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ArrayScan® VTI HCS Reader User's Guide

Version 5.5

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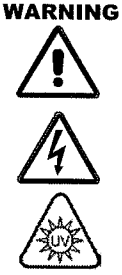
The ArrayScan® V^{TI} HCS Reader is designed to perform automated measurement and analysis of spatial and temporal events in cells by performing automated scans of microplates. The ArrayScan® V^{TI} HCS Reader is designed to be used in a temperature controlled, laboratory environment free of conductive contaminants.

WARNING



Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied in this guide and with accessories 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 Inc. shall not be held liable under any circumstances. Thermo Fisher Scientific Inc. 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!

The ArrayScan® V^{TI} HCS Reader contains voltages that are potentially hazardous. To reduce the risk of electric shock, do not remove covers without proper training. Periodically inspect power cords and plugs for proper condition and replace as necessary. ONLY QUALIFIED SERVICE PERSONNEL may perform testing or repairs.

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Introduction

The Cellomics® ArrayScan® V^{TI} HCS Reader is a fifth generation High Content Screening (HCS) platform designed for multicolor fluorescence assays in standard microplate formats using whole cells. The ArrayScan V^{TI} HCS Reader is based on wide field epifluorescence cell imaging using fluorescently labeled cells. The reader incorporates automated cell identification, focusing, measurement, and analysis capabilities for multiple cellular targets and processes occurring over space. Multiple fluorescence markers are detected by fully automated successive recording of each fluorophore with optimized optical filter sets to achieve efficient color separation. Incorporation of a pre-aligned lamp with a 1500 hour lifetime bulb decreases instrument down-time and enables unattended functionality.

The reader also includes image analysis software algorithms that afford immediate and powerful screening capabilities, with the flexibility to customize assays easily and quickly. Each biological software application (BioApplication) comprises a collection of optimized algorithms that automate the extraction and quantification of assay-specific biological information from cell images collected on the ArrayScan V^{TI} HCS Reader.

The ArrayScan V^{TI} HCS Reader provides:

- White light illumination source enabling usage of the broadest range of fluorescent molecules, in contrast to the narrow band width constraints and elevated phototoxicity associated with laser sources
- Ten software driven excitation filter positions and five dichroic/emission positions increasing assay fluorophore selection flexibility and information yield
- Magnified, detailed images of intact cells and cell components with standard objectives from 5x, 10x, 10x High NA, 20x, and optional objectives (20x High NA, 40x, and 40x High NA)
- Automatic cell identification, focusing, exposure, acquisition, image processing analysis, and data storage
- Low noise, high dynamic range, CCD camera detection
- Flexible optical resolution from the submicron level for subcellular spatial measurements with field sizes of hundreds of microns to millimeters for large population measurements
- Optimized hardware and software for faster automated image acquisition and feature extraction, combined with faster data/image retrieval and storage, yields significant reduction in scan times and increased screening throughput
- Optional robotic plate handlers enabling barcode tracking with multiple protocol assignment and "walk-away" operation of the system

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About this Guide

This guide is a useful reference tool and contains important safety instructions. The chapters that follow this introduction are described below.

Chapter 1 provides an overview of the ArrayScan V^{TI} HCS Reader hardware and software.

Chapter 2 describes safety precautions relating to the operation of the ArrayScan V^{TI} HCS Reader.

Chapter 3 provides instructions on system startup and shutdown.

Chapter 4 describes the process of setting up the ArrayScan V^{TI} HCS Reader for a scan.

Chapter 5 provides instructions on starting and monitoring a plate scan.

Chapter 6 describes the process of creating a new Assay Protocol and working with existing Assay Protocols.

Chapter 7 describes how to manage and upgrade protocols using the Protocol Manager.

Chapter 8 outlines procedures for interactively setting Assay Protocol parameters.

Chapter 9 describes how to reanalyze plates that were previously scanned.

Chapter 10 describes how to use the Acquire-Only assay.

Chapter 11 describes how to use the Illumination Correction Image Acquisition Wizard.

Chapter 12 outlines ArrayScan V^{TI} HCS Reader maintenance procedures.

Chapter 13 describes the features of the Database Configuration Utility.

Terminology, Conventions, and Symbols

This guide assumes that you have a basic knowledge of computers using the Microsoft® Windows® operating system and that you have experience working with windows, menus, commands, buttons, tabs, dialog boxes, and other Microsoft Windows elements. If you are unfamiliar with these terms, please refer to Microsoft Windows documentation.

Throughout this guide certain terminology, conventions, and symbols are used consistently. These conventions are described below.

Terminology

Term	Definition
Click	This term means to place the mouse pointer over the item, then depress and release the primary mouse button (usually the left button) in one quick motion
Right-click	This term means to place the mouse pointer over the item, then depress and release the secondary mouse button (usually the right button) in one quick motion
Double-click	This term means to place the mouse pointer over the item, then depress and release the primary mouse button twice in quick succession.
Drag	This term means to place the mouse pointer over the item, depress and hold down the left mouse button, move the pointer (and the object) to some target location, then release the mouse button.
Press	This term means to push and release a key on the keyboard. For example, press the Tab key.
Shift Ctrl Alt	When any of these terms appear before any of the above terms, it means to hold down the specified keyboard key while taking the hyphenated action. Thus, Shift-click means to hold down the Shift key while clicking an item.
Shortcut menu	This is a menu that appears when you right-click an item.

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- For online help, provide the product name and version number.
- Indicate page number for printed manuals or topic title for online help.
- Comments: Please feel free to comment on the organization, clarity, content, utility, index, search capabilities, or physical characteristics such as the manual size, binding, fonts, etc.
- Suggestions: Please provide suggestions, if possible, on how to correct or improve the documentation or online help.

Email feedback to: **documentation@cellomics.com**

Please use this email address for documentation feedback only. If you have a technical question or comments on the product software or hardware, please contact Technical Support.

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Chapter

1

Overview of the ArrayScan V^{TI} HCS Reader

The ArrayScan V^{TI} HCS Reader is an automated system for imaging fluorescently-labeled cells within a microplate. It is designed for performing quantitative multicolor fluorescence intensity and morphological measurements of cells labeled with one or more fluorescent dyes. Measurements are made on a cell-by-cell or field-by-field basis within each well. The ArrayScan V^{TI} HCS Reader uses fluorescence microscopy and image digitization techniques, as well as proprietary software, to acquire and process images as digital information.

System Overview

The ArrayScan V^{TI} HCS Reader, pictured below, images cells within the wells of standard microplates. In combination with the HitKit series of reagent kits that label specific cellular constituents and BioApplication software modules that analyze the images, the ArrayScan software automatically measures the multiparametric effects of cell treatments in the highly complex environment of the cell.

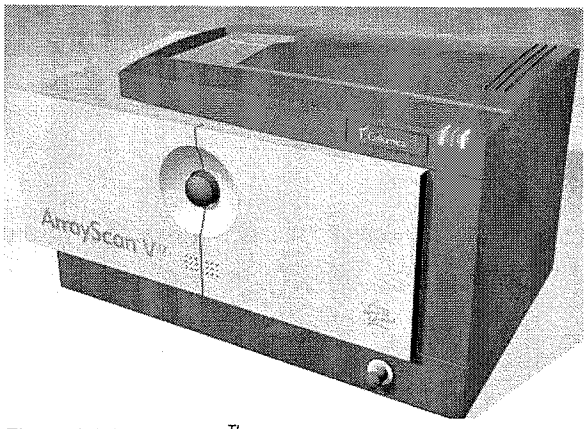


Figure 1.1 ArrayScan V^{TI} HCS Reader

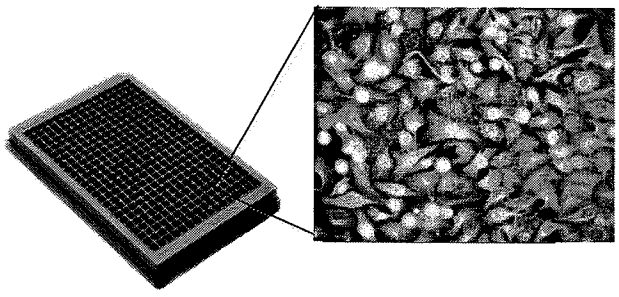


Figure 1.2 Imaging cells within wells of microplates

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ArrayScan Computer

The ArrayScan computer components include a central processing unit connected to a monitor, keyboard, and mouse. The ArrayScan software application runs on the computer, which controls the essential functions on the ArrayScan V^{TI} instrument.

Robotic Plate Handler

When using an optional robotic plate handler with the ArrayScan V^{TI} HCS Reader, you can automatically load plates onto the ArrayScan stage for unattended continuous operation. To increase productivity and maximize instrument use, the robots enable barcode tracking and multiple protocols so that separate BioApplication protocols can be assigned to each plate loaded into the robot during the same run.

Please contact Technical Support for a list of integrated robots and for more information on integrating additional robots. For instructions on using the integrated robots with the ArrayScan V^{TI} HCS Reader, please refer to the *ArrayScan V^{TI} HCS Reader: Robotic Plate Handler User's Guide*.

