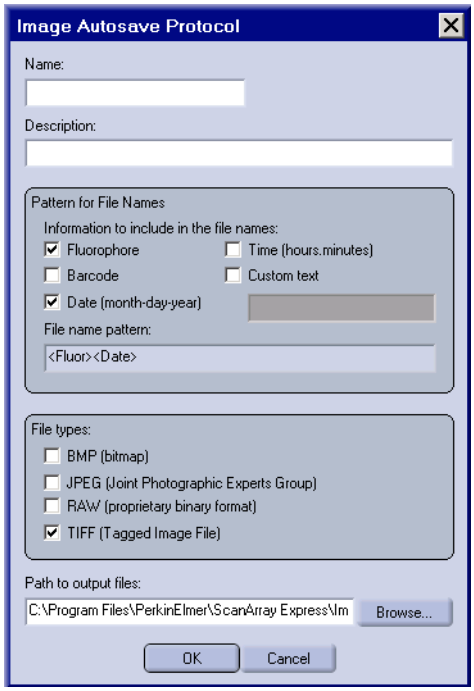


Figure 4–19 Image Autosave Protocol Dialog Box

Note: The items are added to the filename in the order that they were checked, as shown in the File name pattern. If you want to change the order, uncheck all boxes, then recheck them in the order that you want them to appear in the filename.

5. Use the following table:

Item	Description
Name	Enter a name for the image autosave protocol. Use your own name, or some other text that makes the protocol easy to find.
Description	Enter a brief description.
Pattern for File Names	Check the box for each item that you would like to include in the file names. The selections include Fluorophore, Barcode, Date, Time and Custom text. If you check Custom text, the text field becomes active and you can enter up to 64 characters of text. To create a unique filename for each image, you must include the fluorophore, the date, and the time.
File types	Check each file type that you want to use. TIFF is the preferred and default format for storing the output image. Image information is not supplied with .BMP or .JPEG files.

Item	Description
Path to output files	Enter the desired path for output files or use the Browse button to locate the path, which can be a local path or a path on the network. If a directory that doesn't exist is specified in the pathname, a folder will be automatically created for it.

6. Click **OK** to save the changes. Click **Close** to close the *List of Image Autosave Protocols* window.

Quantitating with Protocols

Chapter 5

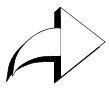
Chapter Summary

5.1 Overview

A *Quantitation Protocol* lets you set up parameters for quantitating, including advanced quantitation methods, and save them to be used again. A *Spreadsheet Autosave Protocol* is used to save the quantitation results -- the protocol specifies the folder, file naming convention and file type to use when saving the results.

Quantitation protocols can also be included in protocol groups or batch sets that are run from the *Scan* window. See Chapter 6, "Automating Scans and Quantitation" for more information on protocol groups and batch sets. To quantitate automatically after a scan, see "Running a Scan Protocol, Automatic Quantitation" in Chapter 4.

This chapter provides instructions for running a Quantitation Protocol and for creating Quantitation Protocols and Spreadsheet Autosave Protocols.



Tip: *If you are a new or infrequent user, you can use Easy Quant to become familiar with the quantitation process before creating and using Quantitation Protocols. See Chapter 3 for information on using Easy Quant.*

For a tutorial that leads you through scanning and quantitation with a sample slide, see Chapter 2, "Getting Started: Scan and Quantitate the Geometric Test Slide."

5.2 Running a Quantitation Protocol

Once a protocol is set up, it is very easy to run. After acquiring your image(s) by scanning or opening previously scanned images (using the *File* menu under *Configure & File* on the *Main Window)*, you select a quantitation protocol to use.

To quantitate an image using a protocol

1. Click **Quantitate** on the *Main Window*. The *Quantitate* window opens, where you can choose a quantitation type.

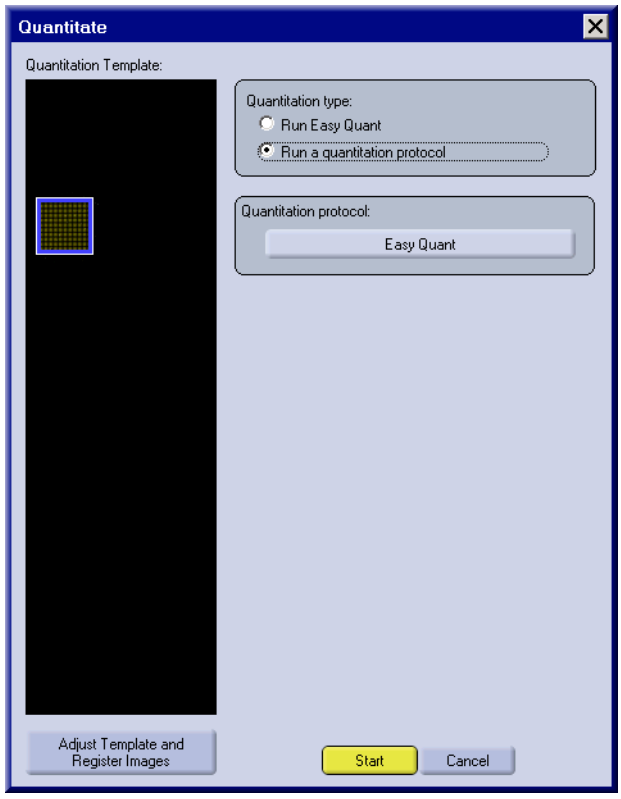


Figure 5–1 Selecting to Quantitate Using a Protocol

2. Select **Run a quantitation protocol**. The settings for performing the quantitation are included in the quantitation protocol. Unlike when using Easy Quant, you cannot change the settings in this window.

3. Select a protocol: click the **Click to Select** button and select a protocol from the *Select a Quantitation Protocol* window that opens. Click **OK**.

   On the *Quantitate* window, the button fills with the name of the selected protocol.

4. Click **Adjust the Template and Register Images** to adjust the template to match the array, and register the images if more than one image is displayed. (See Easy Quantitation in Chapter 3.)

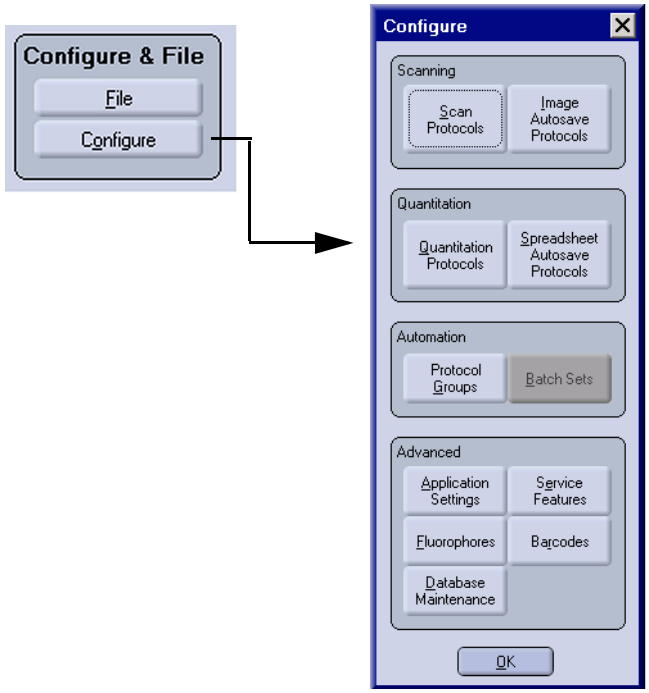
5. Click **Start**. ScanArray Express finds the spots, then immediately quantitates.

After quantitation, the results display on the *Main Window*. See Viewing Quantitation Results in Chapter 3.

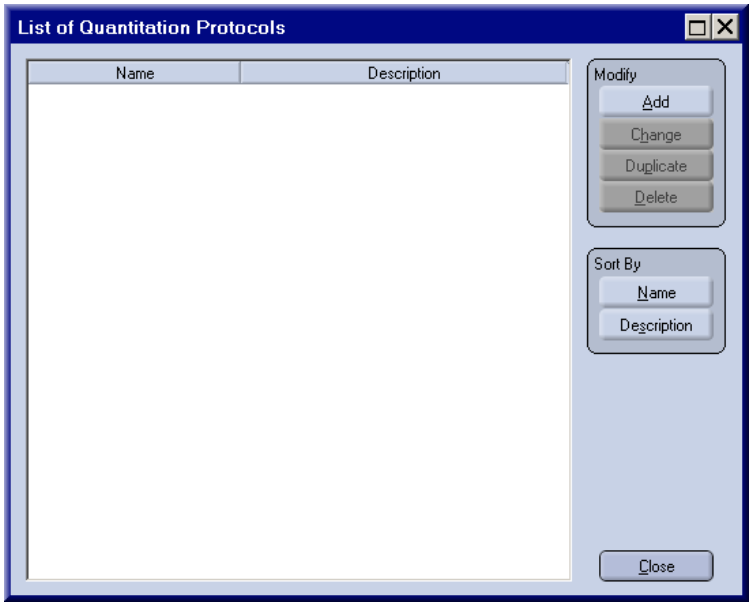
5.3 Creating a Quantitation Protocol

To create a quantitation protocol

1. On the *Main Window*, click **Configure** in the *Configure & File* group. In the *Configure* menu that opens, click **Quantitation Protocols**.



2. The *List of Quantitation Protocols* window opens.



3. Sort the list (optional) by **Name**, **Description**, or **Resolution**.

4. Click one of the following in the *List of Quantitation Protocols* window to open the *Quantitation Protocol* wizard:

   - **Add** to create a new protocol
   - **Change**, after selecting a protocol, to modify an existing protocol
   - **Duplicate**, makes a duplicate of the highlighted protocol, to help you create a new protocol from one that is already close to what you want.
     Or, click:
   - **Delete**, after selecting a protocol, to delete a protocol.

5.4 The Quantitation Protocol Wizard

The *Quantitation Protocol* wizard opens with the *Basic Information* window displayed, as shown below. From this window, using the wizard, you can display and set all of the quantitation parameters.You can move through the windows by clicking the numbers on the left, or by using the buttons on the bottom of the window:

Click the numbers to move from one window display to another.

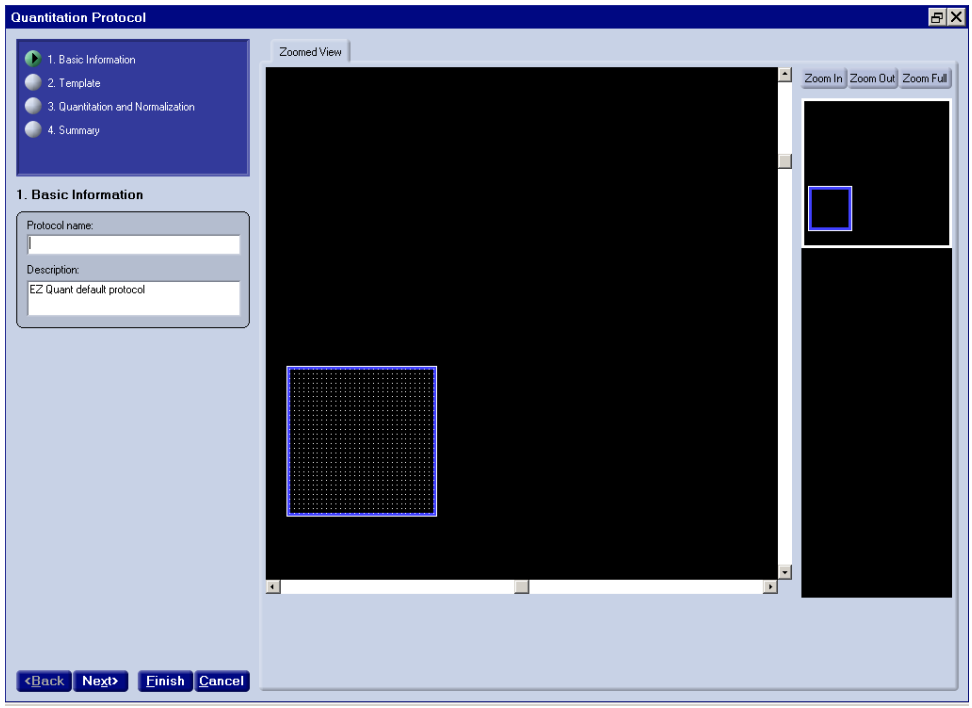


Figure 5–2 The Quantitation Protocol Wizard

- Click **Next** to move to the next window and **Back** to move to the previous window.
- Click **Finish** to save all of your changes and close the wizard.
- Click **Cancel** to close the wizard without saving any of your changes.

You can change the image display, making it larger or smaller, using the **Zoom** buttons:

- **Zoom In**, **Zoom Out**, **Zoom Full** - Click these buttons to zoom in or zoom out the display, or to restore the display to the full slide view. These controls remain visible and selectable throughout the protocol wizard

5.4.1 Basic Information

In the *Quantitate Protocol - Basic Information* window, provide a name and description for the protocol.

To name a quantitation protocol

1. Fill in the information as listed in the following table.

Item	Description
Name	Enter a name for the quantitation protocol so you can recall it easily . You must enter a name.
Description	Enter a description for the quantitation protocol.

2. Click **Next** or click "Image Registration" to go to the next window. If only one image is open, the next window is "Template."

5.4.2 Image Registration

If more than one image is open, **Image Registration** automatically appears in the wizard as Step 2. If only one image is open, this step and the associated window do not appear.

Registration is done from the *Composite* tab, by moving the Source image so it registers to the Control image. That is, the Control image position is fixed, and the Source image is moved relative to the Control. Each image that needs to be registered has to be set as the Source image for registration.

To register the images

1. Open the *Image Registration* window.

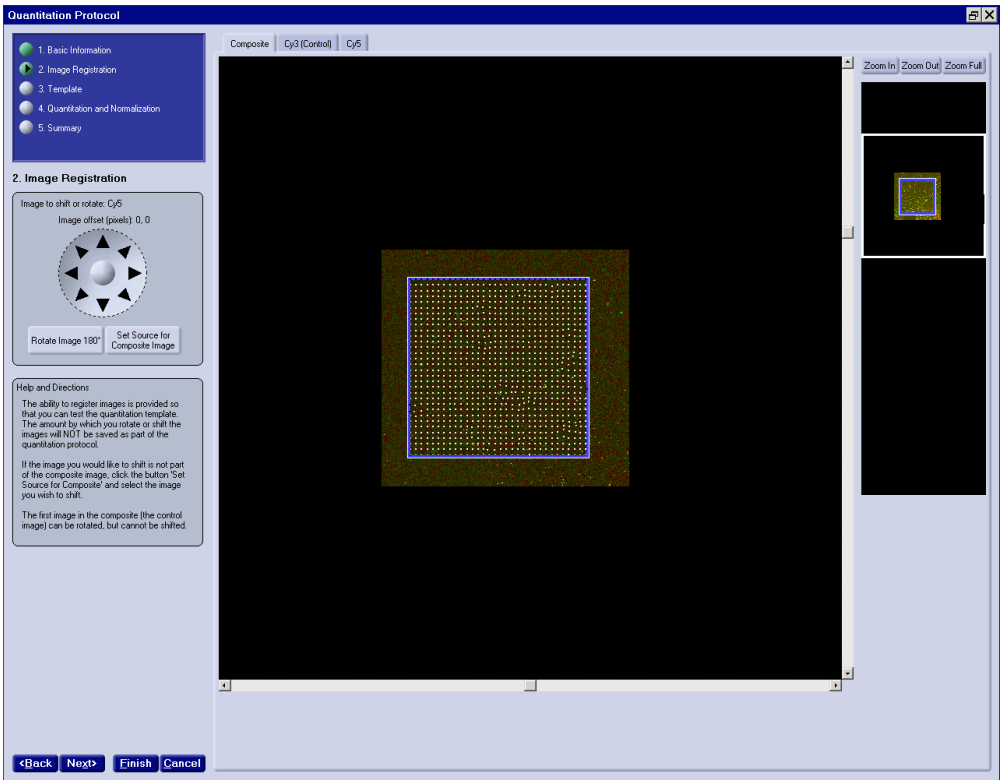


Figure 5–3 The Quantitation Protocol, Image Registration Window

2. Click the *Composite* tab, and adjust the Source (experiment) image to the Control. .

3. To register the next image, click **Set Source for Composite Image** and register it to the Control image in the *Composite* tab. Do this for each image that needs to be registered.

4. Click **Next** or click "Template" to go to the next window.

5.4.3 Template

Specify the template to be used by this protocol. A template must be defined that matches the layout of the array on the scanned image. You can import a template from a .GAL file or you can manually define the array pattern. If you use a .GAL file, it can be a local file or on a network drive, or it can be accessed from ArrayInformatics (for ArrayInformatics users). The advantage of using a .GAL file is that it also imports gene names for the spots from the file.

The defined template overlays onto the scanned image a template of circles that is closely aligned to the microarray spots.

To define a template

1. Open the *Template* window.

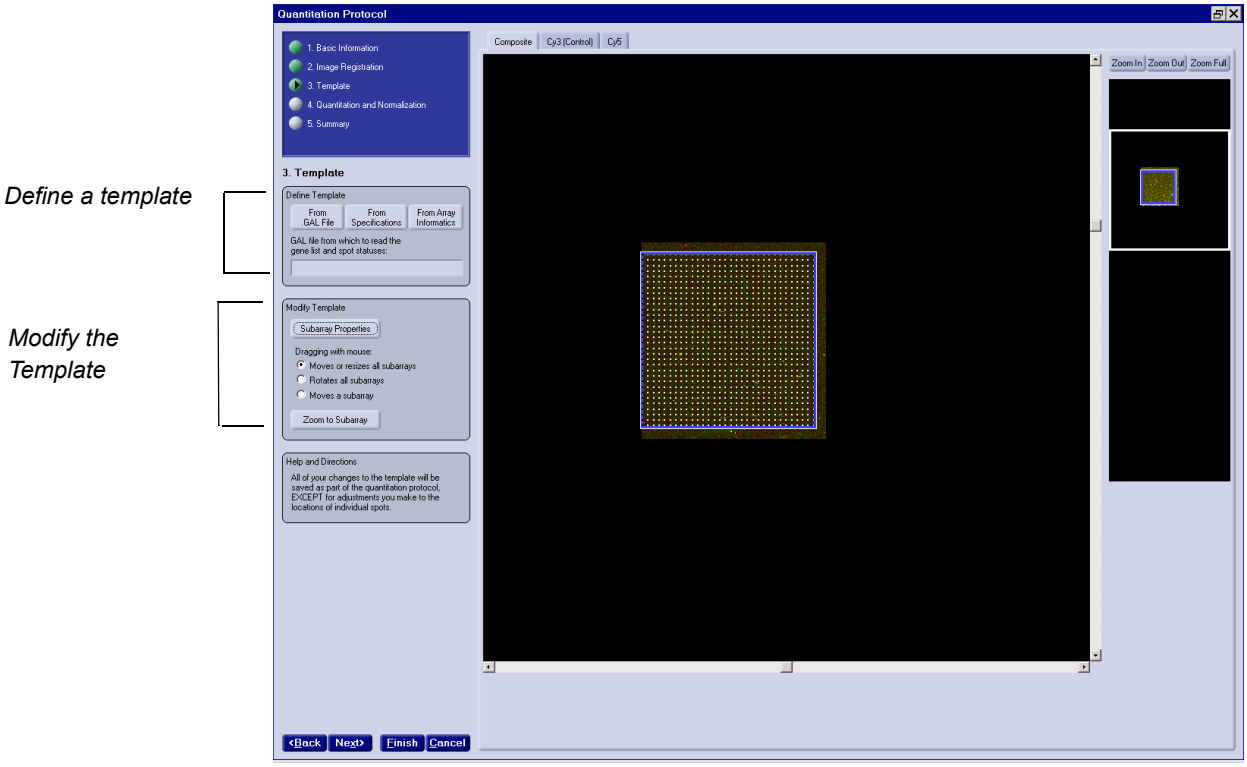


Figure 5–4 The Quantitation Protocol, Template Window

2. Specify the source of the template. Refer to the following table for a description of the possible choices. Instructions for using a .GAL file or specifications are in the sections following the table. (For a description of a .GAL file, see Appendix A.)

