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Find the *Thermo / Cellomics ArrayScan VTI* at our website: ***Click HERE***

II. General Guidelines for Selecting Dyes

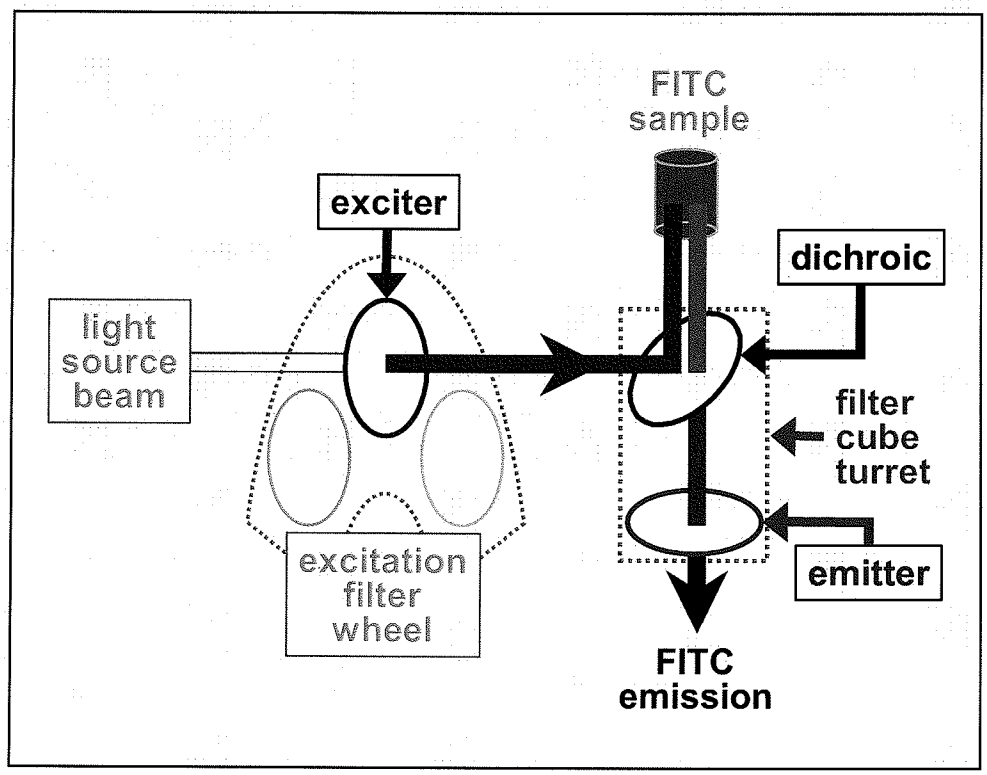
- The excitation filter wheel moves are relatively rapid, whereas turret moves require more time (~0.1 and 1.0 seconds for the excitation filter wheel and turret moves respectively).
- The ArrayScan V^{TI} is shipped with filters and dichroic mirrors from Omega Optical. Typical transmittance spectra of these optical elements are found on the "Curvo-matic" page of the Omega Optical web site and at the end of this appendix.
- For the optimal scan times, select dyes that use the same dichroic-emitter pair (e.g. use XF93 for a 4 color assay that uses Hoechst, FITC, TRITC and Cy5; use XF53 for a 3 color assay that uses Hoechst, FITC, and Texas Red).
- When using a 1 color assay, for the lowest exposure times, select either XF93 –Hoechst , XF100 –FITC (GFP), XF32 –TRITC (sensitive) or Cy5 –XF110 (sensitive).
- When performing a 2 color assay using only Hoechst and FITC (or GFP), select XF100. The XF100 – FITC (GFP) will yield shorter exposure times than the XF93 – FITC when standard FITC fluorophores or EGFP are part of the bioassay.
- When your Cy5 signal is adequate and the assay additionally requires Hoechst, FITC, GFP, and/or TRITC then select XF93 for all channels. This requires no filter turret moves and optimizes scan speed.
- Use the XF32 –TRITC (sensitive) if you need the extra sensitivity to detect your TRITC signal, or if you are not using XF93 and a turret move is needed to collect TRITC.
- Use XF110 Cy5 (sensitive) if you need the extra sensitivity to detect your Cy5 signal, or if you are using dye selections from a filter set other than XF93 and a turret move is needed to collect Cy5.
- Consider the time spent imaging versus the time spent moving the turret. XF110-Cy5 (sensitive) will yield ~4-5 fold shorter exposure times over XF93-Cy5. For example, if the signal level is low and the exposure time is 4 seconds with XF93, it would be ~0.8 seconds with XF110-Cy5 (sensitive). The 3.2 second savings can outweigh the 1 second turret move to place the XF110 into position and the 1 second to remove it from position. Please see Part III, section 3 later in this appendix for more on the order of turret moves during one image acquisition sequence.

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IV. Spectra of Standard Filters and Example Fluorophores

The following sections show Transmission spectra for filters, sometimes overlying spectra of fluorophore excitation (absorbance) and emission (fluorescence at fixed excitation wavelength). All fluorophore spectra are normalized (common practice). Therefore, quantitative inferences based on the figures are not necessarily valid. Filters vary slightly, fluorophore spectra are typically acquired from purified solutions, and fluorophore emission is acquired at a fixed excitation wavelength. The milieu of a fluorophore in a bio-specimen can be quite different (e.g., pH and other components of buffers, natural autofluorescence of the bio-specimen or substrate, quenching, and even potential FRET). Moreover, the relative intensities in a multi-labeled specimen are unlikely to be equivalent and imaging exposure times (CCD integration) will likely differ. Because these factors are always relative for a specimen, there is always cross-talk/bleed-through potential.

The physical configuration of the optical path is schematized in the following figure. The illumination beam from the white light source is filtered by the **exciter** transmitting a narrowed band of wavelengths. The **dichroic** mirror reflects the exciter-filtered beam to the sample, exciting the target fluorophore. Emitted fluorescence is transmitted through the **dichroic** (rather than reflected), and is further filtered by the **emitter**. Thus, specifically intended fluorescence is detected. In such Pinkel[1] sets of filters, the **dichroic** reflects the **exciter**-filtered band, but transmits a band of emitted fluorescence to the **emitter** (below left). In other words, the transmission of the **exciter** and **dichroic** are inversely related, whereas that of the **dichroic** and **emitter** share overlap.



[1] Pinkel sets provide that, given appropriate multi-band-pass dichroics/emitters, additional bands of fluorophore emission can be transmitted without a turret move. Therefore, only different excitation bands (also matched to the dichroic) are needed. These are afforded by a relatively rapid rotation of the exciter wheel, as described in the prior sections.

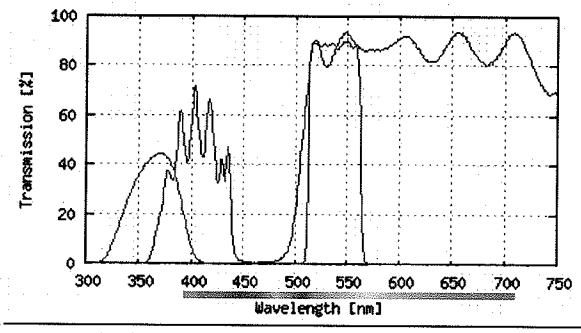
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Spectra for Standard Filter Sets and Fluorophore Examples

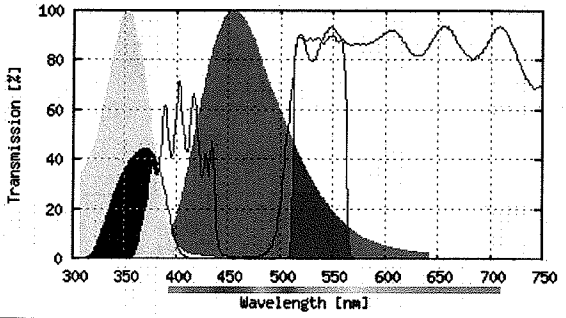
In the following figures, the left column shows the spectra using curves of the color convention described above (blue exciters, green dichroics, and red emitters). The right column shows the same spectral curves as the left, but overlying the spectra (solid) of indicated example fluorophores. In pairs of peaks for a fluorophore, the excitation peak is the left-most, and the emission peak is right-most.

XF100

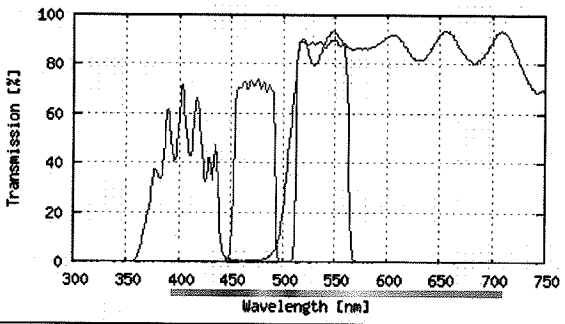
XF100-Hoechst



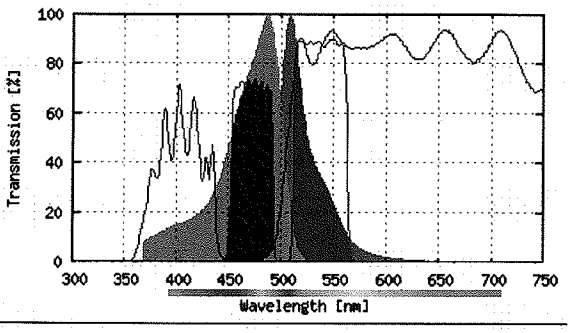
+ Hoechst



XF100-Fitc (GFP)

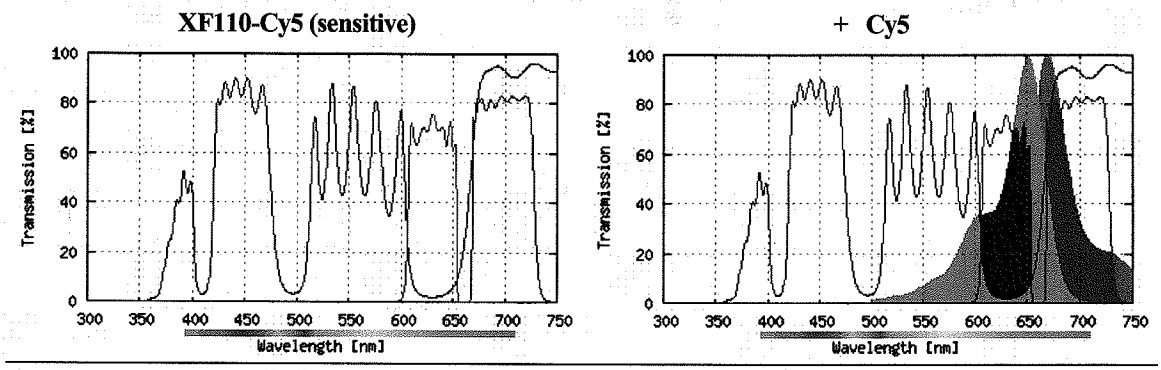


+ EGFP

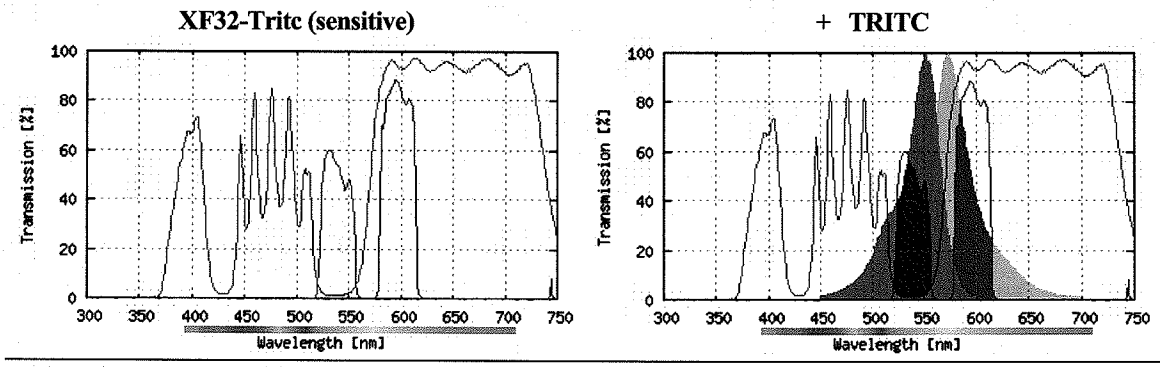


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XF110



XF32



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Appendix

C

Objective and Plate Type Selection

The ArrayScan V^{TI} HCS Reader provides the user flexibility when choosing objectives and microplate types. The ArrayScan V^{TI} HCS Reader uses both standard and high NA objectives from 5x to 40x, and can read the following 96-well and 384-well microplates manufactured with standard plastic bottoms, thin bottoms, or glass bottoms.

Greiner: 96-well plate

Falcon: 96-well clear bottom plate (BD BioCoat®) and 384-well thin bottom plate

Packard View: 96-well plate and 96-well glass bottom plate

Additional plates may also be supported; please contact Technical Support for recommendations.

Choosing a Proper Plate Type with the Objective

Objective – Plate Compatibility

Objective	Standard Plastic Plate	Thin Bottom Plate	Scan Area 96-well Plate	Scan Area 384-well Plate
Standard Lower NA Objectives				
5x (NA 0.25)	X	X	96	384
10x (NA 0.30)	X	X	96	384
20x (NA 0.40)	X	X*	96	384
Higher NA Objectives				
10x (NA .45)		X	96	308
40x (NA .50)	X		96	384
20x (NA .75)		X	60	308
40x (NA .75)		X	60	308

* Image sharpness may be compromised; please contact technical support for more information.

Please refer to the table above and the following information for recommended combinations of supported plate types and objectives. All supported plastic clear-bottom plates, thick or thin bottom, can be read with the three standard lower NA objectives [5x (NA .25), 10x (NA .30), and 20x (NA .40)]. The higher NA 10x (NA .45) objective can be used with the supported thick or thin bottom plates, however, image sharpness is best on thin bottom plates. Similarly the lower NA 20x (NA .40) objective can be used with the supported thick or thin bottom plates, however, image sharpness is best on thick bottom plates. The higher NA 20x (NA .75)

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Appendix

D

Frequently Asked Questions

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.

