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6) After adding the desired Plate IDs to the list, click the **Next** button. The following screen appears.

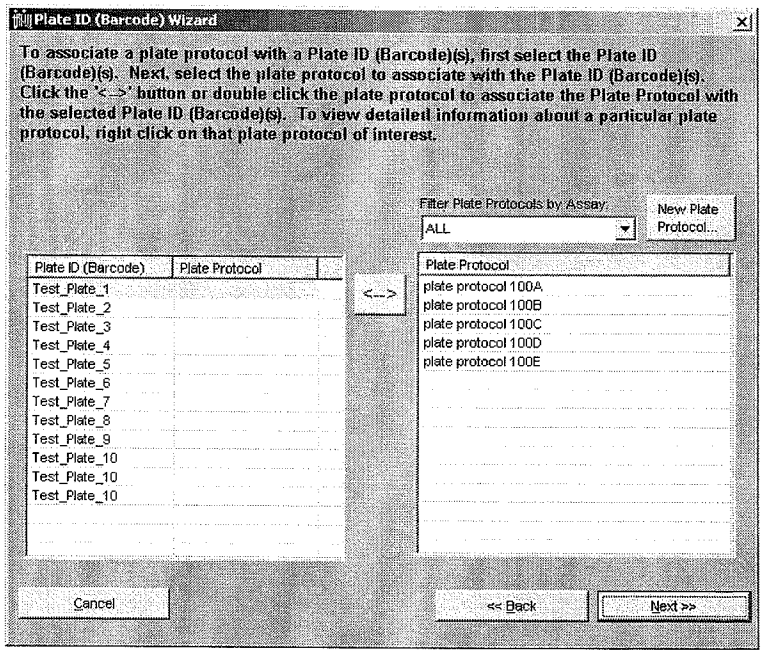


Figure 4.44 Plate ID (Barcode) Wizard: Associate Plate Protocols with Plate IDs

7) You must now associate the Plate IDs with existing Plate Protocols. (Refer to the previous section for more information on creating Plate Protocols.) Follow instructions on screen to associate a single Plate Protocol with a Plate ID. To narrow the list of Plate Protocols, select an assay from the **Filter Plate Protocols by Assay** list. If the desired Plate Protocol does not exist, click the **New Plate Protocol...** button. The Plate Protocol Wizard opens. Refer to the previous section for instructions on creating Plate Protocols.

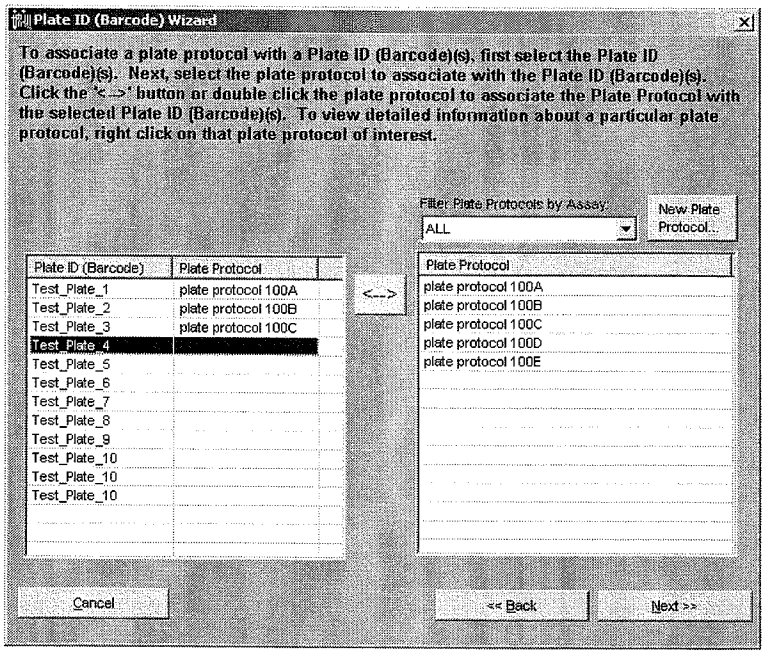


Figure 4.45 Plate ID (Barcode) Wizard: Associate Plate Protocols with Plate IDs

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Plate ID Example: Adding Plate IDs for Scanning a Stack of Plates

The following example demonstrates how you would scan a stack of ten plates and repeat the scan of the tenth plate three times with different assays. This example assumes that you have already created the Plate Protocols for each plate, including the three separate Plate Protocols for the tenth plate. See the previous section, *Creating a New Plate Protocol,* for more information.

To begin, you must first run the Plate ID Wizard in order to create the Plate IDs and associate them with the Plate Protocols for the ten plates.

1) From the main screen of the Plate ID Wizard, click the **Add Plate ID** button.

2) Enter the Plate ID in the box provided starting with the first plate to be scanned. If you are using barcodes, enter the Plate ID from the barcode label. If you are using the robotic plate handler batch numbering feature, enter the number corresponding to the first plate to be scanned. In this example, we are using sequential Plate ID numbering. Therefore, we have used the Fill Pattern option to simplify creating the plate IDs (refer to the figure below). After entering the starting Plate ID number and specifying the Fill Pattern, click the **Add** button.

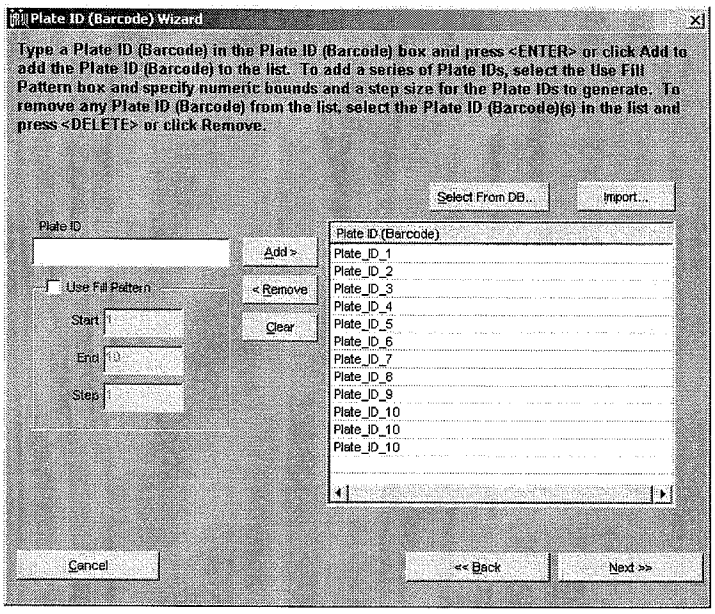


Figure 4.47 Plate ID (Barcode) Wizard: Entering Plate IDs

3) Notice that Plate IDs one through ten have been entered and added to the Plate ID list on the left. But also notice that the tenth Plate ID appears three times. This repeated Plate ID is necessary because we want to scan the tenth plate three times. **In order to scan the same plate more than once, you must create separate but identical Plate ID entries**.

4) Click the **Next** button. You are now prompted to associate each Plate ID entry with a Plate Protocol. To begin, click on the first Plate ID in the list on the left, then select the desired Plate Protocol from the list on the right. Click the <--> button or double-click the Plate Protocol. Repeat this procedure with each plate ID, including the last three identical Plate IDs. By assigning a different Plate Protocol to each of three identical Plate IDs, you are instructing the system to scan the tenth plate three times with three different Plate

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system detects that the plate was entered more than once in the Plate ID Wizard, the system will leave the plate in the instrument and scan it with each Plate Protocol specified before it removes the plate from the instrument and continues with the next plate.

Editing a Plate ID

Use this feature to change the Plate Protocol that is associated with the Plate ID.

To edit a Plate ID,

1) Click the Edit Plate ID button. The following screen appears.

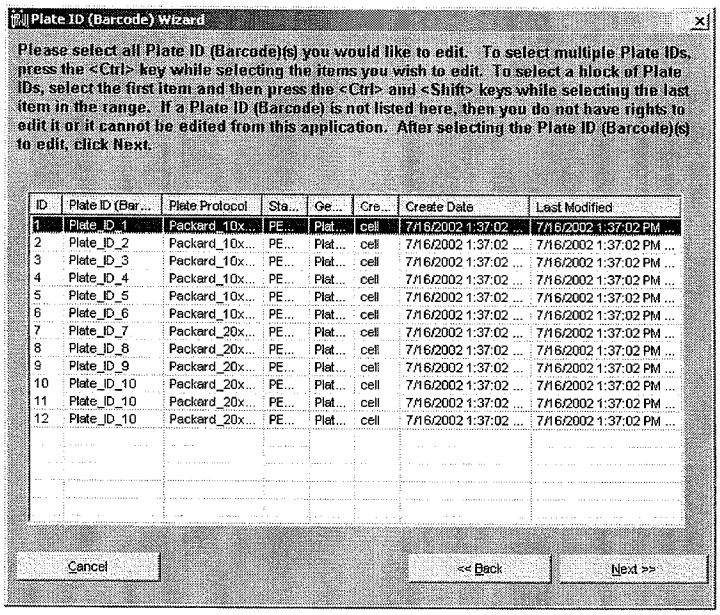


Figure 4.50 Plate ID (Barcode) Wizard: Select Plate IDs for editing

2) Select one or more Plate IDs that you wish to edit and click the Next button. The following screen appears.

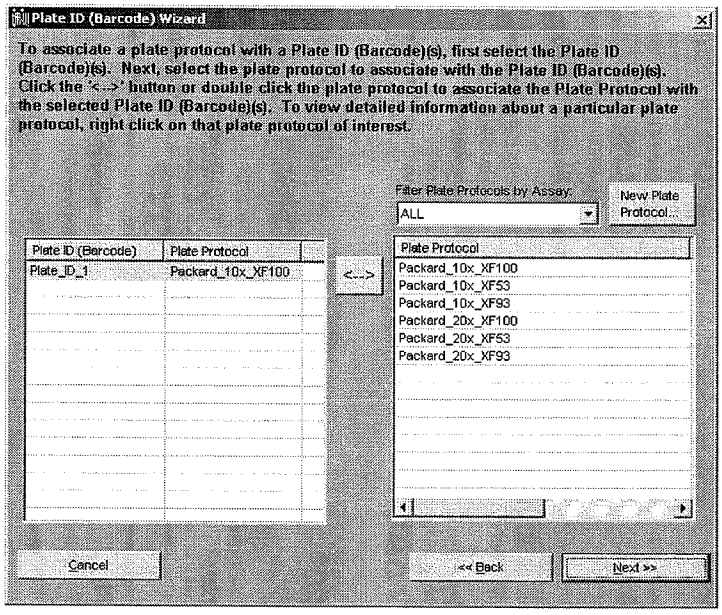


Figure 4.51 Plate ID (Barcode) Wizard: Change Plate Protocol associations

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2) Select one or more Plate IDs that you wish to delete and click the **Next** button. The following screen appears.