   After loading a template, you may need to modify it in Step **3**.

Item	Description
Template	Click a button to specify the source of a template.
From .GAL file	Click this button to open the *Open GAL File* window, where you can browse for and select a GAL file to use. Below the button, the .**GAL file from which to read the gene list and spot statuses** field fills with the name of the selected file. See ***Defining a template Using a GAL File*** on page 5-8.
From specifications	Click this button to manually define the array pattern. The *Template Specification* window opens, where you enter the values. See ***To define a template from specifications*** on page 5-9.

Item	Description
From ArrayInformatics	Click this button to select template information from the ArrayInformatics database, if your ScanArray Express system is integrated with ArrayInformatics. Refer to the *Microarray Laboratory Integration Guide* that ships with the ArrayInformatics software. If your system is not integrated with ArrayInformatics, this button appears dimmed and is unavailable to select.
Modify Template	
Dragging with Mouse	Select one of the behaviors for the mouse: - Moves or resizes all subarrays - Rotates all subarrays - Moves a subarray
Subarray Properties	Opens a dialog box where you can enter the following parameters, and then copy them to all subarrays: Subarray top left corner, x Subarray top left corner, y Straighten spot rows and columns in this subarray.
Zoom to Subarray	Click to zoom the display on a subarray.

3. Modify the template, using the buttons in the *Modify Template* group.

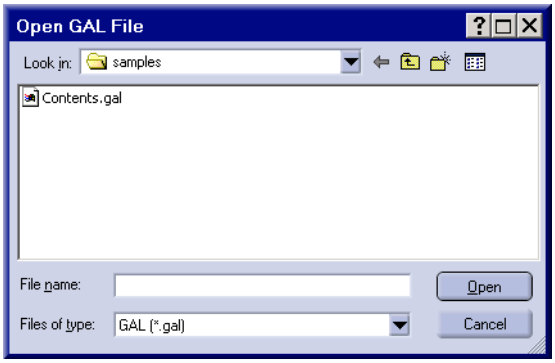
4. Click **Next** or click "Quantitation and Normalization Method" to go to the next window.

5.4.3.1 Defining a Template Using a GAL File

A .GAL file, output from the SpotArray, includes a gene list and information about each spot on the microarray.

To define a template using a .GAL file

1. In the *Quantitation* window under Template, click **From .GAL File**. The *Open GAL File* dialog box opens.



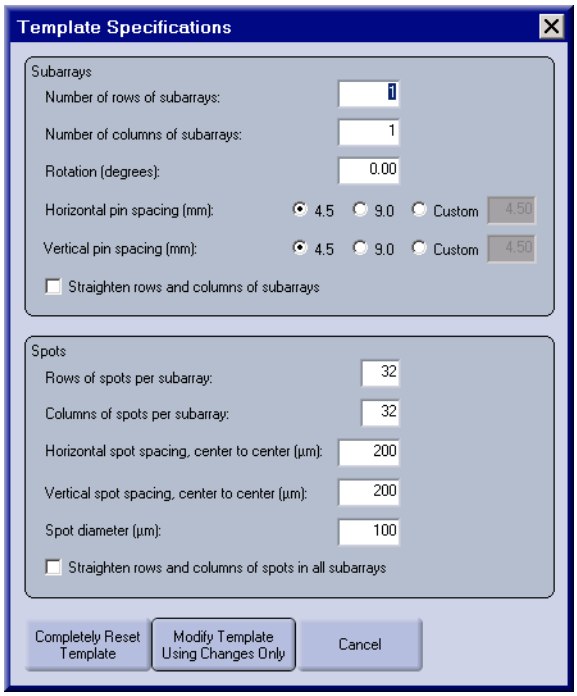
2. Highlight the desired file and click **Open**.
   The dialog box closes and the name and path of the selected .GAL file displays under the **From .GAL File** button in the *Protocol Wizard Template* window.

5.4.3.2 Defining a Template From Specifications

Using specifications, you establish the array pattern by entering numerical data that defines the subarrays and spots.

To define a template from specifications

1. In the *Protocol Wizard Template* window, click **From Specifications** to enter values that define the geometry of the array. The *Template Specifications* window displays.



2. Provide the information for the array pattern, as described in the following table.

Item	Description
Subarrays	Describes the geometry of the microarray.
Number of rows and number of columns of subarrays	Enter the number of subarray rows and the number of subarray columns.
Rotation	If the microarray is skewed on the substrate, you can rotate the template to align the template to the microarray.

Item	Description
Pin spacing, horizontal	Enter the horizontal and vertical pin spacing that was used to create the microarray:
Pin spacing, vertical	4.5 if 384-well plates were used to print the microarray
	9.0 if 96-well plates were used to print the microarray
	Custom - to enable the edit boxes where you can enter values for custom pin spacing.
Straighten rows and columns of subarrays	This forces the subarrays into straight rows and columns for quantitation.
	The straightening does not display on the image, but affects the quantitation results.
Spots	This describes the position and size of each spot in a subarray.
Rows of spots and Columns of spots per subarray	Enter the number of rows and the number of columns of spots that are in each subarray.
Horizontal spot spacing (center to center (μm)	Enter the horizontal and vertical spot spacing, measuring from the center of a spot to the center of the spot in the next row or column.
Spot diameter	Enter the spot diameter in μm.
Straighten rows and columns of spots in all subarrays	This forces the spots into alignment for quantitation. The straightening does not show on the displayed image but affects the quantitation results.

3. Click **Completely Reset Template** to the previous settings or click **Modify Template Using Changes Only** to save your changes.

5.4.4 Quantitation and Normalization Method

Specify the quantitation method and normalization method to be used by this protocol. ScanArray Express uses the *quantitation method* to construct a pixel by pixel map that indicates the property of each pixel in the image. *Normalization* corrects the intensity of each spot for variations in the overall intensity of the images with respect to the control image.

You can use the defaults: **Adaptive Circle** quantitation method and **LOWESS** normalization.

To choose a quantitation method

1. Open the *Quantitation and Normalization* window.

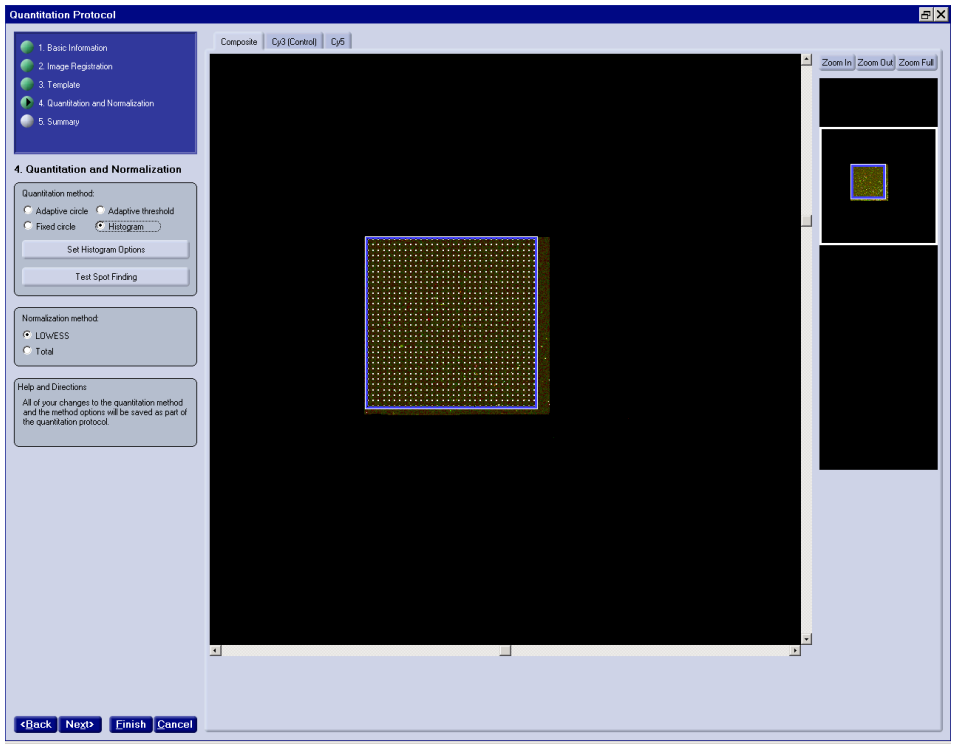


Figure 5–5 Quantitation Protocol Quantitation and Normalization Window

2. Select a quantitation method:

   - **Adaptive circle** (the preferred method) uses a minimum and maximum spot diameter (percentages of the nominal spot diameter) to construct a mask. See ***Adaptive Circle*** on page 5-12.
   - **Fixed circle** uses the parameters of the spot diameter and background inner and outer dimensions to create a spot mask and background mask. See ***Fixed Circle*** on page 5-13.
   - **Adaptive threshold** uses the parameters of the spot diameter and background inner and outer dimensions to create a spot mask and background mask, then refines the mask on a pixel-by-pixel basis. See ***Adaptive Threshold*** on page 5-15.
   - **Histogram** uses the same mask for both the spot and background and creates a graph of each pixel quantity versus the pixel intensity. See ***Histogram*** on page 5-16.

3. Select a normalization method: **LOWESS** or **Total**.

- **LOWESS** (Locally Weighted Scatter Plot Smoothing) carries out robust locally-weighted scatter plot smoothing for both equally spaced and non-equally spaced data.
- **Total**, uses the intensity of each spot in relation to all spots.

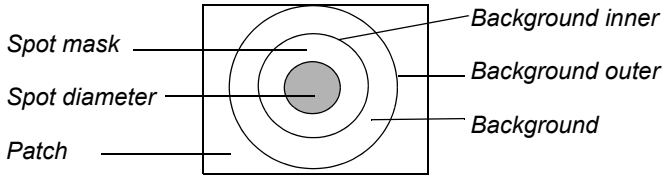
See ***Normalization Methods*** on page 5-17.

4. Click **Test Spot Finding** to ensure that the template you have set up will find the spots.
5. When you are finished, click **Summary** to see a summary of the quantitation protocol.

5.4.5 Quantitation Methods

After a template is imported and adjusted in the protocol wizard *Template* window, the ScanArray Express determines the center for each spot, and determines the corresponding *patch*. The patch is a rectangle that is constructed around the center of the spot with the dimensions indicated in the template.

Both the spot and the background must be defined with the patch. The quantitation method selected then constructs *masks* for the spot and the background. A *mask* is a pixel by pixel map that indicates the property of each pixel.

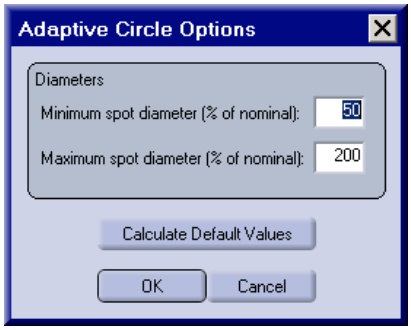


5.4.5.1 Adaptive Circle

The *Adaptive Circle* method fits all spots in the image with circles; the circle diameter is estimated separately for each spot on the microarray, with a minimum and maximum spot diameter as specified in the *Adaptive Circle Options* dialog box. Adaptive Circle is the best method to use when all circles are not the same size. This is the default method.

To use the adaptive circle method

1. Select **Adaptive circle** and click **Adaptive Circle Options**.



2. Enter the minimum and maximum spot diameters. These are not absolute values, but are a percentage of the nominal spot diameter as specified in the .GAL file or template.

   - OR -

   Click **Calculate Default Values** to calculate the default values based on the array pattern selected in the microarray. The defaults for your microarray depend on the template that is loaded.

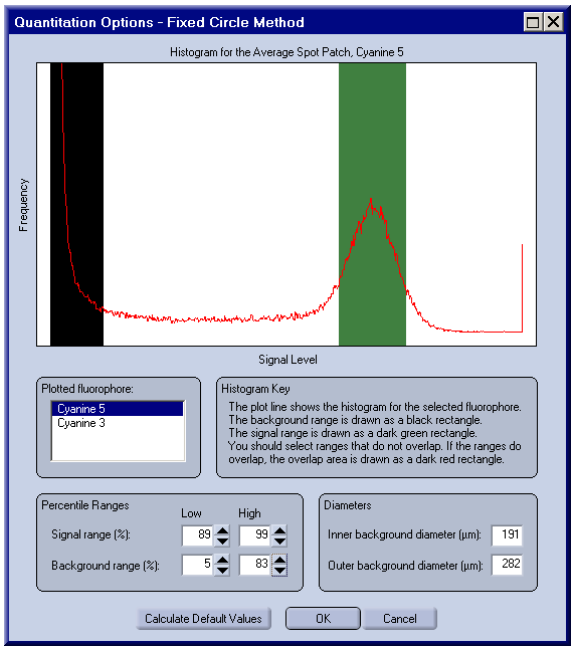
3. Click **OK**.

5.4.5.2 Fixed Circle

The *Fixed Circle* method fits all spots in the image with circles of fixed diameter. All spots must be of the same size and shape. Using the Fixed Circle Method, the spot mask and the background mask are constructed using the parameters of the spot diameter, and the background inner and outer dimensions.

To use the fixed circle method

1. Select **Fixed Circle** and click **Set Fixed Circle Options.**



2. Refer to the following table and specify your settings.

Item	Description
Plotted fluorophore	The highlighted fluorophore will be plotted. Select the fluorophore.
Percentile Ranges	Enter the minimum and maximum percentile values for calculations, where percentile is the percentage of pixels in the sample.
Signal Low	The default is 5; valid values are from 0 to100.
Signal High	The default is 95; valid values are from 0 to 100.
Background Low	The default is 5; valid values are from 0 to 100.
Background High	The default is 95; valid values are from 0 to 100.
Inner Background Diameter (µm) and Outer Background Diameter (µm)	Inner and outer background diameter depends on the array pattern.
Calculate Default Values	Calculates the default values based on the array pattern of the microarray. The defaults depend on the template that has been loaded.

3. Click **OK**.

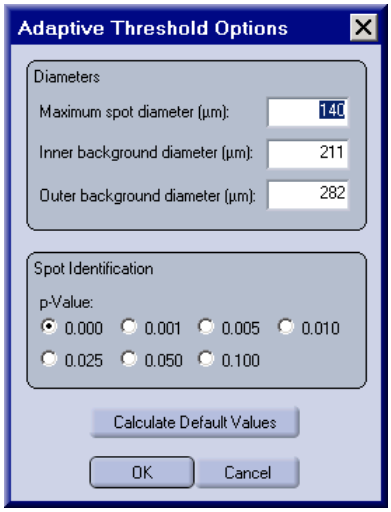
5.4.5.3 Adaptive Threshold

Using the A*daptive Threshold* method, the initial spot mask and background mask are constructed in the same manner as in the Fixed Circle method, but this method will quantitate low intensity spots better. The Adaptive Threshold is refined pixel by pixel using a modified statistical testing process (Mann Whitney test). During the test process an adaptive threshold is determined by comparing eight (8) sample pixels inside the spot mask with the eight (8) median background pixels in the background mask. The process is repeated with successively brighter pixels in the spot sample until a statistically significant difference is found between the background pixels and the spot pixels.

The main advantage of the Adaptive threshold method is that it can identify all spot pixels adaptively and can compensate for any morphological change in spots (i.e., if a spot is not exactly round). A statistical algorithm is used to define the spot. The disadvantage is that background quantitation may become unstable if a large maximum spot diameter is set to compensate for spot size variation.

To use the adaptive threshold method

1. Select **Adaptive threshold** and click **Set Adaptive Threshold Options.**



2. Refer to the table below and set the parameters.

Item	Description
Dimensions	
Maximum spot diameter	Enter the maximum spot diameter in microns.
Inner background diameter	Enter the inner background diameter in microns This must be larger than the maximum spot diameter.
Outer background diameter	Enter the outer background diameter in microns. This must be larger than the inner background diameter. *IMPORTANT*: The inner and outer background dimensions can never be equal.

Item	Description
Spot Identification p-Value	The p-value parameter controls the confidence for testing whether some spot pixels differ statistically from the eight median pixels in the background mask. The p-value represents a probability that the sampled eight spot pixels associated with an adaptive threshold are from a different population than that of the eight median background pixels. The smaller the p-value, the more confident the derived spot threshold. All pixels above the threshold are used for the spot intensity calculation.
Calculate Default Values	Calculates the default values based on the array pattern selected in the microarray. The defaults for your microarray depend on the template that is loaded.

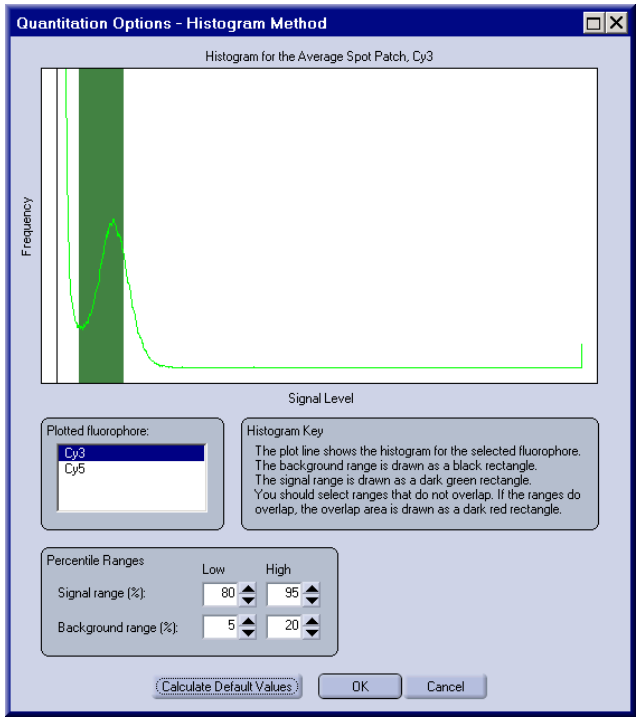
3. Click **OK.**

5.4.5.4 Histogram

Using the *Histogram* method, the histogram for each spot is calculated. Use caution with this method if you don't have experience using it, to ensure that undesirable pixels are not included in the results. You should first run some quantitations and look at the **View Spot Pixels** in the *Spreadsheet* tab to understand what is being included in your quantitation results.