Software Applications

The ArrayScan V^{TI} HCS Reader software includes the following software applications:

- ArrayScan Control Software
- Plate Protocol/Plate ID Wizards
- Illumination Correction Tool
- Acquire-Only Application
- Target Activation BioApplication
- vHCS™: View module
- Optional Robotic Plate Handler software
- Optional HCS BioApplications

ArrayScan Control Software

The ArrayScan control software runs the essential functions of the ArrayScan V^{TI} instrument. This application uses three main views to organize its functions.

- The **Create Protocol View** is used for creating and modifying Assay Protocols. An Assay Protocol includes all of the settings and parameters required to scan a plate.
- The **Protocol Interactive View** is used to interactively determine parameters for an Assay Protocol.
- The **Scan Plate View** is used for starting, controlling, and monitoring scans.

Plate ID/Plate Protocol Wizards

The Plate ID and Plate Protocol Wizards provide you with the capability of setting up multiple protocols in a screen run so that separate Assay Protocols can be assigned to each plate loaded into a robotic plate handler during the same run. Therefore, you can allow multiple user access, which increases productivity of your instrument. The Plate ID and Plate Protocol Wizard also allows you to run plates with multiple protocols. Therefore, you can perform a series of runs

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Optional HCS BioApplications

Several HCS BioApplications are also available. Your system may include one or more of the following HCS BioApplications:

1. Cell Cycle BioApplication
2. Cell Health Profiling BioApplication
3. Cell Motility BioApplication
4. Cell Spreading BioApplication
5. Compartmental Analysis BioApplication
6. Cytoplasm - Cell Membrane Translocation BioApplication
7. Cytoplasm - Nucleus Translocation BioApplication
8. Extended Neurite Outgrowth BioApplication
9. GPCR Signaling BioApplication
10. Micronucleus BioApplication
11. Molecular Translocation BioApplication
12. Morphology Explorer BioApplication
13. Multiparameter Cytotoxicity BioApplication
14. Neurite Outgrowth BioApplication
15. Spot Detector BioApplicaton

A library of screening application packages is available and additional BioApplications are developed on a continuous basis. *Please contact your sales representative for more information.*

vHCS Discovery ToolBox

When you purchase an ArrayScan V^{TI} HCS Reader, you also receive two licenses for the Cellomics vHCS:View software module. The vHCS:View software is one of the modules of the full vHCS: Discovery ToolBox, available to purchase separately. The vHCS View software is a client PC application allowing users to perform the following:

- Rich visualization of HCS data down to the cell level
- Interactive visual analysis of multi-feature, cell-based data down to the image
- Link to Spotfire® DecisionSite for further visualization and data mining (with full vHCS Discovery ToolBox license)
- Multi-plate, multi-well, multi-feature comparisons
- Make HCS data available to anyone, anywhere in your company with a PC and a network connection

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Chapter
2

Safety Precautions

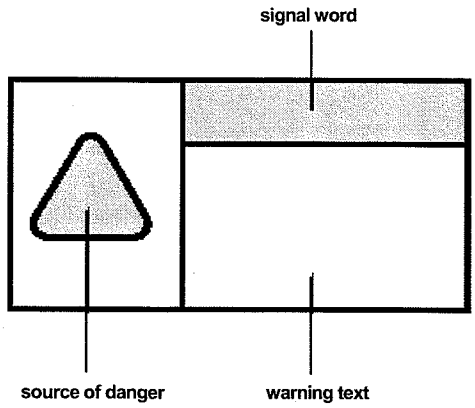
This chapter presents a series of safety precautions related to the operation and maintenance of the ArrayScan V^{TI} HCS Reader. The ArrayScan V^{TI} HCS Reader has been designed, produced, and tested in compliance with EN 61010-1 (IEC 1010-1) and left the factory in a perfect state with regard to its safety requirements. To ensure that this remains the case and to guarantee the safe operation of the equipment, the instructions and warnings contained in the guide must be observed.

Please read these instructions carefully before operating or servicing the instrument. In addition, please read the instructions, warnings, and precautionary measures supplied with the accessories before you start up these items.

Equipment Safety Labels

The product safety labels are structured as follows:

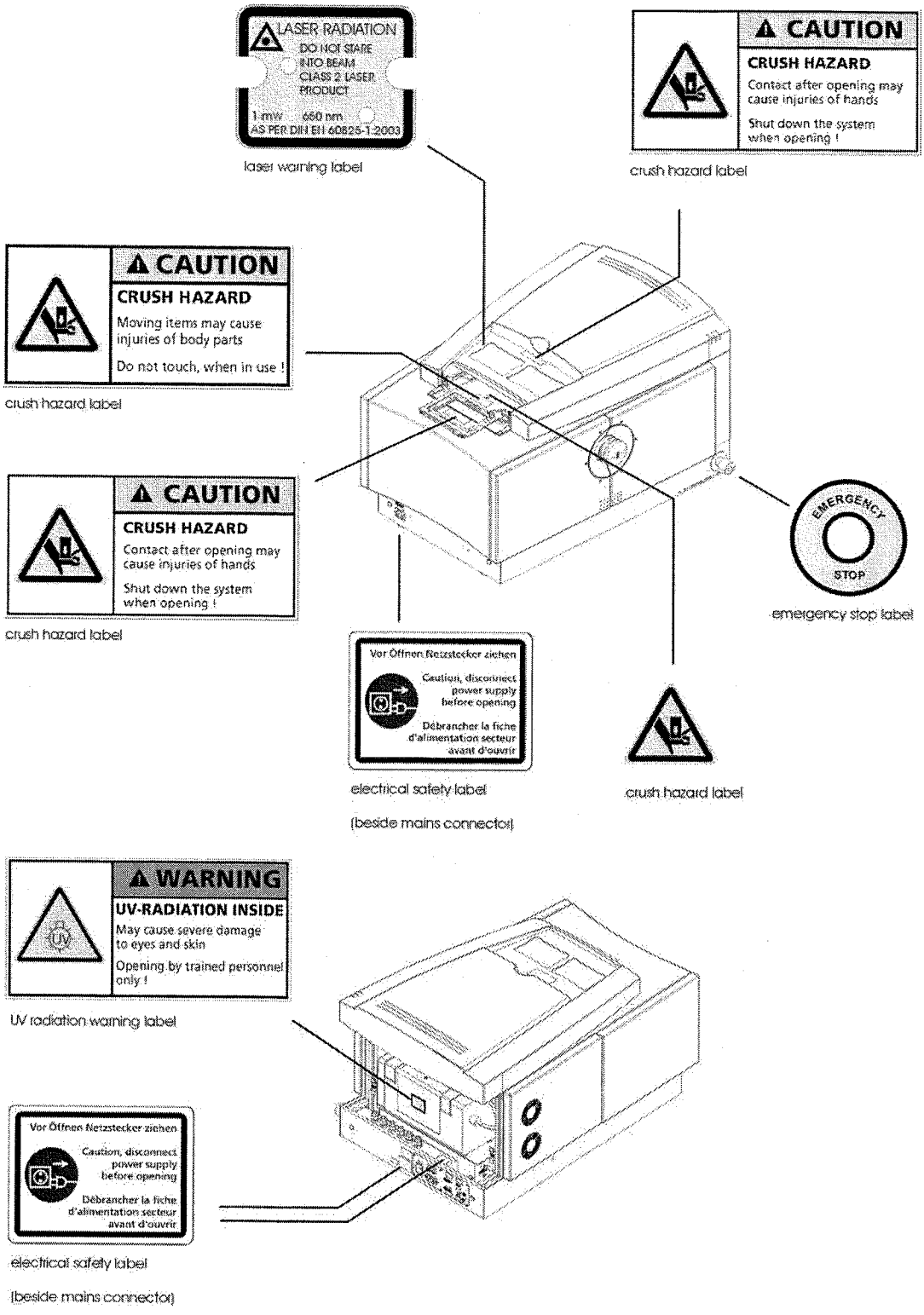
- The left panel contains a warning symbol to indicate a **source of danger**.
- The upper right panel contains the prescribed **signal word** with the safety alert symbol.
- Below the signal word, you will find the corresponding **warning text**.



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Safety Labels

The following drawing shows the location of the safety labels on the ArrayScan V^{TI} instrument. Additional safety precautions are described in the remaining sections of this chapter.



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ArrayScan V^{TI} HCS Reader Operation and Maintenance Precautions

ArrayScan V^{TI} Instrument

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 V^{TI} 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 V^{TI} 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.

WARNING



The ArrayScan V^{TI} instrument is equipped with an Emergency Stop switch. The red switch is located on the front side at the base of the instrument. Pressing the Emergency Stop switch will disconnect the instrument and the robotic plate handler (if applicable) from both mains wires. Note that releasing the mechanically locked Emergency Stop switch immediately powers the instrument on again. Before releasing, turn the main power switch off and take all necessary measures to avoid damage due to unexpected operation or movement.

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CAUTION



Electrostatic Discharge

ArrayScan V^{TI} HCS Reader electronics are sensitive to electrostatic discharge (ESD) and transient voltage spikes.

To avoid permanently damaging the system, please observe the following precautions:

- Use caution when triggering high-current devices near the system. Electrically noisy devices should be powered from an isolated, conditioned power line or dedicated isolation transformer.
- Never connect or disconnect any power cord or data cable while the system is powered on.