This section provides answers to frequently asked questions related to ArrayScan V^{TI} HCS Reader operation. If you have a technical question that you are unable to answer after consulting the documentation, please contact Technical Support.

Image Acquisition Questions

I changed the objective magnification and now my results are incorrect. Why?

Assays are designed for particular magnifications and changing objectives without making corresponding changes to assay algorithm parameters will adversely affect most assays. This is usually because changing the magnification changes the area covered by each pixel. Since some 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 is changed, the effective ring width (in real microns) will be cut in half. This can cause undesirable effects on the assay measurements.

How can I compensate for magnification changes?

In many cases, adjusting pixel unit parameters proportional to the change in magnification can compensate for magnification changes. For example, changing a ring width size from 2 to 4 in the Nuclear Translocation assay can compensate for changing the objective from 10X to 20X. Alternatively, increasing or decreasing the camera resolution inversely proportional to the change in magnification can compensate for these effects. For example, changing from a 10X to a 20X objective and changing the acquisition resolution from 1024x1024 to 512x512. Note that when decreasing objective magnification, you may also decrease the signal to noise ratio, as the areas used to make some measurements may become smaller than a statistically meaningful sample.

My image is in focus but it is too bright/too dim. How can I fix it?

First, verify that the appropriate dyes are selected. Next, select the Fixed option in the Exposure section of the Protocol Interactive View and capture an image. If the image is too bright, decrease the exposure time. If the image is too dim, increase the exposure time. Acquire another image and observe the results. Repeat this process until you are satisfied with the image exposure. Alternatively, use one of the available AutoExpose methods.

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Automation Controller Error Messages

Error Message	Description
I'm sorry, but there was a problem that I could not automatically correct. The motion control subsystem failed initialization	An error occurred while initializing the stage. Contact technical support.
I'm sorry, but there was a problem that I could not automatically correct. The motion control subsystem requires calibration or is malfunctioning	An error occurred while initializing the stage. Contact technical support.
I'm sorry, but there was a problem that I could not automatically correct. The operation failed because the motion control system must be initialized first	An error occurred while initializing the stage. Contact technical support.
I'm sorry, but there was a problem that I could not automatically correct. The last command to the motion control system failed	An error occurred while initializing the stage. Contact technical support.
I'm sorry, but there was a problem that I could not automatically correct. The clockwise (CW) Z axis limit switch wasn't detected	An error occurred while initializing the stage. Contact technical support.
I'm sorry, but there was a problem that I could not automatically correct. The counterclockwise (CCW) Z axis limit switch wasn't detected	An error occurred while initializing the stage. Contact technical support.
I'm sorry, but there was a problem that I could not automatically correct. Both of the Z axis limit switches are malfunctioning	An error occurred while initializing the stage. Contact technical support.
I'm sorry, but there was a problem that I could not automatically correct. The operation failed because of a communications timeout with the ArrayScan. Please make sure your ArrayScan is powered ON and the cables are properly connected and try again	Power may not be on, or a cable may not be connected

Protocol Validation Error Messages

Error Message	Description
Protocol Validation Failed: No Assay Selected	Select an assay from the Assay list.
Protocol Validation Failed: Invalid Assay ASSAY_NAME	Either a problem exists with the listed assay, or the Assay Protocol references an assay that is no longer installed. If the listed assay should be installed, contact technical support. Otherwise, choose a different Assay Protocol or assay.
Protocol Validation Failed: No Camera Selected	The Camera Drop Down List is left empty. Select a valid camera type.
Protocol Validation Failed: Could not create camera.	Contact technical support.
Protocol Validation Failed: Camera Failure	Contact technical support.
Protocol Validation Failed: focus mode is not valid for camera	Contact technical support.
Protocol Validation Failed: acquisition mode is not valid for camera	Contact technical support.
Protocol Validation Failed: Invalid Number of Channels.	The selected number of channels is greater than the max number or lower than the min number supported by the assay. Choose a number of channels within this range.

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Error Message	Description
Protocol Validation Failed: Missing Well Feature FEATURE_NAME	This is most likely to occur when using an older Assay Protocol. FEATURE_NAME is the missing feature. Create a new Assay Protocol for the same assay and re-enter all the parameters.
Protocol Validation Failed: Autofocus Field interval needs to be at least 0.	Change the Intra-Well AutoFocus Interval to be greater than zero.
Protocol Validation Failed: Coarse Focus Plane Count needs to be 3 or greater.	Change the Focus Config parameter, Coarse Focus Plane Count to be 3 or greater.
Protocol Validation Failed: Coarse Focus Step Size needs to be 1 or greater	Change the Focus Config parameter, Coarse Focus Step Size to be 1 or greater
Protocol Validation Failed: Protocol Validation Failed: Fine Focus Plane Count needs to be 3 or greater	Change the Focus Config parameter, Fine Focus Plane Count to be 3 or greater
Protocol Validation Failed: Fine Focus Step Size needs to be 1 or greater	Change the Focus Config parameter, Fine Focus Step Size to be greater than 3, (the error message should say 3 or greater). Recommended to be 10 for 10x and 5 for 20x objectives.
Protocol Validation Failed: Unknown Focus Parameter FLAG_NAME	FLAG_NAME is an invalid name for a focus flag. Change the Focus Config parameter, Focus Flags, by selecting the desired flags and pressing accept.
Protocol Validation Failed: FocusScoreMaxRatio must be between 0 and 1 inclusive	Change the Focus Config parameter, Focus Score Max Ratio to be no greater than 1 and no less than zero.
Protocol Validation Failed: FocusScoreMidRatio must be between 0 and 1 inclusive	Change the Focus Config parameter, Focus Score Mid Ratio to be no greater than 1 and no less than zero.
Protocol Validation Failed: FocusScoreMinRatio must be between 0 and 1 inclusive.	Change the Focus Config parameter, Focus Score Min Ratio to be no greater than 1 and no less than zero.
Protocol Validation Failed: Focus Interp Frac must be between 0 and .3 inclusive	Change the Focus Config parameter, Interp Frac, to be no greater than 0.3 and no less than zero.
Protocol Validation Failed: Focus Score Dist must be between 0 and 2 inclusive	Change the Focus Config parameter, Pixel Scoring Distance , to be no greater than 2 and no less than zero.
Protocol Validation Failed: Focus Edge Threshold must be between 1 and 40 inclusive	Change the Focus Config parameter, Focus Edge Threshold, to be no greater than 40 and no less than 1.
Protocol Validation Failed: Focus Initial Exposure Time must be between 0 and 15 inclusive	Change the Focus Config parameter, Focus Exposure Time, to be no greater than 15 and no less than 0.
Protocol Validation Failed: Smart Focus Plane Count must be between 3 and 100 inclusive	Change the Focus Config parameter, Smart Focus Plane Count, to be no greater than 100 and no less than 3.
Protocol Validation Failed: Focus Tag Point Retries must be between 0 and 12 inclusive	Change the Focus Config parameter, Tag Point Retries, to be no greater than 12 and no less than 0.
Protocol Validation Failed: Invalid Protocol Name	Assay Protocol name cannot be blank, enter an Assay Protocol name.

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Appendix

F

Replacement Parts

All consumable parts can be purchased through customer support. To order replacement parts, please contact your local sales representative or contact technical support directly.

Optional objectives and filter sets are also available. Please call customer support for more information.

ArrayScan Instrument

Part Name	Part Description	Part Number
Transport Handle	1 Handle: Four handles are required for safe transport	**000000-0236-611**
Plastic plug	1 plug covers transport handle thread	**000000-0232-258**
OptiTracker	Optical Performance Monitoring Kit	**K10-0099-1**

Lamp Unit

Part Name	Part Description	Part Number
Replacement Bulb, EXFO X-Cite lamp unit	1 Replacement Bulb	**012-63000**

Fuses

Part Name	Part Description	Part Number
ArrayScan Instrument Fuses	2, T10.0A H 250V 5x20 UL/CSA-rec	**000000-0149-695**

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Appendix

G

Technical Specifications

NOTE

Technical specifications are subject to change without notice.

ArrayScan Instrument

Input Voltage/Frequency	120 V AC ± 10%, 50-60 Hz -or- 230V AC ± 10%, 50-60 Hz with voltage retrofit option kit
Current	10A maximum, approximately 5A typical
Supported Microplates	Refer to the *Objective and Plate Type Selection* appendix for a list of supported microplates.
Plate and Stage Scan Region	235 mm x 115 mm
Illumination	120 W metal halide arc lamp
Focus Resolution	Harmonic Drive, 25 nm step width
Wavelength Range	Approximately 330-700 nm
Filter Wheel	10-fold software controlled motorized filter turret
Objective Turret	Automated 6 position objective turret. 5x, 10x, 10x high NA, and 20x objective lenses Optional objective magnifications of 20x (NA .75), 40x (NA .50), and 40x (NA .75) are available.
Max# of Channels	6 (in a single Assay Protocol)
Camera	High-resolution, thermoelectrically cooled, digital camera with 12-bit dynamic range.
Optics	Infinite corrected system, W 0.8" x 1/36" thread
Weight	86 kg

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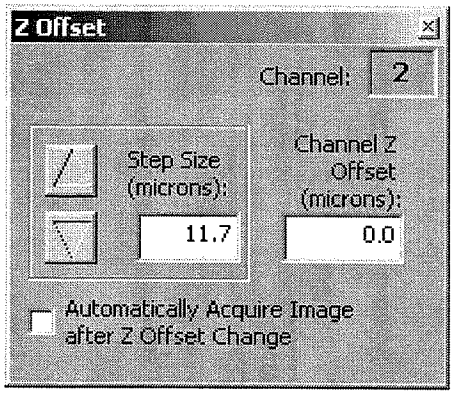


Figure 8.6 Z Offset dialog box

4) Type in a Z Offset value in the Offset box and press Enter. Positive values move the objective away from the microplate; negative values move the objective closer to the microplate. To obtain the image with the new Z Offset value, either select the checkbox labeled, Automatically Acquire Image after Z Offset Change, or click the **Acquire Image** button.

NOTE

You can use Image Popup windows to display several channel images while comparing different Z Offset values per channel. Please refer to **Working with Image Popup Windows** on page 155 for more information.

5) If the Z Offset value that you enter does not yield the desired image, you can use the Focus arrow buttons to continue to move the objective closer or further from the microplate. Enter a new value in the Step Size box to change the objective movement (in microns) each time the Focus arrow buttons are clicked. If the checkbox labeled, Automatically Acquire Image after Z Offset Change, is checked, a new image will be acquired each time you click the Focus arrow buttons. If the checkbox is clear, then you must click the **Acquire Image** button to acquire a new image. Each time you click the Focus arrow buttons, the Z Offset box is updated to reflect the current Z Offset position.

6) When the desired image is displayed, click the Close button to close the Z Offset dialog box.

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5) Edit the Auto expose options as necessary, click OK, and click the **Auto Expose** button. Refer to "Specifying AutoExpose Options" in Chapter 6 for instructions on setting these options. Repeat steps 3 through 5 as necessary until an acceptable image is achieved.

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.

6) Repeat these steps for the remaining channels.

After you set the camera exposure, the Exposure section of the Protocol Interactive View displays the following items:

Display Item	Description
Dynamic Range Bar	Shows the dynamic range used by the most recently acquired image with respect to the available dynamic range of the camera. The bar also displays the actual percentage used. The bar is color coded as described below.
	Blue The dynamic range is below the nominal range obtained from the ***assay***.
	Green The dynamic range is within the nominal range obtained from the assay.
	Red The dynamic range is above the nominal range obtained from the assay.
Exposure Time	Displays the calculated camera exposure time in seconds.

Modifying the Fixed Exposure Time

To modify the Fixed Exposure Time,

1) Focus the image by following instructions outlined in "Focusing Images" above.

2) If the Exposure options are not displayed, click the **More>>** button located in the Channel Parameters section.

3) In the Exposure section, click the **Fixed** button.

4) Enter the exposure time in seconds in the box provided.

5) Click the **Acquire Image** button.

6) Edit the exposure time if necessary and click the **Acquire Image** button again. If the image is too bright, decrease the exposure time. If the image is too dim, increase the exposure time. Repeat this step until a desirable exposure time is achieved.

7) Repeat these steps for the remaining channels.

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Choosing Image Display Options

You can modify the image display options using the **Display Options** button in the Protocol Interactive View. Note that the overlay options are unique to each assay. Refer to your BioApplication Guide to determine which options are available.

To modify Image Display options,

1) Select the desired channel number.

2) Click the **Display Options** button . The Image Display dialog box opens as shown in the following figure.

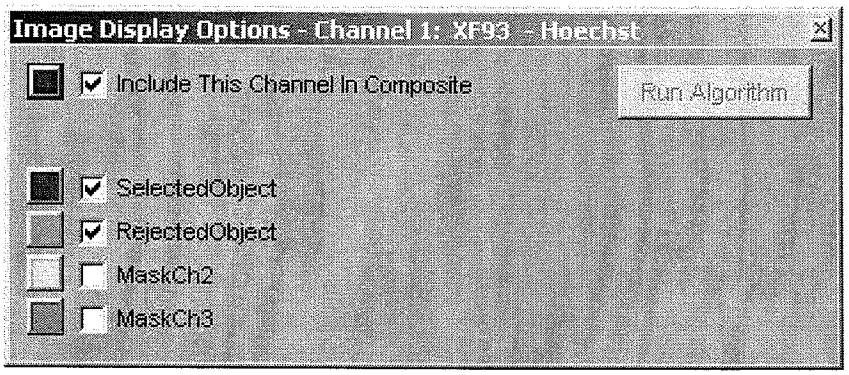


Figure 8.9 Image Display dialog box.