To use the histogram method

1. Select **Histogram** and click **Set Histogram Options**.



2. Set the signal range and background range. Refer to the following table.

Item	Description
Signal range %	Enter the percentile for the following values, where percentile is the percentage of pixels in the sample.
Low	Enter the minimum percentile value for the calculation of the spot statistics.
High	Enter the maximum percentile value for the calculation of the spot statistics.
Background range % Low	Enter the minimum percentile value for the calculation of the background statistics.
High	Enter the maximum percentile value for the calculation of the background statistics.

3. Click **OK**.

5.4.6 Normalization Methods

Normalization corrects the intensity of each spot for variations in the overall intensity of the images with respect to the control image.

- The *LOWESS* method (Locally Weighted Scatter Plot Smoothing) carries out robust locally-weighted scatter plot smoothing for both equally spaced and non-equally spaced data. For more information on the LOWESS method, refer to the following paper:

  Y.H. Yang, S. Dudoit, P. Luu and T.P. Speed. *Normalization for cDNA Microarray Data. SPIE BiOS 2001,* San Jose, California, January 2001.

  The paper can be found at the following web address:

  http://stat-www.berkeley.edu/users/terry/zarray/Html/normspie.html

- The *Total* method uses the ratio of total of all spot pixels.

5.4.7 Quantitation Protocol Summary

The Summary lists all of the parameters you have selected for quantitation and their values.

To view a summary of the protocol settings

1. Open the *Summary* window, where you can review the settings for this protocol.

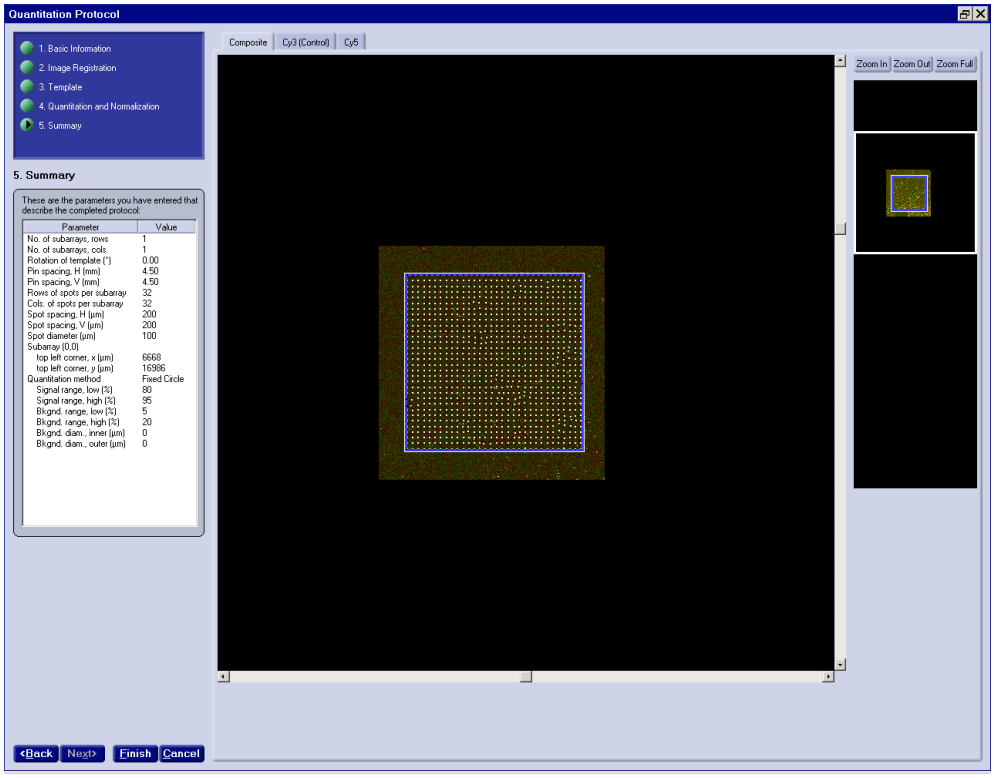


Figure 5–6 Quantitation Protocol Summary Window

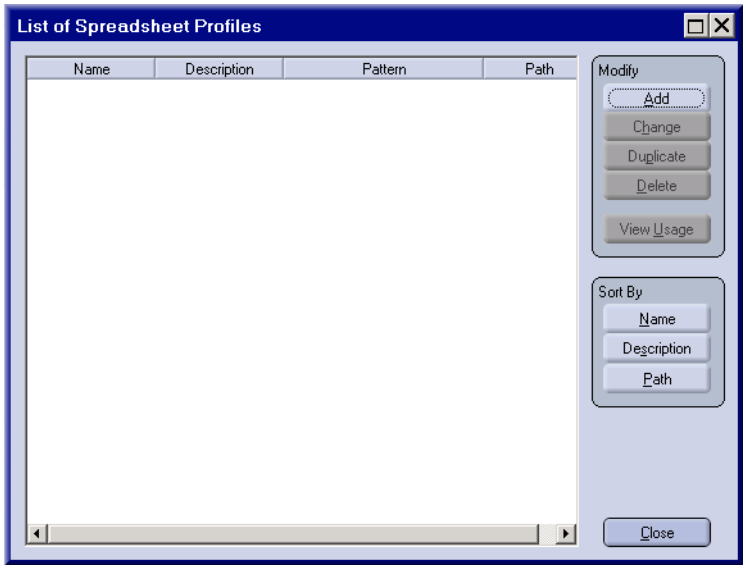
2. Click **Finish** to save your protocol.

5.5 Creating a Spreadsheet Autosave Protocol

Spreadsheet Autosave Protocols specify the filenaming conventions, file type, and file location to use when automatically saving the quantitation results.

To create a spreadsheet autosave protocol

1. On the *Main Window*, click Configure in the *Configure & File* group. In the Configure menu that displays, click **Spreadsheet Autosave Protocol**. The *List of Spreadsheet Autosave Protocols* window opens.



2. Sort the list (optional) by **Name**, **Description**, or **Path**.

3. Click one of the following in the *List of Spreadsheet Autosave Protocols* window:

   - **Add** to create a new protocol.
   - **Change**, after selecting a protocol, to modify an existing protocol.
   - **Duplicate**, makes a duplicate of the highlighted protocol to help you create a new protocol from one that is already close to what you want.
   - **Delete**, after selecting a protocol, to delete it.
   - **View Usage,** after selecting a protocol, to display a list of protocol groups and batch sets which include the selected protocol.

4. Click **Add.**

5. The *Spreadsheet Autosave Protocol* dialog box opens. To create a unique filename, you must include the date and time; if a barcode is available, it should be included in the filename.

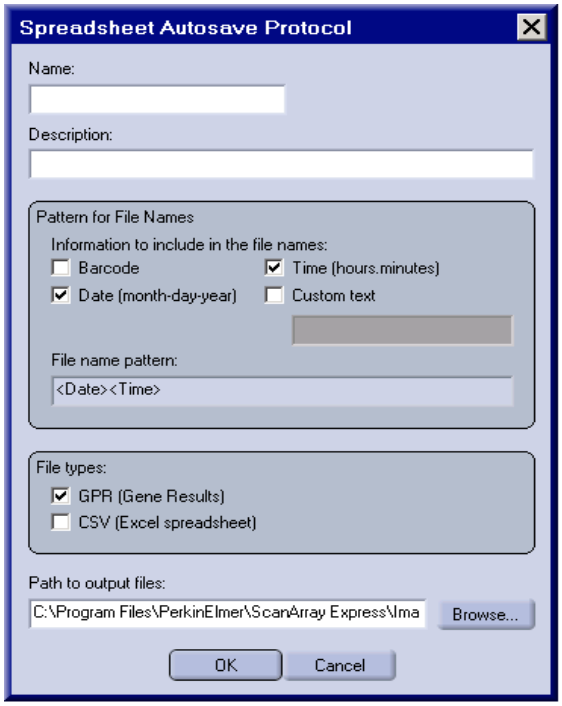


Figure 5–7 Spreadsheet Autosave Protocol Dialog Box

6. Fill in the information as listed in the following table.

Item	Description
Name	Enter a name for the spreadsheet autosave protocol. Use your own name or some other text that makes the protocol easy to find.
Description	Enter a description (optional)
Pattern for File Names	Check the box for each item you want to include in the file names. The information you select will be included in the filename: For example, in the figure above the boxes are checked for Date and Time. The fields are included in order selected, resulting in a filename such as: 080702_0819.GPR
Custom Text	Check this box to enable the edit field and enter custom text, such as descriptive information, to be included in the filename. You can enter up to 64 characters.
File name pattern	Shows the fields selected, their order, and the resulting pattern.

Item	Description
File Types	Select either one or both file types to save the data: GPR - saves the quantitation results as a Gene Results file .CSV - saves the quantitation results as an Excel spreadsheet.
Path to output files	Enter the desired path for output files or use the Browse button to locate the path, which can be a local path or a path on the network.

7. Click **OK** to save the spreadsheet autosave protocol.

8. Click **OK** to close the *List of Spreadsheet Autosave Protocol*s window.

Automating Scans and Quantitation

Chapter 6

Chapter Summary

6.1 Overview

Automating ties together all of the steps for scanning and analyzing your microarrays. Using *Protocol Groups* or *Batch Sets*, you can set up the ScanArray Express to automatically scan, quantitate, and save your results, without intervention.

Note: This is in addition to some of the automatic file saving, and automatic quantitation you can implement when running a single Scan Protocol, as described in Chapter 4.

Protocol Groups is a powerful new ScanArray Express feature. A protocol group lets you run a series of scan protocols on a microarray, one after the other, without intervention, and automatically save the results to a specified location. In addition, you can include quantitation protocols in the protocol group, allowing you to scan, then quantitate, and save all scanned images and quantitation results to a specified location --again, without user intervention.

Scanning *From a batch set* lets you run a scan protocol, different protocols, or a protocol group, on up to 20 slides in a cassette. To use a batch set requires a ScanArray Express system with the HT autoloader option.

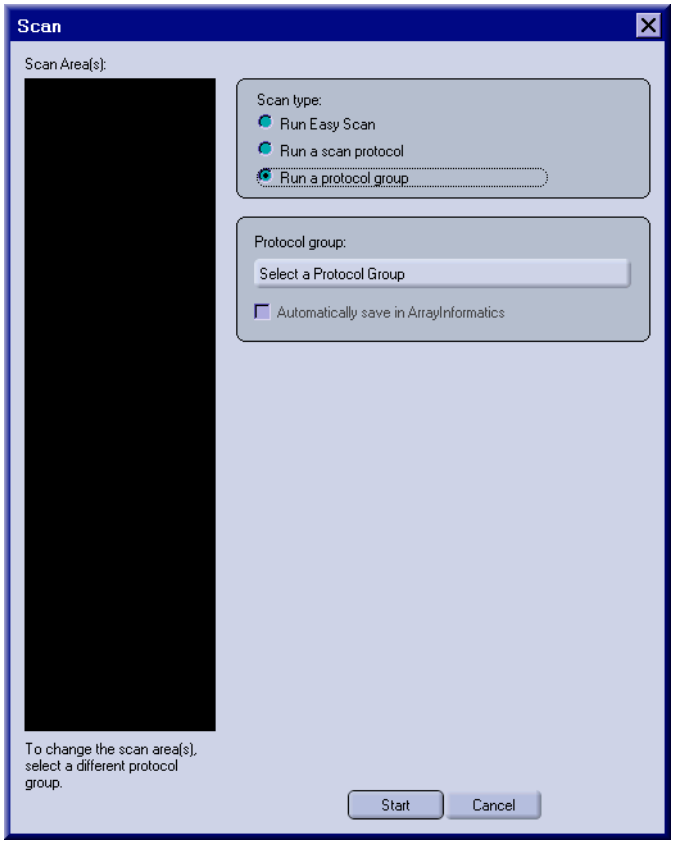
This chapter provides instructions for running a Protocol Group or creating a new one, and for running a scan from a batch set or creating a new Batch Set.

6.2 Running and Creating Protocol Groups

Once a protocol group is set up, it is easy to select and run. To create a Protocol Group, see Section 6.2.1.

To run a protocol group

1. On the *Main Window*, click *Scan*. The *Scan* window opens, where you can select **Run a protocol group**.



2. Click **Select a protocol group** and select a group from the *List of Protocol Groups* window that opens, and click **OK**. The window closes, and returns you to the *Scan* window; the button fills with the protocol group name.

3. Click **Start**.

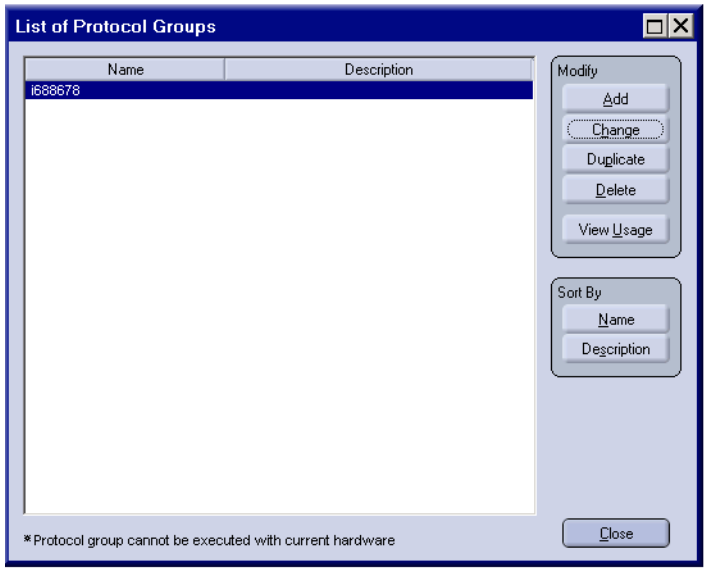
The ScanArray Express loads the microarray, and runs the first protocol in the Protocol Group. The microarray is scanned and the image(s) are saved to the location defined in the image autosave protocol. If quantitation was included in a step of the Protocol Group, ScanArray Express begins spot finding, then quantitates and saves the results to the location defined in the spreadsheet autosave protocol. The images and results files are closed, and ScanArray Express runs the next protocol step.

This process continues until all protocols in the group have run. The images and results from the last protocol are saved as well, but remain displayed on the screen. You can close these files, or open any of the other images/results that have been saved.

6.2.1 Creating a Protocol Group

To create a Protocol Group

1. From the *Main Window*, select **Configure** in the *Configure & File* group, then click **Protocol Groups**. The *List of Protocol Groups* window opens.



2. Sort the list (optional) by **Name** or **Description**.

3. Click one of the following to open the *Protocol Groups* window:

   - **Add** to create a new protocol group.
   - **Change**, after selecting a protocol group, to modify an existing group. If you change and save a protocol group that is currently in use, the ScanArray Express uses the updated protocol group the next time that protocol group is called. The protocol group currently being run continues with the original settings.
   - **Duplicate**, makes a duplicate of the highlighted protocol group to help you create a new group from one that is already close to what you want.

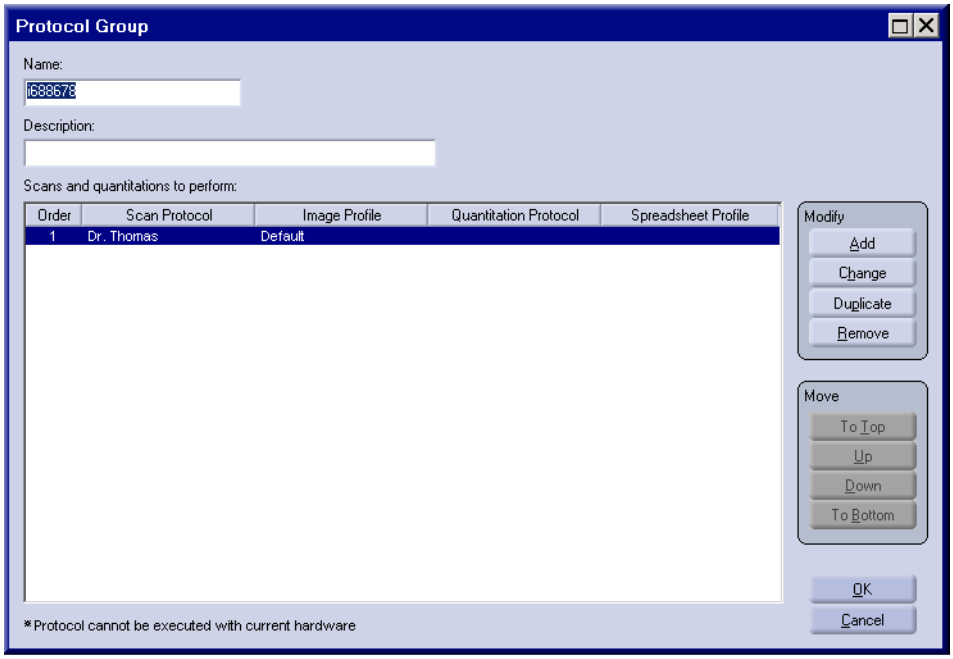
   Or, click:

   - **Delete,** after selecting a protocol group, to delete the group.
   - **View Usage** to see where the protocol group is being used.

To create a protocol group

1. Click **Add**. The *Protocol Group* window opens. A protocol group is organized into a series of steps; each step may include protocols for scanning, or protocols for both scanning and quantitation.

Each line is a *Step* in the Protocol Group.



2. Specify a **Name** and **Description** for the Protocol Group. Use a name that will help you identify the group later.

   In this window, you can **Add**, **Change**, **Duplicate**, or **Remove** a *Step* from the group. A step is a selection of protocols to run; each line in this window is a step.

3. Click **Add** to add a Step. The *Protocol Group - Step 1* window opens.
   See ***To select scan protocols for a group*** on page 6-5.
   See ***To select scan and quantitation protocols*** on page 6-6.

4. Once you have added all of the steps you want to include in the group, you can re-order them if you wish, using the buttons in the *Move* group.

5. When all protocols have been selected, and they are in the order of steps in which you want the steps to run, click **OK** to save the protocol group.

To select scan protocols for a group

1. In the *Protocol Group - Step* window, select **A scan protocol** to create a step for scanning only. The available scan protocols and image autosave protocols display.

   The following example shows the selection for scan protocols only; the fields for quantitation are dimmed and unavailable for selection.