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 V^{TI} 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.

ArrayScan Computer

CAUTION



Loading unrelated software on the ArrayScan computer may cause system damage including but not limited to data corruption and/or software incompatibility. Do not load any software application that has not been provided or approved by Thermo Fisher Scientific. If problems occur as a result of such activity, Thermo Fisher Scientific has no liability and will not be held responsible for damages and repair fees.

CAUTION



If magnetic field disturbance of the computer components is suspected, move the equipment away from the field source or provide a suitable shielded enclosure. For additional information, contact Technical Support.

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WARNING





HOT SURFACE!
Prior to opening the unit and handling the lamp module, allow the lamp module to cool down completely (at least 20 minutes).

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



The operational life of the lamp bulb can be significantly shortened if it is handled incorrectly. Do not touch the bulb's glass envelope or the inside surface of the reflector. Skin oils can cause the lamp module to fail prematurely. Be sure only to handle the bulb flange. A protective glove must be worn to help prevent skin oil contamination.

WARNING



HG – LAMP CONTAINS MERCURY, Manage in Accord with Disposal Laws, see: www.lamprecycle.org or 1-800-668-8752.

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CAUTION



Handle the light guide with care. Light guides are delicate and may break if dropped or if bent to an excessive angle. Do not pull, crush, or place heavy objects on the light guide. Do not touch the end of the light guide with bare hands. When accessing the side panel of the lamp unit, you may need to move the coiled light guide out of the way. Do not disconnect the light guide from the retaining clamp. Be sure to maintain the original coiled radius.

WARNING





RISK OF ELECTRIC SHOCK!

Disconnect the 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.

WARNING



Clean exterior of the unit with a water dampened cloth and simple detergent only.

System Components

Additional safety precaution statements can be found in the supplied documentation for the following system components:

- Computer
- Monitor
- UPS

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Chapter

3

System Startup and Shutdown

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.

System Startup

If you are using a robotic plate handler with the ArrayScan V^{TI} HCS Reader, please refer to the *ArrayScan HCS Reader: Robotic Plate Handler User's Guide* for system startup instructions.

Starting the ArrayScan V^{TI} HCS Reader Hardware

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.

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- If the instrument is turned off and an attempt is made to turn it back on before the lamp has fully cooled, a flashing bulb with the "no" symbol may appear on the lamp display. The lamp will automatically re-strike when the lamp has cooled.
- Check the ArrayScan V^{TI} instrument fuse and change if necessary. Please refer to the *Maintenance* chapter in this guide for instruments.

Viewing Accumulated Lamp Hours

The accumulated lamp hours are continuously shown on the LCD display on the front panel of the unit. The lamp hours are automatically updated.

To view accumulated lamp hours,

1) Open the right front door of the ArrayScan V^{TI} instrument to view the front of the lamp unit.

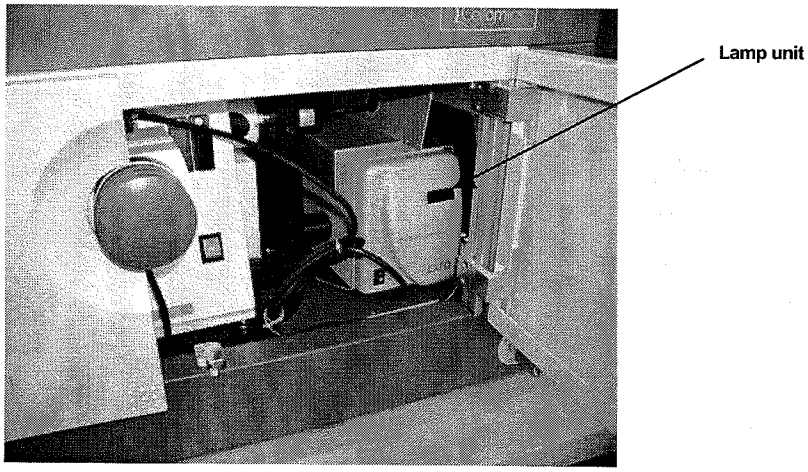


Figure 3.1 View of lamp unit in ArrayScan V^{TI} instrument

2) The accumulated lamp hours are displayed on the lamp LCD as shown in the following figure.

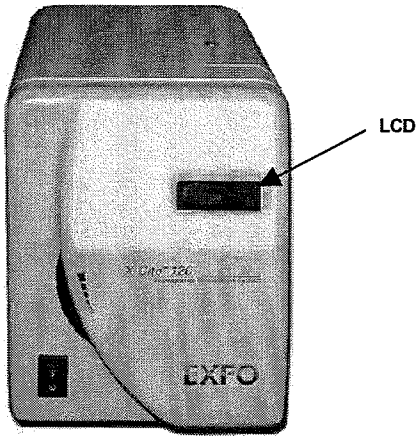


Figure 3.2 Front view of lamp unit

NOTE

Lamp bulbs should be replaced after 1500 hours of operation. To replace the lamp bulb, please refer to the supplied *EXFO X-Cite 120 User's Guide* for instructions. We recommend that you have a spare bulb at all times.

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Software Overview

The ArrayScan software application provides three main windows or 'views'. Each view is used to perform a series of specific tasks. The three views are as follows:

- Scan Plate View
- Create Protocol View
- Protocol Interactive View

Working in Scan Plate View

You can scan a plate when working in Scan Plate View, shown in the following figure. A plate is scanned in a serpentine style as the software automatically moves the motorized microplate stage from well to well (and field to field within each well).

You can display either color-coded wells or numeric well feature values during the microplate scan. Refer to **Chapter 5 – Scanning a Microplate** for details on scanning a microplate.

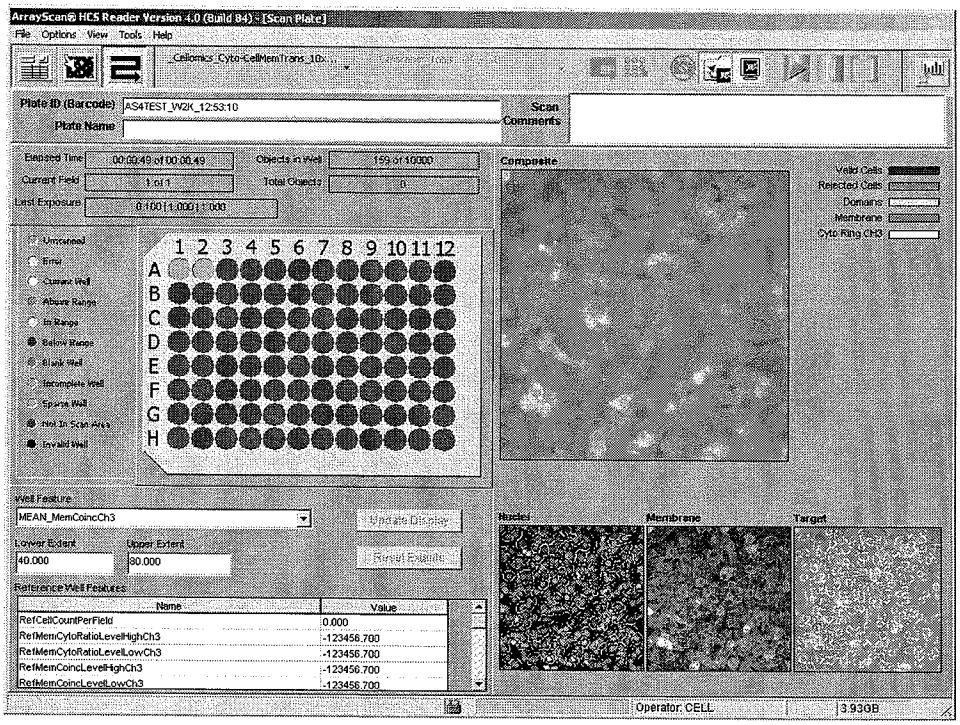


Figure 3.4 Scan Plate View

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Working in Protocol Interactive View

You can acquire images and interactively adjust Assay Protocol parameters when working in Protocol Interactive View, shown in the following figure.

The Protocol Interactive View provides interactive tools to help you determine appropriate parameter values and settings. This view allows you to acquire images and "train" the ArrayScan V^{TI} HCS Reader on the types of objects to analyze. For example, you can simply click on nuclei of interest to determine the range of morphological limits (size, shape, intensity, etc.) for sample cells. The ArrayScan V^{TI} HCS Reader uses these limits during the scan to automatically reject objects that do not fit within the range. Refer to **Chapter 7 –Setting Assay Protocol Parameters Interactively** for details on acquiring images and interactively adjusting Assay Protocol parameters.