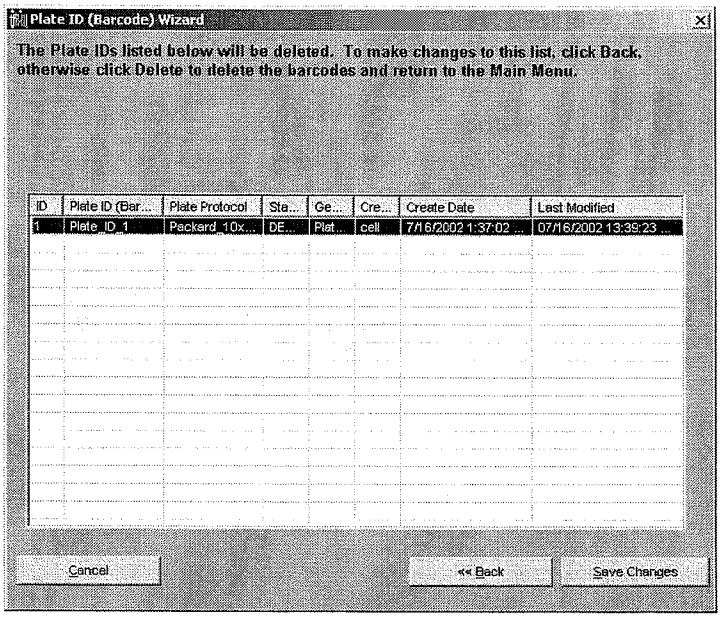


Figure 4.54 Plate ID (Barcode) Wizard: Confirm deletion

3) Review the list of Plate IDs that are selected for deletion. If you want to delete all Plate IDs listed, click the **Save Changes** button. If you want to change the selection, click the **Back** button. Once you click the **Save Changes** button, the main screen of the Plate ID (Barcode) Wizard appears.

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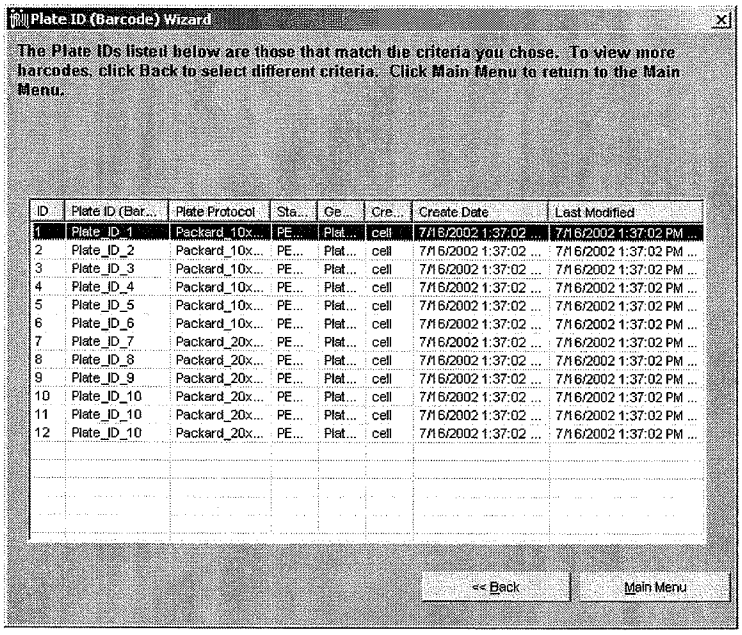


Figure 4.56 Plate ID (Barcode) Wizard: Review Plate IDs

3) All Plate IDs with the specified barcode status will appear in the list. After reviewing the list, click the **Main Menu** button, or click the **Back** button to change the Barcode Status selection.

Exiting the Plate ID Wizard

To exit the Plate ID Wizard,

- Click the **Exit Wizard** button.

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Chapter

5

Scanning a Microplate

WARNING



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A scan is the process of moving from well to well within a microplate and from field to field within each well. During a scan, the ArrayScan V^{TI} HCS Reader acquires and processes images to extract features. The system then saves these features to the database, along with the calculated feature statistics and the raw images.

This chapter provides instructions for scanning a microplate by manually placing a single microplate in the ArrayScan V^{TI} instrument stage. For instructions on scanning one or more microplates using a robotic plate handler, please refer to the *ArrayScan V^{TI} HCS Reader: Robotic Plate Handler User's Guide*.

Before following the procedures outlined in this chapter, you must have a valid Assay Protocol or Plate Protocol/Plate ID for your scan. An Assay Protocol specifies all system settings and parameters needed to scan a microplate. If an Assay Protocol has not been created for the scan, please refer to **Chapter 6 – Creating and Working with Assay Protocols** for instructions. A Plate Protocol specifies the Assay Protocol plus additional system settings including the scan area, store images option, field offset, plate type, and display mode. A Plate ID is needed to associate the plate with the Plate Protocol. Refer to *Chapter 4, ArrayScan V^{TI} HCS Reader Setup Procedures* for more information on creating Plate Protocols and Plate IDs.

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Scanning a Single Microplate

NOTE

The procedures in the section are based on manually inserting a single microplate in the ArrayScan V^{TI} instrument. However, if you wish to scan a single plate using a robotic plate handler, please follow the instructions outline in the *ArrayScan V^{TI} HCS Reader; Robotic Plate Handler User's Guide.*

Two options are available when scanning a single microplate:

- You can perform a single scan using a single assay protocol
- You can scan a single plate using multiple Plate Protocols. You must use multiple Plate Protocols when you want to perform several scans on a single plate using different assays, or when you want to scan different parts of the plate with different assays (or the same assay).

Setup procedures vary depending on the single plate scan option you wish to perform. Follow the instructions outlined below for the appropriate scan option.

NOTE

Prior to scanning, verify that system setup procedures have been performed including loading the plate in the ArrayScan V^{TI} instrument. Refer to **Chapter 4 – ArrayScan V^{TI} HCS Reader Setup Procedures** for instructions.

NOTE

Before starting the scan, verify that sufficient disk space is available. The free disk space indicator on the ArrayScan status bar indicates the amount of disk space remaining.

Preparing for a Single Plate Scan using one Assay Protocol

To set up the scan,

1) Verify that all system setup procedures, outlined in Chapter 4 – ArrayScan VTI HCS Reader Setup Procedures, have been performed. These setup procedures include the selection of a valid Assay Protocol.

NOTE

Alternatively, you can select a valid Plate Protocol. From the Scan Plate View, select **Load Plate Protocol** from the **File** menu.

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Preparing for a Single Plate Scan using Multiple Plate Protocols

To set up the scan,

1) Verify that all system setup procedures, outlined in Chapter 4 – ArrayScan VTI HCS Reader Setup Procedures, have been performed. These setup procedures include the creation of valid Plate Protocols and Plate IDs.

NOTE

When running more than one Plate Protocol on a single plate, you must create identical Plate ID numbers and associate them with the Plate Protocol. Refer to Chapter 4 for more information on creating and associating Plate IDs with Plate Protocols.

2) In the Scan Plate View, select the **Plate ID (User Defined)** option and enter the Plate ID that is associated with each of the Plate Protocols you wish to run. The Plate ID entered here must be identical to the Plate ID created using the Plate ID Wizard.

3) Optional: Enter a name for the microplate in the Plate Name box. You can enter a maximum of 255 characters.

4) Optional: Enter comments that you wish to make about the scan in the Scan Comments box. You can enter a maximum of 4000 characters. These comments are stored with the scan data.

Starting and Monitoring the Scan

NOTE

Prior to starting a scan, Thermo Fisher Scientific recommends that you perform the Illumination Correction procedure. Please refer to **Chapter 10, Using the Illumination Correction Tool** for instructions.

Starting the Scan

To start and monitor the scan,



- Click the **Start Scan** button on the ArrayScan toolbar. If the system detects that the Plate ID you entered is associated with a Plate Protocol, the system will display a message asking if you wish to run the Plate Protocol.

After clicking the Start Scan button, the following actions occur:

- The system preprocesses the plate for focus optimization,
- the stage moves to the first field of the first well to be scanned,
- the camera focuses and acquires an image for each channel,
- the channel images, along with the color composite image, are displayed in the Images area of the Scan Plate View,
- graphics are overlaid on these images to show the results of the selected assay,
- images and analysis data are stored in the database.

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Viewing Scan Images

Displaying and Working with Enlarged Views of Scan Images

During the scanning process, it is possible to pause the scan to display an enlarged view of a scan image. The image is displayed in an Image Popup window as shown in the following figure. The Image popup window provides many options for viewing and saving the scanned images. Please refer to Working with Image Popup Windows on page 155 for more information.

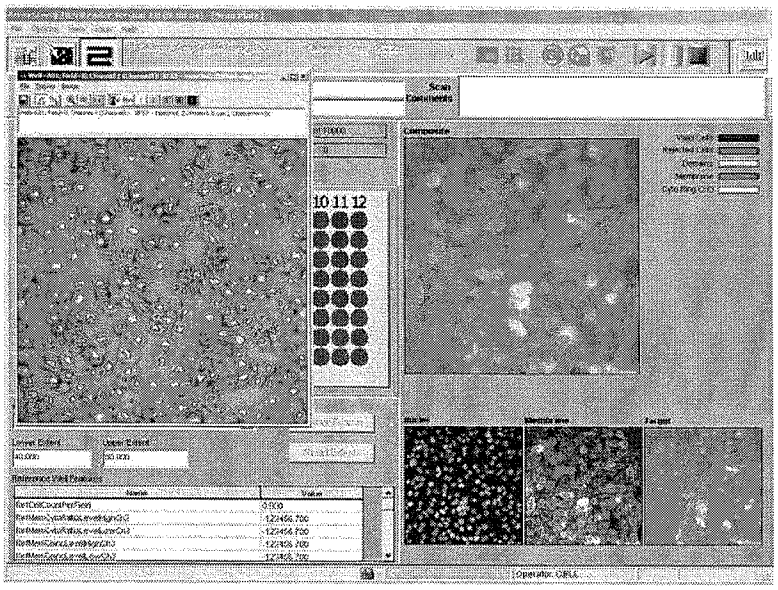


Figure 5.3 Image Popup window in Scan Plate View.

To restart the scan exactly where it left off,

- Close the image window by selecting **Exit** from the **File** menu.

