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ArrayScan® HCS Reader: ApoTome® User's Guide

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Introduction

The ApoTome® optical sectioning hardware and software is a flexible yet easy to use module of the Cellomics® ArrayScan® V^{TI} HCS Reader that enhances assay development and improves the imaging and analysis of difficult samples such Jurkat cells, fibers, clumps of cells, and confluent cells. The ApoTome allows you to improve image quality by eliminating out-of-plane light from the sample, thereby expanding the range of assays to which you can apply HCS technology.

About this Guide

The chapters that follow this introduction are described below.

Chapter 1 provides an overview of using the ApoTome on the ArrayScan V^{TI} HCS Reader.

Chapter 2 describes the ApoTome calibration and verification tool.

Terminology, Conventions, and Symbols

This guide assumes that you have a basic knowledge of computers using the Microsoft® Windows® 2000 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 in the following sections.

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Contacting Technical Support

If you have a technical question that you are unable to answer after consulting the documentation, please contact Technical Support.

Thermo Fisher Scientific
100 Technology Drive
Pittsburgh, Pennsylvania 15219
U.S. Toll-free: (800) 432-4091
Main: (412) 770-2200
Fax: (412) 770-2450
e-mail: techsupport@cellomics.com
www.cellomics.com

For European assistance, contact:
Main: +44 118 9880 262 Fax: +44 118 9880 362

Before contacting Technical Support for service or support, it is helpful if you are prepared to answer the following questions:

- What were you doing when the problem occurred?
- Can you reproduce the problem?
- Did you try to solve the problem? If so, what steps did you take and what did you observe?
- Which error messages, if any, appeared?

Having these answers will help us provide you with a solution as quickly as possible.

Documentation Feedback

Thermo Fisher Scientific is committed to providing you with the highest quality product documentation and online help and we welcome comments and suggestions for improvement. Before emailing your feedback to us, it is helpful if you include the following information:

- For printed manuals, provide the document title and part number. The part number can be found at the bottom of the Copyright Notices page.
- 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

Using the ApoTome with the ArrayScan HCS Reader Software

WARNING

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This chapter describes how to use the ApoTome with the ArrayScan HCS Reader software. The following topics are described in detail in this chapter:

- Engaging the ApoTome
- Determining the Status of the ApoTome
- Creating an Assay Protocol Using the ApoTome
- Scanning a Plate Using ApoTome Assay Protocols
- Running a Disk Scan

Please refer to Chapter 2 for instructions on calibrating the ApoTome. ApoTome calibration is recommended once every three months and any time a change is made to the objectives or filters installed on the system, and after the system has been serviced for any reason.

For troubleshooting information, please refer to Chapter 3.

NOTE

Be sure that the Reader has been powered for at least 30 minutes before calibrating the ApoTome or scanning microplates. (The ApoTome turns on automatically when the Reader is powered.)

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Determining the Status of the ApoTome

When the ApoTome is installed in the ArrayScan HCS Reader, the status bar will display an icon that indicates the status of the ApoTome. The following table describes the meaning of each icon. Note that tool tips are also available and display the status of each icon.

NOTE

The icon appearing in the status bar will refresh periodically. Therefore, when the ApoTome status changes, it may take a few moments for the status bar to display the appropriate icon.

ApoTome Icon	Description
	ApoTome is available and engaged.
	ApoTome is available and engaged but the current Assay Protocol selected does not require the ApoTome.
	ApoTome is available but not engaged.
	ApoTome is available but not engaged and the current Assay Protocol selected requires an ApoTome.
None	ApoTome is not available. Check for installation of the ApoTome.

Creating an Assay Protocol Using the ApoTome

When you want to apply optical sectioning using the ApoTome, you must specify this option in the Assay Protocol using either the Create Protocol View or the Protocol Interactive View.

NOTE

You cannot create an Assay Protocol that requires the ApoTome when working on an instrument that does not have the ApoTome installed.

Create Protocol View

To create an Assay Protocol that uses the ApoTome,

1) Open the Create Protocol View.