3) If you want to include the image for the selected channel as part of the composite image, check the **Include this Channel in Composite** checkbox. To change the color assigned to the composite, click the color button located next to the checkbox. Click on one of the available colors or click **Define Custom Colors>>** for more options. The color composite image is shown in the Scan Plate and Protocol Interactive Views.

4) To display a particular graphic overlay, check the associated overlay check box. To choose not to display the overlay, clear the overlay checkbox.

5) To change the color for a particular graphic overlay, click the color button next to the desired option. The Color dialog box appears. Click on one of the available colors or click **Define Custom Colors>>** for more options.

6) Click the **Run Algorithm** Button.

7) Repeat these steps for the remaining channels.

Displaying Raw Images

You can display a raw image (without overlay graphics) in the Image Display area using the Raw Image display option.

To display Raw Images,

1) Acquire a single image or Image Set.

2) Select Raw Image from the drop down list located beneath the Image Display area as shown in the following figure.

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Identifying Objects in the Image and Acquiring an Object Selection Set

Most cell-based assays provide a number of object selection parameters that are used to detect relevant cells for further processing and feature extraction. Each object selection parameter has an associated range. When running an assay algorithm, the value for each of these parameters is calculated for each object in the image. If any of the values are outside of the associated range, then that object is rejected. Otherwise, the object is determined to be a cell and is processed further.

The object selection parameters should be set so that cells with desirable characteristics (usually size and shape) are selected, while cells with undesirable characteristics are rejected. The Protocol Interactive View provides tools to help you determine the optimal values by interacting directly with images of cells, rather than working only with numbers.

The general process is to acquire an image of sample cells, interactively select those cells that are desirable (i.e., create a Selection Set), and expand or reduce the ranges for each object selection parameter in the Assay Protocol to encompass the range of the selection set. This process can be repeated with multiple sample images.

After you have acquired an Image Set and the images are in focus, you can now start to select objects within the image for analysis. The object's statistics can be added to the Object Selection Statistics table, shown in the following figure. This table contains a list of feature ranges for the selected objects within the image using the currently defined channel parameters.

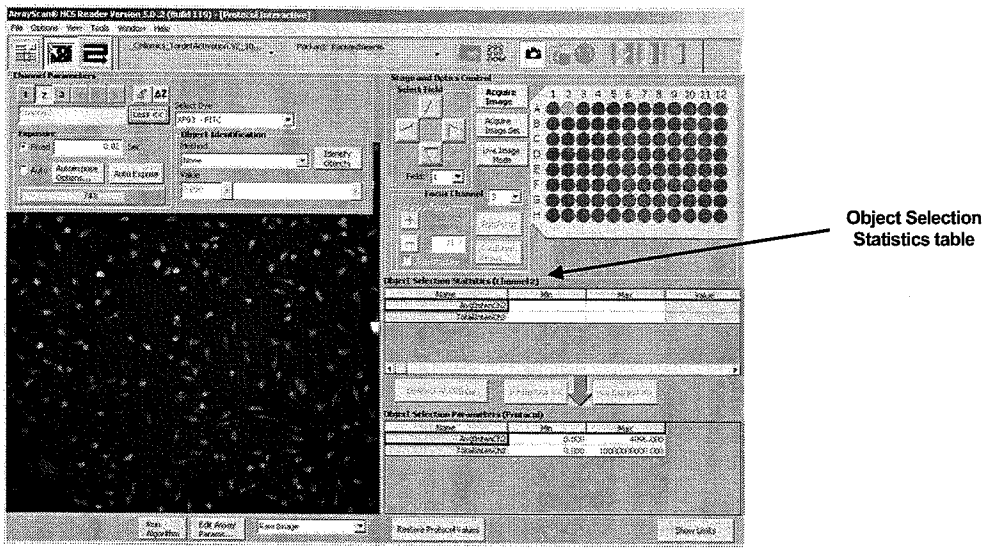


Figure 8.11 Object Selection Statistics table and Identify Object button in Protocol Interactive View.

The Object Selection Statistics table contains the following columns:

Column	Description
Name	The Name column lists the features for the currently selected channel and assay. These features are unique to each assay. For a description of each feature, please refer to the BioApplication Guide for the selected assay.
Min	The Min column is the minimum value of the associated feature within the object selection set.
Max	The Max column is the maximum value of the associated feature within the object selection set.
Value	The Value column lists the values for the object currently under the mouse pointer.

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2) Click the **Run Algorithm** button.

Notice that **Overlay Graphics** appear in the Image Display area (see following figure) if the current assay supports overlays.

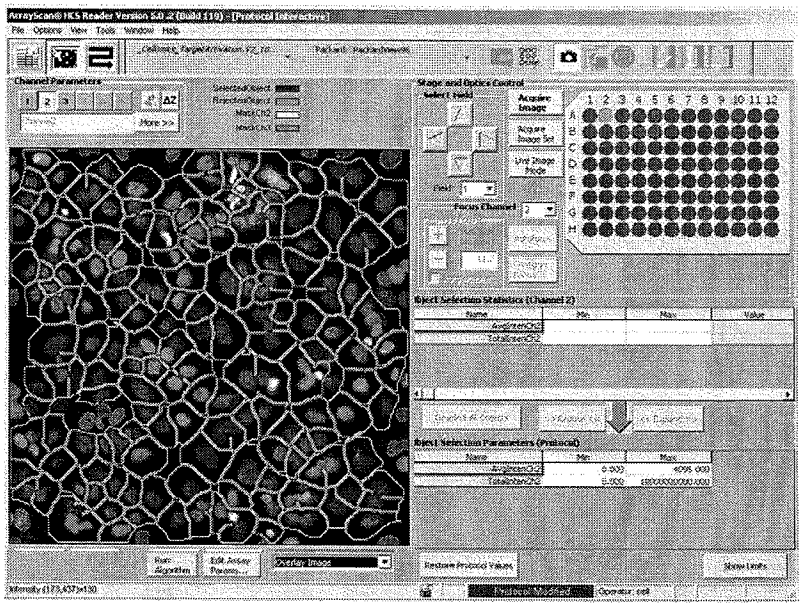


Figure 8.12 Overlay Graphics shown in the Image Display area of the Protocol Interactive View.

Viewing Algorithm Outputs

After running the assay algorithm on the acquired image set, you can view tables containing feature results in the Image Display area using the feature display options. The following feature display options are available:

Feature	Description
Cell Features	Displays feature values for each cell in the currently selected field within the well. Note that Cell Features are available for most assays. Refer to the BioApplication Guide for the selected assay to determine if the assay supports Cell Features and to obtain a description of each Cell Feature.
Field Features	Displays feature values for the currently selected field within the well. Note that Field Features are available for only a few assays. Refer to the BioApplication Guide for the selected assay to determine if the assay supports Field Features and to obtain a description of each Field Feature.
Well Features	Displays feature values for the currently selected well. Refer to the BioApplication Guide for the selected assay to obtain a description of each Well Feature.

To display the features,

- Select the desired feature display option from the drop down list located beneath the Image Display area. A message will appear if the current assay does not support the feature display option. Cell Features are shown in the following figure.

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Working with Image Popup Windows

The Image Popup window provides the ability to view one or more raw images, overlay images, or composite images on the screen simultaneously. When viewing more than one image, you can compare and assess the impact of various parameter adjustments.

Opening One or More Image Popup Windows

To open one or more windows,

1) Display the desired raw, overlay, or composite image in the Image Display area of the Protocol Interactive View.

2) Double-click anywhere within the image area
-or-
From the **File** menu, select **Image Display**.
-or-
While pressing the **Shift** key, click anywhere within the image area.

3) If you wish to display another popup window, display the desired channel image in the Image Display area of the Protocol Interactive View and repeat step 2. The number of popup windows you can display is dependent on the computer memory.

Popup Window Attributes

The raw, overlay, or composite image is displayed in a separate window as shown in the following figure.

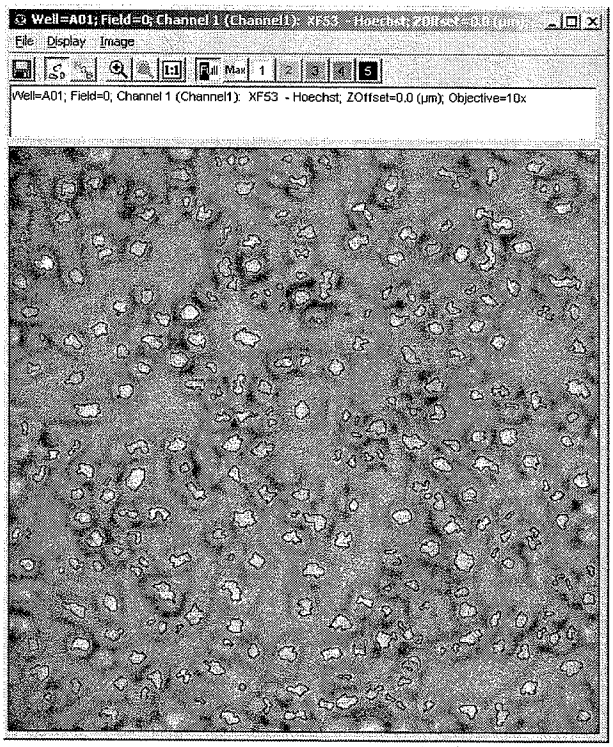


Figure 8.14 Image Popup Window

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Image	Toolbar Button Name	What it Does
2	Show Bits 2 to 9	Bits 2 to 9 are displayed, with image intensities greater than or equal to 511 displayed as white.
3	Show Bits 3 to 10	Bits 3 to 10 are displayed.
4	Show Bits 4 to 11	Bits 4 to 11 are displayed.
5	Show Bits 5 to 12	Bits 5 to 12 are displayed.

Working with Image Popup Windows

The following commands are available from the Image Popup window menus:

- Choose Image Display Type
- Choose an Image View Option
- Zoom in and out
- Save Images

Each command is described in detail in the following sections.

Displaying Well, Cell, and Field Features

The well, cell, and field features associated with the current image can be displayed in the Image Popup window, next to the image.

To toggle the Well Features on or off,

- From the **Display** menu, choose **Well Features**,
  -or-
  Select the **Well Features** toolbar button .

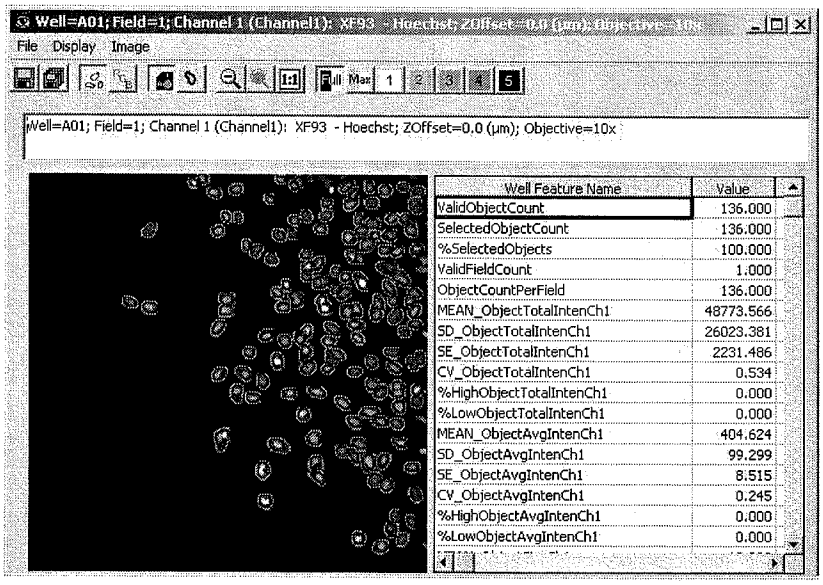


Figure 8.15 Image Popup Window with Well Features Displayed

To toggle the Cell Features on or off,

- From the **Display** menu, choose **Cell Features**,
  -or-

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If the following image was displayed in the Protocol Interactive View:	You will have access to the following image types when opening the Image Popup window:		
	Raw Image	Overlay Image	Composite Image
Raw Image	✓	✓ *	
Overlay Image	✓	✓	
Composite Image			✓

* The Overlay image will only be available in the Image Popup window if you ran the algorithm in the Protocol Interactive View.

To choose an Image Display type,

- From the **Display** menu, choose the desired Image Display type.

Choosing an Image View Option

Two image view options are available: Fit to Window and Max Fit (Auto Scroll). The default option, Fit to Window, displays the entire image in the Image Popup window. The image will resize to fit the window size. The Max Fit option displays as much of the image as will fit within the Image Popup window. If the entire image is too large in either the vertical or horizontal direction, then scroll bars will appear so that you can view the entire image.

To choose an Image View option,

- From the **Image** menu, select **View Options** and choose the desired Image View option.

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Saving Images

You can save images appearing in the Image Popup window as Bitmap (.bmp), TIF (.tif), or Cellomics 16 bit (.dib).

To save the currently displayed image,

1) From the File menu of the Image Popup window, select Save Image As…
The Save Image As dialog box appears as shown in the following figure.

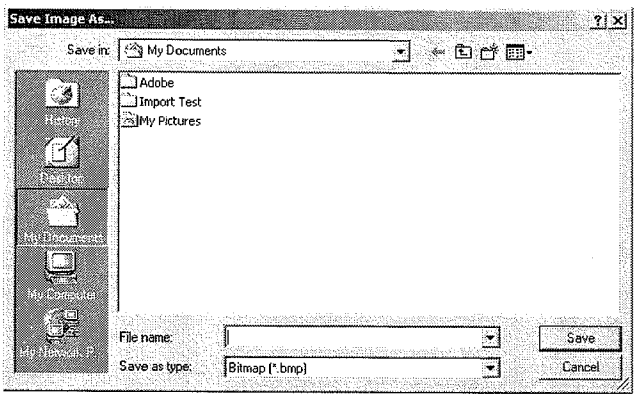


Figure 8.18 Save Image As dialog box

2) Select the desired folder location and file type.

3) Click the Save button.

To save the entire image set for the currently displayed image,

1) From the File menu of the Image Popup window, select Save Image Set …
The Save Image Set dialog box appears as shown in the following figure.