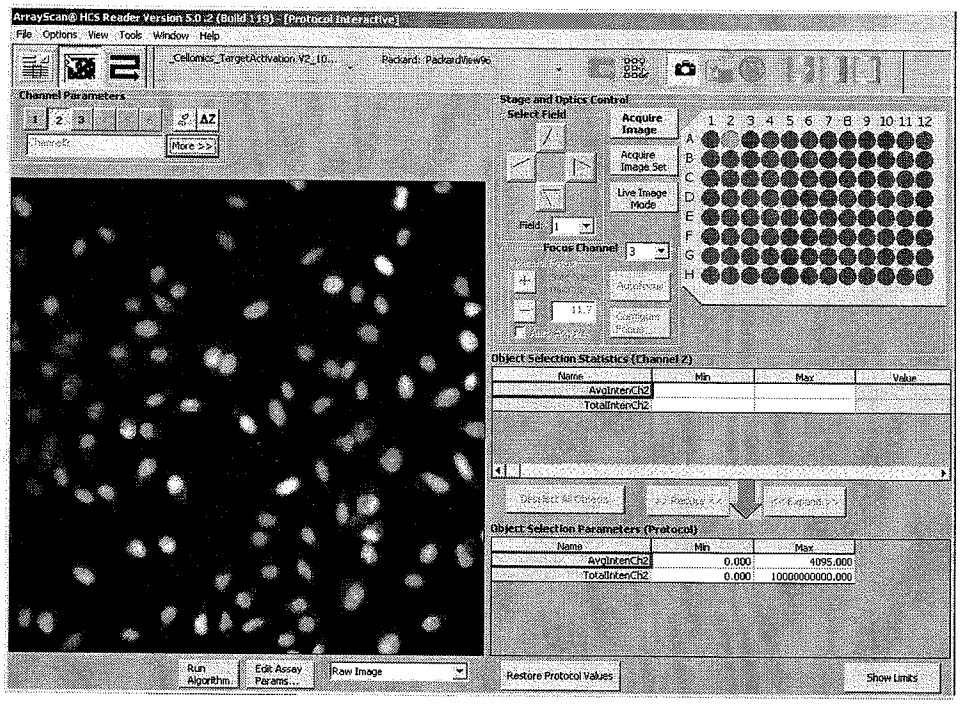


Figure 3.6 Protocol Interactive View

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Toolbar Button Name	What it Does
Live-Mode Scan	Clicking this button toggles between Live Scan Mode and Disk Scan Mode. When running in Disk Scan mode, the scan is run using images stored on a disk rather than live from the camera. This feature is also available from the Options menu.
Store Images	Clicking this toggle button turns the Store Images option on and off. When the Store Images option is turned on, the system stores images generated from a scan in the database along with the plate data. Note that if you do not store images, you cannot rescan them at a later time nor can you view them in the Cellomics vHCS:View application. This feature is also available from the Options menu.
Display More Mode	Clicking this toggle button turns the Display More Mode on and off. When the Display More Mode is turned ON, the system provides more visual feedback during scanning including the display of composite and individual channel images without the overlays. Scan times will be longer when the Display More Mode button is ON. The default setting in OFF. When the Display More Mode is OFF, the system will process plates at a higher rate of speed by limiting the visual feedback during scan time. The quality of the images and the biological data collected is not affected. The same images and data will be available in vHCS:View regardless of the mode setting.
Start Scan	Starts a scan of a microplate.
Pause Scan	Pauses a scan.
Stop Scan	Stops a scan in progress. A stopped scan cannot be restarted from where it was stopped.

ArrayScan Status Bar

The ArrayScan status bar is located across the bottom of the ArrayScan window.



Figure 3.8 ArrayScan Status Bar

The status bar displays the following items.

- **Pixel Location and Intensity** In the Protocol Interactive View, the pixel location and corresponding intensity value is displayed when you place the mouse cursor over the image display area.
- **Protocol Locked/Unlocked** The protocol unlocked symbol displays when the protocol is set to read/write status. The protocol locked symbol displays when the protocol is set to read only.
- **Apotome Used in Protocol** This icon appears when the Apotome is used in the protocol.
- **Protocol Modified** This statement appears when you make a change to an Assay Protocol.
- **User Name** This area displays the name of the currently logged in operator.
- **Apotome Engaged** This icon turns blue, with no slash through the middle when the Apotome is pushed in. When the Apotome is out, the icon has a red slash through the middle. If the Apotome is out, but the loaded protocol uses the Apotome, then an exclamation point also appears.

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Chapter

4

ArrayScan V^{TI} HCS Reader Setup Procedures

Before scanning a microplate on the ArrayScan V^{TI} HCS Reader, you must first perform a series of setup procedures to ensure that the ArrayScan V^{TI} HCS Reader is properly configured to scan the microplates. This chapter describes the required ArrayScan V^{TI} HCS Reader hardware and software setup procedures as outlined in the checklists below.

Robotic Plate Handler Setup Procedures

- ☑ If using a robot, refer to the *ArrayScan V^{TI} HCS Reader: Robotic Plate Handler User's Guide*

ArrayScan V^{TI} Hardware Setup Procedures

- ☑ Label Microplates for Barcode Reader, if desired
- ☑ Insert Microplates in the ArrayScan V^{TI} instrument
- ☑ Specify Barcode Settings
- ☑ Verify that Sufficient Disk Space is Available

ArrayScan Software Setup Procedures

- ☑ If using a Robotic Plate Handler, refer to guide for setup instructions
- ☑ Choose Basic or Advanced Mode of Operation
- ☑ Choose Image Display Method
- ☑ Choose Color Preferences, if desired
- ☑ Change Reference Wells, if applicable
- ☑ Run Illumination Correction Tool, if necessary, after lamp warmup

If running a Single Assay Protocol Plate Scan:

- ☑ Select the Assay Protocol. See Chapter 6 – Creating and Working with Assay Protocols
- ☑ Select the Plate Manufacturer and Type
- ☑ Choose the Scan Area and Direction
- ☑ Specify the Starting Field Offset, if desired
- ☑ Choose the Disk Scan option, if desired
- ☑ Choose the Store Images option, if desired
- ☑ Turn on the Display More Mode setting, if desired

If running a Multiple Assay Protocol Plate Scan:

- ☑ Create Plate Protocols
- ☑ Create Plates IDs using the Plate ID Wizard

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Inserting a Microplate in the ArrayScan V^{TI} Instrument Stage

This section describes how to manually insert a single microplate in the ArrayScan V^{TI} instrument stage without the use of a robotic plate handler. If you are using the barcode reader, follow the labeling procedures described above. For instructions on inserting microplates in a robotic plate handler, please refer to the *ArrayScan V^{TI} HCS Reader: Robotic Microplate Handler User's Guide*.

WARNING





BIOHAZARD!

- Use proper laboratory practices when handling any hazardous materials.
- Make sure that biohazards do not contaminate ArrayScan V^{TI} HCS Reader components.
- In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.

CAUTION



MOVING MECHANICAL PARTS!

The ArrayScan V^{TI} instrument incorporates moving parts. The cover flap in the instrument's cover may open unexpectedly and the sample stage may move without warning. Please note the crush hazard signs applied to potentially dangerous parts. Before opening doors or removing an ArrayScan V^{TI} instrument panel or cover, verify that the system is totally inactive. After verification, wait for a minimum of 30 seconds before opening door or removing panel or cover.

To insert the microplate,

1) From the ArrayScan software application, choose the **Tools** menu, then select **Maintenance, Move to Plate Load Position**. This places the microplate stage in a position that provides easy access to the microplate holder.

WARNING





Risk of Eye 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. Never look directly into an illuminated objective or microplate well.

2) To insert the microplate into the microplate stage, rotate the microplate so that well A1 is located in the lower left corner as viewed from the top, front of the instrument (see following figure)

3) Insert the microplate into the stage. Refer to the following figure.

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Removing a Microplate from the ArrayScan V^{TI} Instrument Stage

This section describes how to remove a single microplate from the ArrayScan V^{TI} instrument stage.

WARNING





BIOHAZARD!

- Use proper laboratory practices when handling any hazardous materials.
- Make sure that biohazards do not contaminate ArrayScan V^{TI} HCS Reader components.
- In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.

CAUTION



MOVING MECHANICAL PARTS!

The ArrayScan V^{TI} instrument incorporates moving parts. The cover flap in the instrument's cover may open unexpectedly and the sample stage may move without warning. Please note the crush hazard signs applied to potentially dangerous parts. Before opening doors or removing an ArrayScan V^{TI} instrument panel or cover, verify that the system is totally inactive. After verification, wait for a minimum of 30 seconds before opening door or removing panel or cover.

To remove a microplate from the stage,

1) From the ArrayScan software application, choose the **Tools** the menu, then select **Maintenance, Move to Plate Unload Position**. This places the microplate stage in a position that provides easy access to the microplate.

WARNING





Risk of Eye 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. Never look directly into an illuminated objective or microplate well.

2) When the plate is fully removed from the stage, remove the plate.

3) From the **Tools** menu, select **Maintenance, Retract Stage**. This command places the stage inside the ArrayScan V^{TI} instrument. This is recommended in order to protect the stage from dust and dirt.

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Barcode Testing

The Barcode Reader Testing options allows you to test the barcode label on an individual plate.

To test the barcode reader,

1) Place a labeled microplate in the ArrayScan stage and retract the stage door (see instructions appearing earlier in this chapter).

2) From the **Tools** menu, select **Maintenance** > **Barcode Configuration**.

3) Specify the check digit options, if applicable.

4) Click the **Read Barcode** button.