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In the case of "Above Range", "In Range", and "Below Range", the well colors indicate well status for the feature that is currently selected in the Well Feature drop down list. This list contains all of the well features that the scan is measuring. The Upper and Lower Extent values are shown for the currently selected feature.

To display well status for other features,

- Select the desired feature from the Well Feature drop down list.

To change the Upper and Lower Extent values of a Well Feature,

1) Select the desired feature from the Well Feature drop down list.
2) Enter the new value(s) in the Upper Extent and/or Lower Extent boxes.

NOTE

You can modify the Lower and Upper Extent values only after a scan has started.
Changes to the Upper and Lower Extent values are valid for the current scan only. These values are not saved with the Assay Protocol. (Use the Create Protocol View to permanently change the default values in the Assay Protocol.)
Error messages resulting from entering invalid upper or lower extent values appear in the Information panel of the Status bar.

3) Click the **Update Display** button to update the color-coded wells to reflect the well status for the new extent values.

To restore the default Upper and Lower Extent values stored in the Assay Protocol,

1) Select the desired well feature from the Well Feature drop-down list.
2) Click the **Reset Extents** button.

NOTE

If this button is not available, the lower and upper extent values have not been modified and are identical to the default values set in the Assay Protocol.
The Reset Extents button only resets values for the selected Well Feature. To change extent values for other well features, you must first select the well feature from the drop-down list.

Viewing Well Feature Data

In addition to the color-coded well view, you can also view the actual feature data values for each of the wells.

To view the well feature data values for the entire microplate,

1) Select the desired feature from the Well Feature drop down list.
2) Right-click anywhere in the microplate representation. A popup menu appears.
3) Select **View Well Data** from the popup menu. The color-coded wells are replaced with a table of the actual data values for the selected feature.
4) Use the horizontal and vertical scroll bars to view the values for the entire plate.

To return to the color-coded well view,

- Right-click on the microplate representation again and select **View Color Coded Plate** from the popup menu.

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Chapter

6

Creating and Working with Assay Protocols

WARNING

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An Assay Protocol specifies all of the system settings and parameters needed to scan a microplate. You can create your own Assay Protocols based on a selected assay, or you can use one of the pre-configured Assay Protocols supplied with the ArrayScan software.

This chapter describes the following Assay Protocol topics:

- Modifying existing ArrayScan Assay Protocols
- Managing Assay Protocols
- Using Advanced Assay Protocol settings

Use the Create Protocol View to modify existing Assay Protocols.

To display the Create Protocol View,



- Click the **Create Protocol** button located on the ArrayScan toolbar.
  -or-
  From the **View** menu, select **Create Protocol**.

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When running in Advanced Mode, you can create new Assay Protocols and specify additional Create Protocol settings and parameters. Please refer to "Advanced Create Protocol Settings" later in this chapter for more information.

Specifying Scan Limits

The ArrayScan V^{TI} HCS Reader uses scan limits to control the scanning of a microplate. You can specify plate, well, and field limits for processing a microplate in the Scan Limits section. These limits tell the system when to move on to the next field or well, or when to abort processing of a well or plate. The system checks the limits after each field is processed.

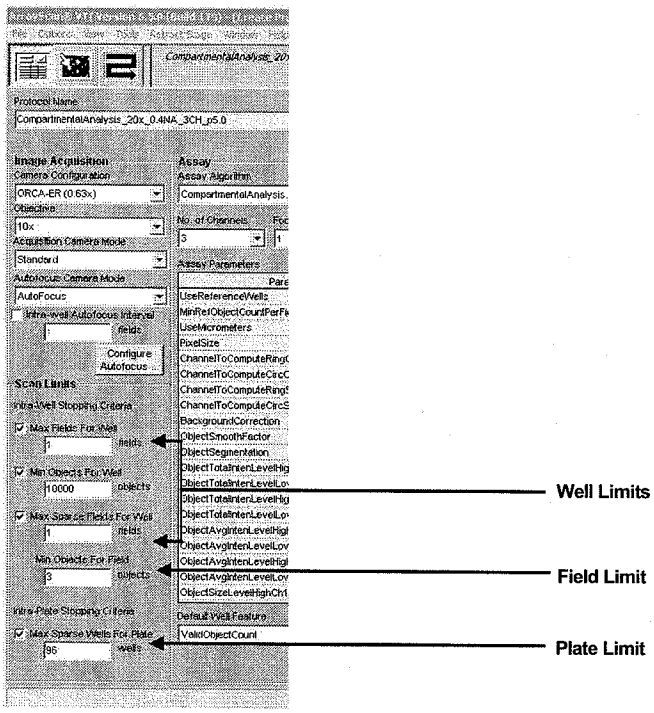


Figure 6.2 Scan Limits section of the Create Protocol View.

Scan Limits Terminology

Before describing the procedures for specifying scan limits, it is necessary to define the terms used within the Scan Limits section.

A **well** is a circular or square area in a microplate containing samples for analysis. The number of wells is dependent on the plate type.

A **field** is a sub-region within a well representing the field of view of the camera. Within each well, a number of fields are available. The ArrayScan HCS Reader scans fields in an outward, counter-clockwise, square spiral pattern as shown in the following figure. The number of available fields is dependent on the objective setting as well as the size of the well.

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To set field limits,

- Check the **Min. Objects for Field** checkbox and enter the minimum number of objects per field in the **objects** box.

Specifying Intra-Plate Stopping Criteria (Plate Limits)

Plate Limits are limits that apply to an entire microplate. The following plate limit option is available.

Plate Limit	Description
Max. Sparse Wells For Plate	Maximum number of sparse wells per plate, including blank wells. If the number of sparse/blank wells exceeds this number, the system stops the scan of the plate. This limit should be set so that it will be exceeded if the plate preparation is inadequate (i.e., there are too many sparse wells).

To set plate limits,

- Check the **Max. Sparse Wells for Plate** checkbox and enter the maximum number of sparse wells per plate in the **wells** box.

Entering Assay Protocol Comments

You can enter comments related to the Assay Protocol in the Protocol Comments box. A maximum of 4000 characters can be stored with the Assay Protocol data. Comments are stored with the results for the plate.

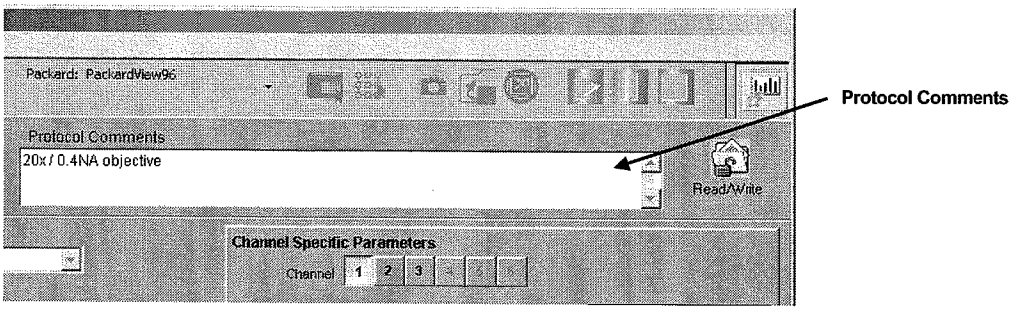


Figure 6.4 Protocol Comments section of the Create Protocol View.

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Intra-well Autofocus Interval = 2 (with an odd number of fields)

49 10	**48** 10	**47** 8	**46** 8	**45** 7	**44** 7	**43** 7
26 2	**25** 2	**24** 8	**23** 8	**22** 7	**21** 7	**42** 7
27 2	**10** 2	**9** 1	**8** 1	**7** 1	**20** 6	**41** 6
28 2	**11** 2	**2** 1	**1** 1	**6** 1	**19** 6	**40** 6
29 3	**12** 3	**3** 1	**4** 1	**5** 1	**18** 5	**39** 5
30 3	**13** 3	**14** 3	**15** 4	**16** 4	**17** 5	**38** 5
31 9	**32** 9	**33** 4	**34** 4	**35** 4	**36** 5	**37** 5

Figure 6.6 Intra-Well Autofocus Interval Setting = 2

Autofocus Interval = 2

KEY

12 3	Scanned Field # Focus #	**16** 4	Focus #
Autofocused Field		**Non-Autofocused Field**	

In this example, the 16th field, a non-autofocused field, is scanned with the autofocus setting established during the 4th autofocus.

→ Arrows show the direction of a scan that covers a well of a 96-well microplate using an odd number of fields, starting at field 1. Note that if you specify a Field Offset, the starting field will be different. If you choose a different objective, the scan direction may be different, depending upon whether there are an odd or even number of fields in the well.

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Autofocus Interval = 3 (with an odd number of fields)

49 5	**48** 5	**47** 5	**46** 4	**45** 4	**44** 4	**43** 4
26 5	**25** 5	**24** 1	**23** 1	**22** 4	**21** 4	**42** 4
27 5	**10** 1	**9** 1	**8** 1	**7** 1	**20** 1	**41** 4
28 2	**11** 1	**2** 1	**1** 1	**6** 1	**19** 1	**40** 3
29 2	**12** 1	**3** 1	**4** 1	**5** 1	**18** 3	**39** 3
30 2	**13** 2	**14** 2	**15** 1	**16** 1	**17** 3	**38** 3
31 2	**32** 2	**33** 2	**34** 2	**35** 3	**36** 3	**37** 3

Figure 6.9 Intra-Well Autofocus Interval Setting = 3

NOTE

When running in Advanced Mode, you can specify all other Image Acquisition settings. Please refer to "Advanced Create Protocol Settings" later in this chapter for more information.

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Specifying Channel Specific Parameters

You can specify channel parameters and image display options for each channel in the Channel Specific Parameters section.

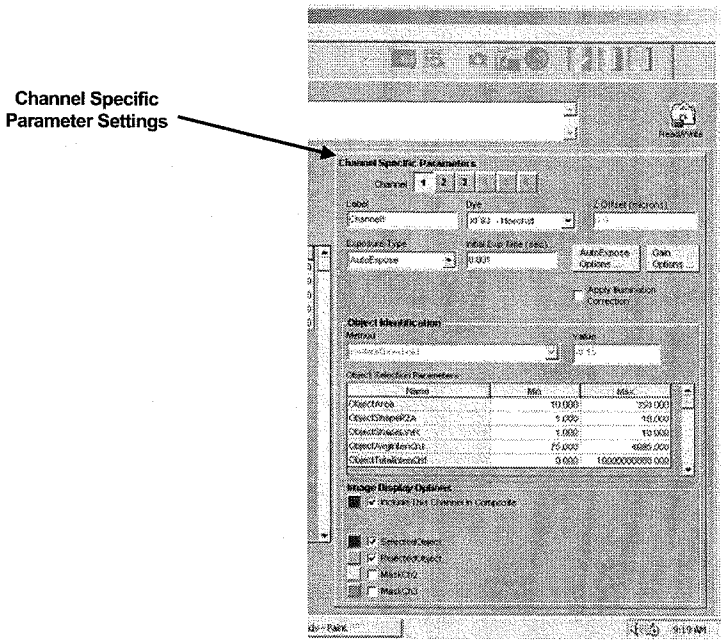


Figure 6.12 Channel Specific Parameters section of the Create Protocol View.