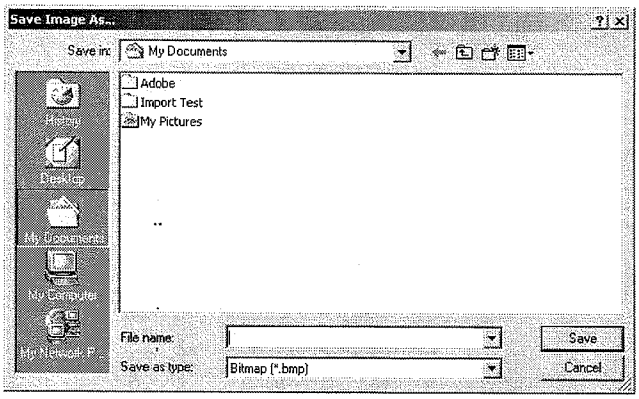


Figure 8.19 Save Image As dialog box.

2) Select the desired folder location and file type.

3) Enter a name into the file name box. The name of each image will be the name entered with an "_CHn" appended to the end of the name. n represents the channel of the image set.

4) Click the Save button.

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To run in Advanced Mode,

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

Selecting a Dye

With the desired channel selected, you can choose the fluorescent dye used to label biological structures in the channel image.

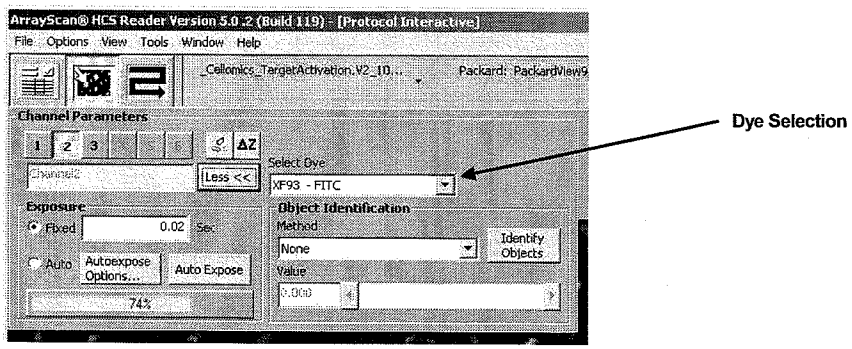


Figure 8.21 Dye selection. (If Dye Selection is not shown, click the More>> button.)

To select a dye,

1) If the Select Dye list is not displayed, click the **More>>** button located in the Channel Parameters section. The Select Dye list appears.

2) Select a dye from the **Select Dye** drop down list. The dye chosen here should match the dye used to label the biological marker on the microplate.

Changing Object Identification Settings

When running in Advanced Mode, you can change the Object Identification settings in the Object Identification section to interactively evaluate the effect on the image.

To change Object Identification settings,

1) Select a channel.

2) If the Object Identification options are not displayed, click the **More>>** button located in the Channel Parameters section.

3) Click the **Acquire Image** button.

4) Under Object Identification, select a threshold method from the Method drop down list.

5) Enter an intensity value in the **Value** box or use the slider bar to increase or decrease the intensity value.

6) Click either the **Identify Objects** button or the **Run Algorithm** button to evaluate the effect.

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Transferring Object Selection Parameters to the Assay Protocol

After you acquire a desirable object selection set (refer to "Identifying Objects in the Image and Acquiring an Object Selection Set" earlier in this chapter for instructions), you can now incorporate these values into the Assay Protocol when running in Advanced Mode.

Notice that the Protocol Interactive View contains two feature tables (refer to the following figure). The feature table on top is titled "Object Selection Statistics". The feature table directly beneath is titled "Object Selection Parameters". The "Object Selection Parameters" table represents the range of each feature for the current object selection set. The "Object Selection Parameters " table represents the feature limits that are currently stored in the Assay Protocol. Once the Object Selection Parameters table contains a representative sample of feature ranges, you can transfer these values to the Object Selection Parameters table and update the Assay Protocol with these new values. You can transfer values for all Object Selection Parameters at once or you can select the parameters from the table for which you wish to transfer ranges.

The Min and Max values displayed in the Object Selection Parameters table are used during scanning for object selection. Only objects whose features are within this range are considered in calculating assay features. The values for the Upper and Lower Limits, displayed when you click the Show Limits button, specify the valid range of the Min and Max values. These limits are set by the assay developer and are generally based on hard or physical limits.

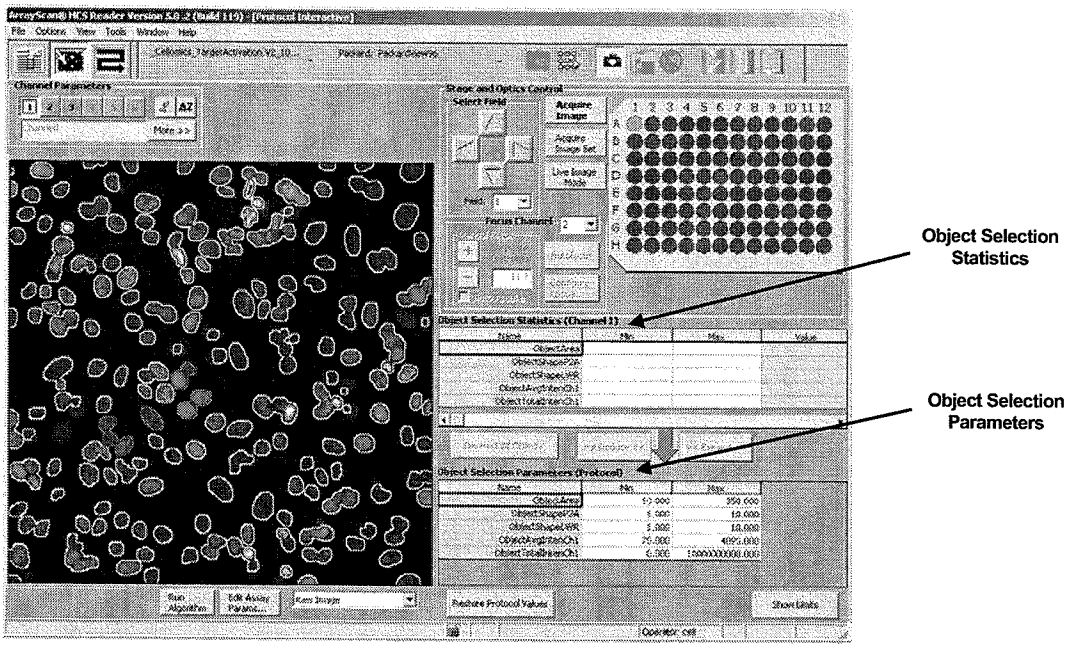


Figure 8.23 Transferring Object Selection Statistics to Object Selection Parameters in the Assay Protocol.

To transfer object selection statistic values into the Assay Protocol using the Expand and Reduce buttons,

1) When running in Advanced Mode, do the following:

2) To transfer ranges for all parameters at once, skip ahead to step 2. Otherwise, if you want to transfer a selection of parameters, click on the parameter name in the Object Selection Statistics table that you want to transfer. Notice that the Min and Max values for the other parameters are dimmed. Repeat until all desired parameters are included in the transfer.

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Chapter
9

Running a Disk Scan

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A Disk Scan allows you to reanalyze, with the Scan Plate or Protocol Interactive Views, images from microplates that were previously scanned. While reanalyzing these images, you can optimize or correct assay parameters to determine the effect on your results. Any parameter affecting the analysis of the image can be altered; altering parameters that directly affect the raw image acquisition (e.g., exposure time, dye name, etc.) will have no effect.

This chapter discusses the following Disk Scan topics:

- Preparing for a Disk Scan
- Starting and Monitoring Disk Scans from the Scan Plate View
- Starting Disk Scans from the Protocol Interactive View
- Optimizing Parameters

Preparing for a Disk Scan

You can run a Disk Scan on any microplate that has been previously scanned with the Store Images option selected. In order to run the disk scan, you must first prepare for the Disk Scan and instruct the system to run a Disk Scan.

Three Disk Scan options are available:

- Running a Disk Scan on one or more plates using a single Assay Protocol
- Running a Disk Scan on a single plate using multiple Plate Protocols
- Running a Disk Scan on multiple plates using multiple Plate Protocols

Setup options vary depending on the Disk Scan option you are running. Follow the instructions outlined below for the appropriate Disk Scan option.

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Running a Disk Scan on Multiple Plates using Multiple Plate Protocols

Setup procedures for this Disk Scan option are identical to the previously described setup procedures for running a Disk Scan on a single plate using multiple Plate Protocols.

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3) If you selected the **Store Database** option, the following screen appears.

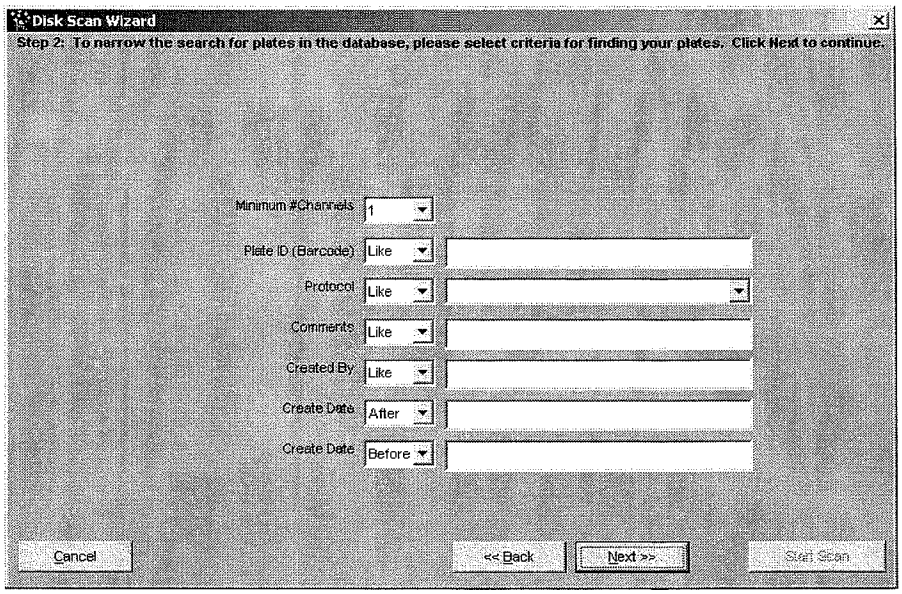


Figure 9.2 Disk Scan Wizard: Find plates

4) To narrow your plate search, enter a value in one or more of the fields provided. Choose **Like** before a search option to find results similar to the value entered. Choose = before a search option to find results exactly as entered. A description of each search option follows. After specifying the search criteria, click the **Next** button.

Option	Description
Minimum # Channels	The number of channels in the image set of the desired plate.
Plate ID (Barcode)	The name that is in the Plate ID box of the ArrayScan software application, or if a robotic plate handler is used, the name that you specified in the robot software, or the barcode read by the system.
Protocol	The Assay Protocol that was used to scan the plate.
Comments	The comments that were typed into the Scan Comments box of the ArrayScan software application.
Created By	The user name that was entered to log on to the system.
Create Date	The date the plate was scanned. **If you want to find plates scanned on a certain date,** enter the date in one of the two Create Date boxes using the = sign before. **If you want to find plates scanned before or after a certain date,** enter the date in one of the two Create Date boxes using either the **Before** or **After** option before the date. **If you want to enter a date range,** enter the starting date in the first Create Date box using the **After** option and then enter the ending date in the second Create Date box using the **Before** option.

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the **Use default scan area for each plate** checkbox. This option will use the full set of available images. If you want to specify a different scan area for each plate, clear the **Use default scan area for each plate** checkbox. Click the **Next** button to continue.

7) When choosing a folder location for plates stored in a directory or CD-ROM, the following screen appears:

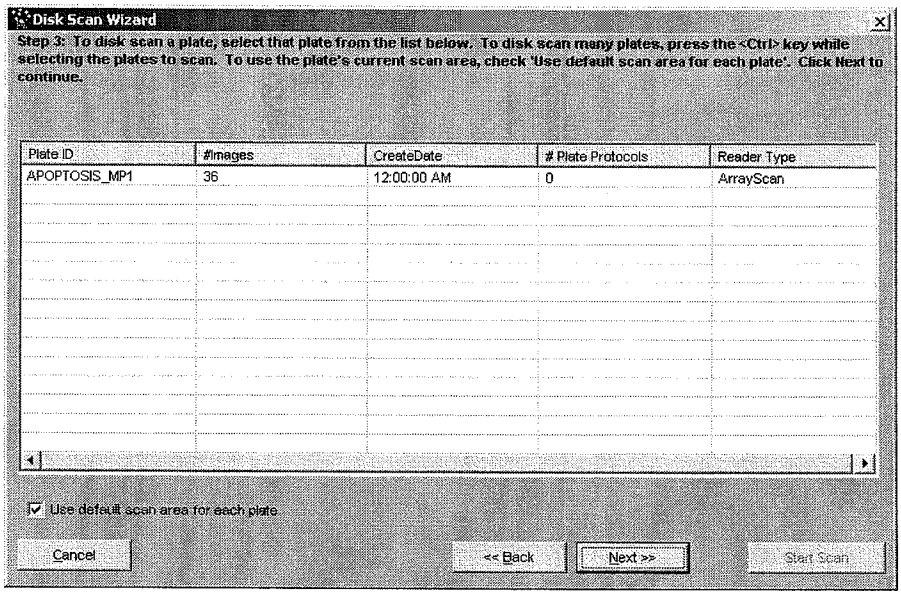


Figure 9.5 Disk Scan Wizard: Select Plates

Note that the images are automatically categorized in the folder into Plate IDs. The Plate ID, number of images, create date, number of Plate Protocols, and Reader type is listed.

If you wish to use the default scan area for each plate that you have chosen, select the **Use default scan area for each plate** checkbox. This option will use the full set of available images. If you want to specify a different scan area for each plate, clear the **Use default scan area for each plate** checkbox. Click the **Next** button to continue.

