Incucyte Live-Cell Analysis Systems User Manual
Incucyte SX5
Incucyte S3
Incucyte SX1

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Incucyte Live-Cell Analysis Systems User Manual

Preface

Welcome to the *Incucyte Live-Cell Analysis Systems User Manual*. The purpose of the *Incucyte Live-Cell Analysis Systems User Manual* is to answer your questions and guide you through the procedures necessary to use Incucyte efficiently and effectively.

Using the manual

You will find the *Incucyte Live-Cell Analysis Systems User Manual* easy to use. You can simply look up the topic that you need in the Table of Contents or the Index. Later, in this Preface, you will find a brief discussion of each chapter to further assist you in locating the information that you need.

Special information about the manual

The *Incucyte Live-Cell Analysis Systems User Manual* has a dual purpose design. It can be distributed electronically and then printed on an as-needed basis, or it can be viewed online in its fully interactive capacity. If you print the document, for best results, it is recommended that you print it on a duplex printer; however, single-sided printing also works. If you view the document online, a standard set of bookmarks appears in a frame on the left side of the document window for navigation through the document. For better viewing, decrease the size of the bookmark frame and use the magnification box to increase the magnification of the document to your viewing preference.

If you do print the document using a single-sided printer, you might see a single blank page at the end of some chapters or appendices. This blank page has been added solely to ensure that the next chapter begins on an odd-numbered page. This blank page in no way indicates that your book is missing information.

Conventions used in the manual

The *Incucyte Live-Cell Analysis Systems User Manual* uses the following conventions:

- Information that can vary in a command—variable information—is indicated by alphanumeric characters enclosed in angle brackets; for example, <Vessel #>. Do not type the angle brackets when you specify the variable information.

- A new term, or a term that must be emphasized for clarity of procedures, is *italicized*.

- Page numbering is "online friendly." Pages are numbered from 1 to x, *starting with the cover* and ending on the last page of the manual.

Although numbering begins on the cover page, this number is not visible on the cover page or front matter pages. Page numbers are visible beginning with the first page of the table of contents.

- This manual is intended for both print and online viewing.

    - If information appears in blue, it is a hyperlink. Table of Contents and Index entries are also hyperlinks. Click the hyperlink to advance to the referenced information.

Assumptions for the manual

The *Incucyte Live-Cell Analysis Systems User Manual* assumes that:

- You are familiar with Windows-based applications and basic Windows functions and navigational elements.

- References to any third-party standards or third-party software functions were current as of the release of this version of Incucyte, and might have already changed.

Organization of the manual

In addition to this Preface, the *Incucyte Live-Cell Analysis Systems User Manual* contains the following sections, chapters, and appendices:

- Section 1, "Scheduling and Acquiring Scans," on page 13 provides detailed instructions for configuring and scheduling vessel scans in the Incucyte application. This section contains the following chapters:

  - Chapter 1, "Getting Started With Incucyte," on page 15 explains how to start and log in to Incucyte and provides an overview of the major navigational elements for the application. It also explains how to log out of Incucyte.

  - Chapter 2, "Managing Scans," on page 21 details all the procedures and considerations that are necessary for image acquisition, including configuring settings and scheduling acquisitions. It also details how to edit the acquisition settings for a vessel, and how to remove a vessel from the scanning schedule.

- Section 2, "Viewing Images," on page 53 provides detailed instructions for selecting the vessel in the database that contains the images that you are analyzing and for viewing, comparing, and ultimately selecting the images in the vessel that you are analyzing. This section contains the following chapters:

  - Chapter 2, "Selecting a Vessel," on page 55 details the Scanned Vessels window, which displays all the vessels that have ever been scanned on your Incucyte system by any user, as well as all the options that are available on the window for selecting the correct vessel.

  - Chapter 3, "Viewing and Working With Images," on page 65 details the Vessel View window, which displays the scanned vessel that has been selected for image analysis, and all the options that are available on the window for viewing, comparing, and ultimately selecting the images that you are analyzing.

- Section 3, "Analyzing Images and Visualizing Results," on page 87 details how you define or select your image analysis parameters, and then define your image analysis as well as how to visualize and investigate your analysis results. This section contains the following chapters:

  - Chapter 2, "Defining the Image Analysis," on page 89 details the Launch Analysis wizard, which you use to define or select your image analysis parameters, and then launch the analysis of the images.

  - Chapter 2, "Visualizing the Analysis Results," on page 107 details the various ways that you can visualize your analysis results so that you can access and interpret your data and identify the keys areas of your assay that worked well as well as the areas that need attention or improvement.