   The stage moves to the barcode read position and scans the barcode label on the plate. Once the barcode reader scans the label, the barcode data from the scan is displayed in the Barcode Data box. (This is the Plate ID displayed in the ArrayScan software application.) The barcode symbology type will display in the Barcode Type box.

5) Verify that the barcode reader is reading the label correctly. If the barcode label cannot be read, the Status box will display "NO BARCODE". Verify that the plate is in the stage and the label is placed in the proper location.

6) Click the **OK** button to apply the settings and close the dialog box. Click the **Apply** button to apply the settings without closing the dialog box.



Please contact Technical Support if you wish to discuss barcode options not provided in the Barcode Configuration dialog box.

Verifying Disk Space

Before starting a scan, you should verify that sufficient disk space is available. The free disk space indicator on the ArrayScan status bar indicates the amount of disk space (in gigabytes) remaining.



Although we recommend storing images from all scans, you can greatly reduce the amount of disk space required by opting not to store the images. However, this will prevent you from viewing the actual images for interesting wells or later running a Disk Scan on that microplate (see **Chapter 8 – Running a Disk Scan**).

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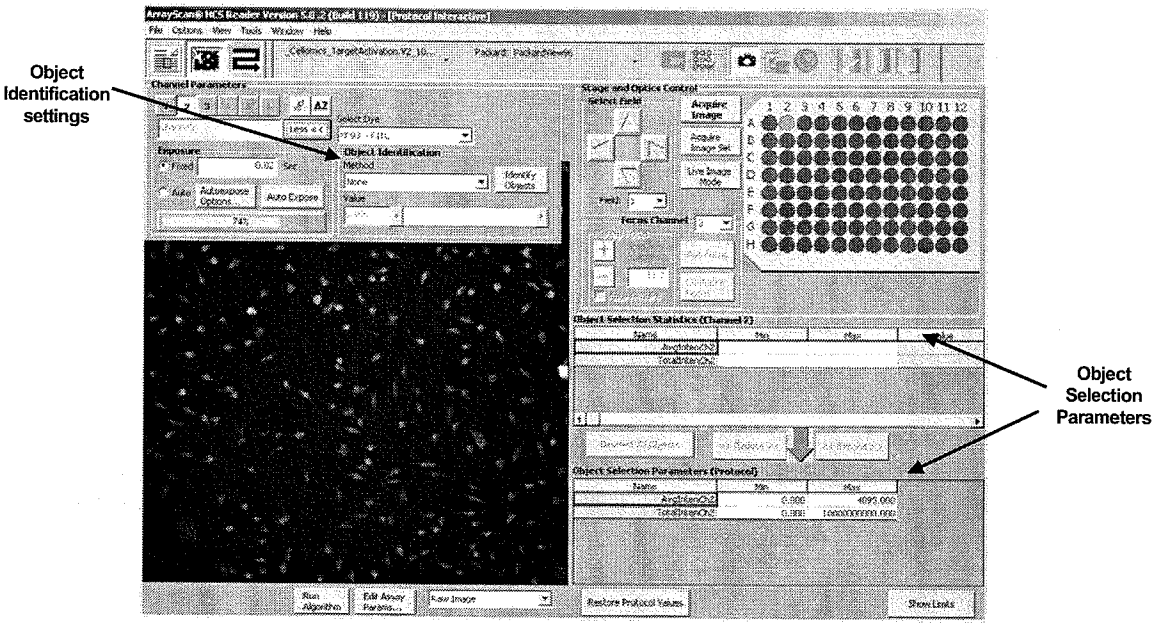


Figure 4.5 Protocol Interactive View when Advanced Mode is selected.

By default, the ArrayScan V^{TI} HCS Reader runs in Advanced Mode unless you select the Basic Mode option. When you restart the ArrayScan V^{TI} HCS Reader, the system returns to Advanced Mode until you re-select Basic Mode.

To turn off Advanced Mode and return to Basic Mode,

- From the **Options** menu, select **User Options**, then select **Advanced Mode**. The checkmark next to the Advanced Mode menu item will disappear.

Choosing the Image Display Method

You can use the Image Display Options dialog box to choose the way in which images are displayed on the screen.

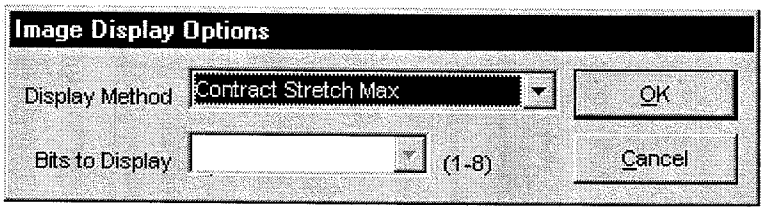


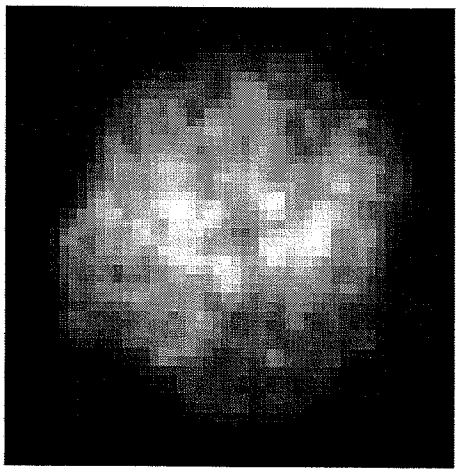
Figure 4.6 Image Display Options dialog box

The ArrayScan V^{TI} HCS Reader uses 12-bits (4096 gray values) when acquiring images. However, the computer monitor is only capable of displaying 8-bits (256 gray values). By default, the ArrayScan V^{TI} HCS Reader displays all images in contrast stretched mode. In other words, the ArrayScan V^{TI} HCS Reader automatically stretches or shrinks the range of pixel values to match the capabilities of the display. This method is suitable under most screening conditions.

In certain cases, however, it is not desirable to automatically contrast stretch the images. For example, it is useful to have a fixed mapping between the 12-bit pixels of the camera and the 8-bit pixels of the display when trying to evaluate the quality of an exposure time, or when trying to compare the intensity of image features between two images. In this case, the

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Contrast Stretch Full



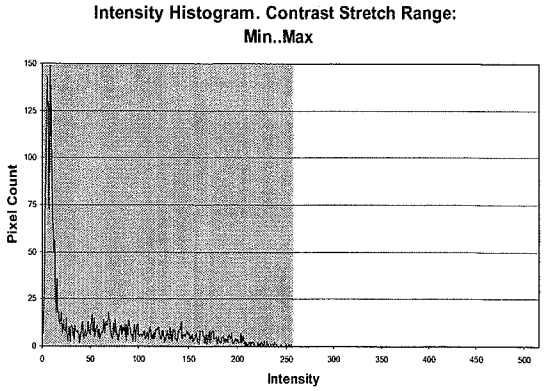
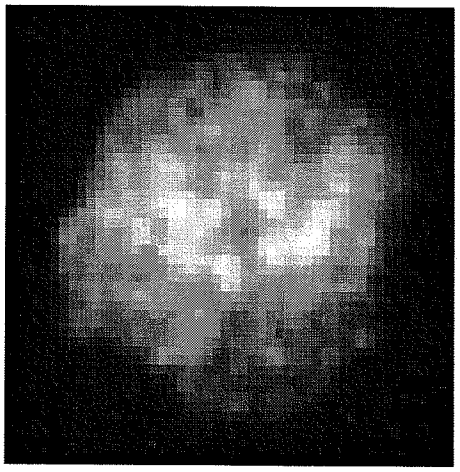


Figure 4.8 Only intensities between Min/max (40 to 515) are mapped into the range from 0 to 255 levels of intensities and are displayed as 255 different shades of gray. Unused background is removed allowing a wider range of intensities to be used for image display.

Contrast Stretch Max



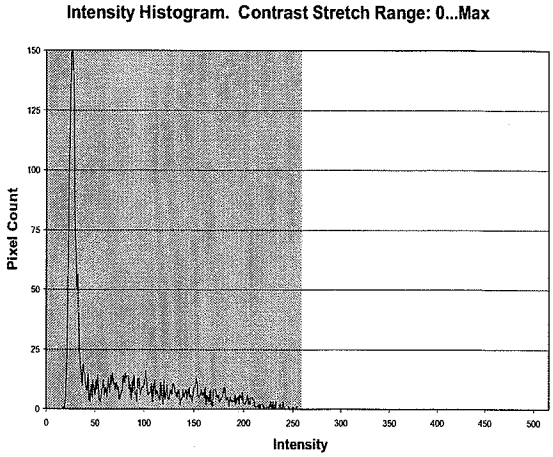


Figure 4.9 Full range of intensities (0 to 515) is mapped into the range from 0 to 255 levels of intensities and is displayed as 255 different shades of gray. All image details can be seen.

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Displaying the Log Window

The Log Window displays general program functions as well as error messages. An example is shown in the following figure. You can turn the display of this window on and off when using the Display Log Window menu item.