The remaining wizard screens that appear will vary depending on whether you choose plates that are associated with plate protocols. This section provides two sets of instructions. Follow the instructions titled, *Running a Disk Scan using Plate Protocols,* when selecting plates that are associated with Plate Protocols. Following the instructions titled, *Running a Disk Scan without Plate Protocols,* when selecting plates that are not associated with Plate Protocols.

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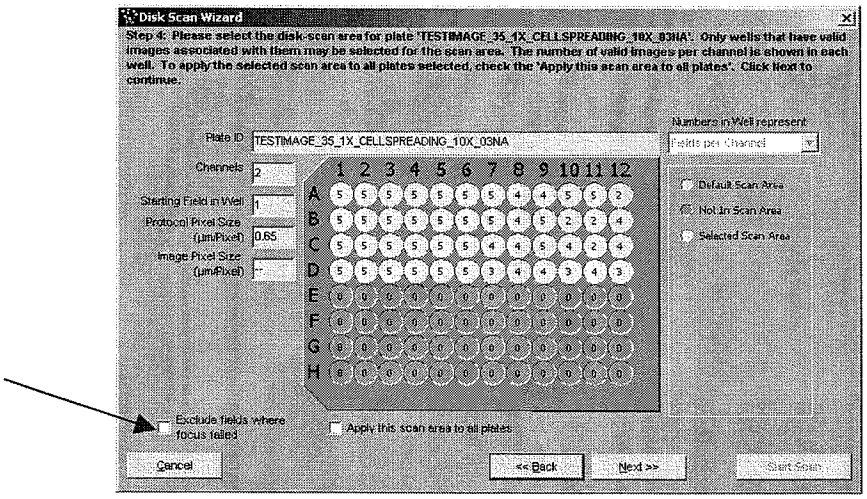


Figure 9.7 Disk Scan Wizard: Select scan area

9) Select the scan area for the specified plate. If you wish to apply this scan area to all the remaining plates, check the Apply this scan area to all plates checkbox. Otherwise, click the Next button. You will be prompted to choose the scan area for each plate.

When selecting wells in the Scan Area, the system uses the following well color coding scheme:

Well Color	Description
Aqua	An aqua circle represents a well that will be scanned.
Light Gray	A light gray circle represents a well that physically exists on the microplate, but will not be scanned.
Dark Gray	A dark gray circle represents a well that physically exists on the microplate but cannot be selected as part of the scan area because of objective limitations. These typically include wells bordering the perimeter of the microplate. For example, when using a 96-well microplate with the 20x High NA or 40x High NA objective, the scan area is reduced from 96 wells to 60 wells. When using a 384-well microplate with either of these objectives, the scan area is reduced from 384 wells to 308 wells.

To specify a contiguous scan area,

- Left-click on the starting well and drag the mouse to the ending well.
  -or-
  Left-click on one corner of the region, then hold down the SHIFT key and click on the opposite corner of the region.

To specify a non-contiguous scan area,

- While holding down the CTRL key on your keyboard, click on the desired wells in the scan area or drag the mouse to the ending well. When you have selected all wells in the desired scan area, release the CTRL key.

After choosing the scan area for the last plate, click the **Next** button.

10) The following screen will appear. This screen provides a summary of all of the specified settings. Review the settings. If they are correct, click the **Start Scan** button. If you want to change the settings, click the **Back** button and repeat the procedure.

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Running a Disk Scan Using Plate Protocols

These instructions assume that you are choosing one or more plates that are associated with one or more Plate Protocols. If Plate Protocols do not exist for the plates that you choose to scan, please skip ahead to the next section titled, *Running a Disk Scan without Plate Protocols*.

11) After selecting the plates that you wish to scan, the following screen appears. This screen indicates the number of Plate Protocols associated with each plate.

If each plate is associated with one or more Plate Protocols and you want the system to use the settings that are specified in the Plate Protocols, verify that the **Use Plate Protocols** checkbox is checked for each Plate ID, click the **Next** button, and skip ahead to the last step in this procedure.

If you would like to specify different settings for one or more plates, uncheck the **Use Plate Protocols** checkbox for each desired Plate ID and click the **Next** button. (Note: Be sure that you did not check the **Use default scan area for each plate** option in the previous wizard screen.)

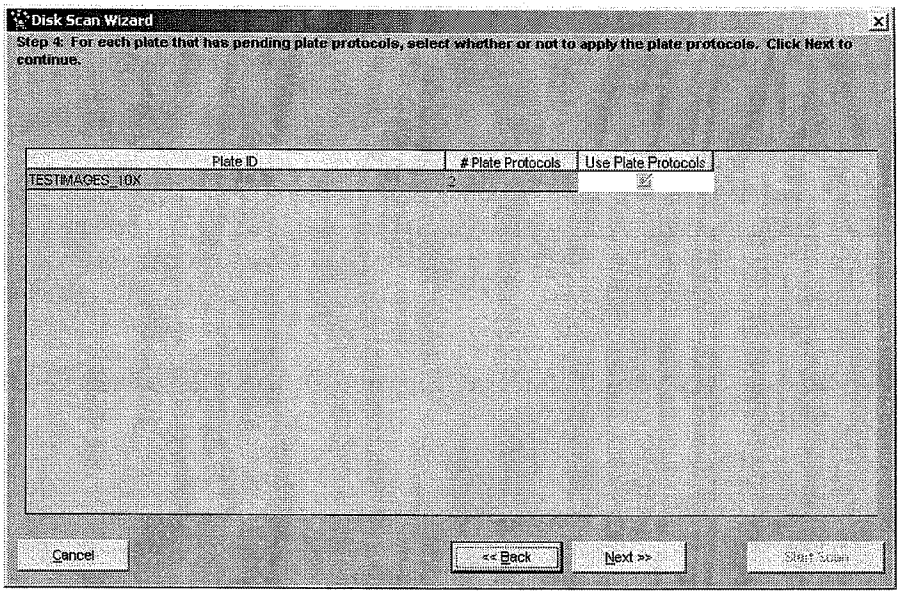


Figure 9.9 Disk Scan Wizard: Use Plate Protocols

NOTE

If you chose to use the Plate Protocols but you also chose the **Use default scan area for each plate** option in the previous wizard screen, note that the scan area specified in the Plate Protocol will override the default scan area setting.

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To specify a non-contiguous scan area,

- While holding down the CTRL key on your keyboard, click on the desired wells in the scan area or drag the mouse to the ending well. When you have selected all wells in the desired scan area, release the CTRL key.

After choosing the scan area for the last plate, click the **Next** button.

14) The following screen will appear. This screen provides a summary of all of the specified settings. (Note that when using Plate Protocols, all columns will display 'N/A' except for the Plate ID column.) Review the settings. If they are correct, click the **Start Scan** button. If you want to change the settings, click the **Back** button and repeat the procedure.

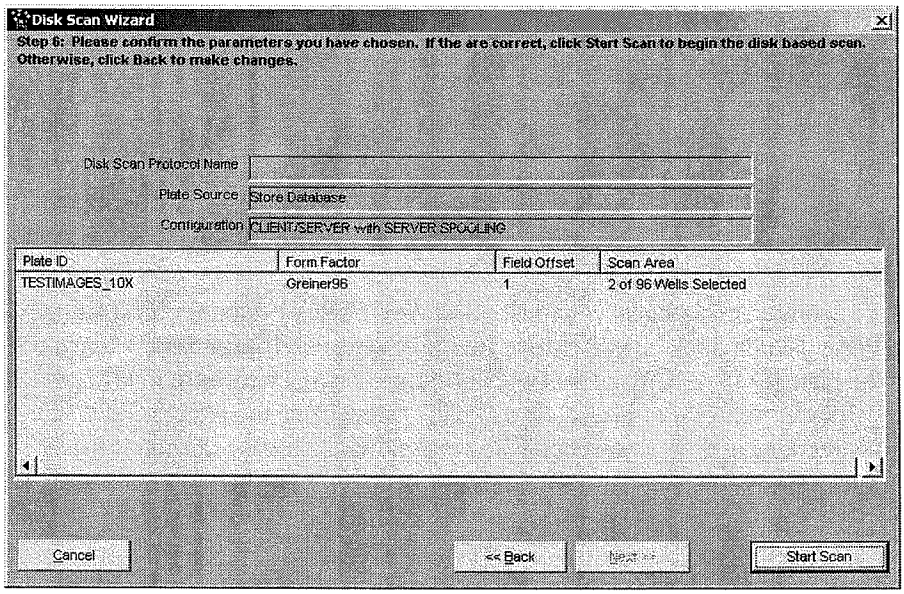


Figure 9.11 Disk Scan Wizard: Confirm settings

You can monitor a Disk Scan in the same manner as you would monitor a physical plate scan (refer to **Chapter 5 – Scanning a Microplate** for instructions).

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Acquiring Images

After clicking the **Acquire Image** or the **Acquire Image Sets** button, you are prompted to select a plate, from the Store or from files, that contains the image of interest to you, or to select test image(s) from a file. After specifying all settings in the Wizard, the Wizard loads the selected images into the Protocol Interactive View.

To run the Disk Scan Wizard,

1) From the Disk Scan Setting in the Protocol Interactive View, select **Add Plate or Acquire Test Images** under **Current Plate**. Click the **Acquire Image** or the **Acquire Image Sets** button. The Disk Scan Wizard appears.

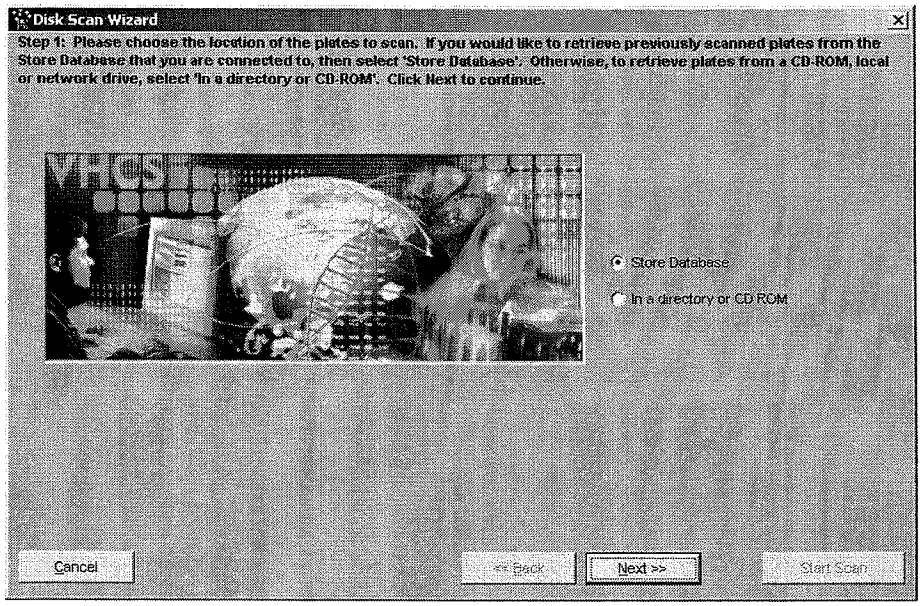


Figure 9.13 Disk Scan Wizard window

2) Follow the directions on screen. If you specified the image source as test images, you will be asked to select an image file (if you clicked **Acquire Image**), or an image file for each channel (if you clicked **Acquire Image Set**).

3) After specifying all settings, click the **Acquire Image** button on the last screen of the Disk Scan Wizard. The test image, or the image for the selected channel of the first field of the first well on the plate appears in the Protocol Interactive image display area.

Selecting Plate Images to View

If you load a set of images associated with a plate, that plate name is added to the list in the Current Plate field.

To view images from a particular plate,

1) Select the plate name from the list. Each well associated with that plate will show the number of fields contained in the well.

2) Select a well. It will be highlighted in light gray.

3) Select or type in the field you want to view from the Field drop-down list.

4) Select the Channel you want to view.

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Chapter
10

Using the Acquire-Only Assay

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When using the ArrayScan V^{TI} HCS Reader Acquire-Only assay, you can acquire images from an ArrayScan instrument using as many channels as necessary without performing any of the image processing steps. This assay is most useful when acquiring images for use with Image-Pro® Plus, or developing your own assays, or when using Cellomics assays under biological conditions that have not yet been evaluated by our laboratories. After acquiring the images with the Acquire-Only assay, you can later process the images by running a Disk Scan using different approaches for optimization of the particular application. When using the Acquire-Only assay with the Disk Scan feature, you can prevent photo-damage of labeled microplates by eliminating the need to scan a plate multiple times.

This chapter discusses the following Acquire-Only assay topics:

- Creating an Acquire-Only Assay Protocol
- Running the Acquire-Only Scan
- Processing Acquire-Only Images

Creating an Acquire-Only Assay Protocol

Prior to creating the Assay Protocol, verify that the following scan settings are specified appropriately for your assay (refer to **Chapter 4 – ArrayScan V^{TI} HCS Reader Setup Procedures** for detailed instructions):

To create an Acquire-Only Assay Protocol,

1) Insert a single plate in the ArrayScan instrument.
2) From the **Options** menu, select **User Options**, then select **Advanced Mode**.
3) From the Assay Protocol list, select the Cellomics AcquireOnly Assay Protocol, _Cellomics_AcqOnly_10x_0.3NA_3CH_p4.0.
4) From the **View** menu, select **Create Protocol**.

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Chapter
11

Using the Illumination Correction Tool

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Illumination Correction on the ArrayScan V^{TI} HCS Reader provides the following:

- Diagnostic indicators of optical conditions
- Flat field illumination correction
- Dark current subtraction

Industrial-scale automation is subject to continuous operation by a range of users and therefore is under continuous stress. Such stresses may subtly effect optical performance and include lamp bulb age, optical filter deterioration, and mechanical perturbations. These stresses effect the performance of all optical systems and are detectable through CCD imaging as variations in the uniformity of field fluorescence. When severe, the lack of uniformity may impact the extraction of spatially resolved data.