2) Open an existing Assay Protocol
-or-
Create a new Assay Protocol

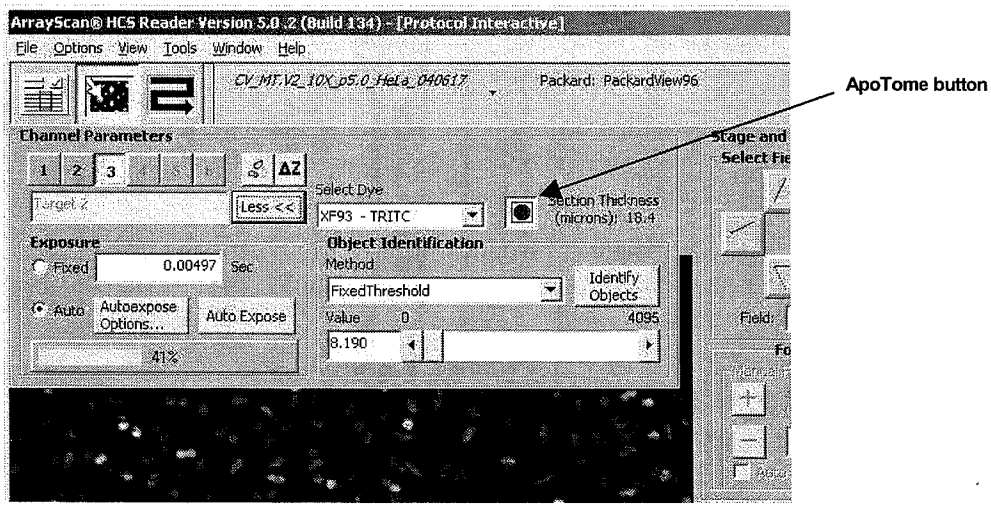
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Protocol Interactive View

To create an Assay Protocol that uses the ApoTome,

1) Display the Protocol Interactive View.

2) Select the desired channel number and then click the ApoTome button (see following figure). Optical sectioning can be turned on or off for each channel.



3) Note the optical section thickness (in microns). Please refer to the table in the previous section listing the optical section thickness for each objective.

Acquiring Images

Clicking the Acquire Image button in the Protocol Interactive View will display the sectioned image. Note that only the sectioned image is displayed and not the individual pre-processed grid projection images.

When you click the Acquire Image Set button, the instrument will acquire ApoTome images for channels that were specified to use the ApoTome and it will acquire conventional (non-sectioned) images for channels that were not specified to use the ApoTome.

NOTE

If the Assay Protocol requires the ApoTome and the ApoTome status is *Available but Not Engaged*, then clicking the Autofocus, AutoExpose, Acquire Image, or Acquire Image Set button will produce a warning message to indicate that the ApoTome is not engaged as required by the protocol. You will have the opportunity to retry, perform the action without the ApoTome, or cancel the action.

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Running a Disk Scan

When using the vHCS™:Scan application on a desktop computer, you can create and load Assay Protocols that require the ApoTome if the vHCS:Scan application is configured to use the ApoTome. If you cannot create or load ApoTome enabled Assay Protocols, please contact Cellomics Technical Support for assistance.

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Chapter

2

Calibrating the ApoTome

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This chapter describes the procedures for calibrating the ApoTome on the ArrayScan HCS Reader. **ApoTome calibration is recommended once every three months and any time a change is made to the objectives or filters installed on the system, and after the system has been serviced for any reason.**

Please note that you must have the following items before proceeding:

- You must have ArrayScan version 5.0.2 or later installed on the Reader.
- You must have the ApoTome calibration plate.

The purpose of the ApoTome Calibration is to provide a means of calibrating the grid focus position. The ApoTome Calibration Tool walks you through the calibration and verification procedures. Detailed instructions are provided in this chapter. For troubleshooting information, please refer to Chapter 3.

Opening the ApoTome Calibration Tool

NOTE

If the ArrayScan software is running, close the application before attempting to open the ApoTome Calibration Tool.

To open the ApoTome Calibration Tool,

1) From the Windows Start menu, select **Programs** > **Cellomics** > **ArrayScan50** > **Customer Tools** > **ApoTome Calibration**

The ApoTome Calibration Tool will appear as shown in the following figure.

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Grid Z Calibration and Verification

To calibrate the ApoTome, you must perform the Grid Z Calibration and Verification procedure. The purpose of this procedure is to calibrate the grid focus position.