The Channel Specific Parameters section provides the following options:

Channel Option	Description
Channel (Number)	Displays the number of channels available for the selected assay. Clicking on each numbered button allows you to set parameters for the corresponding channel number.
Label	User specified name for the channel. This label will be used on the images viewed in Scan Plate View as well as the vHCS:View application.
Dye	Lists all available dyes and corresponding filter sets for the selected channel number. The dye and filter set chosen here should match the dye used to label the biological marker on the microplate. The filter sets displayed are provided by Omega Optical, Inc. Please refer to the **Automated Filter Selection Appendix** for more information on selecting dye and filter set combinations.
Z Offset (microns)	User specified value in microns. Specifying a Z offset value, either positive or negative, allows you to acquire images of structures appearing above or below the focal plane. This value is set relative to the focus channel., therefore, you cannot specify a Z Offset value for the designated focus channel. The default value for each channel is zero. This value is saved as a Plate Feature: ZOffset-Ch*N*.

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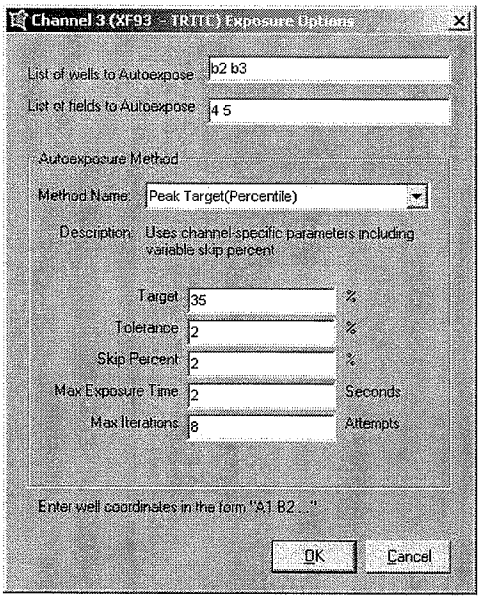


Figure 6.13 Exposure Options dialog box.

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To set Autoexposure Options,

NOTE

Autoexpose Methods and Parameter settings have been optimized by Thermo Fisher Scientific. Applications are strongly dependent on these settings.
Do not change the Autoexpose Method or Parameter settings appearing in the Exposure Options dialog box without first contacting Technical Support.

1) In the **Wells to AutoExpose** box, enter the autoexpose well numbers. A space between well numbers is required.
Example: **F1 E1 D1 C1**

NOTE

- You need to specify the Wells to AutoExpose only once per Assay Protocol. This setting is not channel specific.
- Exposure times determined from individual wells will be averaged over the set.
- If the microplate will be scanned repeatedly, you may change the autoexpose wells between scans to avoid bleaching them.

2) In the **Fields to AutoExpose** box, enter the desired field numbers. A space between field numbers is required.
Example: **2 4 6**

NOTE

- You need to specify these fields only once per Assay Protocol. This setting is not channel specific.
- You should choose a field that will not be scanned. For example, if you specified '5' as the "Max. Fields For Well" Scan Limit setting, you should choose field numbers 6 or higher.

3) Click **OK**.

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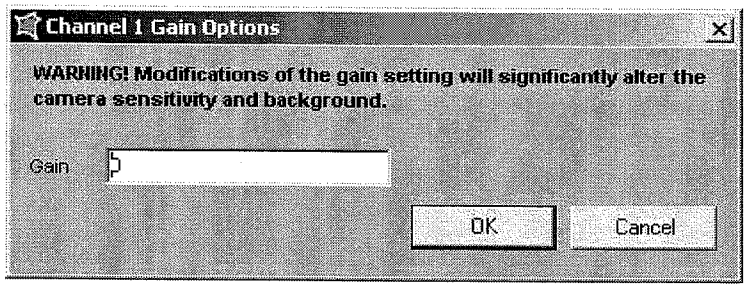


Figure 6.15 Gain options dialog box

4) Enter the gain value in the **Gain** box.

5) Click **OK**.

Applying Illumination Correction

To apply the Illumination Correction settings as specified using the Illumination Correction Image Acquisition Wizard, follow the procedure below. Please refer to **Chapter 10, Using the Illumination Correction Tool** for more information.

To apply the Illumination Correction,

1) Click on the desired channel number.

2) Check the **Apply Illumination Correction** checkbox.

3) The system will prompt you to apply the Illumination Correction to the remaining channels. Select OK is you wish to apply the Illumination Correction to all channels. Otherwise, repeat the steps above for the desired channels.

NOTE

When you add additional channels, Illumination Correction is selected by default. When you deselect Illumination Correction from one channel, the system prompts you to deselect it from the additional channels, if desired.

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Managing Assay Protocols

The following functions can also be performed through the **Protocol Manager** interface. For more detail on the Protocol Manager including saving and managing protocols, please Reference **Chapter 7**, entitled **Using the Protocol Manager**.

Saving an Assay Protocol

When you save an Assay Protocol, you are storing the currently selected Assay Protocol settings in the database. These settings include all of the user specified options, settings, and parameters required to scan the microplate. You can either replace the existing Assay Protocol with the newly changed Assay Protocol or you may save the changes under a new Assay Protocol name.

For more detail on saving and managing protocols, please Reference Chapter 7, entitled "Using the Protocol Manager".

To save the changes using a new Assay Protocol name,

1) From the File menu, select Save Assay Protocol As.

The Save Assay Protocol As… dialog box appears as shown in the following figure.

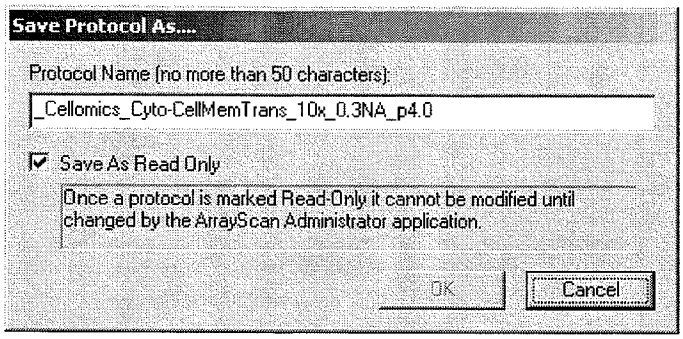


Figure 6.17 Save Assay Protocol As dialog box.

2) Enter a new name, up to 50 characters in length, in the box provided. Do not include apostrophes or pipe symbols (|) in the Assay Protocol name.

3) If you wish to make further changes to the protocol, clear the Save As Read Only checkbox to save the Assay Protocol as a read/write protocol.

4) Click OK.

NOTE

All settings are saved in the Assay Protocol with the exception of the scan area, scan direction, store images option, field offset, plate type, and display mode. If you wish to save these settings as well, select **Save Plate Protocol As** from the **File** menu of the Scan Plate View and specify a Plate Protocol to be saved.

To overwrite the existing Assay Protocol,

■ From the **File** menu, select **Save Assay Protocol**.

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Exporting an Assay Protocol

You can export an Assay Protocol to a protocol text file (in a specific format) using the Export Assay Protocol command. You may want to export an Assay Protocol to save a backup copy of the Assay Protocol.

To export an Assay Protocol,

1) From the File menu, select Export Assay Protocol.

2) The Export Protocol dialog box appears as shown in the following figure.

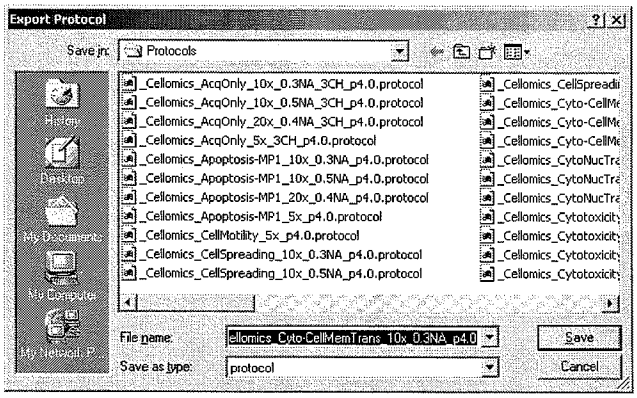


Figure 6.18 Export Protocol dialog box

3) In the **Save in** drop down list, select the drive and folder where you wish to export the Assay Protocol.

4) Type in the name you wish to use for the exported Assay Protocol file or click an existing file.

5) Click the **Save** button.

NOTE

If an Assay Protocol text file of the same name already exists, a confirmation message will appear. Choose **YES** to replace the existing Assay Protocol text file. Choose **NO** to return to the Export Protocol dialog box.

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Advanced Create Protocol Settings

When running in Advanced Mode, you have access to additional functionality, settings and parameters in the Create Protocol View. These additions include the following:

- **Create New Assay Protocols**
- **Import Assay Protocols**
- **Delete Assay Protocols**
- **Specify Assay Parameters**
- **Specify Image Acquisition Parameters**
- **Specify Object Identification Parameters**

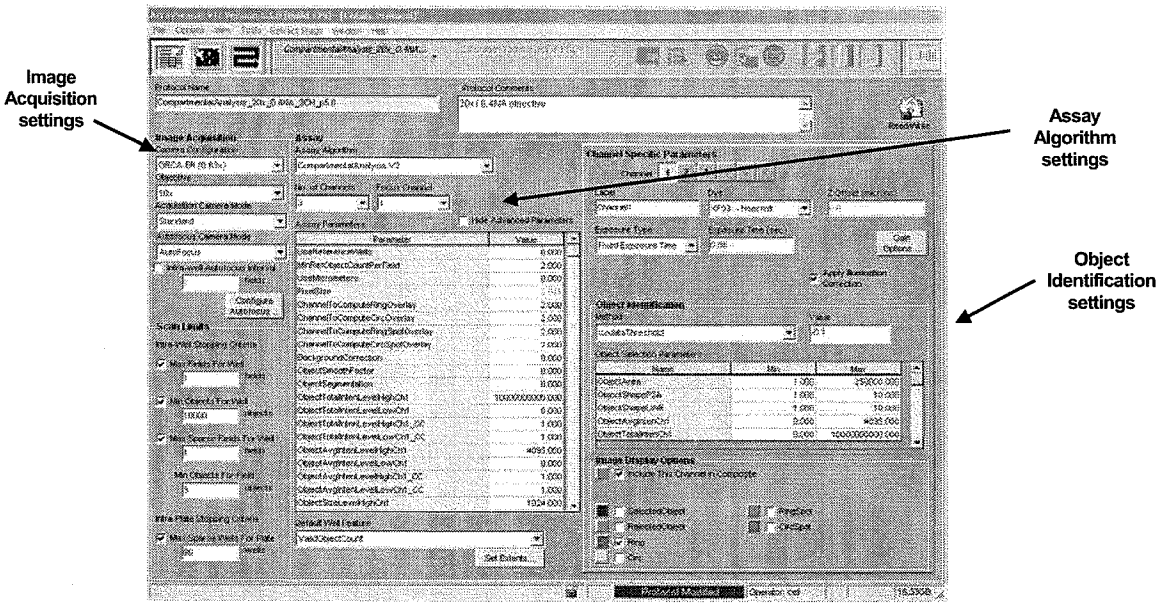


Figure 6.20 Create Protocol View in Advanced Mode.