The illumination correction wizard on the ArrayScan V^{TI} HCS Reader serves as an optical performance monitoring tool and will facilitate awareness of performance changes along with corrective measures to include automated image correction if desired by the operator. The use of a fluorescent standard reference plate enables the analysis and reporting on the extent to which fluorescence intensities vary across an image of a stable homogenous fluorescent cocktail (OptiTracker™ Performance Monitoring Kit). After an automated scan of the fluorescent standard, the operator is presented with an opportunity to print a performance report.

When correction is desired, a dark current subtraction for background camera noise is performed. This subtraction is executed on all images prior to data extraction and prior to flat field illumination correction (see below). This dark current subtraction effectively improves the contrast in the image.

The ArrayScan V^{TI} HCS Reader, when properly configured, produces illumination with variation significantly lower than the biological heterogeneity in a cellular sample. However, results of single cell analyses may be affected by uneven illumination. Illumination correction is designed to correct images for the unevenness in the illumination across a field of view due to the physical limitations inherent in any optical system.

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General Process of Using Illumination Correction

Details of the steps in this process are located in later sections of this chapter and in the directions provided with the Illumination Correction Solution.

- ArrayScan V^{TI} HCS Reader light source **must be on for 10-15 min with Stable light indicator ON**.
- Prepare the Illumination Correction Plate according to the package directions, or use a previously prepared and stored Illumination Correction Plate.
- Run the automated Illumination Correction Image Acquisition Wizard.
- The ArrayScan V^{TI} HCS Reader will now automatically perform Illumination Correction during a plate scan as well as when acquiring images in the Protocol Interactive View, provided the Apply Illumination Correction checkbox is checked.

Overview of the Illumination Correction Feature

Thermo Fisher Scientific recommends using the Illumination Correction feature for all channels of all Cellomics BioApplications when intensity variations (COV) are high (refer to status message table below). You have the option of selecting this option for one or more channels (when optical performance is passed).

The ArrayScan Image Display Mode is set to Image Stretch Max by default. Stretch Max exaggerates pixel differences for visualization purposes. You can see details within cells or at the cell periphery that may not be seen without contrast stretching (see Image Display Mode section). This Image Display Mode will also show gradients of unevenness even though the range of pixel intensity is minimal. It is recommended that Image Stretch Max be maintained to enhance visualization even though subtle changes in intensity may appear more pronounced. All data is extracted from the raw, non-contrast stretched image.

The following examples illustrate these guidelines.

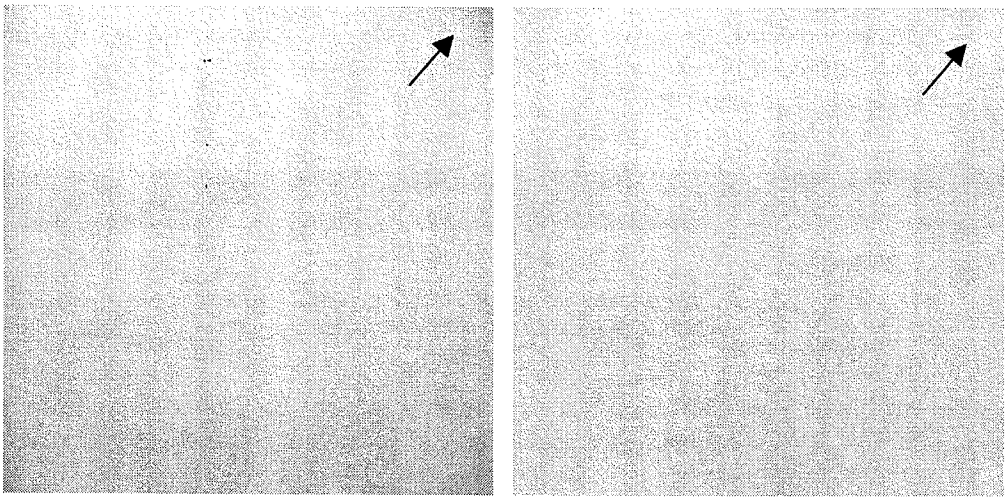


Figure 11.1 The Illumination Correction image in the left panel shows the subtle shading on a Contrast Stretch Max displayed image that is uncorrected with a COV = 7.2%. The right panel has been corrected and now appears more uniform with a COV = 5.2%.

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Running the Illumination Correction Image Acquisition Wizard

The Illumination Correction Image Acquisition Wizard images the OptiTracker plate to capture the illumination pattern for each filter-dichroic set and each objective for which correction is desired.

To run the Wizard,

1) From the ArrayScan Tools menu, select Illumination Correction Wizard.

2) The screen shown in the following figure appears and provides a brief description of the correction method. Select Next to continue.

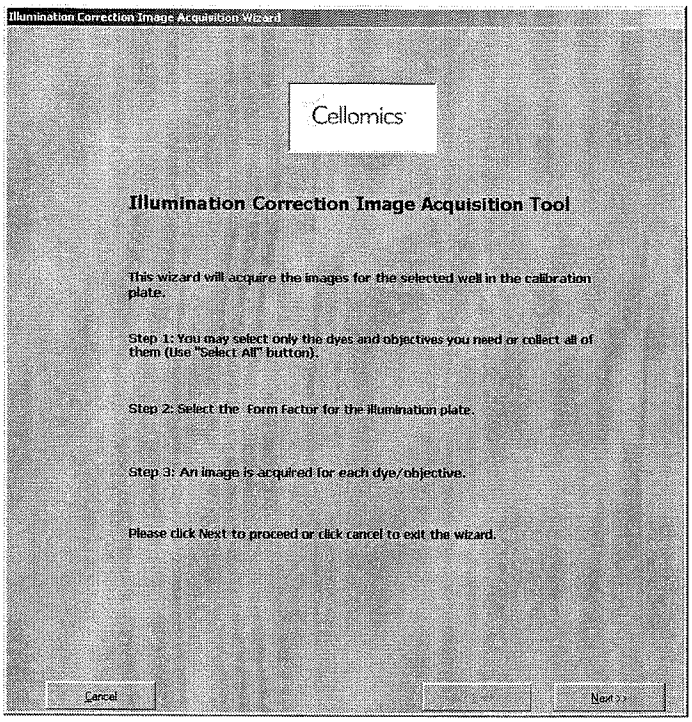


Figure 11.3 Illumination Correction Image Acquisition Wizard

3) The screen shown in the following figure is displayed to caution you NOT TO PROCEED UNTIL THE SYSTEM LIGHT SOURCE BUTTON IS ON-STABLE. You are then prompted to insert the Illumination Correction Plate into the instrument. Select OK to continue.

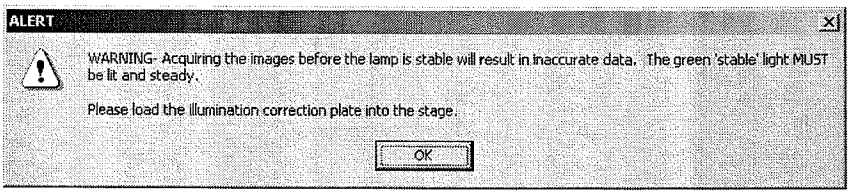


Figure 11.4 Warning message

4) The screen shown in the following figure appears. This screen allows you to specify the filter-dichroic combinations as well as the objective(s) to be included in the correction procedure.

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6) The screen shown in the following figure appears. This screen allows you to specify the Plate Manufacturer and the Plate Type of the Illumination Correction Plate. In the same screen, you also indicate which well contains the reference dye solution. Click on the desired well in the plate representation. Select **Next** to continue.

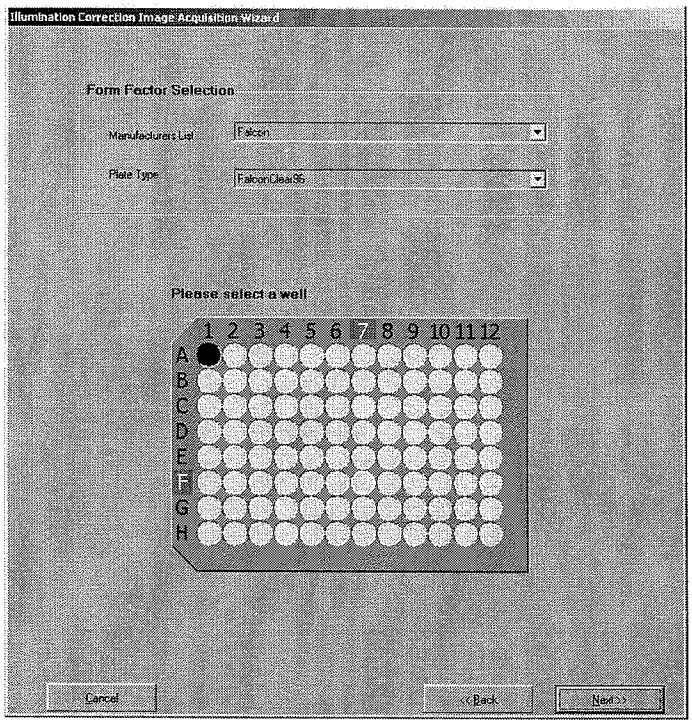


Figure 11.6 Illumination Correction Image Acquisition Wizard: Selecting plate manufacturer, plate type, and well selection.

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8) The screen shown in the following figure appears after the correction process has completed. In this case, the Status indicator, Passed, specifies that the spatial heterogeneity of each filter-dichroic and objective combination lies within a range where application of Illumination Correction is appropriate. Please refer to the Status Message table appearing later in this chapter for a description of all other status messages.

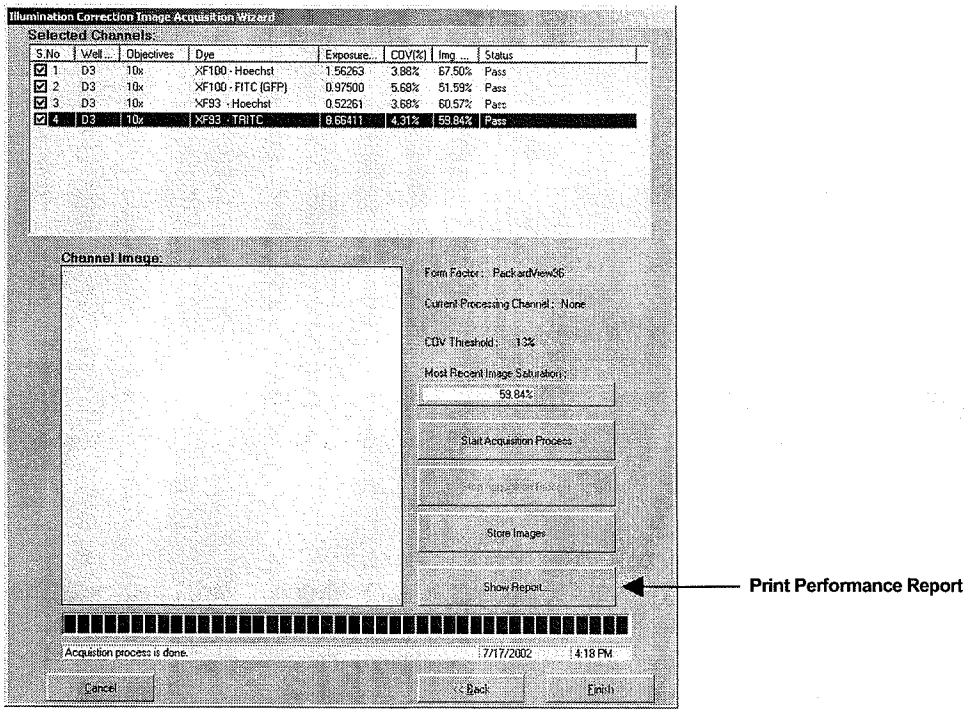


Figure 11.8 Illumination Correction Image Acquisition Wizard: Processing complete

9) If a printed report is required, click the **Show Report** button.

10) A web browser page similar to that shown in the following figure appears. To send a copy to a printer, select Print from the File menu. The window can then be closed if desired.

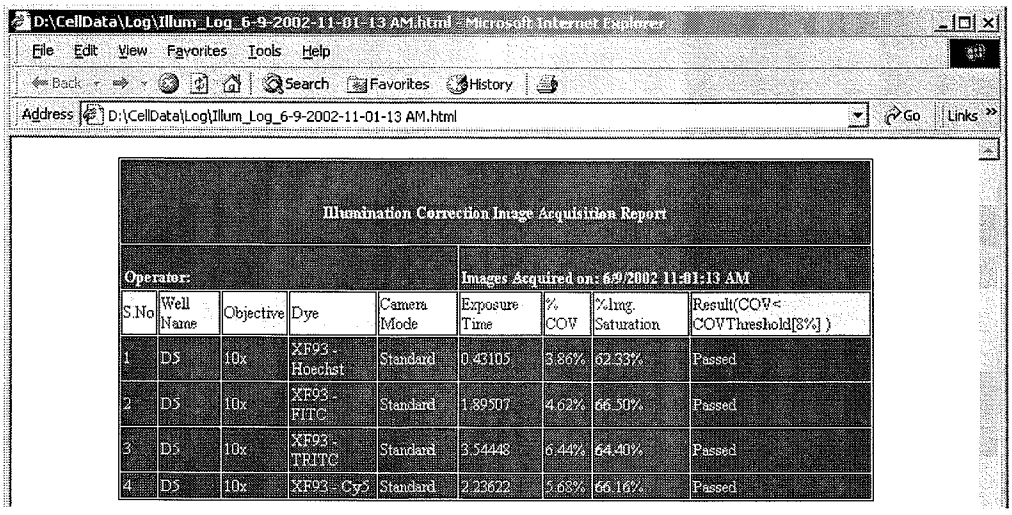


Figure 11.9 Illumination Correction Image Acquisition Report. The % COV describes the intensity variation across the image of the fluorescent standard and is a quantitative description of optical performance.