- Section 4, "Supporting Information," on page 135 details the various tools and options that are available for supporting your image analysis. This sections contains the following appendices:

  - Appendix A, "Plate Map Editor," on page 137 details the use of the Plate Map Editor, which you use for defining a custom plate map for image analysis.

- Section 5, "Incucyte Management and Administration," on page 149 details the various functions that are available for managing and administering your image data as well as the Incucyte system itself. This section contains the following appendices:

    - Appendix A, "Incucyte Data Archives," on page 151 details Incucyte's Archive function, which you use to manage your historical vessel data in Incucyte.

    - Appendix B, "Changing the Incucyte SX1 Objective," on page 165 details how to change the objective for the Incucyte SX1 before you configure and schedule a vessel scan.

    - Appendix C, "Changing the Incucyte SX5 Optical Module," on page 169 details how to change the optical module for the Incucyte SX5 before you configure and schedule a vessel scan.

Preface

Incucyte Live-Cell Analysis Systems User Manual

Section 1
Scheduling and Acquiring Scans

Section Contents

Incucyte Live-Cell Analysis Systems User Manual

Chapter 1
Getting Started With Incucyte

The Incucyte Live-Cell Analysis Systems (referred to as Incucyte in this manual for brevity) enables observation and quantification of cell behavior via real-time, quantitative live-cell analysis. Incucyte is the most current software for your Incucyte Live-Cell Analysis Systems. The application provides enhanced user-friendly control of all aspects of imaging and analysis including powerful and intuitive image processing tools. This chapter explains how to start and log in to Incucyte and provides an overview of the major navigational elements for the application. It also explains how to log out of Incucyte.

This chapter covers the following topics:

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Starting and Logging In to Incucyte

After you install Incucyte, a shortcut icon for the application is placed on the desktop. An option for the application is also available from the Start menu (Start > All Programs > Incucyte > Incucyte).

Figure 1-1:   Incucyte desktop icon

1. Double-click the desktop icon to launch Incucyte, or select the option from the Start menu.

    The Incucyte Open Connection dialog box opens. By default, Connect to Incucyte is selected.

Figure 1-2:   Incucyte Open Connection dialog box
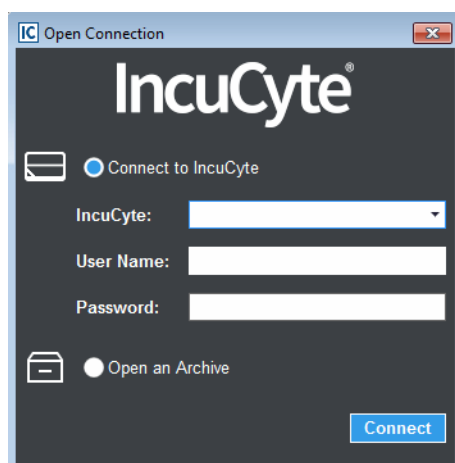
2. Enter the connection information for your Incucyte system.

    The Incucyte value is the instrument identifier that was assigned to your Incucyte system at installation, for example, IC50014. Sartorius establishes an Administrator user name and password with the purchase of the instrument. The Administrator user can create additional user accounts from the Manage main menu option (Manage > User Accounts).

3. Click Connect.

    The Incucyte main window opens. See

The Incucyte Main Window

The Incucyte main window opens after you start and log in to Incucyte. The window is your starting point for accessing all the functionality that is available in Incucyte.