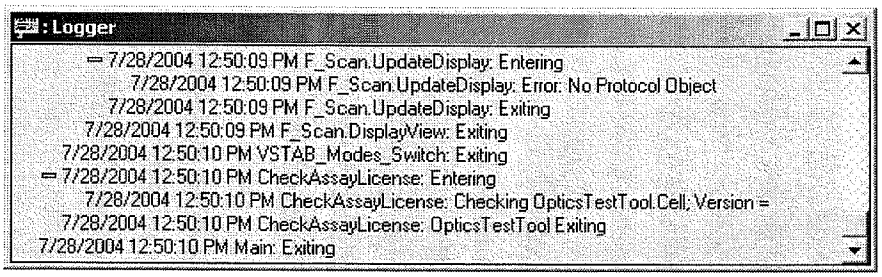


Figure 4.11 Reader Log Window

To display the log window,

- From the **Options** menu, select **User Options**, and then select **Display Log Window**. A checkmark will appear next to the Display Log Window menu item.

To turn off the display of the log window

- From the **Options** menu, select **User Options**, and then select **Display Log Window**. The checkmark next to the Display Log Window menu item will disappear.

Changing Reference Wells

If you are using reference wells (refer to your BioApplication Guide for more information), you can use the Reference Well Configuration tool to change the well settings in the assay. You can also define the reference well type. Depending on the BioApplication, up to three reference well types are available and are defined in the table below.

Reference Well Type	Description
Known	The cells in the "Known" wells have been treated with a compound or condition where their response has a known distribution and/or value. This allows the response from the "Known" reference wells to be characterized and limits on its range be set based on the expected response due to the condition or compound applied.
Min	The cells in the "Min" wells have been treated with a compound or condition so that they give the minimum response of the range of response signals possible. This allows the range for the minimum signal to be determined, and the response of other cells to be characterized in relation to this minimum response.
Max	The cells in the "Max" wells have been treated with a compound or condition so that they give the maximum response of the range of response signals possible. This allows the range for the maximum signal to be determined, and the response of other cells to be characterized in relation to this maximum response.

To change the Reference Wells,

1) Select the Assay Protocol from the Protocol Selection list.

2) From the **Tools** menu, select **Change Reference Wells**. The Reference Well Configuration screen will appear as shown in the following figure. All reference wells appear in the plate representation and are color-coded according to the legend on the right.

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Using the Illumination Correction Tool

The Illumination Correction Tool allows you to monitor the health of your optical system and, if necessary, enhance the precision with which cellular measurements are made. The latter is achieved by correcting the cellular images for spatial heterogeneity before data extraction. Spatial heterogeneities arise from one or more of the components of the optical train of any imaging system including the sample illuminator, lenses, mirrors, spectral filters and dichroics, objectives, and the imaging camera itself. The Illumination Correction Tool is designed to evaluate the severity of the abnormality and correct the ArrayScan HCS imaging system for spatial heterogeneity using background subtraction and flat field division methods.

Please refer to **Chapter 10, Using the Illumination Correction Tool** for more information.

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Specifying the Scan Area and Direction

Before running a scan, you can first specify the scan area and scan direction. The Scan Area Selection feature allows you to select the wells or well regions to be scanned. The wells may be contiguous or non-contiguous. This feature gives you the option of scanning a sub-region of the entire microplate. In addition to selecting the Scan Areas, you can also specify if you want to scan by rows or columns.

Choosing the Scan Area

To access the Scan Area Selection dialog box,



- In Scan Plate View, click the Scan Area Selection button on the ArrayScan toolbar. The Scan Area Selection dialog box appears as shown in the following figure.

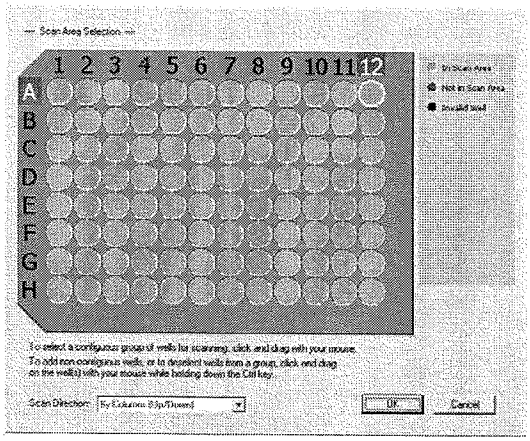


Figure 4.13 Scan Area of the Create Protocol View.

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

Well Color	Description
Light Grey	A light gray circle represents a well that will be scanned.
Dark Grey	A dark gray circle represents a well that physically exists on the microplate, but will not be scanned.
Black	A black 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.

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To scan by rows,

- In the Scan Area Selection dialog box, select **By Rows** from the **Scan Direction** drop down list.

To scan by columns,

- In the Scan Area Selection dialog box, select **By Columns** from the **Scan Direction** drop down list.

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81	80	79	78	77	76	75	74	73
50	49	48	47	46	45	44	43	72
51	26	25	24	23	22	21	42	71
52	27	10	9	8	7	20	41	70
53	28	11	2	1	6	19	40	69
54	29	12	3	4	5	18	39	68
55	30	13	14	15	16	17	38	67
56	31	32	33	34	35	36	37	66
57	58	59	60	61	62	63	64	65

Figure 4.19 Field scan pattern for 81 fields.

324	323	322	321	320	319	318	317	316	315	314	313	312	311	310	309	308	307
257	256	255	254	253	252	251	250	249	248	247	246	245	244	243	242	241	306
258	197	196	195	194	193	192	191	190	189	188	187	186	185	184	183	240	305
259	198	145	144	143	142	141	140	139	138	137	136	135	134	133	182	239	304
260	199	146	101	100	99	98	97	96	95	94	93	92	91	132	181	238	303
261	200	147	102	65	64	63	62	61	60	59	58	57	90	131	180	237	302
262	201	148	103	66	37	36	35	34	33	32	31	56	89	130	179	236	301
263	202	149	104	67	38	17	16	15	14	13	30	55	88	129	178	235	300
264	203	150	105	68	39	18	5	4	3	12	29	54	87	128	177	234	299
265	204	151	106	69	40	19	6	1	2	11	28	53	86	127	176	233	298
266	205	152	107	70	41	20	7	8	9	10	27	52	85	126	175	232	297
267	206	153	108	71	42	21	22	23	24	25	26	51	84	125	174	231	296
268	207	154	109	72	43	44	45	46	47	48	49	50	83	124	173	230	295
269	208	155	110	73	74	75	76	77	78	79	80	81	82	123	172	229	294
270	209	156	111	112	113	114	115	116	117	118	119	120	121	122	171	228	293
271	210	157	158	159	160	161	162	163	164	165	166	167	168	169	170	227	292
272	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	291
273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290

Figure 4.20 Field scan pattern for 324 fields.

The Starting Field Offset feature allows you to specify the starting field number from which the ArrayScan VTI HCS Reader will start the spiral field scan pattern within a well. You may want to use this feature if, for example, you know that your cells are clumped near the well edges and you do not want to scan near the center of the well. You may also want to use this feature to prevent photo-bleaching of fields in the center, or to avoid fields that are already photo-bleached.

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Choosing the Disk Scan Mode

Before starting a scan, you must first specify if you are running a live scan or a disk scan. A live scan refers to the process of scanning a physical microplate on the ArrayScan V^{TI} instrument. This does not necessarily mean that the cells on the microplate are alive. A disk scan is a scan using images stored on a disk rather than images acquired from the ArrayScan V^{TI} instrument.

When running in disk scan mode, you can reanalyze images from microplates that were previously scanned in live scan mode. While reanalyzing these images, you can optimize or correct scan parameters to determine the effect on your results. Any parameter in the Assay Protocol can be altered with the exception of those parameters that directly affect the raw image acquisition (e.g., exposure time, dye name, etc.).

Refer to **Chapter 8, Running a Disk Scan** for more information.

To switch between Live Scan Mode and Disk Scan Mode,



- In the Protocol Interactive or Scan Plate View, click the **Live-mode Scan** toggle button on the ArrayScan toolbar to switch between the two modes. When the toggle appears as shown to the left, the system is in Disk Scan Mode.

Choosing the Store Images Option

The Store Images toggle button on the ArrayScan toolbar provides the option of storing images generated from a scan in the database along with the plate data.

To store images generated from the scan,



- In Scan Plate View, click the **Store Images Setting** toggle button on the ArrayScan toolbar to switch between storing and not storing images. When the toggle button appears as shown to the left, the system will store images.

NOTE



If you do not store images, you cannot rescan them at a later time nor can you view them in the vHCS:View application.

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Running Multiple Assay Protocol Plate Scans

If you are scanning a stack of plates that require different Assay Protocols, or scanning a single plate with more than one Assay Protocol, the following software setup procedures are required before running the multiple Assay Protocol plate scan.

- **Create Plate Protocols**
- **Create Plate IDs**

Working with Plate Protocols

Before scanning either (a) a stack of plates that require different Assay Protocols, or (b) a single plate that requires more than one Assay Protocol, it is necessary to create Plate Protocols. A Plate Protocol specifies several protocol settings including the assay, Assay Protocol, plate manufacturer and type, store images option, display mode, scan area, and starting field. Therefore, it is possible to scan a stack of plates that have different settings for each of these protocol options.