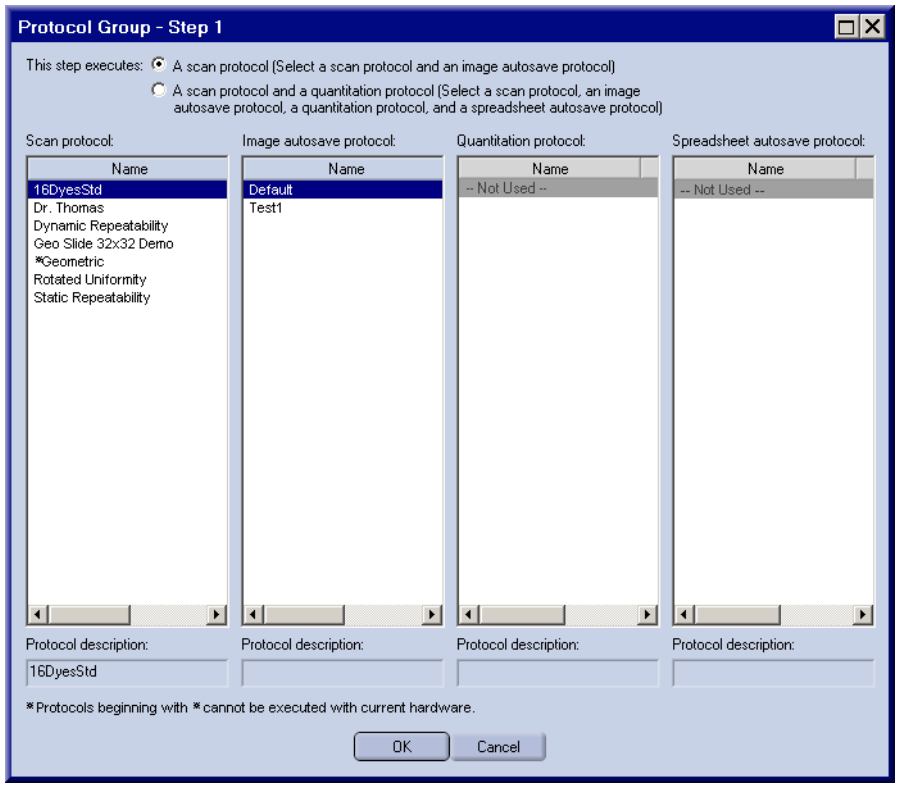


Figure 5–1 Creating a Step for Scanning

2. Under *Scan Protocol*, select the protocol you want to include in this step of the protocol group.

3. Under *Image Autosave Protocol*, select an Image Autosave Protocol to use in this step.

4. Click **OK** to send your selections to the *Protocol Group* window.

You can add as many steps as you want to the Protocol Group.

To select scan and quantitation protocols

1. In the *Protocol Group - Step* window, select the second option, **A Scan Protocol and a Quantitation Protocol**, as shown in the following figure.

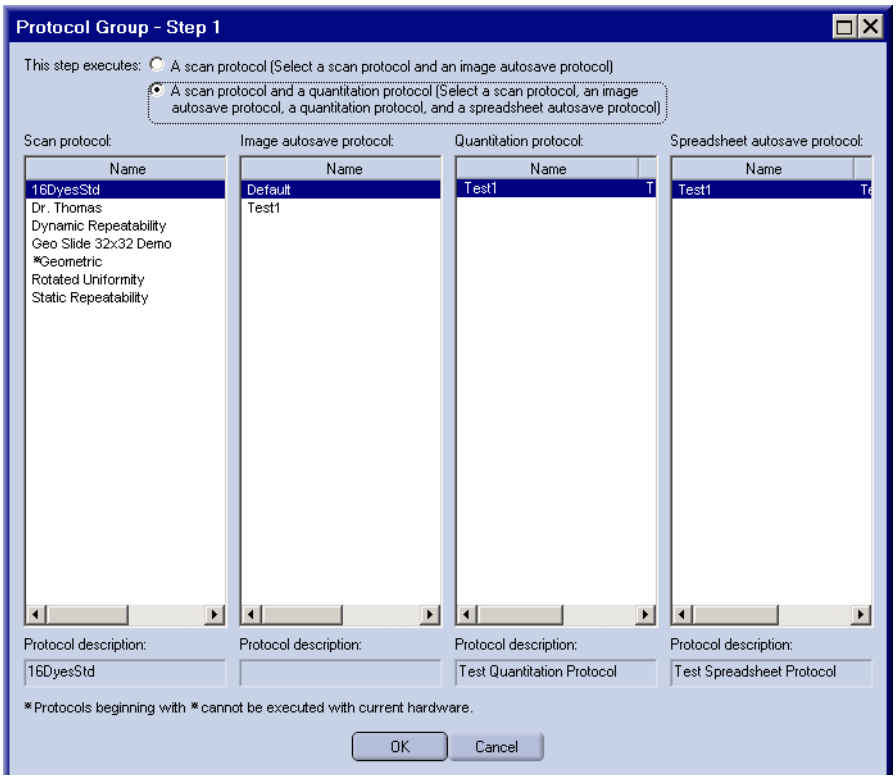


Figure 5–2 Creating a Step for Scanning and Quantitating

2. Select a Scan protocol, Image autosave protocol, Quantitation protocol, and Spreadsheet autosave protocol.

3. Click **OK** to save the step to the Protocol Group.

4. Repeat for each step that you want to add to the protocol group. You can add as many steps as you want.

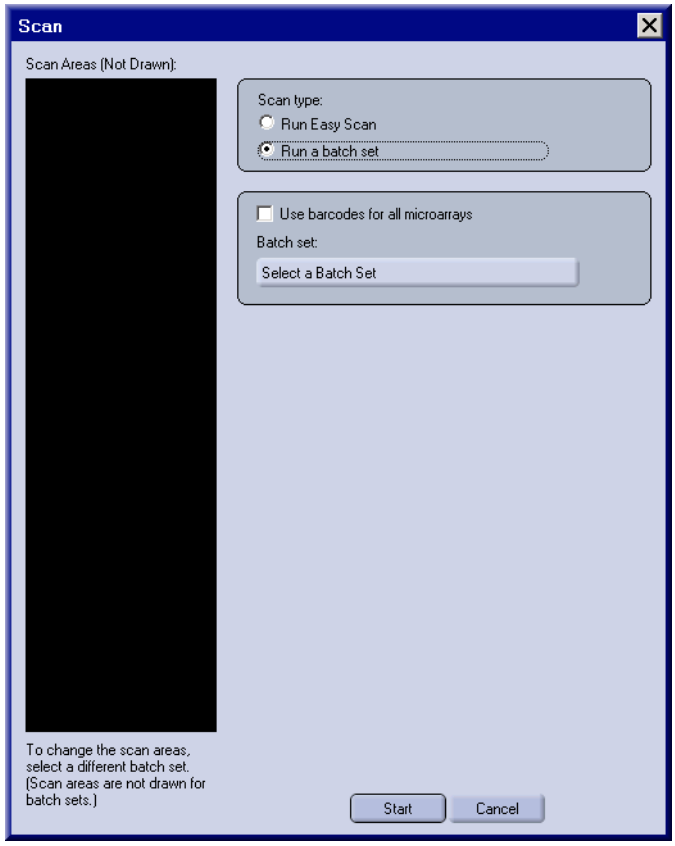
6.3 Running and Creating Batch Sets

Batch Sets perform scans on one to twenty slides in an autoloader cassette.

If you haven't done so already, you need to create a Batch Set definition that specifies which slots in the cassette hold microarrays, and which protocol or protocol group to run on the microarray in that slot. See ***Creating a Batch Set*** on page 6-8.

To run a batch set

1. On the *Main Window*, click **Scan**. The *Scan* window opens, where you can select **Run a batch set**. You cannot changes settings from this window, including the scan area. To make any changes, you need to select a different batch set, or edit the batch set.



2. Click **Use barcodes for all microarrays** to get the protocol information from the microarray barcodes.

   - OR -

   Click **Select a Batch set**. Select a batch set from the window that opens, and click **OK**. The window closes and returns you to the *Scan* window; the button fills with the name of the batch set.

3. Click **Start**.

The ScanArray Express loads the microarray in the first configured slot, and runs the specified protocol group or protocols. The microarray is scanned and the image(s) are saved to the location defined in the image autosave protocol. If quantitation was included for a configured slot, ScanArray Express begins spot finding, then quantitates and saves the results to the location defined in the spreadsheet autosave protocol. The images and results files are closed, and ScanArray Express unloads the microarray and proceeds to the next configured slot.

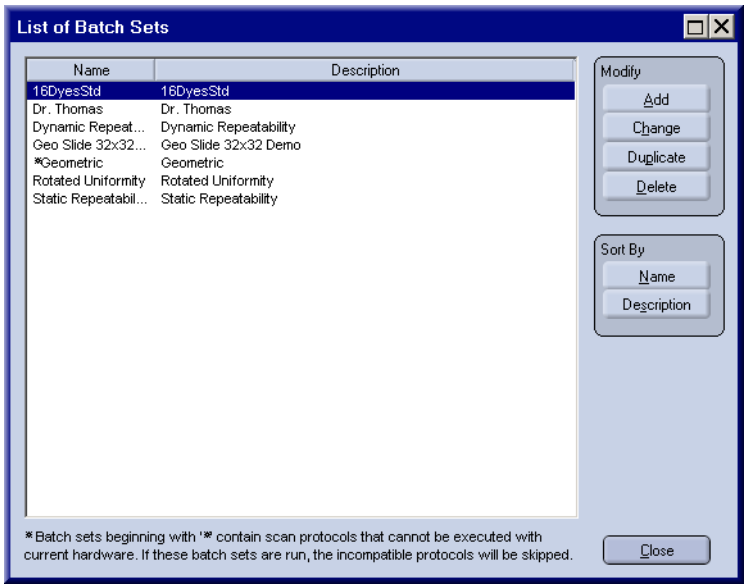
This process continues until all microarrays in the configured slots in the batch set have been processed. The images and results from the microarray in the last processed slot are saved as well, but remain displayed on the screen. You can close these files, or open any of the other images/results that have been saved.

After running a batch set, you can check the log to verify that all microarrays were processed.

6.3.1 Creating a Batch Set

To create a batch set

1. On the *Main Window,* click **Configure** in the *Configure & File* group of the main window, then click **Batch Set**. The *List of Batch Sets* window displays.



2. Sort the batch sets (optional) by **Name** or **Description**.

3. Click one of the following to open the *Batch Set* window:

   - **Add** to create a new batch set.
   - **Change**, after selecting a batch set, to modify an existing set.
   - **Duplicate**, makes a duplicate of the highlighted batch set to help you create a new batch set from one that is already close to what you want.

   Or, click:

   - **Delete,** after selecting a batch set, to delete the set.

4. Click **Add**. The *Batch Set* window opens.

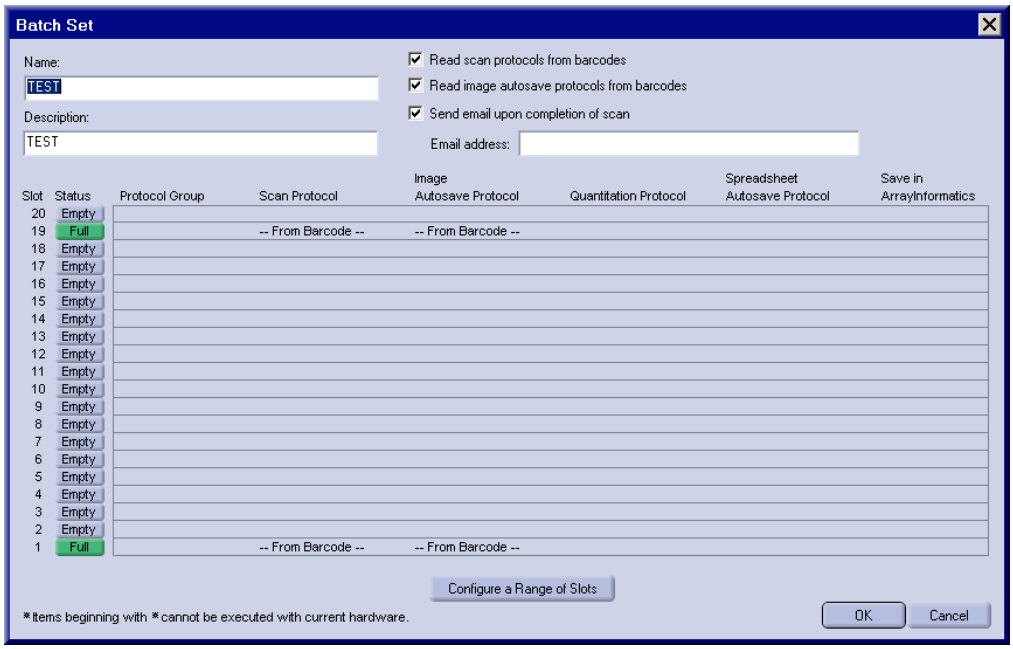


Figure 5–3 Batch Set Window

5. Enter a name for the batch set, and specify parameters for the set, and configure each slot to be used. Refer to the following table for information.

Item	Description
Name	Enter a name for the batch set; use a name that will help you identify the batch set.
Description	Enter a description for the batch set.
Read scan protocols from barcodes	Check this box to read information in the microarray barcode on which protocols to run.
Read image autosave protocols from barcodes	Check this box to read the image autosave protocol information from the microarray barcodes.
Send email upon completion of scan	Check this box to send an email after scanning has completed running the batch set; enter an appropriate email address.
Slot Status	Set or change the status of each slot by clicking the **Status** button next to the slot number. The options are Full, Empty, or Ignored. The default is **Empty.** See ***Configuring a Slot*** on page 6-10.
Configure a Range of Slots	Click this button to open the *Range of Cassette Slots* window where you can set the same parameters for an entire range of slots. See ***Configuring a Range of Slots*** on page 6-11.

6.3.2 Configuring a Slot

To configure a single slot

1. Click the status button for the slot, and change the status to **Full**. The *Cassette Slot (Number)* window opens.

2. Select the button for what you want to execute for this slot:

   - a scan protocol,
   - both a scan protocol and quantitation protocol, or
   - a protocol group. a scan protocol,

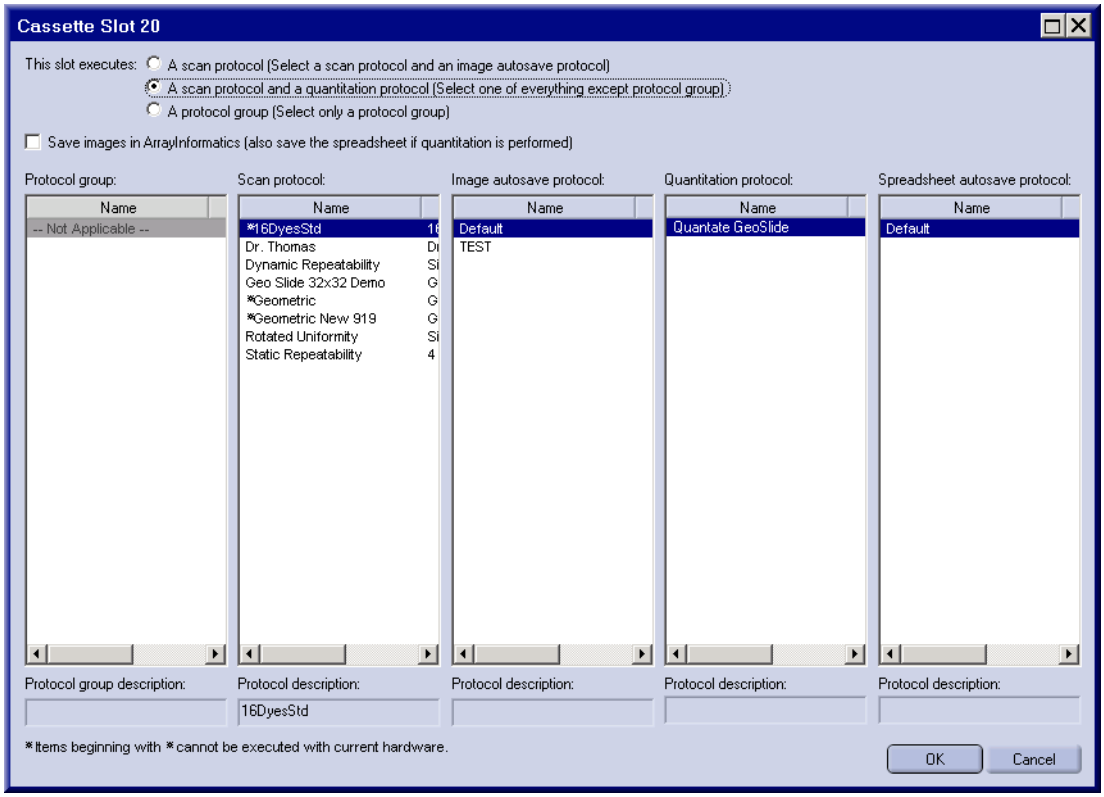


Figure 5–4 Cassette Slot Window

The corresponding boxes are enabled for a protocol group or protocols and display a list of available protocols.

3. If you want to save your results automatically in ArrayInformatics, check the box to save the images (and results if quantitation is performed) in ArrayInformatics. If your ScanArray Express is not integrated with ArrayInformatics this box will be dimmed and unavailable to select.

4. Select the protocol group, or protocols to execute, and click **OK**.

6.3.3 Configuring a Range of Slots

To configure a range of slots

1. Click **Configure a Range of Slots** on the *Batch Set* window. The *Range of Cassette Slots* window opens.

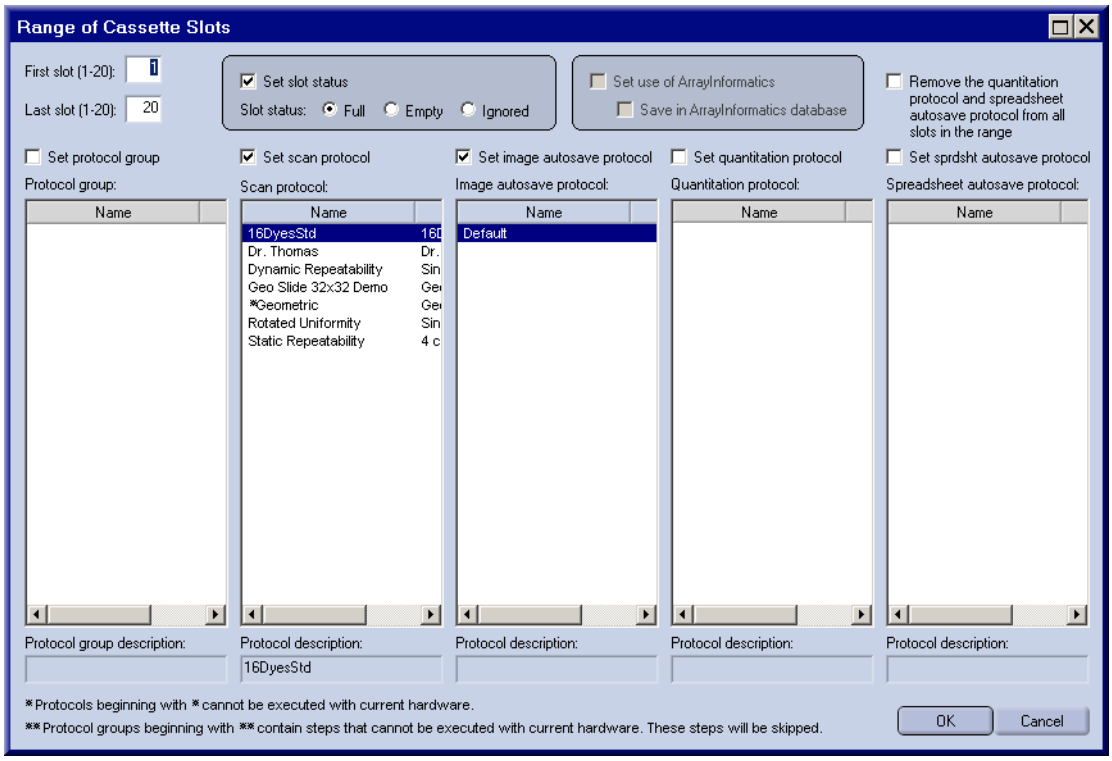


Figure 5–5 Range of Cassette Slots Window

2. Refer to the following table, and set the parameters for the range of slots.

Item	Description
First Slot Last slot	Enter the number of the first slot and the number of the last slot to include in the range.
Set slot state	Check this box to set the status for all slots in the range.
Set use of ArrayInformatics	Check this box to enable the selection box below it for saving in ArrayInformatics. If your system is not integrated with ArrayInformatics, this box is be dimmed and unavailable to select.
Save to ArrayInformatics Database	Check this box to automatically save the scan images and/or quantitation data from this range of slots in the ArrayInformatics database.
Remove the quantitation protocol from all slots in the range	Check this box to easily remove the quantitation protocol and the spreadsheet autosave protocol from all of the slots in the range that you are configuring.