To run in Advanced Mode,

- From the **Options** menu, select **User Options**, then select **Advanced Mode**. The Advanced Mode menu item will be checked.

Creating a New Assay Protocol

When running in Advanced Mode, you can create new Assay Protocols using the New Assay Protocol command.

NOTE

Thermo Fisher Scientific recommends that you always begin with a factory provided Assay Protocol for the algorithm and objective that you wish to use.

To create a new Assay Protocol,

- From the **File** menu, select **New Assay Protocol**.

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Deleting an Assay Protocol

When running in Advanced Mode, you can delete an Assay Protocol from the protocol database using the Protocol Manager. Please refer to **Chapter 7, Using the Protocol Manager** for details on deleting Assay Protocols.

NOTE



In the Delete Assay Protocol dialog box, you must click the Delete button in order to delete the Assay Protocol. If you simply select an Assay Protocol from the list and click the Done button, the Assay Protocol will not be deleted.

Read-only protocols will not appear in the Assay Protocol list and cannot be deleted using the Delete Protocol command.

You cannot delete an Assay Protocol that is currently open.

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Entering Channel Information

The ArrayScan V^{TI} HCS Reader supports up to six channels. Not all assays directly support all six channels. For example, the Nuclear Translocation BioApplication assay reports features in two channels, one that makes measurements of the nucleus and one that makes measurements of the cytoplasm.

To set up channels,

1) In Advanced Mode, select the number of channels for the currently selected assay from the No. of Channels drop down list.

2) Select the channel to use for the Focus channel from the Focus Channel drop down list. The focus channel is the channel used for focusing images. The channel selected should have strong image contrast and a low exposure time.

NOTE

When using certain cell based assays, additional channels can be added to the standard channels supported by the assay. Please refer to the section titled "Performing Subpopulation Analysis" later in this chapter for more information.

Hiding Advanced Assay Parameters

To hide the advanced assay parameters from the Assay Parameters table,

- Check the **Hide Advanced Features** checkbox.

Please refer to your Assay BioApplication Guide to determine which parameters are advanced parameters.

Entering Assay Parameter Values

The Assay Parameters table consists of two columns. The first column contains the names of all required parameters for the selected assay. The second column contains empty cells where you may enter values for each of the parameters. Each assay has a set of parameters that is unique to that assay. Changing the assay selection changes the list of assay parameters. For a description of the available parameters and recommended values, refer to the BioApplication Guide for that particular assay.

NOTE

When using certain cell based assays, additional mask modifier parameters are available. Please refer to the section titled "Performing Subpopulation Analysis" later in this chapter for more information.

Assay Parameters that appear for display purposes only (you cannot change the value of these parameters) are displayed with a gray background in the Value box.

To enter assay parameters,

- In Advanced Mode, enter a value in each of the table cells corresponding to a parameter name. Consult your BioApplication User's Guide for guidance on setting these parameters.

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To enter Image Acquisition information,

1) In Advanced Mode, select the objective from the **Objective** list.

2) Select the acquisition mode from the **Acquisition Camera Mode** list.

3) Select the focus mode from the **Autofocus Camera Mode** list.

4) If you wish to autofocus more than once within a well, check the **Intra-Well Autofocus Interval** checkbox and specify the desired number of fields in the **Fields** box. For example, if you wish to autofocus every three fields within the well, enter '3' in the Fields box.

Changing objectives without adjusting relevant Assay Protocol parameters will adversely affect most assays. This usually stems from the fact that changing the magnification changes the area covered by each pixel. Some of the assays have parameters that are expressed in pixel units, but actually represent an area. These parameters must be adjusted to compensate for the change in magnification. For example, a nuclear translocation assay might have a parameter that adjusts the ring size around the nucleus. If the magnification is doubled and nothing else changed, the effective ring width (in real microns) will be cut in half. This can cause undesirable effects on the assay measurements.

NOTE



For best results, start with a factory supplied Assay Protocol for the algorithm and objective that you wish to use and modify Assay Parameters to fit your biology.

To install a new objective, please refer to the *Objective Installation and Configuration Guide.*

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value. Then the average value of the foreground and the background pixels is calculated and a new threshold value is taken midway between those two values. This process is repeated, based upon the new threshold estimate until the threshold value does not change. In most cases, the IsoData threshold method produces good results. However, if you are working with sparsely populated images, where the area of the background is significantly larger than the area of the objects, the Peak Triangulation method may provide more precise results.

Peak Triangulation Method: The threshold level is determined by the Triangulation algorithm. This algorithm is a method based upon the gray level histogram of the image. The top of the main peak of the histogram and the end tail point of the histogram are connected with a straight line. The point on the histogram curve that produces the maximum distance to the straight line identifies the histogram bin/entry/index of a threshold value. If you are working with sparsely populated images, where the area of the background is significantly larger than the area of the objects, the Peak Triangulation method may provide more precise results.

None: In some BioApplications, you may not want to perform thresholding in a channel. In this case, you must enter a '0' for the threshold value.

Threshold Value

If you select the Fixed Threshold method, enter a pixel intensity value in the Value box. Values can range from 0 to 4095.

If you select one of the autothresholding methods, the system automatically calculates the threshold intensity value for each image. If you want to change the calculated threshold value by a fixed percentage, you can enter the percentage value (as a decimal) in the Value box. This value represents a percentage (positive or negative) whereby the system increases or decreases the threshold intensity value. For example, if you enter -0.15, the system decreases the calculated threshold value by 15%. If you enter 0.15, the system increases the calculated threshold value by 15%. If you enter 0 in the Value box, the system uses the calculated threshold value.

To specify object identification settings,

1) In Advanced Mode, select the desired channel by clicking on the Channel number.
2) Choose an Object Identification method from the Method drop down list.
3) Enter an appropriate value in the Value box for the Object Identification method.
4) Repeat these steps for the remaining channels.

Method	Values Range
Fixed	0 – 4095
IsoData	-0.995 – 0.995*
Peak Triangulation	-0.995 – 0.995
None	0

* some assays may reduce the range to –0.5 – 0.5

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Performing Gating of a Subpopulation

Some cell based assays, such as the Cytoplasm to Nucleus Translocation BioApplication assay, support Gating. When running in Advance Mode, this feature provides selective cell processing based on fluorescence intensity. Therefore, in addition to selecting cells based on nuclear area or nuclear shape, you can also select or reject cells based on fluorescence intensity. You may want to perform Gating if, for example, you have identified cells in the marker channel, and now you want to refine the object selection based on intensity of the objects in a second channel. You can enter intensity threshold values in the Object Selection Parameters table of the Protocol Create View. Alternatively, you can set these parameter values interactively when working in the Protocol Interactive View.

In addition to specifying intensity threshold ranges for one or more channels, you can apply a mask to one or more downstream channels using the Mask Modifier settings in the Assay Parameters table. You may want to use this feature if, for example, you want to dilate the mask of each object to include other cellular markers. The mask can be dilated or eroded but will not overlap with other masks from nearby objects.

In addition to processing cells based on fluorescence intensity in standard assay channels, you can also add one or more additional channels to the assay (up to six channels). In these additional channels, you can apply a mask via the Mask Modifier setting as described above, and you can specify intensity threshold values.

You can view the results of the Gating in the Cellomics vHCS View application when viewing Well Details and Cell Details.

For detailed instructions on performing Gating, please refer to your BioApplication Guide.

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Chapter 7

Using the Protocol Manager

The Protocol Manager window provides a central locations from which you can organize and manipulate protocols.

Opening the Protocol Manager

To open the Protocol Manager,

- From the **File** menu, select **Manage Protocols…** .

-or-

Click the Protocol Selection button on the toolbar.