It is also possible to assign multiple Plate Protocols to a single plate. Therefore, you can perform a series of scans on the same plate, each with a different assay, or you can apply different settings to different parts of the plate. Please refer to **Chapter 5, Scanning a Microplate** for setup instructions.

The Plate Protocol Wizard is used to create Plate Protocols.

To run the Plate Protocol Wizard,

- If the ArrayScan software application is open, select **Plate Protocol Wizard** from the **Tools** menu. Otherwise, from the Windows Start menu, select **Programs>Cellomics>ArrayScan> Plate Protocol Wizard**.

The main screen of the Plate Protocol Wizard appears as shown in the figure below.

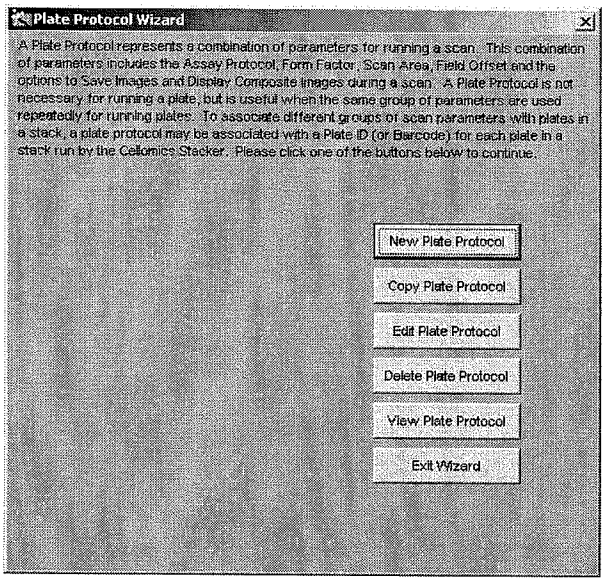


Figure 4.22 Plate Protocol Wizard: Main screen

The Plate Protocol Wizard allows you to create new Plate Protocols, and copy, edit, delete, and view existing Plate Protocols.

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6) Select the Scan Area.

7) To select a contiguous scan area, do one of the following:

- 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

8) To select a non-contiguous scan area, do the following:

- 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.

9) Select the Starting Field from the Starting Field list. For more information, refer to the section titled *Specifying the Starting Field Offset* appearing earlier in the chapter.

10) Click the **Next** button. The following screen appears.

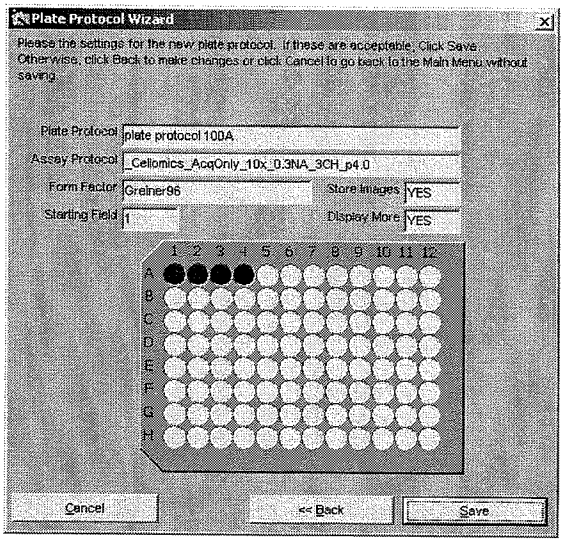


Figure 4.25 Plate Protocol Wizard: Verify Settings

11) Review all Plate Protocol settings. If they are acceptable, click the **Save** button. Otherwise, click the **Back** button to make changes.

12) Once you click the **Save** button, the main screen of the Plate Protocol Wizard appears. To create additional Plate Protocols, click the **New Plate Protocol** button and repeat the procedure. After creating the last Plate Protocol, click the **Exit Wizard** button.

NOTE

Alternatively, you can save a single Plate Protocol when working in the Scan Plate View. Select an Assay Protocol, then specify all other system settings such as the scan area, field offset, etc., and then select **Save Plate Protocol** from the **File** menu.

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4) All settings for the selected protocol appear. In the Plate Protocol Name box, enter a new name for the Plate Protocol. If you wish to change any other settings appearing on the screen, do so at this time.

5) Click the Next button. The following screen appears.

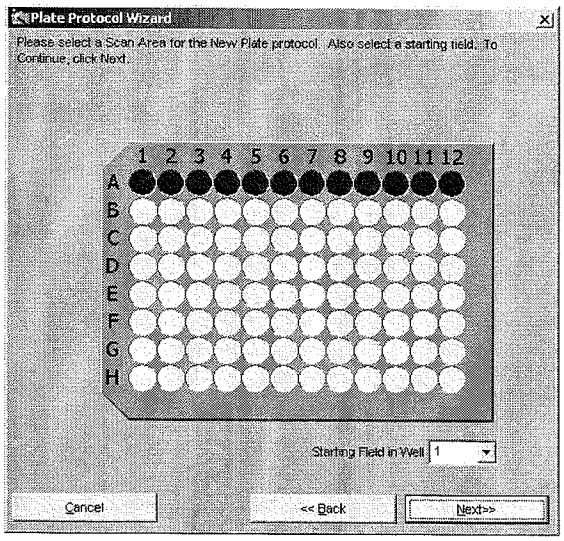


Figure 4.28 Plate Protocol Wizard: Select scan area

6) If you wish to change the Scan Area or Starting Field number for the copied Plate Protocol, do so at this time.

7) Click the **Next** button. The following screen appears.

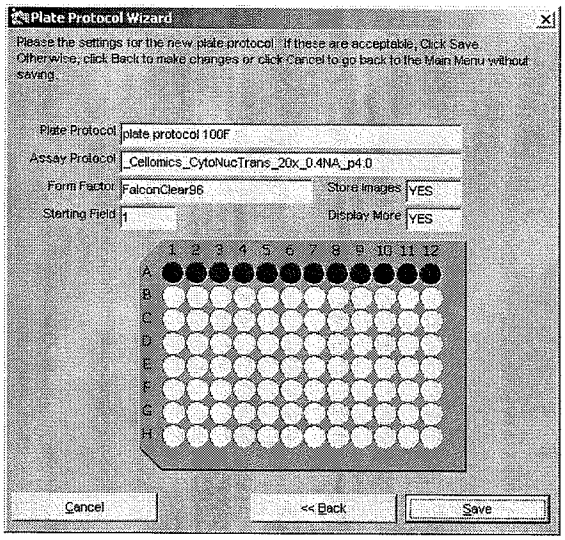


Figure 4.29 Plate Protocol Wizard: Confirm settings for copy

8) Review all Plate Protocol settings. If they are acceptable, click the **Save** button. Otherwise, click the **Back** button to make changes.

9) Once you click the **Save** button, the main screen of the Plate Protocol Wizard appears. To copy additional Plate Protocols, click the **Copy Plate Protocol** button and repeat the procedure. After copying the last Plate Protocol, click the **Exit Wizard** button.

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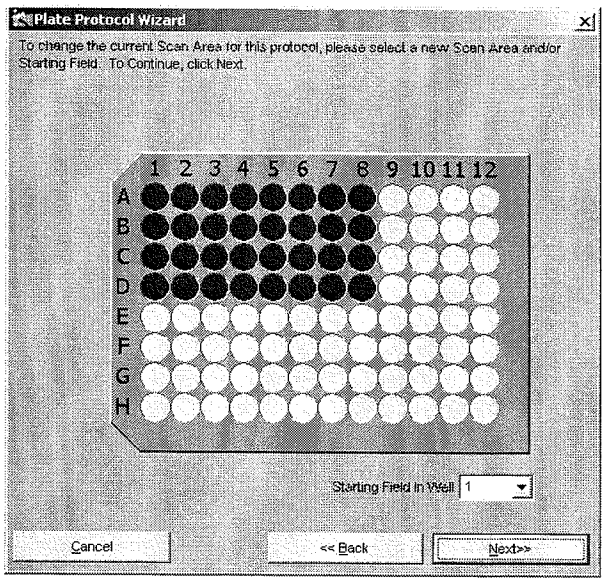


Figure 4.32 Plate Protocol Wizard: Change scan area

4) Change the Scan Area and Starting Field setting as desired and click the Next button. The following screen appears.

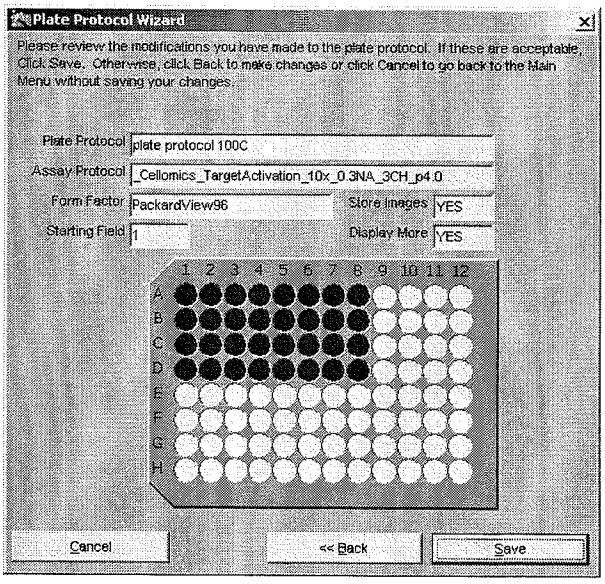


Figure 4.33 Plate Protocol Wizard: Confirm settings for editing

5) Review all settings. If settings are correct, click the Save button. Otherwise, click the Back button to make changes.