Item	Description
Set protocol group	Check this box to select one or more protocol groups to run on the range of slots. Checking this box enables the Protocol Group box, where any configured protocol groups will display.
Set scan protocol	Check this box to select one or more scan protocols to run on the range of slots. Checking this box enables the Scan protocol box, where any configured scan protocols will display.
Set image autosave protocol	Check this box to select an image autosave protocol to save the scanned images automatically. Checking this box enables the Image autosave box, where any configured protocols will display.
Set Quantitation protocol	Check this box to select one or more quantitation protocols to run on the range of slots. Checking this box enables the Quantitation protocol box, where any configured protocols will display.
Set spreadsheet autosave protocol	Check this box to select a spreadsheet autosave protocol to save the quantitation results automatically. Checking this box enables the Spreadsheet autosave box, where any configured protocols will display.

3. When you are finished configuring the range, click **OK** to save your changes and return to the *Batch Set* window.

System Settings

Chapter 7

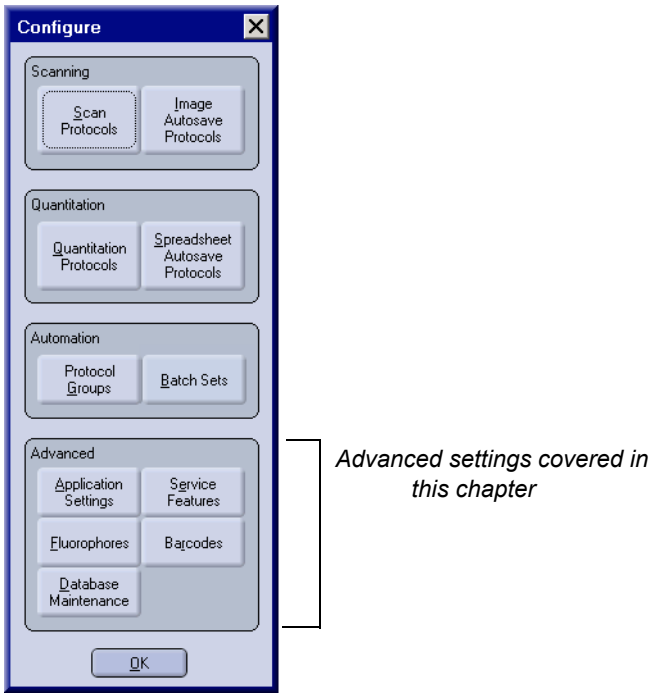
Chapter Summary

7.1 Overview

ScanArray Express settings that are used frequently, or those that affect the operation of the ScanArray Express system, can be specified and stored using the *Configure* menu. Settings include those for configuring fluorophores and barcodes, and the system-wide application settings, such as network connection. For systems that are integrated with ArrayInformatics, the pathnames for automatically saving images and data to an ArrayInformatics database are specified here.

To access the system settings

1. In the *Main Window*, click **Configure** in the *Configure & File* group. The *Configure* menu displays.



7.2 ScanArray Express Settings

You can dynamically change most software settings without restarting the instrument. You must have local administrator privileges to change these settings.

To set or change application settings

1. In the *Configure* menu, click **Application Settings**. The *Application Settings* dialog displays.

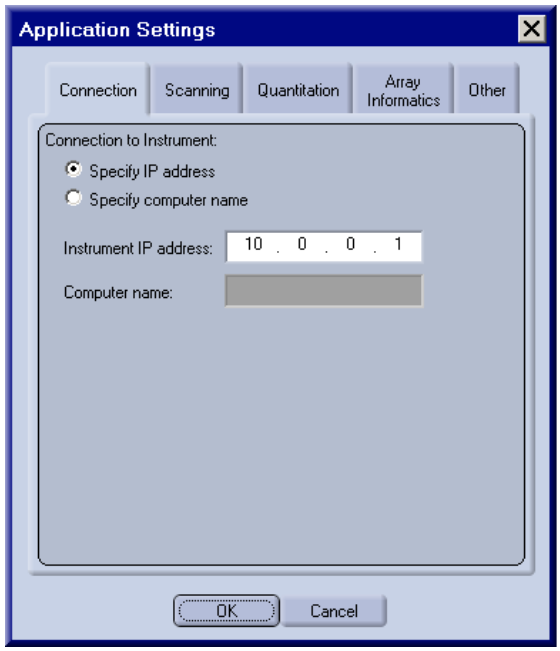


Figure 7–1 Application Settings Dialog Box

2. Click the tab for the settings you want to set or change, and refer to the appropriate section below.

3. Click **OK** when you are finished with all settings that you wish to set or change.

7.2.1 Connection Settings

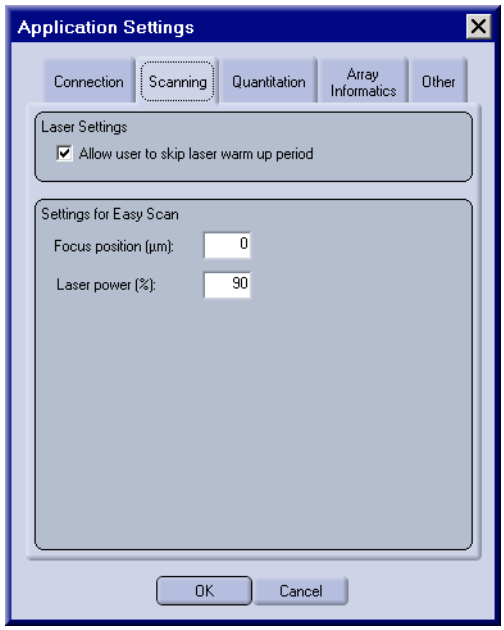
On the *Connection* tab, select either **Specify IP address** or **Specify computer name**. The appropriate field becomes active, where you can enter the IP address or the computer name. The application must be restarted for the new instrument IP address/computer name to take effect.

7.2.2 Scanning Settings

You can allow the user to skip the laser warm-up, and set default focus position and laser power for Easy Scan.

To set the scanning settings

1. Click the **Scanning** tab.



2. Refer to the following table.

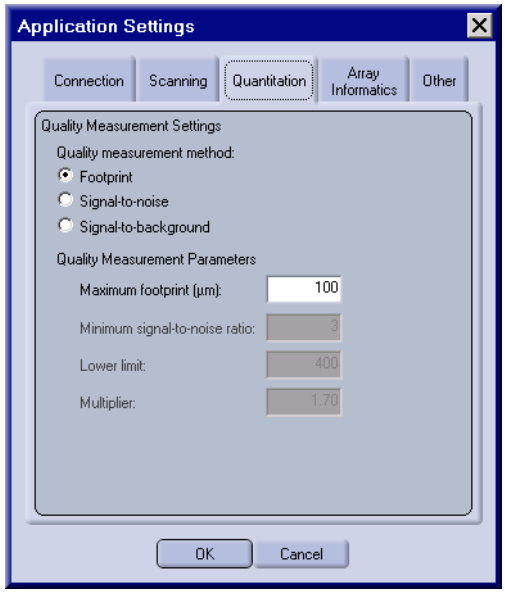
Item	Description
Allow user to skip laser warm up period	Check this box to allow the user to start scanning before the lasers are warmed up (5 or 15 minutes, depending on the type of laser). This does not cause any damage, but the sensitivity of the resulting scans will vary. If the user is concerned with repeatability or uniformity of scanning, they should wait for the lasers to warm up.
Settings for Easy Scan	This defines the settings of the focus position and laser power for all Easy Scans. These settings are not changeable from the *Scan* window.
Focus Position (µm)	The only time the focus position needs to be changed is when the user changes to a different type of slide substrate, such as a gel or membrane slide, where the imaging surface is at a different height than it is with a glass side. The default is 0 (zero). Valid values range from -150 to 2000 (µm).
Laser Power (%)	The default is 90 percent. Valid values range from 0 to 100 percent.

7.2.3 Quantitation Settings

The quantitation settings let you set some default system settings for quantitation.

To change quantitation settings

1. Click the **Quantitation** tab.



2. Refer to the following table and enter your settings.

Item	Description
Quality Measurement Settings	These settings are used for the quantitation methods.
Quality measurement method	Select a method to for quality measurement. Formulas for methods are provided in Appendix D. Footprint Signal-to-noise Lower limit and multiplier
Quality Measurement Parameters	These fields are active or inactive, depending on which Quality measurement method you selected.

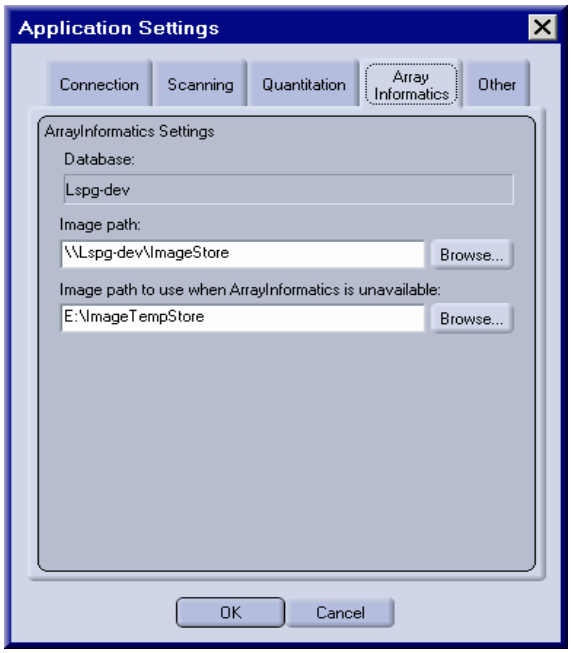
7.2.4 ArrayInformatics Settings

For ArrayInformatics users only, the fields on this tab let you see which ArrayInformatics database the ScanArray Express is connected to, and lets you specify the pathname for saving image files.

Note: Changing the active database must be done using ArrayInformatics Database Tools installed on the ScanArray Express client computer. For more information, refer to the Integration Guide that ships with the ArrayInformatics software.

To set the ArrayInformatics Settings

1. Click the **ArrayInformatics** tab.



2. Refer to the following table and specify your settings.

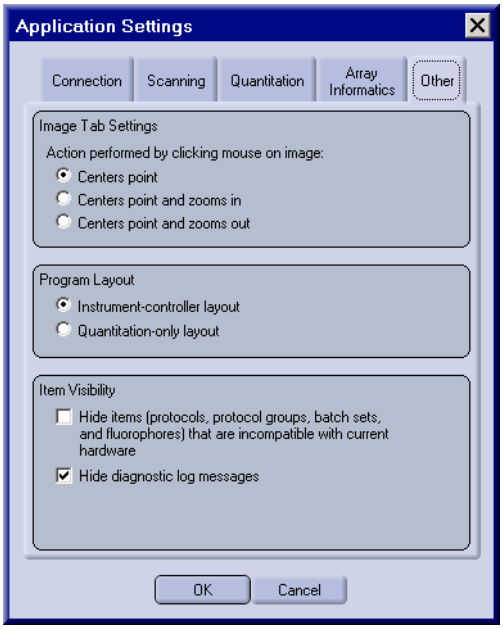
Item	Description
Database	This field displays the database that is currently active for this ScanArray Express system. The database cannot be changed from this window, but must be changed using ArrayInformatics Database Tools.
Image Path	Browse the network to locate and select the directory that has been set up for saving the scan images. Images that are saved to ArrayInformatics are saved to this location.
Image Path to use when ArrayInformatics is unavailable.	This is a local directory where scanned images are saved if the ArrayInformatics is temporarily unavailable, or some other error prevents saving in ArrayInformatics. Images that are saved to this directory can later be sent to the ArrayInformatics database.

7.2.5 Other Settings

Settings on the *Other* tab include those that affect what is seen on the user interface.

To set or change these settings

1. Click the **Other** tab.



2. Refer to the following table and specify your settings.

Item	Description
Image Tab Settings	Select a method to set the behavior of the mouse click on all image tabs.
Action performed by mouse click on image	Centers point Centers point and zooms in Centers point and zooms out
Program Layout	Select whether the ScanArray Express system is to show both scanning and quantitation controls, or only quantitation controls. - Instrument-Controller layout - Quantitation-Only layout - the user see only Quantitation buttons and windows. The scan button does not display.
Item Visibility	Check one or both boxes: Hide items (protocols, protocol groups, batch sets and fluorophores) that are incompatible with current hardware. If this box is not checked, the incompatible items display, marked with an asterisk.
Hide diagnostic log messages	Keeps the diagnostic instrument messages from displaying in the Log tab on the *Main Window*.

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

7.3 Configuring Fluorophores

Fluorophores specify the laser wavelength and emission filter used to scan a microarray. The ScanArray Express ships with a number of pre-defined system fluorophores. These cannot be deleted and only the display palette field assigned to the fluorophore may be changed. User-defined fluorophores may be added, duplicated, changed and deleted as specified below. A fluorophore currently specified in a protocol may not be deleted.

7.3.1 Defining a Fluorophore

To add a new fluorophore to ScanArray Express:

1. On the *Main Window*, click **Configure** in the *Configure & File* group, then click **Fluorophores** . The *List of Fluorophores* window displays.

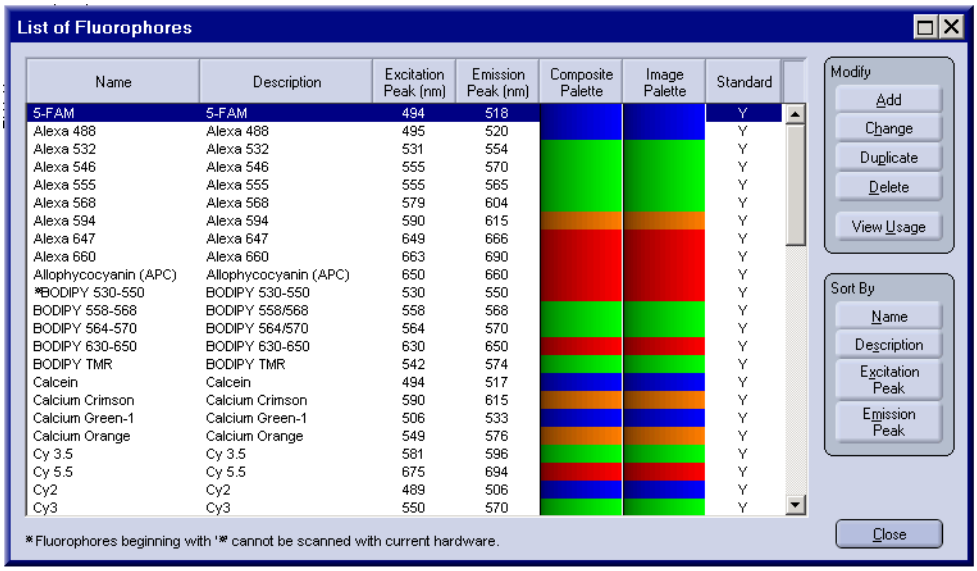


Figure 7–2 List of Fluorophores Window

2. Sort the list (optional) by **Name**, **Description**, **Excitation Peak** or **Emission Peak**.

3. Click one of the following in the *List of Fluorophores* window:

   - **Add** to create a new fluorophore.
   - **Change**, after selecting a fluorophore, to modify an existing one.
   - **Duplicate**, makes a duplicate of the highlighted fluorophore to help you create a new fluorophore from one that is already close to what you want.
   - **Delete**, after selecting a fluorophore, to delete it.

View Usage, after selecting a fluorophore, to display a list of protocols which include the selected fluorophore. You cannot delete a fluorophore that is being used by a protocol.

4. Click **Add**. The *Fluorophore* dialog box displays.

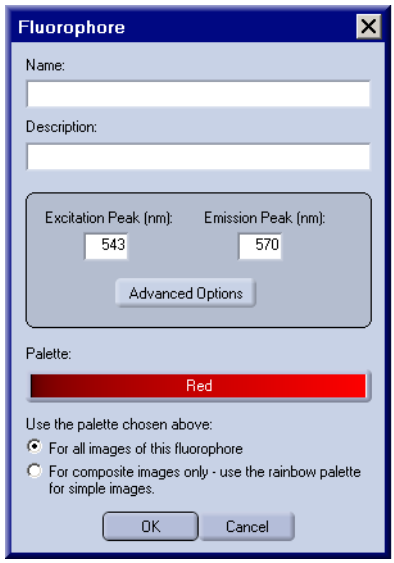
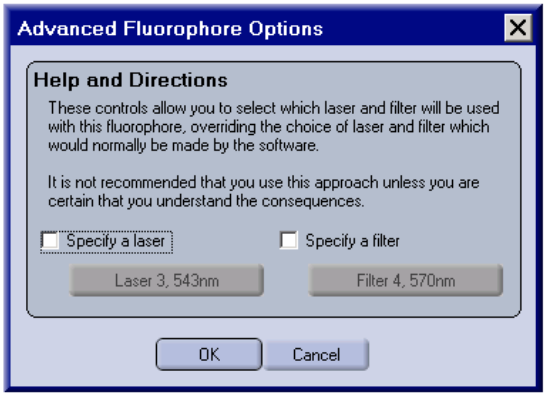


Figure 7–3 Fluorophore Dialog Box

5. Enter the name and a brief description of the fluorophore in the appropriate fields.

6. Enter the **Excitation Peak** and **Emission Peak** of the fluorophore. These values can be obtained from the manufacturer of the fluorophore. Once these values are entered, the ScanArray Express automatically selects the laser and an appropriate filter for the fluorophore.

7. To override the system selection, click **Advanced Options** and enter the desired **Laser** and **Filter,** then click **OK**.



Note: Changing the filter or laser settings is not recommended, as the ScanArray Express has optimized these selections based on the Excitation Peak and Emission Peak entered.

8. Click the **Palette** button to display the palette list. Select the desired palette and click **OK**.

Note: Using the rainbow palette for the simple images provides more visual data by providing a spread of intensities instead of limited shades of just one color. This allows you to differentiate more spots than you can with a single color.