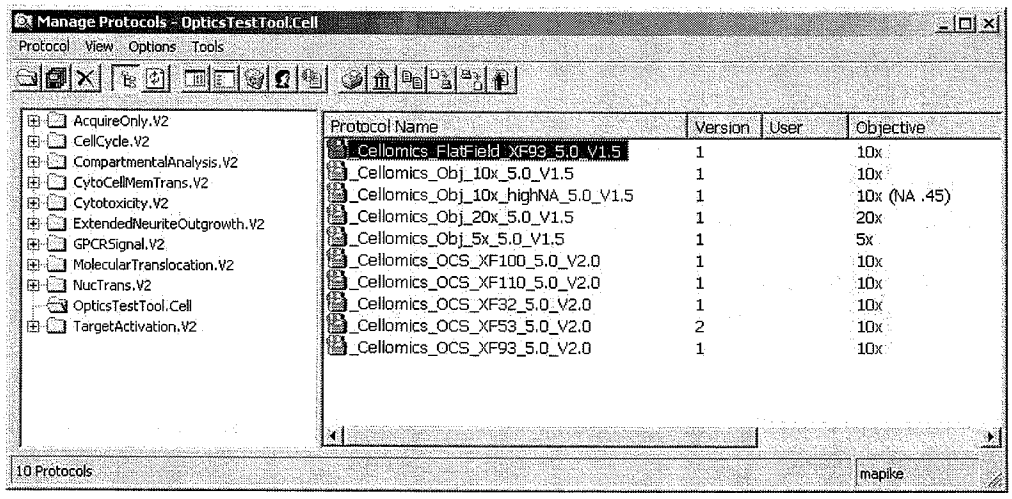
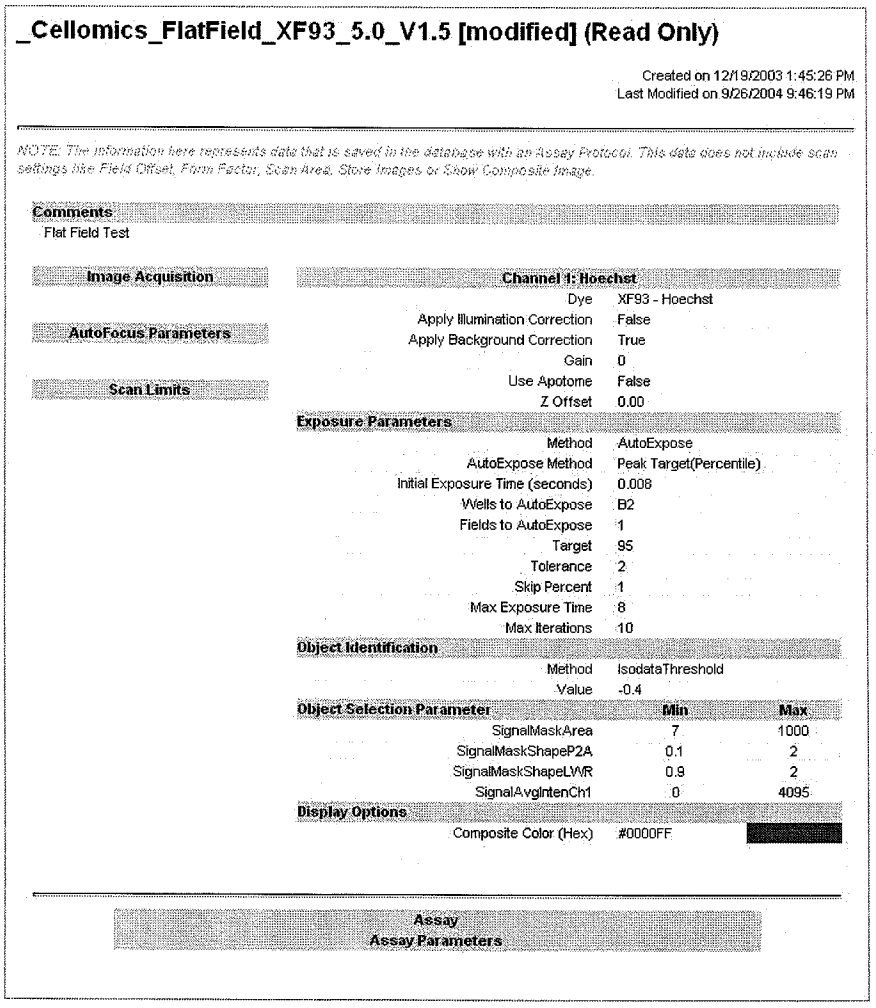


Figure 7.1 The Manage Protocols Window.

The left (navigation) pane of the Protocol Manager window shows the available assay protocols organized in the manner that you specify. When you select a folder that contains assay protocols, the protocols in the folder are displayed in the right pane.

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_Cellomics_FlatField_XF93_5.0_V1.5 [modified] (Read Only)

Created on 12/19/2003 1:45:26 PM
Last Modified on 9/26/2004 9:46:19 PM

NOTE: The information here represents data that is saved in the database with an Assay Protocol. This data does not include scan settings like Field Offset, Form Factor, Scan Area, Store Images or Show Composite Image.

Comments
Flat Field Test

Image Acquisition
AutoFocus Parameters
Scan Limits

Channel 1: Hoechst

Dye	XF93 - Hoechst
Apply Illumination Correction	False
Apply Background Correction	True
Gain	0
Use Apotome	False
Z Offset	0.00

Exposure Parameters

Method	AutoExpose
AutoExpose Method	Peak Target(Percentile)
Initial Exposure Time (seconds)	0.008
Wells to AutoExpose	B2
Fields to AutoExpose	1
Target	95
Tolerance	2
Skip Percent	1
Max Exposure Time	8
Max Iterations	10

Object Identification

Method	IsodataThreshold
Value	-0.4

Object Selection Parameter	Min	Max
SignalMaskArea	7	1000
SignalMaskShapeP2A	0.1	2
SignalMaskShapeLWR	0.9	2
SignalAvgIntenCh1	0	4095

Display Options

Composite Color (Hex)	#0000FF

Assay
Assay Parameters

Figure 7.2 A Protocol Summary Web Page.

The protocol opens in your default web browser. A summary of all of the configured values for the selected protocol will be displayed as a web page.

To save a protocol,

- From the **Protocol** menu, select **Save As**

-or-



Click the **Save As** toolbar button.

-or-

Highlight the protocol you would like to save, right click and select **Save Protocol As…** from the popup menu.

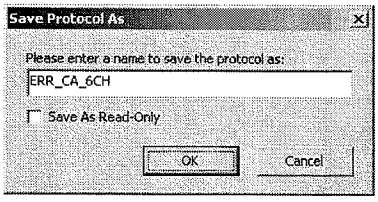


Figure 7.3 Protocol Save As Dialog.

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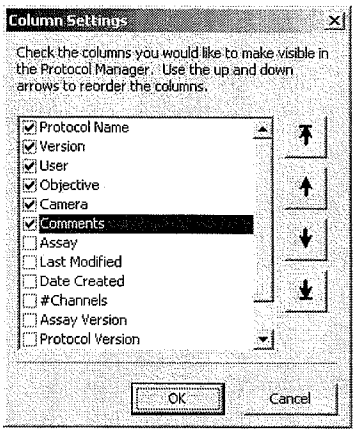


Figure 7.4 Manage Protocol Window Column Selection.

3) Check the boxes of the columns that you would like to have displayed in the Protocol Manager window.

4) Change the positioning of a column by selecting it and pressing the up or down arrow buttons. The column will move one position up or down each time you press the button. The selected column does not have to be checked for display in order to change its position.

5) The top arrow button moves the selected column to the first column position. The bottom arrow moves the selected column to the last column position.

6) Click OK to redisplay the currently displayed protocols with the new columns visible.

Selecting the Type of Protocols Shown

To show only protocols created by the current user,

- From the **Options** menu, select **Show <user> Protocols Only**

-or-



Click the **Show <user> Protocols Only** toolbar button.

The protocols shown in the right-hand window pane will be limited to the protocols created or last modified by the current user.

To show deleted protocols,

- From the **Options** menu, select **Show Deleted Protocols**

-or-



Click the **Show Deleted Protocols** toolbar button.

Protocols that were previously deleted will be displayed in the right-hand window pane, following any other restrictions currently in effect (current user, current version, and so on). A trash basket icon will mark them as deleted protocols. Deleted protocols can only be opened in the Create Protocol window.

To show only compatible protocols,

- From the **Options** menu, select **Show Compatible Protocols Only**

-or-

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6) Click **OK** to reorganize the protocols. The new organization will be shown in the navigation pane of the Protocol Manager.

To view the organized protocols, click on the '+' next to the folder you would like to open. Continue opening subfolders until you reach the lowest level. Click on that folder to see the protocols that meet the organization specified.

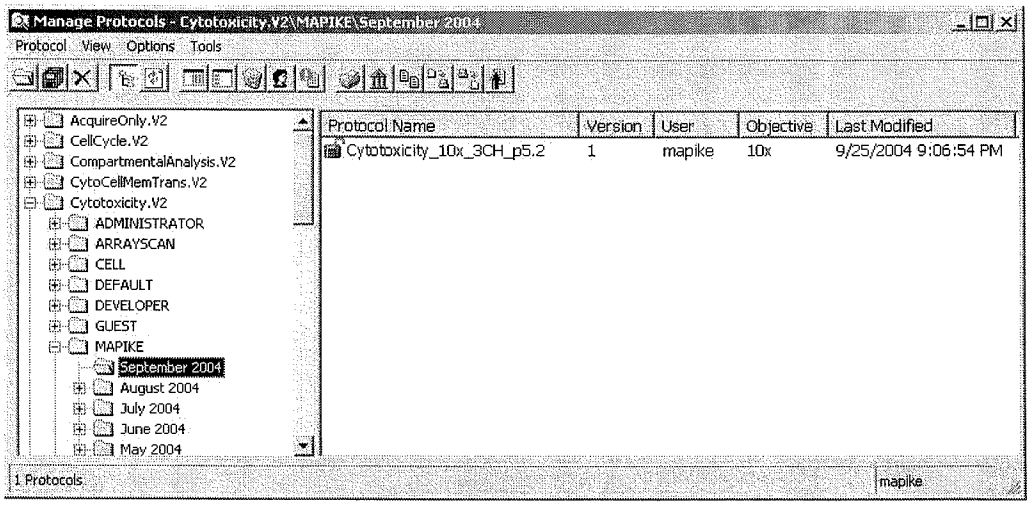


Figure 7.6 Manage Protocols Window with Protocols Organized to Three Levels.

Protocol Manager Tools

The Protocol Manager allows you to manipulate protocol files.

Print Protocols

Protocols can be printed by displaying the protocol information page in your web browser and using your browser's print feature.

To open a protocol for printing,

- From the **Tools** menu, select **Print**

-or-



Click the **Print** toolbar button.

Use your browser's print feature to print a copy of the protocol.

Show Protocol Differences

You can select two protocols and produce a report that lists the differences between the protocols.

To compare two protocols,

1) Use CTRL-click to select two displayed protocols.

2) From the **Tools** menu, select **Show Differences**

-or-

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*<Protocol Name>*_v*<Protocol Version>*.protocol

5) Click OK to save the active versions of the protocols to individual files.

NOTE

Older inactive versions of protocols are not saved when doing a bulk export. Older versions of protocols can be exported from the Version History window.

Show Protocol History

When you change any characteristics of a protocol then save the protocol, a new version of the protocol is created. The old versions are marked as inactive, but are still in the database and can be viewed using the Show Protocol History command. From the Version History window, you can open an older version (in the Create Protocol window only), compare two versions, print a version, export a version, or rollback to a previous version. Older versions of protocols can only be opened in the Create View window of ArrayScan or vHCS:Scan.

To view the protocol history,

1) Select a protocol in the Manage Protocols window.

2) From the **Tools** menu, select **Show History**

-or-



3) Click the Show History toolbar button.