6) Once you click the Save button, the main screen of the Plate Protocol Wizard appears. To edit additional Plate Protocols, click the Edit Plate Protocol button and repeat the procedure. After editing the last Plate Protocol, click the Exit Wizard button.

NOTE

Alternatively, you can edit a single Plate Protocol when working in the Scan Plate View. Select **Load Plate Protocol** from the **File** menu. You can now change all desired settings. When finished, select **Save Plate Protocol** from the **File** menu.

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Viewing Existing Plate Protocols

To view existing Plate Protocols,

1) Click the **View Plate Protocol** button. The following screen appears.

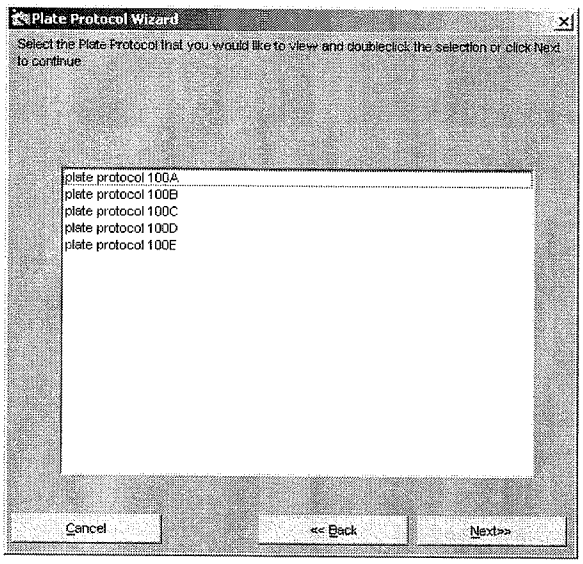


Figure 4.36 Plate Protocol Wizard: Select Plate Protocol to view.

2) Select the Plate Protocol that you wish to view from the list and click the **Next** button. The following screen appears.

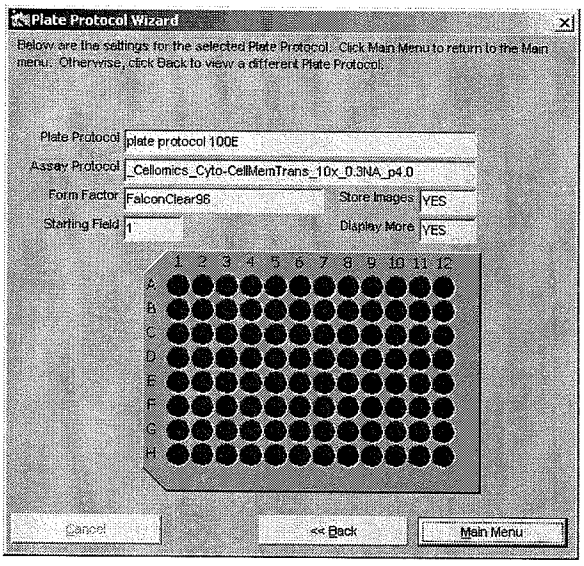


Figure 4.37 Plate Protocol Wizard: View settings

3) View the settings of the selected Plate Protocol. When finished viewing Plate Protocol settings, click the **Main Menu** button. To view settings for a different Plate Protocol, click the **Back** button.

Exiting the Plate Protocol Wizard

To exit the Plate Protocol Wizard,

- Click the **Exit Wizard** button.

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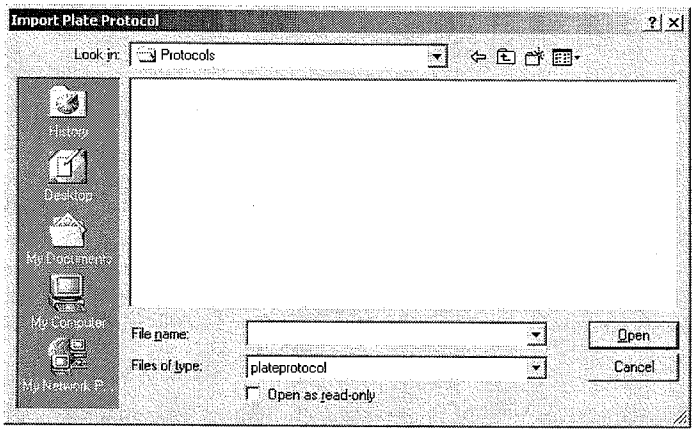


Figure 4.39 Import Plate Protocol dialog box

3) In the **Look in** drop down list, select the drive and folder that contains the Plate Protocol text file you wish to import.

4) In the folder list, navigate to the folder that contains the Plate Protocol file you want to import.

5) Select the Plate Protocol text file.

6) Click the **Open** button.

NOTE

If a Plate Protocol of the same name already exists in the Plate Protocol database, a confirmation message will appear. Choose **YES** to overwrite the existing Plate Protocol with the imported Plate Protocol. Choose **NO** to choose a new name for the imported Plate Protocol.

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Adding a New Plate ID

To add a new Plate ID,

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

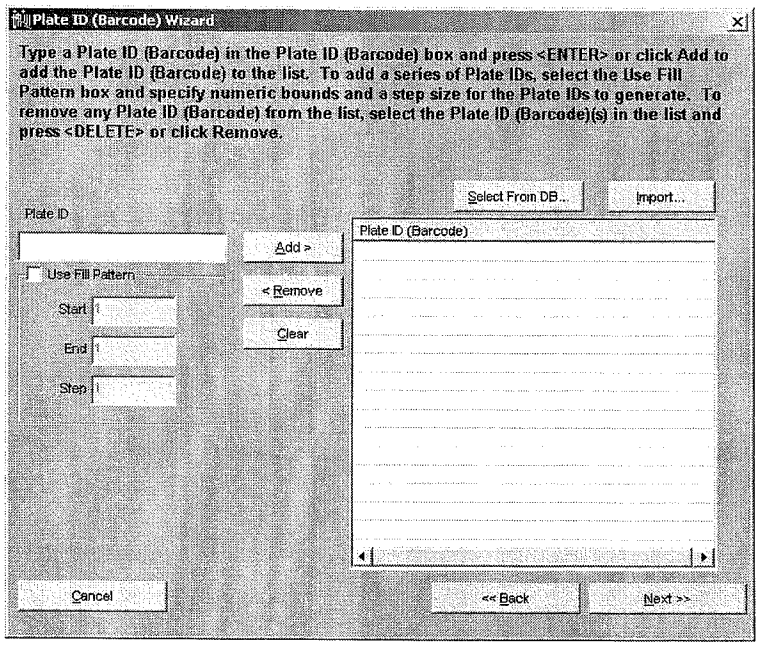


Figure 4.41 Plate ID (Barcode) Wizard: Create Plate ID Entries

2) If you are placing barcode labels on the plates and your barcode label maker generates a file containing the barcodes, follow the procedures below to import the barcodes from the file. Otherwise, skip ahead to step 3. To import the barcode file, click the **Import** button. The Import Barcodes dialog box appears as shown below. Follow the directions on screen and click the **Import** button. The Plate IDs will appear in the Plate ID list. Skip ahead to step 6.

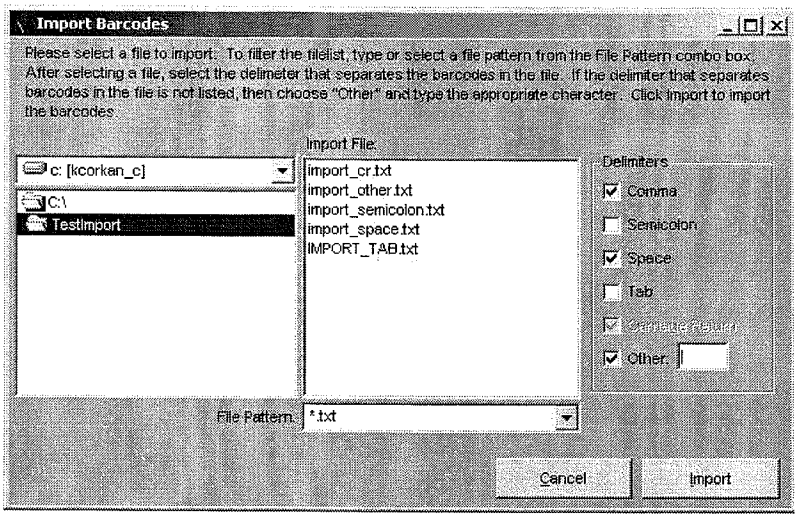


Figure 4.42 Plate ID (Barcode) Wizard: Import barcodes

3) Enter the desired Plate ID in the Plate ID box. To search for existing Plate IDs, click the **Select from DB** button. The Plate Selection Filter dialog box appears. Enter the desired

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