9. Click **OK** to close the *Fluorophore* dialog box and save the new fluorophore.

7.4 Configuring Barcodes

With an optional barcode reader installed, the ScanArray Express can read barcodes on the microarrays. Using barcodes as unique identifiers on individual substrates provides a valuable way for tracking samples and workflow, and automating analysis of microarray experiments. Using barcodes allows a gene sample to be tracked through the entire microarray process including printing, hybridization, scanning, quantitation, and visualization.

For systems that are integrated with ArrayInformatics, unique barcodes on microarrays are required and allow you to retrieve information from and automatically save data to the ArrayInformatics database.

7.4.1 Supported Barcodes

The ScanArray Express barcode reader supports Code 128, Code 39, and Interleaved 2 of 5 (ITF) symbologies by default. The ScanArray Express barcode reader may be configured to support additional symbologies with help from technical support. The maximum number of digits supported by each symbology depends on the minimum element width (the barcode “pitch”) in the printed barcode. Alphabetic and numeric digits can be combined, but alphabetic digits take more space than numbers, thus decreasing the maximum number of allowed digits.

Table 7-1 lists the maximum number of alphanumeric characters for substrate barcodes.

Table 7-1: Substrate Barcodes

Element Width		Number of Characters		
Inches	Mm	Code 128	Code 39	ITF
.010”	.254 mm	6	2	6
.0075”	.19 mm	12	4	10
.005”	.127 mm	22	8	16

Pre-printed barcode labels are commercially available, or you can print your own that conform to the guidelines in Table 7-1. PerkinElmer has qualified the following blank labels:

	Manufacturer	Part Number	Size (inches)	Size (mm)
Substrate	Intermec	E17512	0.920” x 0.550”	23.4 x 14 mm

The label containing the barcode should fit on the first 15 mm of the slide so that it does not tilt the slide in the slide holder, and the label must be centered on the slide.

PerkinElmer has qualified a printer and labels that will withstand hybridization, washing and other processes. The recommended printer is an Intermec 3240 with a 137071206 Super Premium ribbon. The recommended label, also from Intermec is L3203011 2 mil glass poly, with dimensions of 0.920" x 0.550" and a corner radius of 0.04 to 0.06".

Information about the printer and the labels can be obtained from Intermec:

- Internet Address: http://www.intermec.com
- e-mail: info@intermec.com

You must define the barcode parameters, which can include the Image Autosave Protocol and Scan Protocol, as described in the next section.

7.4.2 Defining Barcode Parameters

To define barcode parameters

1. On the *Main Window,* click **Configure** in the *Configure & File* group and then click **Barcodes** to display the *Barcodes* window.

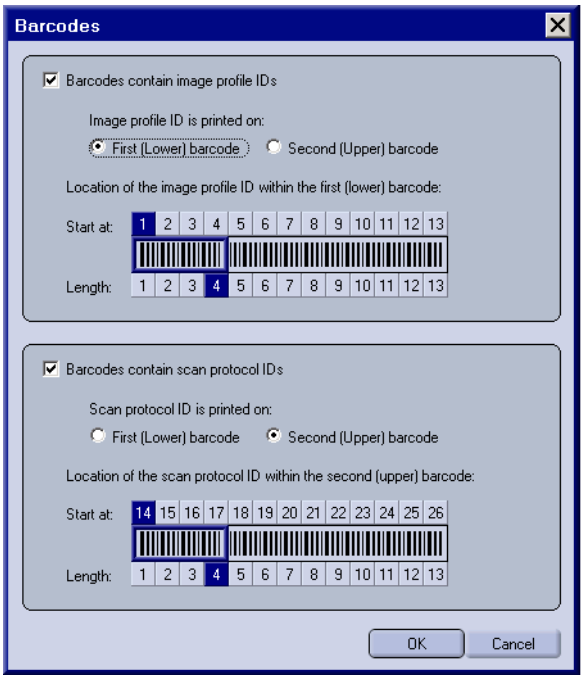


Figure 7–4 Barcode Dialog Box

2. Specify the settings, referring to the following table for information.

Item	Description
Barcodes contain image autosave protocol IDs	Check this box to indicate that the barcode includes the identification of the image autosave protocol to be used when saving the scanned image files. Checking this box enables the barcode fields below it.
Image autosave protocol is printed on	Select **First (lower) barcode** or **Second (upper) barcode** to indicate whether the image autosave protocol information in on the lower or upper barcode on the slide. Enter the starting position on the barcode and the length (1 through 13) of the image autosave protocol information.
Barcodes contain scan protocol information	Check this box to indicate that the barcode includes the identification of scan protocol to be used when scanning this microarray. Checking this box enables the barcode fields below it.

Item	Description
Scan protocol ID is printed on	Select **First (lower barcode)** or **Second (upper) barcode** to indicate whether the scan protocol ID information is on the lower or upper barcode on the slide. Enter the starting position on the barcode and the length (1 through 13) of the scan protocol ID. Barcodes cannot overlap and there must be no common characters.

3. Click **OK** to save the barcode configuration.

7.5 Database Maintenance

The ScanArray Express database is where your protocols, autosave protocols, and data are stored. Make a copy of the ScanArray Express database regularly, to ensure that in the event the database becomes corrupted, a recent backup copy of the database is available to restore. Database maintenance is also used by the database administrator to backup, restore, and repair the database.

Figure 7–5 Database Maintenance Window

Button	Description
Backup Database	Allows you to backup the database.
Restore Database	Allows you to restore the database.
Compact and Repair the Database	Allows you to compact and repair the database. This feature defragments the database in a way that is similar to defragmenting your hard drive for better space utilization, and lets you use the smallest amount of space possible for the database.

7.5.1 Backing up the Database

You should have a set procedure for when to back up the database, and how to name the backup file so that, in the event you need to restore the database, the correct backup file is easily identified.

To back up the database

1. Click **Back Up Database**.
2. Use the *Browse* window to select a folder. A default name for the backup is supplied, and it is recommended to use the default name.

7.5.2 Restoring the Database

Restore the database only if absolutely necessary; that is, if the file has been lost or accidentally deleted or if you have accidentally deleted a protocol.

Note: Avoid restoring from previous versions of the software. If you do restore a backup from a previous software version (for example from v1.1 when running v2.0), the ScanArray Express software will have to be reinstalled.

To restore the database

1. Click **Restore Database.**
2. Use the *Browse* window to find the backup file that was named and saved during backup.

7.6 Service Features

Service features are used for calibration and configuration of the instrument and for controlling the instrument software operation. Certain service features are intended for use by authorized personnel only, or under the direction of service or technical support personnel.

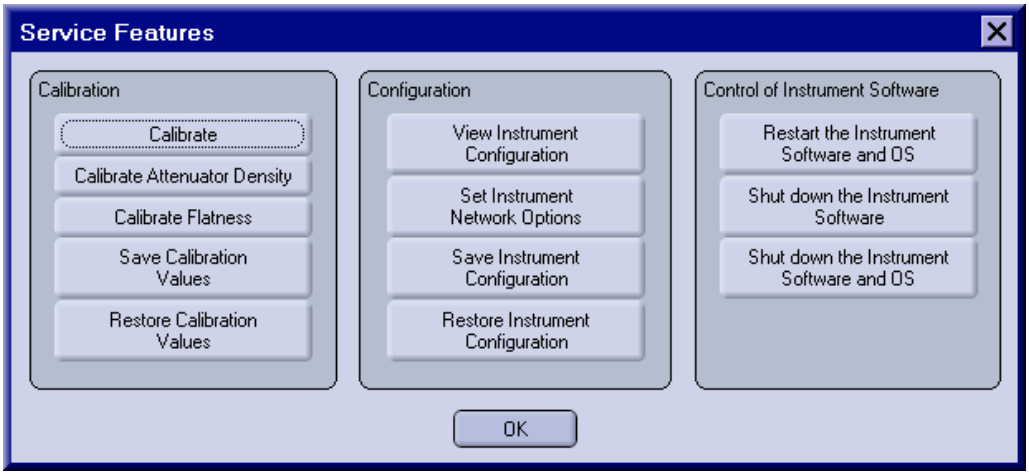


Figure 7–6 Service Features Window

7.6.1 Descriptions

Service features are described in the following table.

Feature	Description
Calibration	
Calibrate	Used by service personnel to calibrate critical instrument parameters; this is password protected to prevent improper usage.
Calibrate Attenuator Density	Used exclusively by service personnel to calibrate the instrument attentuator. Improper usage may cause incorrect operation of the instrument.
Calibrate Flatness	Intended for use under the direction of service or technical support personnel, this feature allows calibration of the X-Y stage flatness. Please consult with technical support before running this feature.
Save Calibration Values	Creates a file containing all instrument-related calibration values. It is recommended to save this file one time in the event of an instrument hard drive failure, although the data is stored in the factory when the instrument is shipped.
Restore Calibration Values	Used exclusively by service personnel. Restoring invalid calibration values can cause hardware damage to the instrument.

Feature	Description
Configuration	
View Instrument Configuration	Information pertaining to the instrument hardware (what is installed, version numbers) and software (version numbers). See Figure 7-7.
Set Instrument Network Options	Allows the user to remotely change the network settings on the instrument. See ***Setting the Instrument Network Options*** on page 7-16.
Save Instrument Configuration	Creates a file containing all instrument-related configuration information. It is recommended to save this file one time in the event of an instrument hard drive failure, although all data is stored in the factory when the instrument is shipped. See Figure 7-7 below.
Restore Instrument Configuration	Used exclusively by service personnel. Restoring invalid instrument configuration information could cause improper operation of the instrument.
Control of Instrument Software	
Restart the Instrument Software and OS	This is used to cause the instrument re-initialize itself. This should be used when the instrument is determined to be in an inoperable state and the user would typically cycle the instrument power to cause the instrument to re-initialize.
Shut down the Instrument Software	Used exclusively by service personnel. Using this feature prevents the user from being able to properly operate the instrument.
Shut down the Instrument Software and OS	This is highly recommended to be used before shutting down the instrument. Before turning off the instrument using the power switch, click this button to observe the instrument's ready LED turn off; wait approximately 30 seconds and then use the instrument's power switch to turn off the instrument.

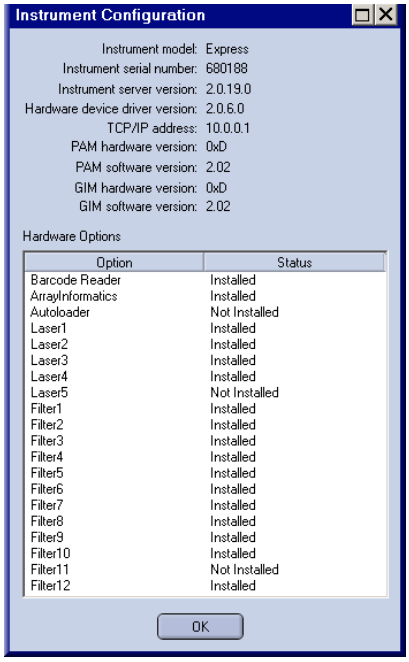


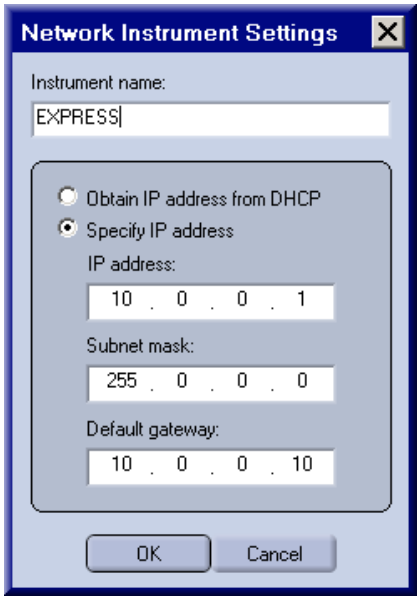
Figure 7–7 The Instrument Configuration Window, from ***Save Instrument Configuration***

7.6.2 Setting the Instrument Network Options

You can change the instrument name (the name seen by the network) and the network settings.

To change the network settings

1. On the *Service Features* menu, click **Set Instrument Network Options**. The following window opens.



2. Select **Obtain IP address from DHCP**, which allows the instrument's network settings to be determined dynamically at startup, or **Specify IP address**, which gives the instrument a fixed set of network settings.

3. Click **OK** to send the changes to the instrument. The instrument must be restarted after the network settings have been changed. The recommended way to restart the instrument is to use **Restart the Instrument Software and OS** from the *Service Features* menu.

Diagnostics and Troubleshooting

Chapter 8

8.1 Overview

This chapter discusses diagnostic tools, and basic troubleshooting and error recovery procedures that are available to help identify problems with the instrument.

8.2 Diagnostic Tools

ScanArray Express diagnostic tools include Start-up Diagnostics. In addition, various LEDs on the instrument panel display the status of the instrument.

8.2.1 Start-up Diagnostics

This diagnostic tool is executed automatically each time the instrument is powered ON. If the interlocks are open when the software is started, diagnostics are performed after the interlocks are closed. A system check is performed to ensure that all major functions are operational. The status can be reviewed by clicking the *Diagnostic* tab in the *Main Window*. Status is indicated using Pass/Fail criteria.

Start-up Diagnostic Test	Purpose
Attenuator Initialization	Includes all initialization and homing of the attenuator.
Attenuator Homing	Moves the hardware to accurately locate the home sensor and applies the appropriate calibration offset.
Filter Wheel Initialization	Includes all initialization and homing of the filter wheel.
Filter Wheel Homing	Moves the hardware to accurately locate the home sensor and applies the appropriate calibration offset.
Objective (Z-axis) Initialization	Includes all initialization and homing of Z-axis hardware.

Start-up Diagnostic Test	Purpose
Objective Homing	Moves the hardware to accurately locate the home sensor and applies the appropriate calibration.
Servo (Y-axis) Initialization	Includes all initialization and homing of Y-axis hardware.
Servo Homing	Moves the hardware to accurately locate the home sensor and applies the appropriate calibration offset.
Galvo Interface Module (GIM) Boot	Downloads operational code to the GIM board.
Galvo (X-axis) Initialization	Includes all initialization and homing of X-axis hardware.
Galvo Homing	Moves the hardware to accurately locate the home sensor and applies the appropriate calibration.
PMT Amplifier-A/D Module (PAM) Boot	Downloads operational code to the PAM board.
Laser Initialization	Verifies presence of 1-5 laser modules, and determines type of laser (internal/external, manufacturer).
Laser Communication	Verifies serial communication exists with serial laser, if one is installed. Serial lasers include internal and external and are located in laser positions 4 and 5. A failure to communicate with internal serial laser 4 indicates a potential hardware problem.
Barcode Reader Initialization	Includes all initialization of the optional barcode reader. This includes establishing serial communication with the barcode reader and downloading of all configuration parameters.
Barcode Reader Communication	Verifies serial communication exists with the barcode reader.
Autoloader Initialization	Includes all initialization of the optional the HT autoloader. This includes programming, downloading configuration parameters and homing of the HT.
Autoloader Program Download	Downloads operational code to the HT controller board.
Autoloader Setup	Downloads configuration/calibration parameters to the controller.
Autoloader Elevator Homing	Moves the elevator hardware to accurately locate the home sensor and applies the appropriate calibration offset.

If an item fails the diagnostic test during Start-up, refer to Section 8.3 for troubleshooting tips.

8.2.2 Instrument Statistics Accumulation

The following statistics are recorded and available for review:

- Laser On time
- Total slides loaded/unloaded

8.3 Troubleshooting

8.3.1 Hardware

8.3.1.1 Ready LED Flashes Yellow

The instrument performs a variety of self-tests of different modules upon initial power up. If any one of them fails to initialize properly the ready light will flash yellow.

1. If an external laser is present, ensure the interlock block is installed on the rear panel of the instrument. If it isn't, power down the instrument, install the interlock block and power up the instrument again.

2. If you do not get a steady green light after a minute or so, try removing the front panel and putting it back on again. This will reset the two safety interlock switches.

3. Power down the instrument and power it up again. If the Ready LED continues to flash yellow, contact PerkinElmer Life Sciences.

8.3.1.2 External Laser Will Not Turn On

1. Ensure the interlock on the rear panel of the external laser is plugged in. If it isn't, power down the instrument, install the interlock block and power up the instrument again.

2. If the laser still does not turn on, contact PerkinElmer.

8.3.1.3 Microarray is Jammed

If a microarray becomes jammed the ScanArray Express attempts to correct the problem with a PURGE sequence.

To run a manual PURGE sequence

1. The HT system includes sensors that monitor the location of the microarray. If a microarray becomes jammed, a PURGE sequence is initiated by the software that locates the jam and places the microarray back into the cassette. An error message displays on the client computer.

2. If a PURGE Sequence does not fully eject the microarray into the cassette, a message displays and you will have to remove the microarray manually by removing the front cover, removing the cassette and extracting the microarray from the system.

8.3.1.4 Poor Image Uniformity

Contact Technical Support at 800-551-2121, or 617-350-9263.

8.3.2 Software

8.3.2.1 Unable to Open Stored .TIFF files

1. Make sure the stored file names do not include dots (.) other than the dot preceding the file extension (e.g., .tif, .csv, .gpr), slashes (/), back-slashes (\), or dashes (-) in the file name.

2. Make sure there is enough free space on the hard disk to open the file. If there is not, locate any unnecessary image files and delete them to free up space.

3. If you are still unable to open the files, contact PerkinElmer.

8.4 Recording the ScanArray Express System Activity

A *system log,* or *event log*, keeps a record of all ScanArray Express activity in a log file. In the file, the ScanArray Express system logs the date and time of the activity, the type and severity of the event, the current user (as logged onto Windows 2000/XP), and a description of the event. This log may be helpful in tracking operations or for use by Service personnel to diagnose any problems.

The system log tracks the following user requests to:

- start or stop a scan
- add, update, and delete parameters
- modify system configuration
- start-up and shut down the ScanArray Express application

and tracks the following information:

- changes in the status of client and instrument software communication
- software and hardware failures

8.4.1 Viewing the System Log

1. On the ScanArray Express *Main Window*, click the **Log** tab.

2. Use the scroll bar to move through the display as needed.

Supported File Formats

Appendix A

Summary

A.1 Overview

The ScanArray Express supports the following files, which are described in the subsequent sections.

.GAL - An array content file is created by the SpotArray Microarray printer after a printing procedure. This file can be called into ScanArray Express as a template for quantitating.

.GPR - A quantitation results file created by the ScanArray Express. A .GPR file supports only two fluorophores and does not save all of the data columns. See the description below.

.CSV - A text file of quantitation results. A .csv file saves all information that is in the quantitation spreadsheet results.

.TIF - the images can be saved as TIF files, as well as bitmap (.BMP), .JPEG, and RAW files.

A.2 Gene Array List (.gal) Format

The gene array list format is written to an ASCII text file that is viewable by applications such as Microsoft® Notepad and Wordpad. The file contains header information and information for each spot in the microarray.

GAL files conform to the ATF format, a standard tab-delimited text file format readable by many downstream scanning and quantitation software applications. They can be created in Microsoft Excel by saving an Excel spreadsheet as Text (Tab delimited).