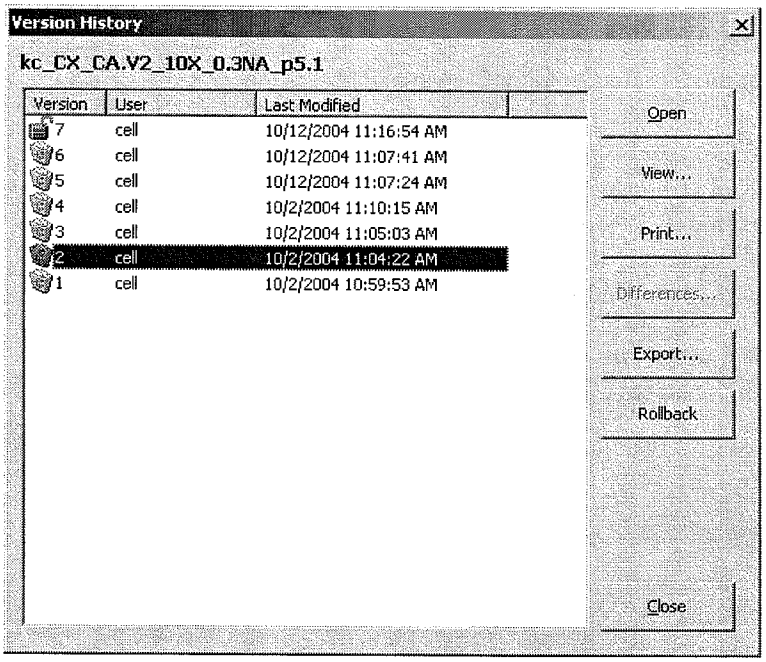


Figure 7.8 Protocol Version History Window.

To open a protocol version,

4) Select a protocol in the Version History window.

5) Click **Open**.

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View Protocol Properties

You can view the system properties of a protocol, and set its status.

To view a protocol's properties,

1) Select a displayed protocol.

2) From the **Tools** menu, select **Properties**

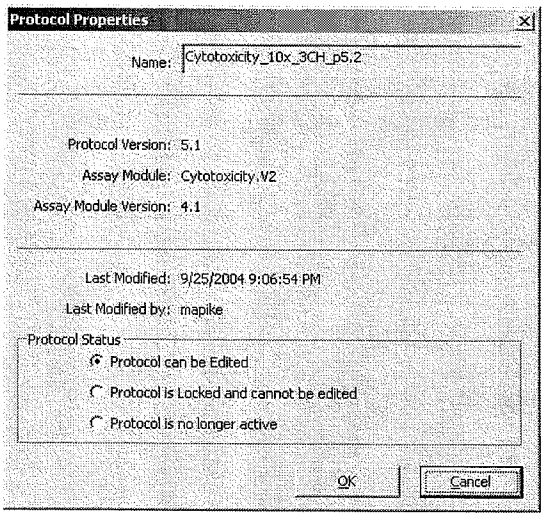


Figure 7.10 Protocol Properties Dialog.

3) The Protocol Properties dialog show you the name of the protocol, the version of the software under which is was created, the Assay Module it uses, the Assay Module version, the date the protocol was last modified and the user who modified it.

4) If you have administrative rights on ArrayScan, you can set the status of the protocol to editable, locked, or inactive. Select the desired status and click OK to set it.

Upgrade Protocols

Purpose

The protocol upgrade process is used to convert protocols to accommodate one or more of the following changes:

- *ArrayScan Software Version* change (from version 3.1/4.0 to 3.5/4.5)
- *BioApplication* upgrade (major or minor)
- *Pixel Resolution* change (due to camera or coupler change)

The use-cases are:

- Upgrade from V4.0 to V5.0 : This will change the ArrayScan Software Version, the BioApplication, and the Pixel Resolution.
- Using vHCS to scan old images with new BioApplication
- Using vHCS to convert protocols to instruments with different couplers

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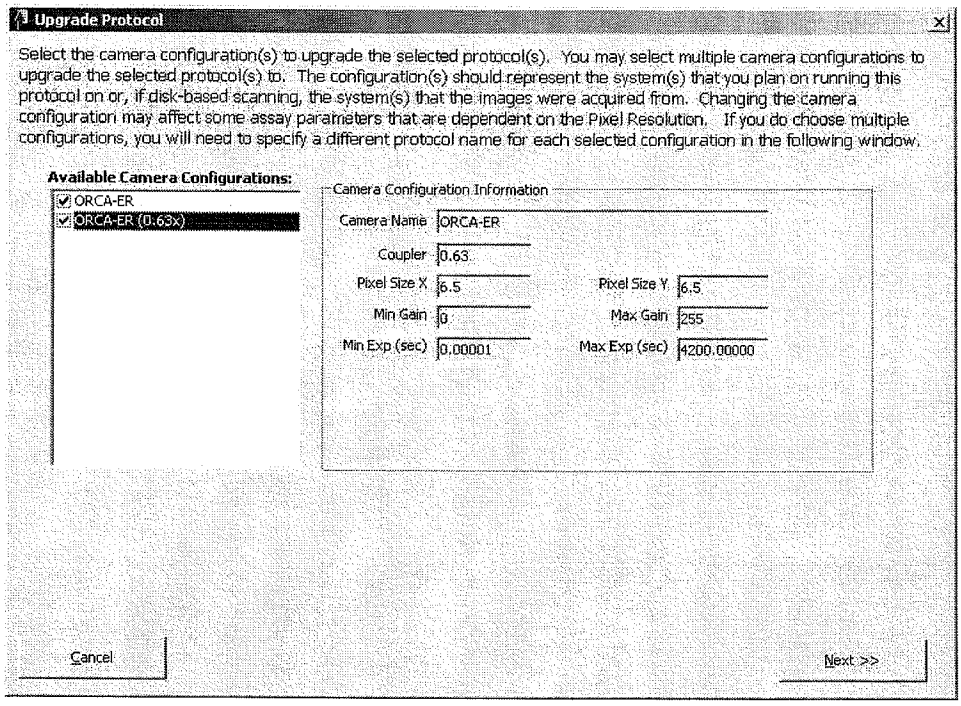


Figure 7.11 Protocol Upgrade Camera Selection Dialog.

5) Select the cameras for which you want upgraded protocols to be produced, and click **Next**. If there are multiple Bioapplication upgrade paths for the protocols you selected, you will see a screen allowing you to specify which Bioapplication upgrade paths to take for any affected Bioapplications. Otherwise, if you have chosen **Upgrade As…**, or you have chosen to upgrade to more than one camera configuration, you will see a screen that allows you to specify names for the new protocols.

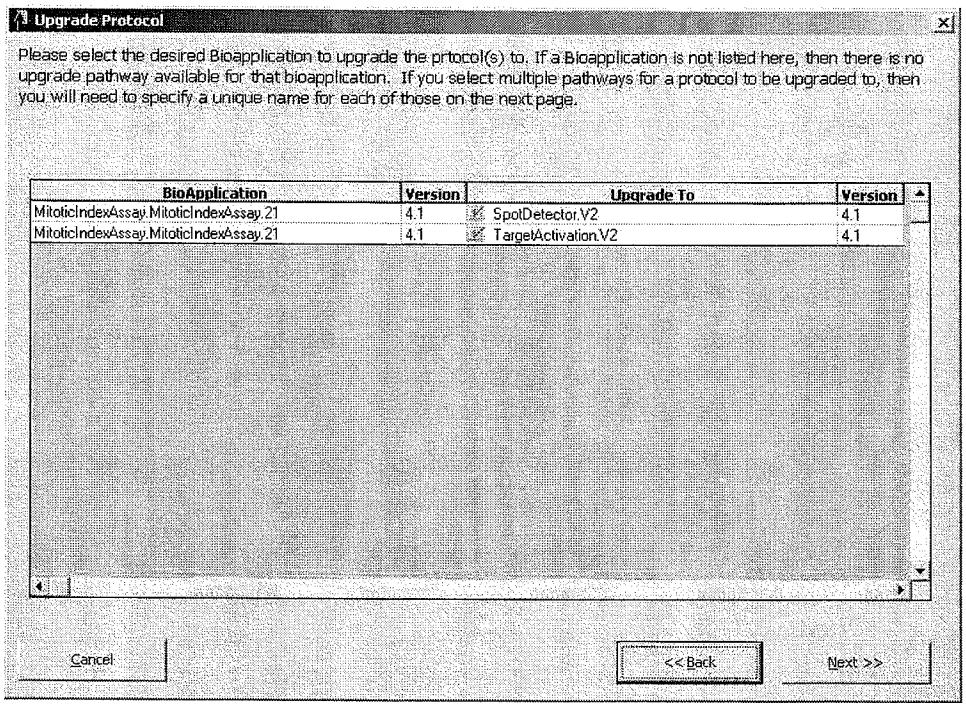


Figure 7.12 Multiple Upgrade Path Dialog.

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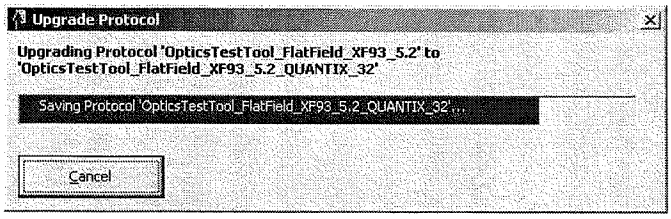


Figure 7.14 Protocol Upgrade Progress Bar.

10) Once the upgrade is finished, a web page appears showing the differences between the original and upgraded protocols.

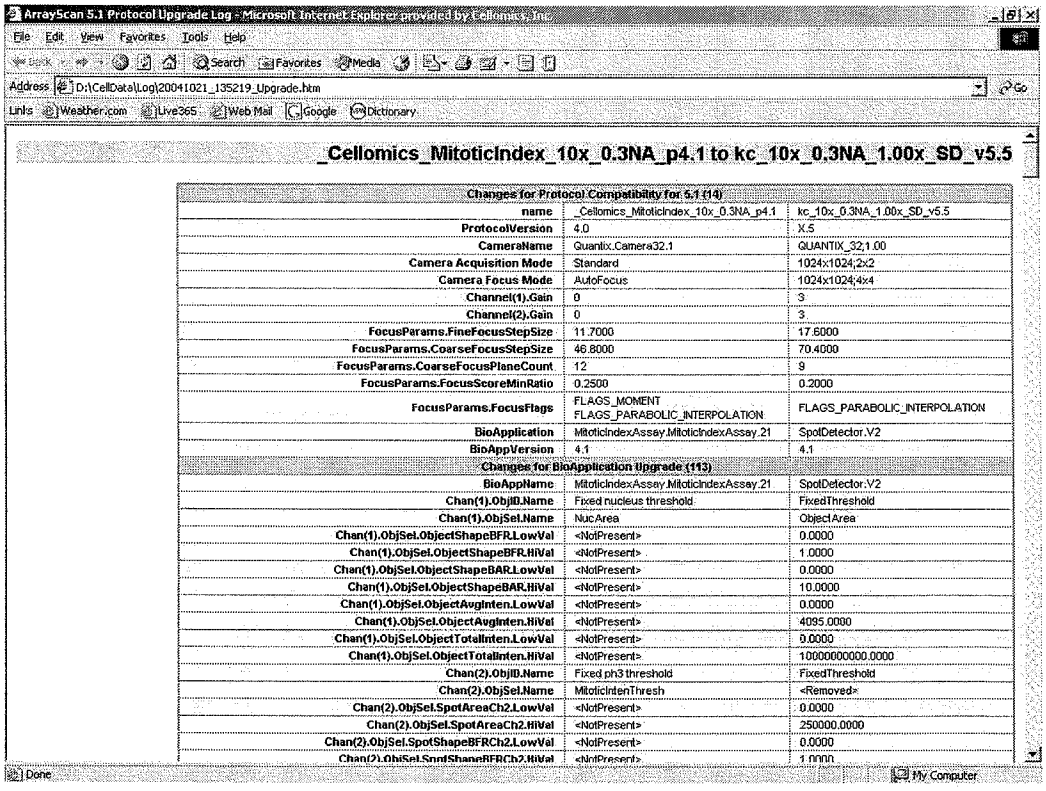


Figure 7.15 Upgraded Protocol Differences Web Page.