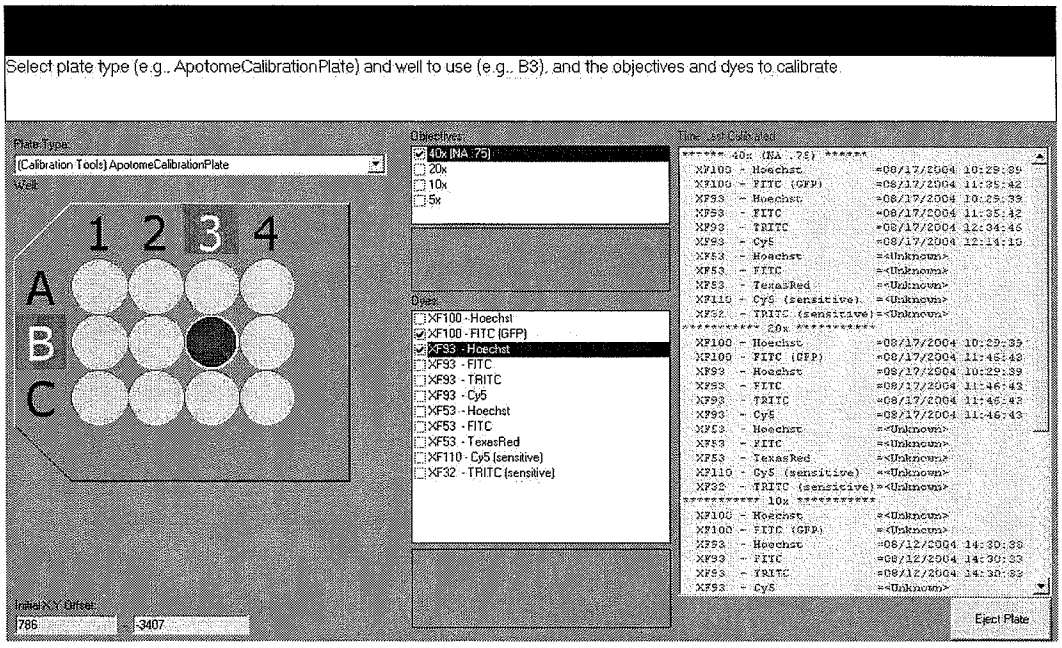
NOTE

The GRID PHASE and CAMERA ORIENTATION Calibration/Verification and Test and Evaluation options are reserved for trained service personnel and/or require the assistance of Technical Support. Please contact Technical Support before attempting to run these tool

To start the Grid Z calibration,

1) Verify that the objective you wish to calibrate is installed. If it is not, run the Objective Change Wizard (refer to the *Objective Installation Guide*).

2) Click the **GRID Z Calibration/Verification** button and click the **Next** button. (Note that this is the default selection). The Selection screen appears as shown in the following figure.



3) In the Plate Type list, select the **ApoTomeCalibrationPlate** option.

4) In the plate representation, click on well B3 if not already selected.

5) Under Objectives, select the objectives to be calibrated.

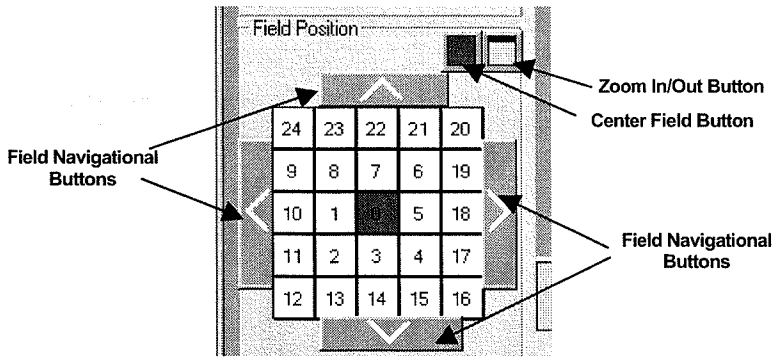
NOTE

Note that the 40x (NA .50) objective is not compatible with the Apotome.