A.2.1 Description of File Format

Gene array list files contain two major sections: the header section and the spot data section.

A.2.1.1 Description of the Header Section

The header section describes basic file information and provides information about each of the blocks. Each line is explained in Table A-1:

Table A-1: GAL File Format

Record	Description
Required Lines	
ATF 1.0	First line of an ATF file; the same in all GAL files: File format (ATF) and version (1.0)
8 5	Second line of an ATF file: 8 (number of optional header lines plus 1). 5 (number of spot data columns).
"Type=GenePix Array List v1.0"	Type of file; the same in all GAL files.
Optional Lines	
"BlockCount=4"	Number of blocks described in the file, 4 in this example.
"BlockType=0"	Type of block described, rectangular in this example. 0 = rectangular. 1 = orange-packing #1. 2 = orange-packing #2.
"URL=..."	The URL for the Go To Web command.
"Supplier=PerkinElmer Life Sciences"	The manufacturer that supplied the array or arrayer.
"ArrayerSoftwareName= SpotArray"	The name of the arrayer software.
"ArrayerSoftwareVersion=2.0"	The version number of the arrayer software.
"Block*n*="	The position and dimensions of each block. There is one record for each block, and each record contains 7 fields. Each field is separated by a comma followed by a space. 1. X position of center of top leftmost spot in the block (in µm). 2. Y position of center of top leftmost spot in the block (in µm). 3. Nominal diameter of spots within the block (in µm). 4. Number of columns of spots in the block.

Record	Description
	5. Column spacing of spots (center to center) in the block (in µm). 6. Number of rows of spots in the block. 7. Row spacing of spots (center to center) in the block (in µm). **Note:** Positions on arrays are measured in microns with respect to the origin, which is the top left corner of the slide.
Required Line	
"Block" "Column" "Row" "Name" "ID"	Last line of the header, containing column titles for the spot data records.

A.2.1.2 Description of the Spot Data Section

The spot data section contains lines that describe each spot in detail. It includes the block, column, and row numbers for spots, as well as descriptive name and identifier information.

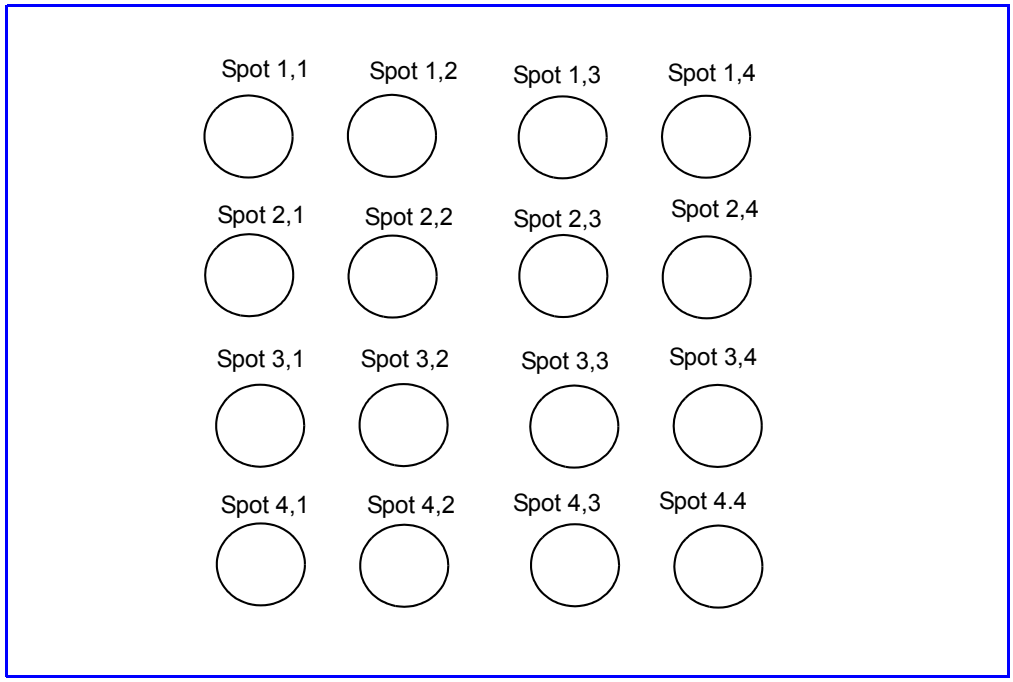
There is one text line for each spot in the microarray, containing a field for each of the descriptive columns. Each line contains tab-delimited fields in the order described in Table A-2.

Table A-2: Spot Data

Field	Description
Block	Block number for the spot.
Column	Column where the spot is located within a block.
Row	Row where the spot is located within a block.
Name	Gene name (limited to 40 characters)
ID	Gene identifier (limited to 40 characters)

A.2.2 Blocks (Subarrays)

A block is a set of spots printed by one pin within a 4.5 mm x 4.5 mm square or a 9 mm x 9 mm square area. Blocks are also called subarrays. Blocks are numbered from top left to bottom right. For example:



A.2.3 Example GAL file

The following simple array list file describes four blocks ("BlockCount=4"), each with 24 columns and 5 rows. For simplicity, the data record information (name, ID, etc.) is included only for the first two spots:

```
ATF        1.0
8          5
"Type=GenePix ArrayList V1.0"
"BlockCount=4"
"BlockType=0"
"URL=http://www.perkinelmer.com/microarray/
"Supplier=PerkinElmer Life Sciences"
"ArrayerSoftwareName=SpotArray"
"ArraySoftwareVersion=1.0"
"Block1= 400, 400, 100, 24, 175, 5, 175"
"Block2= 4896, 400, 100, 24, 175, 5, 175"
"Block3= 400, 4896, 100, 24, 175, 5, 175"
"Block4= 4896, 4896, 100, 24, 175, 5, 175"
"Block"  "Column"  "Row"  "Name"  "ID"
 1         1         1      VPS8    YAL002W
 1         2         1      NTG1    YAL015C
```

A.3 .GPR Results File Format

A .GPR file is a Results file containing general information about image acquisition and analysis, as well as the data extracted from each individual feature.

A.3.1 GPR Header

A sample header file and a description of each entry is included in Table A-3 below.

Table A-3: .GPR File Format

Entry	Description
ATF 1.0	File type and version number.
24 43	Number of optional header records and number of data fields (columns).
"Type=PerkinElmer results 2.0"	Type of ATF file.
"DateTime=2000/02/09 17:15:48"	Date and time when the image was acquired.
"Settings=Geo Slide 32 x 32 Demo"	The name of the settings file that was used for analysis.
"GalFile=C\Program Files\PerkinElmer\ScanArray Express\Samples\GeoSample32x32.gal"	The Array Content List file used to associate Names and IDs to each entry.
"Scanner=Express Serial No.: 680065"	Type of scanner used to acquire the image.
"Comment=hyb 2673"	User-entered file comment.
"PixelSize=10"	Resolution of each pixel in µm.
"ImageNAME=633 nm 543 nm"	Name of each image buffer.
"FileNAME=C:\Program Files\PerkinElmer\ScanArray Express\Samples\GeoSample32x32-Cy5.tif C:\Program Files\PerkinElmer\ScanArray Express\Samples\GeoSample32x32-Cy3.tif	File name for each image buffer. In a multi-image tiff file, the same name is repeated.
"PMTVolts=600 600"	The voltage of the PMTs during acquisition.
"NormalizationFactor:RatioOfMedians=0.819944"	The Ratio of Medians Normalization Factor
"NormalizationFactor:RatioOfMeans=0.848769"	The Ratio of Means Normalization Factor.
"NormalizationFactor:MedianOfRatios=0.843367"	The Median of Ratios Normalization Factor.
"NormalizationFactor:MeanOfRatios=0.427747"	The Mean of Ratios Normalization Factor.
"NormalizationFactor:RegressionRatio=1.0055"	The Regression Ratio Normalization Factor.
"JpegImage="	Name of optional compressed image saved with the results file.
"RatioFormulation=W2/W1 (543/633)"	The ratio formulation of the ratio image, showing which image is numerator and which is denominator.

Entry	Description
"Barcode=00331"	The barcode symbols read from the image.
"ImageOrigin=0, 0"	The origin of the image relative to the scan area.
"JpegOrigin=390, 4320"	the analysis Blocks) relative to the scan area origin.
"Creator=ScanArrayExpress, Microarray Analysis System 2.0.0.0"	The version of the ScanArray Express software used to create the Results file.
"Temperature=1.5"	The temperature of the scanner in volts.
"LaserPower=1 1"	The power of each laser in volts.
"LaserOnTime=5 5"	The laser on-time for each laser in minutes.
Data record column headings	Column titles for each measurement (see below).
Data Records	Extracted data.
GAL FILES	Gene Array List files describe the size and position of blocks, the layout of feature-indicators in them, and the names and identifiers of the printed substances associated with each feature-indicator.

A.3.2 GPR Data

Table A-4 below describes each column in the GPR file.

Table A-4: .GPR Data

Column Title	Description
Block	the block number of the feature.
Column	the column number of the feature.
Row	the row number of the feature.
Name	the name of the feature derived from the Array List (up to 40 characters long, contained in quotation marks).
ID	the unique identifier of the feature derived from the Array List (up to 40 characters long, contained in quotation marks).
X	the X-coordinate in µm of the center of the feature-indicator associated with the feature, where (0,0) is the top left of the image.
Y	the Y-coordinate in µm of the center of the feature-indicator associated with the feature, where (0,0) is the top left of the image.
Dia	the diameter in µm of the feature-indicator.

Column Title	Description
F633 Median	median feature pixel intensity at wavelength #1 (633 nm).
F633 Mean	mean feature pixel intensity at wavelength #1 (633 nm).
F633 SD	the standard deviation of the feature pixel intensity at wavelength #1 (633 nm)
B633 Median	the median feature background intensity at wavelength #1 (633 nm).
B633 Mean	the mean feature background intensity at wavelength #1 (633 nm)
B633 SD	the standard deviation of the feature background intensity at wavelength #1 (633 nm).
% > B633 + 1 SD	the percentage of feature pixels with intensities more than one standard deviation above the background pixel intensity, at wavelength #1 (633 nm).
% > B633 + 2 SD	the percentage of feature pixels with intensities more than two standard deviations above the background pixel intensity, at wavelength #1 (633 nm).
F633 % Sat.	the percentage of feature pixels at wavelength #1 that are saturated.
F543 Median	median feature pixel intensity at wavelength #2 (543 nm).
F543 Mean	mean feature pixel intensity at wavelength #2 (543 nm).
F543543 SD	the standard deviation of the feature intensity at wavelength #2 (543 nm).
B543 Median	the median feature background intensity at wavelength #2 (543 nm).
B543 Mean	the mean feature background intensity at wavelength #2 (543 nm).
B543 SD	at wavelength #2 (543 nm).
% > B543 + 1 SD	at wavelength #2 (543 nm).
% > B543 + 2 SD	the percentage of feature pixels with intensities more than two standard deviations above the background pixel intensity, at wavelength #2 (543 nm).
F543 % Sat.	the percentage of feature pixels at wavelength #2 that are saturated.

Column Title	Description
Ratio of Medians	the ratio of the median intensities of each feature for each wavelength, with the median background subtracted.
Ratio of Means	the ratio of the arithmetic mean intensities of each feature for each wavelength, with the median background subtracted.
Median of Ratios	the median of pixel-by-pixel ratios of pixel intensities, with the median background subtracted.
Mean of Ratios	intensities, with the median background subtracted.
Ratios SD	the standard deviation of pixel intensity ratios.
Rgn Ratio	the regression ratio.
Rgn R^2	the coefficient of determination for the current regression value.
F Pixels	the total number of feature pixels.
B Pixels	the total number of background pixels.
Sum of Medians	the sum of the median intensities for each wavelength, with the median background subtracted.
Sum of Means[	the sum of the arithmetic mean intensities for each wavelength, with the median background subtracted.
Log Ratio	log (base 2) transform of the ratio of the medians.
Flags	the type of flag associated with a feature.
Normalize	the normalization status of the feature (included/not included).
F1 Median - B1	the median feature pixel intensity at wavelength #1 with the median background subtracted.
F2 Median - B2	the median feature pixel intensity at wavelength #2 with the median background subtracted.
F1 Mean - B1	the mean feature pixel intensity at wavelength #1 with the median background subtracted.
F2 Mean - B2	the mean feature pixel intensity at wavelength #2 with the median background subtracted.
SNR 1	the signal-to-noise ratio at wavelength #1, defined by (Mean Foreground 1- Mean Background 1) / (Standard deviation of Background 1)
F1 Total Intensity	the sum of feature pixel intensities at wavelength #1
Index	the number of the feature as it occurs on the array.
"User Defined"	user-defined feature data read from the GAL file.

A.4 .CSV Results File Format

The .CSV Results file contains sections enclosed by BEGIN SectionName and END SectionName. Each section contains zero, one, or multiple rows between the BEGIN and END. Each row contains data items separated by commas.

The major sections of a .CSV file are described in Section A.4.1. Each field in the file are described in the Section A.4.2.

A.4.1 Description of the File Format

Table A-5 lists the major sections of the ScanArray Express .CSV file and a brief description of each.

Table A-5: Description of Major File Sections

Section	Description
Header	Contains information about the product and version number, and the ArrayInformatics database name, if the data is to be saved to ArrayInformatics.
General	Contains date, instrument information, user log-in name, and other
Quantitation Parameters	Contains values for the quantitation method selected.
Quality Measurement Critieria	Contains the values for quality measurement.
Arary Pattern Information	Contains descriptive information about the spot size, spacing, and array layout.
Image Information	Contains information about the image, for example the fluorophore, the barcode, etc.
Normalization Information	Contains values for the normalization method.
Data	The first row contains the labels and the following rows are the measurements, one for each spot. There may be up to four Channels. Note: Chx in the following columns represents Channels x, (x = 1,2,3,4)

A.4.2 Description of the File Fields

The following is a description of each field in the .CSV file.

BEGIN HEADER

PerkinElmer Life Sciences. //name of the company

ScanArrayCSVFileFormat,2.0//name and version of the file format

ScanArray Express,2.0 //name and version of the software

AIDBServerName, SomeName //Added for AIDB, this row is available only if the data was intended for AIDB

Number of Columns, 62 (Actual Number of columns here) // number of Data columns, number of data records

END HEADER

BEGIN GENERAL INFO

DateTime,12/12/01 8.00 //Date and time when the image was acquired in 24 hour format

GalFile,c:\images\test.gal //gal file which associates names and ids

Scanner, //type of scanner used, Model and serial number

User Name, //Windows Logged in user name.

Computer Name,//Computer Name

Protocol, //Scan Protocol used to scan the images

Quantification Method//Quantiation method

Quality Confidence Calculation, //Quality criteria user

User comments, //user comments

Image Orgin,0,0 //image origin relative to the scan area

Temperature, //temperature of the scanner in volts

Laser Powers,10,10 //laser power

Laser On Time, // Laser on time for each laser in minutes

PMT Voltages,10,12 //voltages

InstrumentID,//Added for AIDB this is the AIDB instrument token returned by AIDB, present only if the data is intended for AIDB

END GENERAL INFO

BEGIN QUANTIATION PARAMETERS

Values in this section depend on the quantitation method selected.

For Adaptive Circle (Express) method:
Minimum Percentil, value
Maximum Percentile, value
For Histogram Method:
Low Signal %, Value

High Signal %, Value
Low Background %, value
High Background %,value
For Fixed Circle:
Low Signal %, Value
High Signal %, Value
Low Background %,value
High Background %,value
Nominal Spot Diameter, value
Inner Background Diameter, value
Outer Background Diameter, value
For Adaptive Threshold Method:
Maximum Spot Diameter, value
Inner Background Diameter, value
Outer Background Diameter, value
p-value, value

END QUANTITATION PARAMETERS

BEGIN QUALITY MEASUREMENT CRITERIA

Selected Criteria, FootPrint/SignalToNoiseRatio/SignalToBackground.
MaximumFootprint,value
or
MinSignalToNoise,value
Or
LowerLimit, value
Multiplier, value

END QUALITY MEASUREMENT CRITERIA

BEGIN ARRAY PATTERN INFO

Units,Microns
Array Rows,1
Array Columns,1
Spot Rows,32
Spot Columns,32
Array Row Spacing,2000.00
Array Column Spacing,2000.00
Spot Row Spacing,201.04
Spot Column Spacing,200.52
Spot Diameter,100.00
Interstitial,0,0 is off 1 is first one missing; 2 is second one missing
Spots Per Array,1024
Total Spots,1024

END ARRAY PATTERN INFO

BEGIN IMAGE INFO

The first row contains the labels and the following rows are the information, one for each image.
Lables:
ImageID Channel,Image,Fluorophor,Barcode,Units,X Units Per Pixel,Y Units Per PixeL,X Offset,Y Offset,Status
An example of the following rows:
ch1,F:\Program Files\Packard BioChip\DSOMA\ExperimentSets\qa-4\Images\TAMRA.TIF,TAMRA04030270 ,E000CS,Microns,20.00, 20.00, 0, 0,Control Image

END IMAGE INFO

BEGIN NORMALIZATION INFO

Normalization Method = //Normalization method
Normalization Factor = //normalization Factor

END NORMALIZATION INFO

BEGIN DATA

The first row contains the labels and the following rows are the measurements, one for each spot.

Note: Chx in the following columns represents Channels x, (x = 1,2,3,4)

The labels of the columns:

Following columns present for each spot

Index
Array Row
Array Column
Spot Row
Spot Column
Name
ID
X
Y
Diameter
F Pixels
B Pixels
chx Footprint
Flags
Following columns Repeated for each channel
Chx Median
Chx Mean

Chx SD
Chx B Median
Chx B Mean
Chx B SD
Chx % > B + 1 SD
Chx % > B + 2 SD
Chx F % Sat.
Chx Median – B
Chx Mean – B
Chx Signal Noise Ratio

Following columns Repeated for Each Non-Control channel

Chx Ratio of Medians
Chx Ratio of Means
Chx Median of Ratios
Chx Mean of Ratios
Chx Ratios SD
Chx Rgn Ratio
Chx Rgn R^2
Chx Log Ratio

Following 2 columns are present if there 2 or more channels quantitated

Sum of Medians
Sum of Means

Following Columns Repeated for each Channel if there are 2 or more channels quantitated.

Chx N Median //normalized median
Chx N Mean
Chx N (Median–B)
Chx N (Mean–B)

Following Columns Repeated for each Non Control Channel

Chx N Ratio of Medians
Chx N Ratio of Means
Chx N Median of Ratios
Chx N Mean of Ratios
Chx N Rgn Ratio
Chx N Log Ratio

END DATA

A.5 .TIF Files

The .TIFF (.TIF) file format -- **T**ag(ged) **I**mage **F**ile **F**ormat -- is an industry standard, 16-bit file format for storing images. The .TIF files can be transferred between different platforms.

Declaration of Conformity

Appendix B

This device conforms to the requirements of US 21CFR1040.10 and 1040.11 at date of manufacture.