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Status Messages when Using Illumination Correction

The Illumination Correction Image Acquisition Wizard Report provides status conditions for operation and diagnostics. The status indicates if you should continue to operate your ArrayScan V^{TI} HCS Reader, or if you should contact Technical Support for assistance. You can operate your ArrayScan V^{TI} HCS Reader when you receive either a Pass or Pass/Illumination Correctable condition. All possible status messages and recommended actions are outlined in the table below. Please perform the recommended actions before contacting Technical Support.

Status Message	COV*	Correction Images Saved	Recommended Action
Pass	0-13%	Yes	None
Pass/ Illumination Correctable	>13-25%	Yes	None needed, but issue should be brought up at next available Service visit or Preventative Maintenance visit. You may continue to operate your ArrayScan V^{TI} HCS Reader. It is recommended that you review Diagnostic list below to return the COV back to an unconditional Pass.
Fail/ Illumination Issue	>25%	No	Review the Diagnostic Checklist below. If this does not reduce the COV to above Pass conditions, contact Technical Support. **Thermo Fisher Scientific recommends that you do not use your instrument with any filter/objective combinations that fail due to this issue.**
Fail/ Exposure Too Short	-	No	Determine if you are imaging the correct well and the correct dilution in the plate. Review the Diagnostic Checklist below. If this does not restore images to Pass conditions, contact Technical Support.
Fail/ Exposure. Too Long	-	No	Determine if you are imaging the correct well and the correct dilution in the plate. Review the Diagnostic Checklist below. If this does not restore images to Pass conditions, contact Technical Support.

*Coefficient of Variation within the OptiTracker solution image used for diagnostic pass/fail criteria and illumination image correction for data extraction during scanning.

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Guidelines on the Use of the OptiTracker Plate

- **NEVER** start the Illumination Correction Image Acquisition Wizard before the lamp is on and ready for use (as indicated by the constant, non-blinking bulb symbol on the lamp front panel). The lamp must be stable. Taking images before the lamp is stable can lead to using inappropriate image correction.
- The OptiTracker solution and the sealed OptiTracker plate (see OptiTracker Insert) are to be stored at room temperature, away from light.
- The seal on the plate should be intact to prevent evaporation or leaks. Approximately 200µl should remain in each ICS-containing well.
- The sealed plate can be used for scanning all filters and objectives for at least 1 month.
- The same well can be used for illumination correction imaging at least 20 times.
- A label on the plate can record the number of times a well has been used. **Do Not** place the label over any of the wells containing solution.
- Thermo Fisher Scientific recommends that you use illumination correction for all channels in your assay.
- When running the Illumination Correction Image Acquisition Wizard, select the channels and objectives that you will use during your single or multi-plate scan.
- Follow proper procedures and safety precautions when preparing and using the Illumination Correction plate.

Frequently Asked Questions

Can I use the illumination images that I acquired two weeks ago for use with the scan that I want do today?

No, the ArrayScan V^{TI} HCS Reader prompts you to collect a new set of images before scanning a plate if greater than 48 hours has passed since the last set of illumination correction images was collected.

How long does it take to acquire the illumination correction images?

- If you choose to take images for only one objective and one filter set, the Wizard will complete the task in less than 1 minute.
- If you choose to take images for all filters with all objectives, the Wizard will complete the task in about 10-15 minutes. For systems with automated objectives and filters, you can walk away and return later, click the Store Images button, and then start your scan. Note that this is possible if the specified filters and objectives require a single dilution of the cocktail. If you require multiple dilutions of the cocktail, you must run the Wizard for each dilution. If you have a 3.1 system, more interaction is required but the process generally requires approximately five minutes.

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Chapter
12

ArrayScan V^{TI} HCS Reader Maintenance

This chapter describes routine maintenance and cleaning procedures. **Please be sure to read and understand all safety precautions appearing in this chapter and Chapter 2 of this guide before performing these procedures.**

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.

WARNING



RISK OF ELECTRIC SHOCK!

- ONLY QUALIFIED SERVICE PERSONNEL may perform testing or repairs. The instrument may only be opened by trained personnel and if it has been disconnected from the mains.
- The ArrayScan V^{TI} HCS Reader contains voltages that are potentially hazardous. The ArrayScan instrument may only be connected to power outlets that are properly grounded and free from electrical or mechanical defects. The grounding effect must not be nullified by an extension cable which does not have a protective ground wire.
- Check whether the local line voltage and the operating voltage of the ArrayScan instrument and all the other electrical components are identical. Incorrect voltage settings which do not meet the specifications may damage the instrument or impair its functions.
- If it is established that the protection measures are no longer being met, the instrument must be switched OFF and safeguarded against inadvertent operation.
- The maximum power consumption of 1000 VA of the instrument must be considered.
- An independent grounding of the instrument is required for 230V operation.
- Periodically inspect power cords and plugs for proper condition and replace as necessary.
- Disconnect the mains power cord before changing the fuses. To reduce the risk of fire or shock, replace fuses only with fuses of the same type and rating. The use of makeshift fuses and the short-circuiting of fuse holders are not permitted. Fuses have been provided for both the Neutral and the Line wire. When operating the instrument at a fixed power line of 115 volts, please note that the Neutral wire is also fused. According to local regulations, the removal of the N-side fuse may be necessary.

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WARNING



Always handle the equipment with care when moving. The ArrayScan V^{TI} HCS instrument weighs approximately 190 lbs. (86 kg). At least two people are required for transport. Take the proper lifting precautions to avoid injury and always lift the ArrayScan instrument using the four transport handles. When mounting them before transport, make sure that all four handles are completely screwed in and tightened properly before lifting the instrument.

DANGER







The lamp used in this product contains HIGH VOLTAGE components. ONLY QUALIFIED SERVICE PERSONNEL should perform any testing or repairs. Disconnect the AC power cord from the unit before opening the lamp cover or performing any maintenance procedures.

All cover screws must be replaced prior to applying power to the unit or safety of the unit will be impaired.

DANGER







RISK OF EYE AND SKIN INJURY!
Wear safety glasses designed to filter dangerous ultraviolet wavelengths. Wear protective gloves and clothing to filter infrared light.

Eye damage may result from directly viewing the light produced by the lamp. The level of UV energy supplied by the lamp used in this product is sufficient to ignite flammable surfaces.

- Do not attempt to disconnect the light guide. Turning power on to the lamp unit without the light guide properly installed can increase your risk of exposure to UV light.
- Shut off lamp power if light leakage is observed.
- Never look into the light emitting end of the light guide. The light could severely damage the cornea and retina of the eye if the light is observed directly.
- Never allow light from the light guide to radiate onto flammable material.
- Never look directly into an illuminated objective or microplate well.
- Turn the lamp off and disconnect the AC power cord from the unit before removing cover.

Skin damage may result from exposure to infrared light emanating from the lamp unit. The irradiation will cause heat generation and may cause sunburn-grade inflammation. Always wear safety glasses, gloves, and other appropriate protective gear when operating the equipment.

CAUTION



Dust and dirt can impair the performance of optics and moving parts. When performing any maintenance procedures, do not leave the ArrayScan instrument doors and panels open for an extended period.

WARNING





The sample stage is equipped with a laser barcode scanner. This scanner incorporates a laser device of laser safety class 2. The wavelength of the laser is 650 nm, the output power is 1 mW (continuous wave). During common operation, laser radiation outside the instrument is unlikely. Intentional deflection of the beam by means of tools or mirrors may be possible but constitutes an improper use of the instrument.

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- Never use acetone. Some optics are fitted using acetone-soluable cement and might fall out if cleaning with acetone is attempted.
- Do not use isopropyl alcohol that is sold for household or technical cleaning purposes. Technical IPA contains residues that leave a white, chalk-like film on cleaned surfaces.

Methods for Cleaning

Dry Cleaning

Always try first to dry clean optical surfaces with a blowbulb, i.e., without any liquid. Use cleaning liquids only if dirt cannot be blown off.

1) Blow dust off with a rubber blow bulb. The blowing direction is always toward the outside, or from top to bottom. Avoid using a blowbulb in environments where additional dust may be stirred up and deposited on optical surfaces. For example, to clean an emission filter, remove the filter from the reflector turret prior to cleaning and do not blow into the turret.

2) Check whether this dry cleaning is sufficient.

Liquid Cleaning

If dry cleaning is not sufficient, follow the procedures below for liquid cleaning. Always start with the mildest solvent first.

1) Wrap lens tissue around a wooden stick. To wrap the tissue, do not touch your skin with the tissue or cotton as this will contaminate the material with microscopic amounts of grease and sweat. Optimally, wear clean cotton gloves.

2) Dip the end into solvent to wet it slightly. The stick must not drip. Excessive amounts of solvent may creep into tiny gaps between lenses in compound optics and permanently destroy them. Dab off excessive solvent onto a piece of optics cleaning tissue.

3) Clean small round surfaces by circular wiping, starting at the center and proceeding toward the periphery. Large surfaces are cleaned strip by strip. Do not apply pressure. Optics surfaces are usually coated with extremely thin anti-reflection coatings that are not as hard as those known from spectacle lenses.

4) Turn the stick slightly while wiping so as to wipe every part of the surface with a fresh piece of cotton. Ideally, every part of the swab comes in contact with the surface not more than one time. Change swabs frequently. It is normal to use three to five swabs to clean one side of a filter.

- Whenever possible, do not clean optics in built-in condition. Usually optics are mounted by means of screwed in retaining rings. Touching the thread with the swab may dilute grease applied to the thread and accidentally distribute it across the optical surface. Fibers from the thread may be torn off by microscopic sharp edges of the thread and remain visible in the optical image.

5) If the adhering dirt cannot be removed with one kind of solvent, try another. For example, grease will not dilute in distilled water.

6) Leave cleaned components to dry completely and check again for cleanliness (see below) before installing them. Do not blow on the surfaces as this might contaminate them again.

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System Schematics

System Connection Diagram – Data

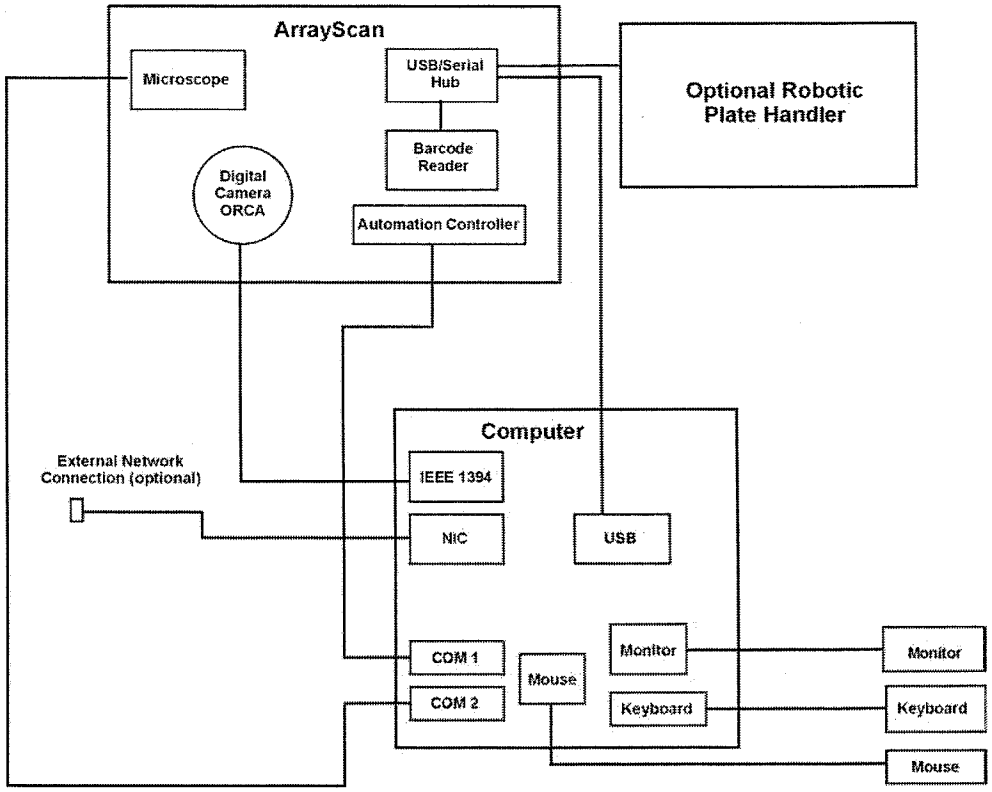


Figure 12.3 ArrayScan V^{TI} HCS Reader Connection Diagram – Data

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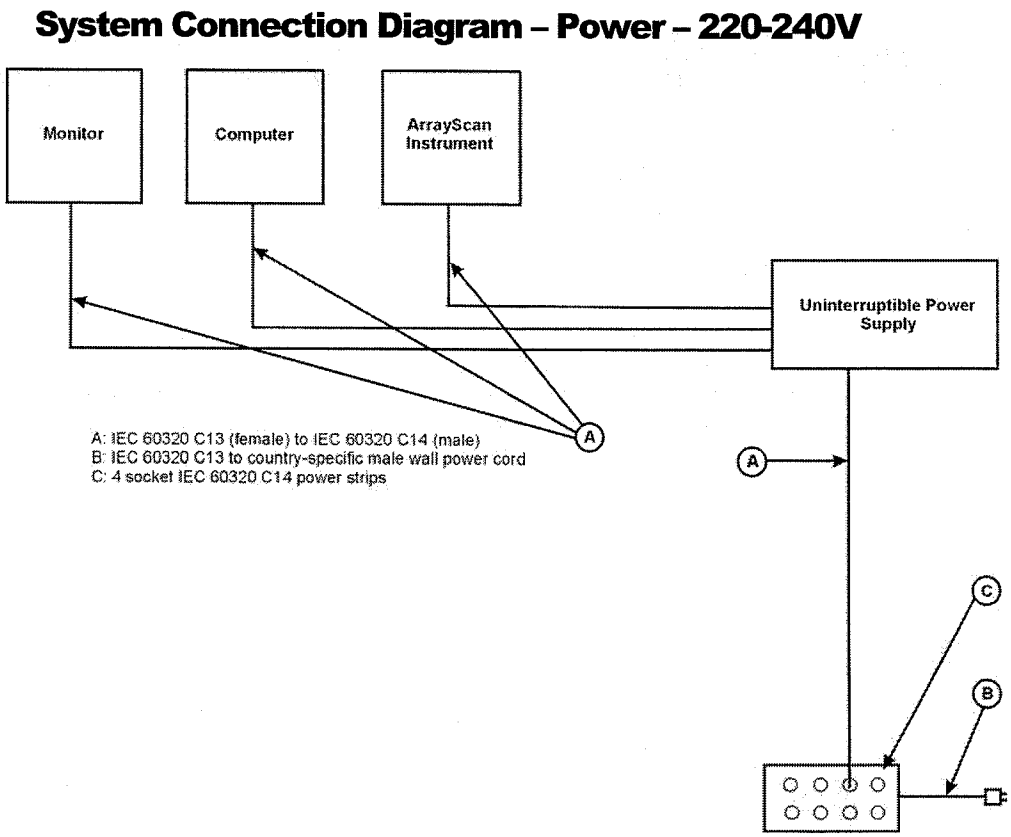


Figure 12.5 ArrayScan V^{TI} HCS Reader Connection Diagram – Power 220-240V

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ArrayScan V^{TI} HCS Reader Computer and Monitor

Computer Monitor Cleaning

CAUTION



Make sure the computer and monitor are turned off before cleaning.