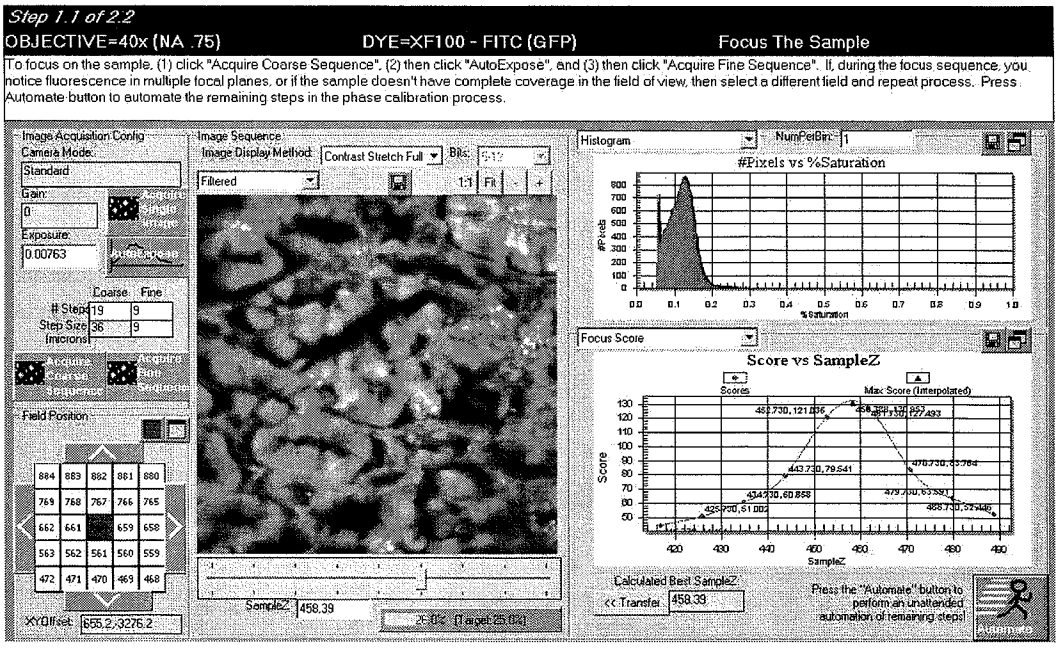
6) Under Dyes, select the dyes to be calibrated.

7) Click the **Eject Plate** button located in the lower right corner. Insert the ApoTome calibration plate so that the marker for well A1 is located at the near left corner as viewed

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Continue to choose different field numbers until you see the sample image. Once you've displayed the sample image, click the **Automate** button located in the lower right corner. The software will automatically acquire a single image from the selected field. The software will then attempt to focus the image by using the coarse sequence. It will then run autoexpose and run the fine focus sequence. During this process, you should notice an image that looks similar to the image shown in the following figure.



If the Automate process fails to focus the image, you can manually focus the image. To do so, click the **Acquire Coarse Sequence** button. You will notice the image focusing sequence on screen. Notice that the slider bar located beneath the image moves with each acquired image. The tool should display the best focused image. If it does not, move the slider bar until the optimal image is displayed on screen. Now click the **Autoexpose** button. Next click the **Acquire Fine Sequence** button. Again, if the tool does not choose the best image, move the slider bar to display the desired image. If you are unable to find and focus the sample image, please contact Technical Support for assistance.

10) If you manually acquired and focused the images, you must click the **Next** button to continue (the Automate procedure automatically displays the next screen). The "Focus the Grid" screen appears. Click the **Automate** button located in the lower right corner. The software will automatically attempt to focus the image by using the coarse sequence

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This step completes the ApoTome Calibration. The main screen will now appear. Click the **Close** button to close the tool.

NOTE

If you are installing optional objectives on the ArrayScan instrument, you must run the calibration tool again for each optional objective. When running the calibration tool, select the optional objective only, but select all dyes. Note that the 40x (NA .50) objective is not compatible with the Apotome.

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Chapter

3

Troubleshooting

WARNING



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Please refer to this chapter if you are unable to calibrate the ApoTome using the instructions provided in Chapter 2. You can also refer to this chapter if you find that the image quality of the ApoTome images is not ideal.