This instrument is designed and certified to meet the following regulatory and safety standards:

B.1 Electrical and Mechanical Safety Standards

- The product is UL listed (USA and Canada) to the following standard:
- UL 3101-1:1993 Electrical Equipment for Laboratory Use (USA & Canada).
- The product is TÜV certified to the following standard:
- EN 61010-1/A2:1995 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use (Europe).

B.2 Laser Safety Standards

- CRDH Title 21, CFR 1040.10 Class I Laser Product (USA).
- EN 60825-1:1994 Laser Equipment- Classification Requirements and Users Guide for Laser Safety, (IEC 825), Class I Laser Product.

B.3 Electromagnetic Emissions Standards

- FCC Part 15 Class A, Radiated and Conducted (USA).
- EN55011:1991 Class A, Radiated and Conducted (Europe).
- ICES-003, Industry Canada, Interference-Causing Equipment Standard, Digital Apparatus, Class A

B.4 Electromagnetic Immunity Standards

- EN50082-1, Electromagnetic Compatibility - Generic Immunity Standard, Part 1. Residential, Commercial, and Light Industry
- EN61000-4-2, Electrostatic Discharge
- EN61000-4-3, Radiated Electromagnetic Fields
- EN61000-4-4, Electrical Fast Transient/Burst

- EN61000-4-5, Surge Immunity Requirements
- EN61000-4-6, Conducted Disturbances Induced By Radio-Frequency Fields
- EN61000-4-11, Voltage Dips, Short Interruptions and Voltage Variations Immunity Tests

B.5 FCC Label for Class A Products

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:

- This device may not cause harmful interference,
- This device must accept any interference received, including interference that may cause undesired operation.

B.6 ICES-003 Label for Class A Products

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouiller du Canada.

B.7 Other EU Conformance

The product is CE marked in conformance with the following directives:

- 89/336/EEC, A92/31/EEC, A93/68/EED EMC Directive (Europe).
- 72/23/EEC, A93/68/EEC Low Voltage Directive (Europe).

Specifications

Appendix C

The following specifications are for ScanArray Lite, ScanArray Express and ScanArray HT. The specifications are the same for each model except where noted.

Table C-1: Specifications

ScanArray Specifications	Description
Sample Size	Standard or metric slides: Width 0.97 - 1.02" (24.6 - 25.5 mm) Length 2.95 - 3.0" (75.0 - 76.2 mm) Thickness 0.037 - 0.051" (0.93 - 1.29 mm)
Scanning Field	22 mm x 60 mm Norm; 22 x 73 allowed
Pixel Resolution	User selectable for each acquisition: 5,10, 20, 30 and 50 microns
Excitation Wavelengths	User selectable, depending on the model: Lite: 543 and 633 nm Express and HT: Up to 5 of 488, 514, 543, 594, 612 and 633 nm
Emission Wavelengths	User selectable, depending on the model: Lite: 570 and 670 nm Express and HT: 508, 522, 530, 549, 570, 578, 592, 614, 660, 670 and 694 nm
Sensitivity	Lite: 0.05 fluorescent molecule/μm^2 Express and HT: <0.1 fluorescent molecule/μm^2
Scan Speed	20 scan lines/second <2.5minutes for a 20 x 30 mm area at 10 microns
Dynamic Range	16-bit dynamic range over 4 orders of magnitude
Repeatability and Uniformity	Both less than 5% CV
Image File Formats	TIFF, BMP, JPEG or Raw
Palette Selections	8 user selectable: rainbow, gray, red, orange, yellow, green, blue and purple
Workstation (minimum specs)	Windows, Pentium III 1 GHz processor; 40 GB hard drive; 512 MB RAM; 19" color monitor; CD-RW; 1024 x 768 x 65K color; two 10/100 Mbs Ethernet network interface card (NIC); Windows 2000 SP1; Internet Explorer 5.0 or later
Size	
Scanner	**Lite**: 30" L x 13" W x 14" H (75 cm x 32 cm x 36 cm) **Express**: 30" L x 16" W x 16" H (76 cm x 41 cm x 41 cm) **HT**: 38" L x 16" W x 16" H (97 cm x 41 cm x 41 cm)
External Laser	29" L x 13" W x 12" H (74 cm x 33 cm x 30 cm)

ScanArray Specifications	Description
Weight	
Scanner	**Lite**: 70 lbs (32 kg) **Express**: 82 lbs (37 kg) **HT**: 108 lbs (50 kg)
External Laser	55 pounds (25 kg)
Electrical	
ScanArray Instrument	Autoselecting from 100 - 240 V, 3.5 A, 50/60 Hz
External Laser, (additional requirements)	Autoselecting from 100 - 240 V, 15 A, 50/60 Hz
Safety Agency Approvals	
Electrical Safety	UL: UL 3101-1:1993 Electrical Equipment for Laboratory Use (USA & Canada). TUV: EN 61010-1/A2:1995 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use (Europe).
Laser Safety	CRDH Title 21, CFR 1040.10 Class I Laser Product (USA). EN 60825-1:1994 Laser Equipment- Classification Requirements and Users Guide for Laser Safety, (IEC 825), Class I Laser Product
Electromagnetic Emissions	FCC Part 15 Class A, Radiated and Conducted (USA). EN55011:1991, Class A, Radiated and Conducted (Europe). ICES-003, Industry Canada, Interference - Causing Equipment Standard, Digital Apparatus, Class A
Electromagnetic Immunity	EN50082-1 Electromagnetic Compatibility - Generic Immunity Standard, Part 1. Residential, Commercial, and Light Industry EN61000-4-2, Electrostatic Discharge EN61000-4-3, Radiated Electromagnetic Fields EN61000-4-4, Electrical Fast Transient/Burst EN61000-4-5, Surge Immunity Requirements EN61000-4-6, Conducted Disturbances Induced By Radio-Frequency Fields EN61000-4-11, Voltage Dips, Short Interruptions and Voltage Variations Immunity Tests
FCC Label for Class A Products	This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) This device must accept any interference received, including interference that may cause undesired operation.
ICES-003 Label for Class A Products	This class A digital apparatus meets all requirements of the Canadian Interference-Causing Regulations. Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouiller du Canada.

ScanArray Specifications	Description
Other EU Conformance	The product is CE marked in conformance with the following directives. 89/336/EEC, A92/31/EEC, A93/68/EED EMC Directive (Europe) 72/23/EEC, A93/68/EEC Low Voltage Directive (Europe).

Specifications as of September, 2002. PerkinElmer Life Sciences reserves the right to change these specifications without notice.

Formulas

Appendix D

D.1 Overview

This chapter lists the formulas used by ScanArray Express for Quantitation.

D.1.1 Quality Measurement Formulas

ScanArray Express calculates a set of raw measurements and marks spot quality based on the method and corresponding threshold values specified in the application settings (see Chapter 7), and reports them in the export file.

Next to each quality measurement below are its raw measurement and criterian for marking spots as "good."

Table D-1: Quality Measurement Formulas

Quality Measurement	Raw Measurement Calculation	Criterian for marking spots as "Good"
Footprint	For each subarray, calculate the difference between the center of the nominal spots and the center of the found spot, then shift the nominal spots by the difference. Let the shifted nominal position be (X,Y), the found position to be (x,y), the footprint is: $(X - x)^2 + (Y - y)^2$	Spots with a calculated footprint less than the maximum specified in the application settings are good.
Signal to Noise	Spot intensity/standard deviation of background intensity.	Spots with a signal to noise ratio greater than the minimum specified in the application settings are good.
Signal to background	Multiplier = M Lower Limit = L r_1 = M x background mean r_2 = L + background mean.	Spots with a mean greater than r_1 and a mean greater than r_2 are marked good.

Warranty

PerkinElmer Life Sciences warrants to the customer that the ScanArray Express Microarray Analysis System will be free from defects in material and workmanship and will meet all performance specifications for a period of one year from the date of shipment. This warranty covers all parts and labor.

In the event that the instrument must be returned to the factory for repair under warranty; the instrument must be packed in the original packaging. The outside of the package must indicate the Returned Material Authorization Number (RMA) provided by PerkinElmer Life Sciences

PerkinElmer Life Sciences shall not be liable for any incidental, special or consequential damage loss or expense directly or indirectly arising from the use of the ScanArray Express Microarray Analysis System. PerkinElmer Life Sciences makes no warranty whatsoever in regard to products or parts furnished by third parties, such being subject to the warranty of their respective manufacturers. Service under this warranty shall be requested by contacting your nearest PerkinElmer Life Sciences office.

This warranty does not extend to any instruments or parts that have been subject to misuse, neglect or accident, or have been modified by anyone other than PerkinElmer Life Sciences or have been used in violation of PerkinElmer Life Sciences instructions. The warranty is null and void if the cover has been removed from the system.

The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties, of merchantability or fitness for a particular purpose or otherwise expressed or implied in fact or in law and state PerkinElmer Life Sciences entire and exclusive liability and buyer's exclusive remedy for any claims or damages in connection with the furnishing of goods or parts, their design, suitability for use, installation or operation. PerkinElmer Life Sciences will in no event be liable for any special, incidental or consequential damages whatsoever and PerkinElmer Life Sciences liability under no circumstances will exceed the contract price for the goods for which liability is claimed.

This warranty shall be governed by, and construed and enforced with the substantive laws of the Commonwealth of Massachusetts.

This warranty may vary outside of the United States. In other countries, please contact your local PerkinElmer Life Sciences office for the exact terms of the warranty.

Glossary

Term	Definition
Accuracy	Is the maximum positioning error that can occur between the commanded position of a motion-control device and the actual position. Accuracy is a measure of absolute error. For example, if the motion-control device is commanded to move to position X1,Y1, Z1, but actually moves to position X2, Y2, Z2, the maximumdifference between corresponding values is the accuracy.
Administrator	The person in your organization that manages the facility network and/or sets up your Windows NT/2000 workstation.
AI	See ArrayInformatics.
Application Software	Software designed to perform a specific function directly for the user. ScanArray's application software communicates with the embedded instrument software.
Array	A collection of spots printed on a substrate, arranged in a pattern of regular rows and columns.
ArrayInformatics	A microarray gene expression database and visualization software available from PerkinElmer. ArrayInformatics can share data with ScanArray Express systems that are configured for integration with ArrayInformatics.
HT	An accessory device in scanner that uses a motor to automatically load substrates into and unload substrates out of a cassette. The cassette holds several substrates, 20 in the case of the ScanArray Express® HT
Barcode	A set of letters and/or numbers encoded into a pattern of bars that can be read and interpreted by a device called a barcode reader. Barcodes are most often printed on labels, with the labels affixed to objects that need to be identified and tracked (plates and substrates, in this case).
Batch Set	Set of scanning protocols for use with an HT.
Bi-directional Jitter	Jitter is the deviation in or displacement of some aspect of the pulses in a high-frequency digital signal. The deviation can be expressed in terms of amplitude, phase timing or the width of the signal pulse. When the lines and dots in a scanned image appear jagged there is bi-directional jitter. The amount of allowable jitter is less than ±1pixel. To adjust jitter, contact PerkinElmer Life Sciences Support.
BMP	Microsoft Windows image file format.
Calibration File	Calibration allows normalization of all ScanArray Express instruments. A calibration file is a text file that includes the calibration data of an instrument. This file is created at the factory and should not be changed by the user.

Term	Definition
Client	A software application program, running on a computer workstation, that provides the GUI and allows the user tocalibrate, configure, control, and monitor the instrument. The client software communicates with the instrument software to send configuration and control commands to and receive status and configuration information about the instrument.
Configuration File	A configuration file is a text file that specifies certain system parameters.
Crossover Cable	Cable used for the direct connection of two workstation Network Interface Cards (NICs). NICs have dedicated send and receive lines. A crossover cable connects from the send line of one NIC to the receive line of the other NIC.
Daisy-chain	Daisy-chain is a serial connection of several devices.
Ethernet	Ethernet is the most widely installed Local Area Network (LAN). A LAN is a network of interconnected workstations sharing the resources of a single processor or server within a relatively small geographic area (i.e. an office building). Ethernet is a set of hardware and signaling standards for used for LANs. The most commonly installed systems are 10/100 BaseT.
Facility Network	A facility network is the network at the facility or institution where you work that links all of the workstations and hubs together.
Fiberoptic Cable	Cable made of strands of thin fibers (glass or plastic wire) that transmits pulses of light. Optical fiber carries much more information than conventional copper wire and is in general not subject to electromagnetic interference. In ScanArray Express, fiberoptic cable is used to deliver excitation light from the external blue laser to the instrument.
Fluorophore	A dye used to mark DNA. It emits light at a given wavelength when excited. The scanner needs to know its excitation wavelength and emission wavelength to perform a scan.
FTP	File Transfer Protocol, a standard Internet protocol, is the simplest way to exchange files between computers. It is used to upload and/or download files from your workstation to the instrument.
Gateway Address	A gateway is a network point that acts as an entrance to another network. It is used to control traffic on your facilty's network. A gateway address is the address of a particular gateway on a network.
Geometric Sample Slide	A test sample made of photoresist printed on glass, it is supplied with a new system. When scanned, it displays geometric images on your workstation monitor. It is used to test image geometry, jitter, and basic function.
GUI	See Graphical User Interface
Graphical User Interface	The portion of an application program that provides a visual interface between the program and the user. A GUI consists of windows, dialog boxes, icons, buttons, etc. that allow a user to control the execution of the program and view its status.
Homing	An initialization process for motion-control devices where the motion-control device is driven to one particular position to calibrate the control mechanism.
Image	The output of a scan of one fluorophore.

Term	Definition
Image information	All of the information associated with an image, including all system settings at the time a scan was started, date and time the scan was performed, instrument SN and revision, instrument software revision, client software revision and user comment field.
Image autosave protocol	The information used to determine how images are stored. This includes format and path.
Instrument	The hardware that comprises the entire Scanner, including the scanner module and the external laser.
Instrument Software	This is the internal instrument software necessary to operate the instrument.
IP Address	Network address of a workstation or network. IP Addresses are always in the form of 4 number fields separated by dots. Each number field represents 1 byte Values can be in the range of 0-255. The numbers on the left of the string define the network, the numbers on the right define the individual workstation or Network Interface Card (NIC).
Label	A thin, flexible, adhesive-backed piece of plastic or paper which a barcode or other identifying information may be recorded.
Laser	A device that amplifies light. ScanArray Express has 2-5 lasers at different wavelengths which excite specific fluorophores.
Microarray	A substrate with an array of spots printed on it; the end product of a spotting instrument. The "microarray" is the physical object; an "array" is an ordered collection of spots on the microarray.
NIC - Network Interface Card	A network interface card (NIC) is a computer/workstation circuit board or card that is installed in a computer so that it can be connected to a network. Workstations on local area networks (LANs) typically contain a network interface card specifically designed for the LAN transmission technology, such as Ethernet.
Palette	A range of colors assigned to image pixels based on the intensity value for the pixel.
Photo Bleaching	The loss of fluorescent signal due to intense laser power. This can be minimized by lowering laser power and by subjecting the sample to the laser power for less time. The lower the resolution, the less time the microarray is subjected to the lasers. At a 10 micron scan, with good quality dyes, and 95% laser power, typical signal loss is less than 2 to 3% per scan.
Pixel	Images are made up of a 2-dimensional array of numbers called picture elements, or pixels. The resolution of an image is determined by the size of pixels. For example, 5µm pixels is higher resolution than 10µm.
PMI	Periodic Maintenance and Inspection. Planned maintenance, calibration, and inspection to correct for expected wear in the system.
PMT	Photo Multiplier Tube. A light sensitive vacuum tube that controls current based on the amount of light being received by the tube. A PMT has high gain allowing it to have a high currentto Light ratio.
PMT (Photomultiplier Tube) Board	The PMT absorbs low level fluorescence light and converts it to an electrical signal. The signal is amplified, filtered, and converted to a digital value for each pixel.
PMT Gain	The ratio of light energy to current for the PMT output. The higher the Gain the smaller the amount of light needed for a given amount of current.

Term	Definition
Protocol	A formula that defines an experiment or portion thereof. See Scanning protocol. QuantArray.
Regedit	In Microsoft Windows 95, Windows 98, Windows NT and Windows 2000 and Windows XP operating systems, the Registry is a single place for keeping information such as, what hardware is attached, what system options have been selected, how computer memory is set up, and what application programs are to be present when the operating system is started. Regedit is an editing program used to update installed and uninstalled application programs. It is also used to update the Registry file.
Repeatability	The change in light level detected at a given point on the slide between two scans. Mechanical, electronic and optical changes all affect repeatability.
Resolution	The pixel size that is being scanned for one data point. ScanArray Express can scan as small as 5 microns.
Saturation	A fluorescence signal that is strong enough to drive the PMT detector to its full-scale maximum voltage and output signal is said to cause saturation. Lowering the PMT Gain or Laser Power setting will avoid this.
Scanning protocol	A formula that defines how the scanner hardware and Software execute the scanning portion of a microarray experiment. A scanning protocol, consisting of a set of parameters, allows the user to define the parameters one time and then repeatedly execute the same protocol to scan multiple microarrays in an identical manner.
Spot	The dried remains of sample fluid printed onto a substrate.
Subnet Mask	Also called sub-networks, they are used to partition network addresses for efficiency and security. Subnet masks work by “masking” some number of the less significant address bits on all of the workstations in the subnetwork.
Substrate	The piece of glass upon which the array is printed. These take the form of microscope slides, which have chemically active coatings on the glass that allow the printed spots of sample to bind to the substrate.
TCP/IP	Transmission Control Protocol/Internet Protocol is the basic communication language or protocol of the internet. It is also used in private networks called intranets and extranets. TCP manages the disassembling of a message or file into smaller packets that are transmitted over the internet and received by a TCP layer that reassembles the packets to the original message. The IP layer handles the address part of each packet so that it gets to the right destination.

Term	Definition
Virtual Memory	The concept of using hard disk space to extend the amount of RAM available to a program. Windows 2000 supports virtual memory and allows you to specify the amount of hard disk space to use as virtual memory. Windows 2000 also manages the swapping of data from RAM and the hard disk space so that it appears to your application program(s) as though the virtual memory is RAM. This reduces the amount of physical RAM required. ScanArray Express uses RAM to save image data. The following chart provides a guide for the amount of additional virtual memory to allocate on your workstation when running other applications concurrently with ScanArray Express.

#Scans/ Acquisition	Scan Resolution	Additional Virtual Memory Required
1	5 microns	185 MB
1	10 microns	45 MB
2	5 microns	245 MB
2	10 microns	60 MB
3	5 microns	305 MB
3	10 microns	75MB
4	5 microns	365 MB
4	10 microns	90 MB
5	5 microns	425 MB
5	10 microns	105 MB

To change Virtual Memory:

1. Login as Administrator.
2. Select Start/Settings/System.
3. Select the Performance tab.
4. Click the Virtual Memory **Change** button.

XYZ axis	The three axes associated with the sample. X is across the narrow dimension of the slide. Y is the length of the slide. Z is perpendicular to the slide. In ScanArray Express, lines are in the X direction, Lines are selected by moving the slide in Y and focus is lens movement in Z.

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