The upgraded protocols will appear in the Protocol Manager window.

11) If you selected **Upgrade** from the **Tools** menu, and have only one camera configuration, you will see only the progress bar and the upgrade log web page.

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Chapter

8

Setting Assay Protocol Parameters Interactively

WARNING

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Failure to adhere to safety precautions and/or procedures outlined in this document may result in system failure, personal injury, or death. Thermo Fisher Scientific shall not be held liable under any circumstances. Thermo Fisher Scientific does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories.

Occasionally, you may want to view images from the microplate wells in order to effectively set Assay Protocol parameters. In the Protocol Interactive View, you can acquire these images, and interactively adjust Assay Protocol parameters while evaluating the effects on the image.

This chapter covers the following Protocol Interactive View topics:

- Choosing Channel Parameters
- Displaying and Focusing Images
- Adjusting Camera Exposure Time
- Working with Images and Image Sets
- Using Advanced Protocol Interactive Settings

Each of the parameters in the Protocol Interactive View is also available in the Create Protocol View. Changing the parameter in either view changes the parameter stored in the Assay Protocol. The Protocol Interactive View provides a variety of tools to assist you in determining the appropriate values for these parameters.

To display the Protocol Interactive View,



- Click the **Protocol Interactive** button located on the ArrayScan toolbar
  -or-
  From the **View** menu, select **Protocol Interactive**.

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Acquiring and Focusing Images

Stage and Optics Control Options

You can use the Stage and Optics Control area to manipulate the stage and the camera in order to acquire live images from the camera.

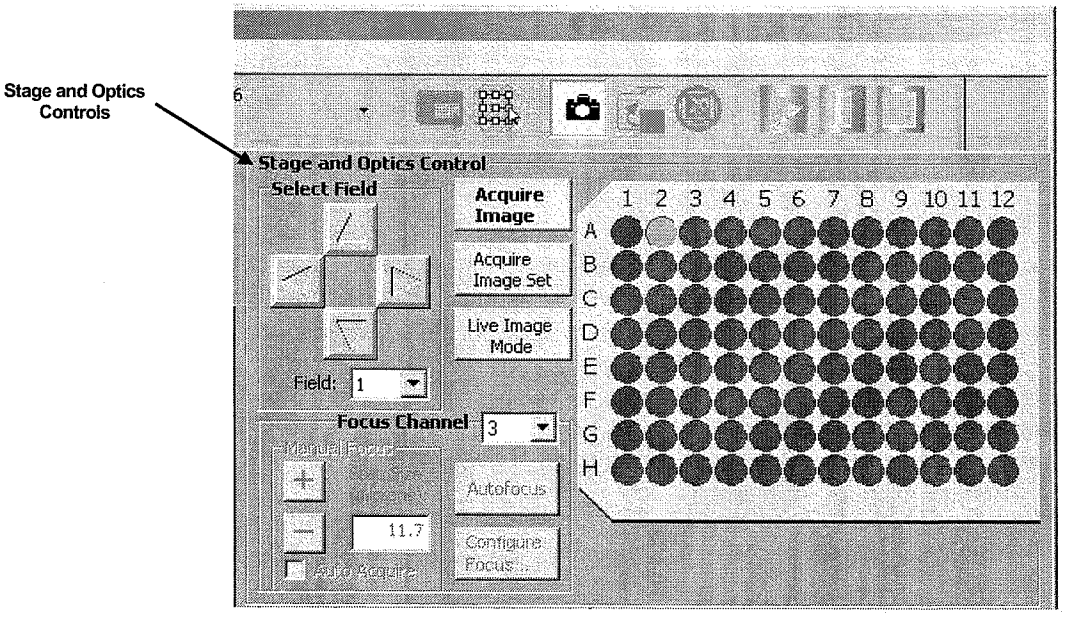
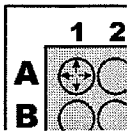


Figure 8.3 Stage and Optics Control section of the Protocol Interactive View

The following stage and optics controls are available.

Option	Description
Select Field arrow buttons	Moves the objective relative to the microplate well as shown in the figure on the right. Movement is one field at a time. The field number that is currently selected is displayed above the Field arrow buttons. 
Field list	Displays the field numbers from which to choose.
Manual Focus arrow buttons	Moves the objective relative to the microplate. The up arrow button moves the objective away from the microplate. The down arrow button moves the objective closer to the microplate.
Step Size	Represents the minimal objective movement (in microns) each time the Manual Focus arrow buttons are clicked. This value defaults to the theoretical depth-of-focus for the selected objective.
Microplate Representation	Displays a representation of the microplate. Click on a microplate well to move the objective to the selected well.
Autofocus button	The ArrayScan V^{TI} HCS Reader uses a proprietary autofocusing algorithm to produce sharp images while scanning. Each Assay Protocol has its own set of focusing parameter values. This button will run the autofocus algorithm at the current position on the microplate.
Acquire Image button	Acquires an image for the currently selected well and channel and displays it in the Image Display area.
Acquire Image Set button	Acquires one image from each of the available channels and stores them in memory. The image from channel 1 is displayed in the Image Display area.

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Changing Autofocus Settings

When using an Assay Protocol that was created for use with a different objective, it will be necessary to change the autofocus settings. Guidelines for changing the required settings based on the desired standard objective are provided below.

NOTE

For best results, start with a factory supplied Assay Protocol for the algorithm and objective that you wish to use and modify the Assay Parameters to fit your biology.

To change the autofocus settings,

1) Click the **Configure Autofocus** button. The Autofocus Options dialog box appears as shown below.

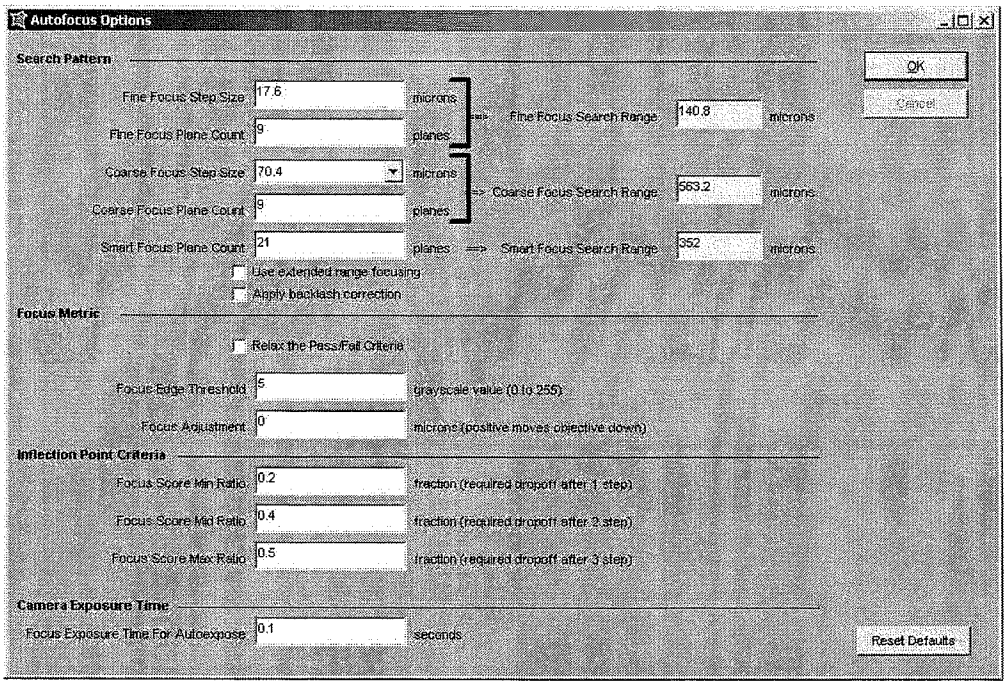


Figure 8.4 Autofocus Options dialog box

2) Change the settings using the guidelines in the following table and click **OK** when finished. If you decide you do not want to save the changes, click the **Reset Defaults** button before clicking the **OK** button. Reset Defaults will set the autofocus parameters to the defaults for the chosen objective. Reset Defaults will not revert the autofocus parameters to their previous settings.

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Manual Focusing

Manual focusing allows you to control the position of the objective in order to focus the image.

To manually focus an image,

1) Under Channel Parameters, select the desired channel number.
2) In the Stage and Optics Control area, click on the desired well. The microplate stage moves to the center of the well. Wait for this motion to complete before proceeding.
3) Click the **Acquire Image** button. The image appears in the Image Display area.
4) Enter a value in the **Step Size** box. This value represents the minimal objective movement (in microns) each time the Manual Focus arrow buttons are clicked.
5) Click the **Manual Focus** arrow buttons to move the objective relative to the microplate. The down arrow button moves the objective closer to the microplate. The up arrow button moves the objective away from the microplate.
6) Click the **Acquire Image** button to obtain another image.
7) Adjust the Step Size, if necessary.
8) Repeat steps 4 and 5 until the image is in focus.

NOTE



To view images from another field, click the **Select Field** arrow buttons until the desired field is displayed or select the desired **Field** number from the Field drop down list.

An alternative manual focusing method involves the use of the Live Image Mode button. You can use the Live Image Mode button to continually acquire images from the camera as fast as the currently set exposure time allows. These images are reduced resolution images of the current dye. Because the images are reduced in resolution, they transfer faster and can often be used for manual fine focusing of the camera. Click the Live Image Mode button again to return to normal mode.

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