Never spray or pour any liquid directly onto the screen or case.

To clean the monitor screen,

1) Wipe the screen with a clean, soft, lint-free cloth. This removes dust and other particles.

2) If still not clean, apply a small amount of non-ammonia, non-alcohol based glass cleaner onto a clean, soft, lint-free cloth, and wipe the screen. Always use an anti-static screen cleaner.

To clean the monitor case,

1) Wipe with a soft, dry cloth.

2) If still not clean, apply a small amount of non-ammonia, non-alcohol based mild non-abrasive detergent onto a clean, soft, lint-free cloth, and wipe the case.

Computer Case Exterior Cleaning

Periodically use a clean, dry, soft cloth to wipe the exterior surfaces. Use a mild, non-abrasive detergent and clean, soft, lint-free cloth if further case exterior cleaning is needed.

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4) Shutdown the application software and the computer and monitor.

5) Disconnect all cables between the instrument and the computer as well as the robotic microplate handler, if present. All cables are clearly marked and also differ by their pinout, so there is no risk of improper connection at the new location. Coil and tie the cords. Unplug the terminal strip from the wall socket. If you are using a robotic plate handler, please refer to the robotic plate handler manufacturer documentation for instructions on preparing the robot for a move.

6) Remove the four plastic plugs from the threads in the instrument base and insert the four transport handles. These handles are usually stored in a plastic bag within the instrument. Always use the handles provided with the instrument. If the handles are not available, please refer to the *Replacement Parts* appendix for ordering information.

7) Four transport handles are required for safe transport. Make sure that all four handles are completely screwed in and tightened properly before lifting the instrument. Note that the weight of the ArrayScan instrument is 190 lbs. (86 kg). At least two persons are required for transport.

8) At the new location, place the instrument on the workbench.

9) Remove the instrument cover located beside the fans. The back of the camera can be seen. Underneath the camera, part of the instrument's base plate is visible. Place a water level on the base plate (see following figure).

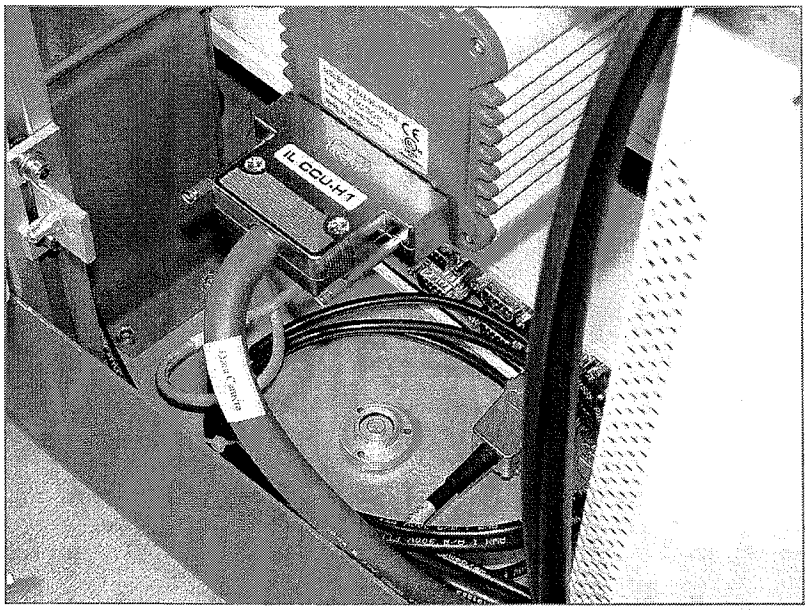


Figure 12.6 View of ArrayScan instrument base plate behind camera

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Chapter
13

Working with the Database Configuration Utility

The Cellomics Database Configuration Utility is a utility used to configure the database parameters used for the ArrayScan application in the cellomics.ini file and the app.mdb database.

Configuring the database parameters is necessary because the ArrayScan application either can work with a local database or be connected to a client/server database, such as Microsoft SQL Server® 2000. The Database Configuration Utility allows the applications to work with any database configuration.

Starting the Database Configuration Utility

To start the Database Configuration Utility,

1) In Windows Explorer, navigate to the Cellomics installation folder. The default location is **C:\Program Files\Cellomics\ArrayScan50\Tools**.

2) Double-click **dbconfig.exe**.

The Data Storage Configuration dialog box appears as shown below.

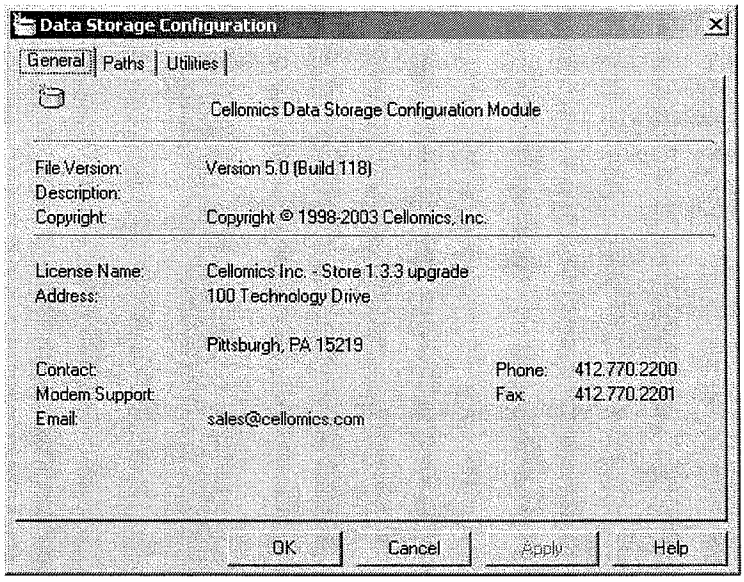


Figure 13.1 Data Storage Configuration dialog box.

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Utilities Tab

The Utilities tab is shown in the figure below.

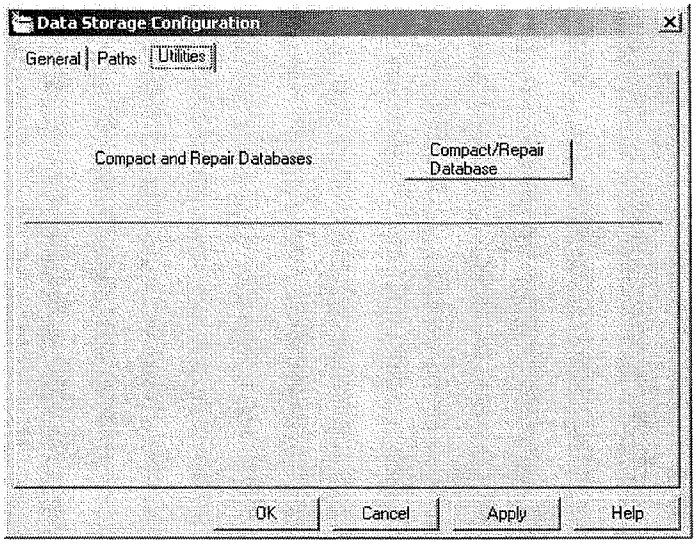


Figure 13.3 The Utilities tab of the Data Storage Configuration dialog box.

This tab provides access to the database compact and repair utilities.

NOTE

These options should only be used when assisted by Technical Support.

For stand-alone versions, these options allow you to repair and/or compact the databases used by the ArrayScan V™ HCS Reader. For more information on their functions, refer to the help for Microsoft Access® on "Repair and Compacting Databases". For client/server configurations, please refer to your database administrator's guide for help with diagnosing and repairing server database problems.

Exiting the Database Configuration Utility

To exit the Database Configuration Utility,

- Click the **OK** or **Cancel** button in the Database Configuration Utility dialog box.

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Appendix

A

Glossary

Algorithm

Computer based analysis of images to identify, isolate, and quantify specific features in the image.

Assay

An assay is the combination of an appropriate biological test system with an image processing algorithm designed to extract data from images that produces meaningful results about the cells being examined.

Assay Protocol

An Assay Protocol is a collection of all the ArrayScan application parameter settings needed to perform a high content screening assay on a single plate. The Assay Protocol includes the information needed to scan and acquire images from the plate, the hardware selected to be used in the scan, the dyes used to measure each biological marker, the object identification parameters used to define a valid object, and the image processing algorithm used to extract data from the images and return results related to the biological process being examined.

BioApplication

BioApplications are software modules that analyze images collected on an HCS reader and make measurements that reflect the biological properties of the cell. Features such as size, shape, amount of fluorescent label, and pattern of fluorescence are measured and recorded for each cell. The BioApplication also calculates the average values for these measurements and reports on the cell population at the whole-well level. The data is written to the Cellomics Store database for future viewing by vHCS:View and analysis by vHCS:Build and vHCS:Scan.

Blank Field

A blank field is a field that contains no identifiable objects in the channel.

Blank Well

A blank well is a well whose number of contiguous blank fields exceeds the "Max. Blank Fields" limit.

Channel

A channel is used to acquire an image with a specific configuration of optical filters and channel specific parameters. A typical assay uses different fluorescent dyes to label different biological structures. These dyes should emit light at characteristic wavelengths. Different channels are used to acquire wavelength specific images from each of these dyes. The assay can then process images from each channel in different ways to extract the desired biological

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Object

An object is an area identified within the image. An object can be a single biological cell, a sub-region of a cell, or a cluster of cells. For example, a nucleus, a pair of touching cells, or a clump of lysosomes may be treated as an object by the assay.

Object Selection Parameter

A feature used to define objects (cells, subcellular components, or multi-cellular clusters)of interest in the channel. Only objects within the user-specified range of values for all selection parameters are included in the measurements.

Percent Saturation

The percent saturation indicates the fraction of the available gray levels that are represented in the image (after ignoring the allowable saturated pixels). The allowable number of saturated pixels is set in different ways, depending upon the particular autoexpose algorithm chosen.

Pixel

Short for "picture elements", pixel is the smallest piece of an image. The two dimensional image is divided into several rows and columns. The intersection of a row and a column is known as a pixel. A value representing the average brightness in the square is assigned to every pixel. This is known as signal quantization. The number of quantization levels depends on the digital camera being used. For the ArrayScan V^{TI} HCS Reader camera (12 bit), there are 4096 levels.

Plate

Plate is an abbreviation for microplate. It is a flat, shallow dish that is used to contain samples for analysis. A 96-well microplate consists of 12 columns of wells labeled from 1 through 12 and 8 rows of wells labeled from A through H, for a total of 96.

Plate Data

Plate data is a set of all of the well data from a plate, including the aggregate statistical measurements and any measurements derived from all of the well data on that plate.

Plate ID

A character string used to identify a specific microplate or image set. The Plate ID is equal to the character string on the plate's barcode, if the barcode reader is used. A Plate ID is unique to an image set so that reanalysis of an image set will have the same Plate ID but a different Plate Name (see below).

Plate Name

A character string used to identify an individual analysis of a plate or image set. A Plate Name can be entered for both a live scan and a Disk Scan.

Plate Protocol

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.

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Appendix

B

Automated Filter Selection

The ArrayScan V^{TI} HCS Reader contains a ten-position automated excitation filter wheel and a five-position automated turret for changing the dichroic-mirror and emission-filter pair.

The word "dye" in this appendix is used in the same way it is used in the documentation describing the ArrayScan software. A "dye" is a set of (a) an excitation filter and (b) a dichroic-mirror and an emission-filter pair. For example, the XF93 – Hoechst dye is a combination of the Hoechst excitation filter and the XF93 dichroic-mirror and emission-filter pair. Dyes are saved with the Assay Protocol.

The following topics are discussed in this chapter:

1) Features of the dichroic-mirror and emission-filter pairs

2) General guidelines for selecting dyes

3) Recommendations of dyes to use with a typical set of fluorophores to optimize scan speeds

4) The transmission spectra of the filters and dichroic mirrors

I. Features of the Dichroic-Mirror Emission-Filter Pairs

NOTE

From this point forward, the phrase "dichroic-mirror and emission-filter pair" will be referred to simply as a "dichroic-emitter pair".

The ArrayScan V^{TI} uses filters and dichroic mirrors provided by Omega Optical, Inc. Omega refers to a complete set of excitation filters and dichroic-emitter pair with "XF" followed by a number (e.g., XF93). Each set is optimized for a specific number of fluorophores and a typical set of fluorophores.

The following table lists the dichroic-emitter pairs available in the turret on the ArrayScan V^{TI}. The configuration includes multi-band pass filter sets to allow plates and slides to be scanned without requiring a turret change. Moving between dichroic-emitter pair turret positions adds approximately 1 second per change.

The system is optimized to image fluorophores that have fluorescent properties similar to these fluorophores: Hoechst 33342, FITC, TRITC, Texas Red, and Cy®5.

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