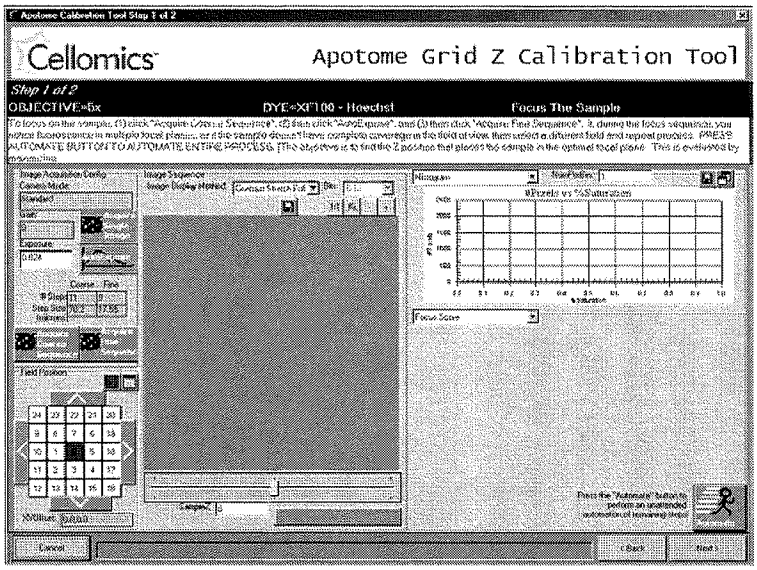
The troubleshooting information is divided into the following four categories:

- Troubleshooting problems that you experience while attempting to calibrate the grid focus position using the ApoTome Calibration Tool.
- Troubleshooting low image quality when reviewing ApoTome sectioned images generated by the ApoTome Calibration Tool.
- Troubleshooting low image quality when using the ArrayScan HCS Reader scanning software to generate ApoTome sectioned images.
- Troubleshooting miscellaneous problems when creating an ApoTome sectioned image.

If you are unable to solve the problem using the following troubleshooting information, please contact Technical Support for assistance.

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Focus the Sample Screen of the ApoTome Calibration Tool



Symptom	Cause	Solution
Spots or distortions in the sample image	Dirt on the sample cover-slip	Clean calibration slides using the methods described for cleaning optics in the ArrayScan V^{TI} HCS Reader User's Guide.
Unable to find sample	An incorrect Plate Type has been selected for the plate being used	Verify that the selected Plate Type (Selection screen) is "ApoTomeCalibrationPlate".
	The plate is incorrectly inserted in the stage	Insert the plate in the stage so that the marker for well A1 is located at the near left corner as viewed from the front doors of the ArrayScan instrument.
	An incorrect field is selected	Check that the field selected matches the field labeled on the slide in the holder. Also try the adjacent fields.
	Reader stage is not calibrated properly	Use the System Calibration Wizard to verify and correct the stage calibration.
Fails during automated focusing of sample	Sample contains fluorescence in multiple focal planes	Move to another field and/or focus grid manually rather than in the automated mode.

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Low Image Quality of ApoTome Images Generated by the Reader Scanning Software

Symptom	Cause	Solution
Grid lines appear in sectioned images.	Sample is photo-bleached.	Try another field or another well. Try reducing the integration time, including anti-fading reagents in sample prep, or using another fluorophore.
	Z-offset may not be optimal.	Try a different Z-offset.
	Calibration parameter, other than grid focus position, is incorrect.	Contact Technical Support for assistance.
	Hardware failure	Contact Technical Support for assistance.
Grid lines appear in non-sectioned images.	Calibration of grid position for conventional fluorescence is off or hardware failure.	Contact Technical Support for assistance.
	Hardware failure	Contact Technical Support for assistance.
Unable to reach desired saturation percent or failed auto-expose.	Target saturation value is too high for the sectioning conditions.	Use a lower target saturation percent.
Increasing exposure time decreases intensity.	Pre-processed images are being saturated, causing the sectioned image to be lower in intensity.	Reduce exposure time or reduce target saturation percent (for auto-expose).
Dark areas are appearing in the sectioned images and the number of identified objects is too low.	Plate is not flat enough and thus some cells are out of the optical section.	Increase the number of fields per well.
		Switch to higher magnification objective, if possible.
Dark areas are appearing in the sectioned images but the number of identified objects is satisfactory.	Plate is not flat enough and thus some cells are out of the optical section.	A solution is probably not needed because scan times and integration times will not be affected.
The exposure time used for non-sectioned image does not work well for sectioned images.	Because the sectioned image eliminates light from background objects, the integration time typically needs to be longer.	Increase exposure time.

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Appendix

A

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.

ApoTome Replacment Part

Part Name	Part Description	Part Number
ApoTome Grid VH	Transmission grid	**1151-002**
ApoTome Instrument Calibration Plate	Calibration slides and holder	**IC500505**

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