Figure 1-3:   Incucyte main window
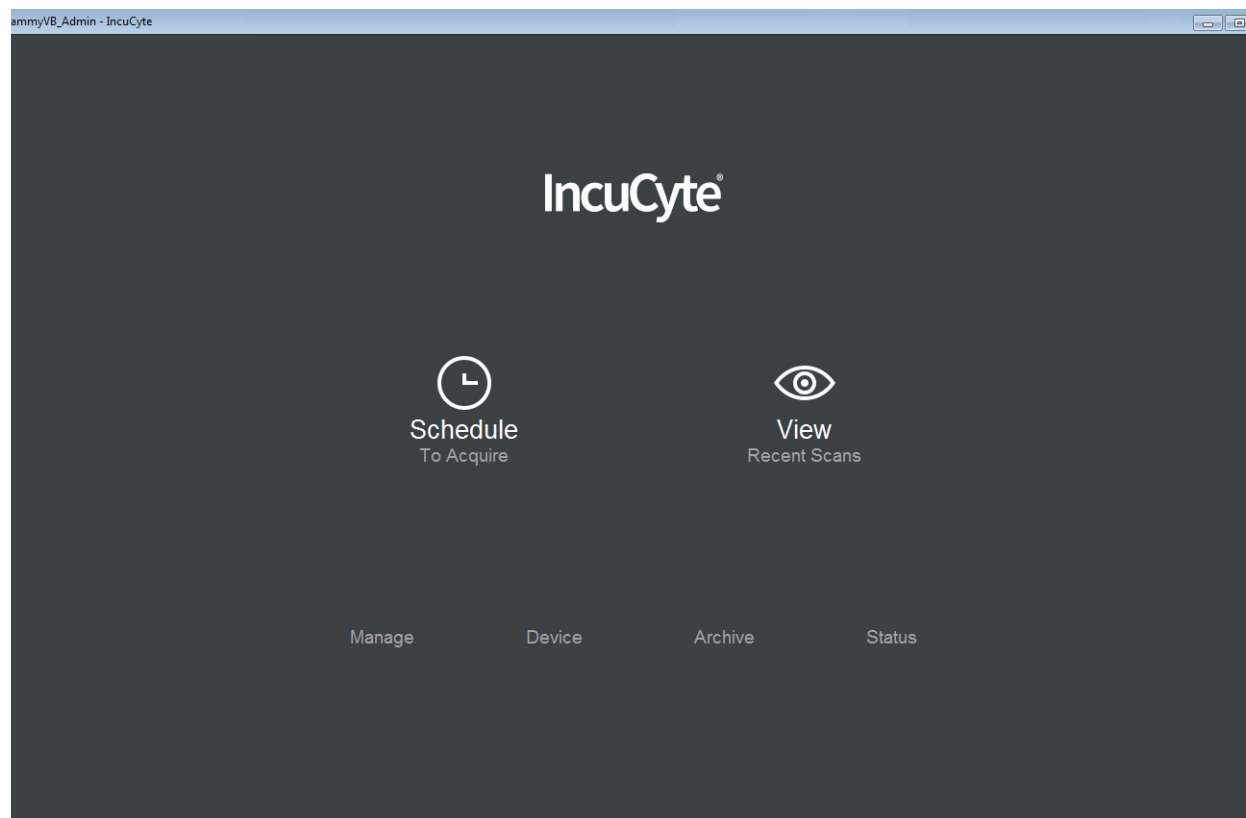
From top to bottom, the Incucyte main window has the following components: the title bar, the Scan icons, and the main menu.

Title bar

The Incucyte main window title bar displays your User Name in the left corner. The right corner displays the standard Windows icons for minimizing, maximizing, and closing the window.

Scan icons

The two Scan icons—Schedule To Acquire and View Recent Scans—provide the links to the two primary functions of Incucyte: acquiring scans and viewing and analyzing scans. The Schedule To Acquire icon provides the link to the scheduling and acquiring scanned vessel functions. The View Recent Scans icon provides the link to the viewing scanned vessel functions. The Schedule and View options are also available in the menu that is displayed at the top of every window in Incucyte.

Main menu

The main menu displays the top level of basic choices for Incucyte. The same options are also available in the menu that is displayed at the top of every window in Incucyte.

Option	Description
Manage	Opens the Manage window, which provides the options for managing critical components (analysis definitions, vessel types, and user accounts) for Incucyte.
Device	Opens the Device window, which provides the options for managing Incucyte support for scan diagnostics.
Archive	Opens the Archive window, which provides the options for managing your data archives. See Appendix A, "Incucyte Data Archives," on page 151.
Status	Opens the Status page, which provides the options for viewing and managing the current status (system, temperature, archive, and analysis) for your Incucyte Live-Cell Analysis Systems as well as the corresponding system logs.

Logging out of Incucyte

When you log out of Incucyte, the Incucyte system remains active. This ensures you that your scans will be still be acquired according to schedule even if you are not logged in to the application.

To log out of Incucyte

1. From any window other than the Main window in Incucyte, click the Menu bar to open a dropdown menu.

2. On the dropdown menu, select Connection.

3. On the Connection sub-menu, click Close.

Chapter 2
Managing Scans

The Incucyte software is divided into two major parts: acquiring scans and the viewing and analysis of the scans.

- Acquiring scans involves configuring vessel settings, scheduling acquisitions, acquiring images, and storing vessel data in a database. You can also edit the acquisition settings for a vessel and remove a vessel from the scanning schedule.

- Viewing and analysis consists of selecting, measuring, assessing, and managing the acquired images and vessel data.

This chapter details all the procedures and considerations that are necessary for acquiring images, including configuring vessel settings and scheduling acquisitions. It also details how to edit the acquisition settings for a vessel, and how to remove a vessel from the scanning schedule.

This chapter covers the following topics:

Incucyte Live-Cell Analysis Systems User Manual
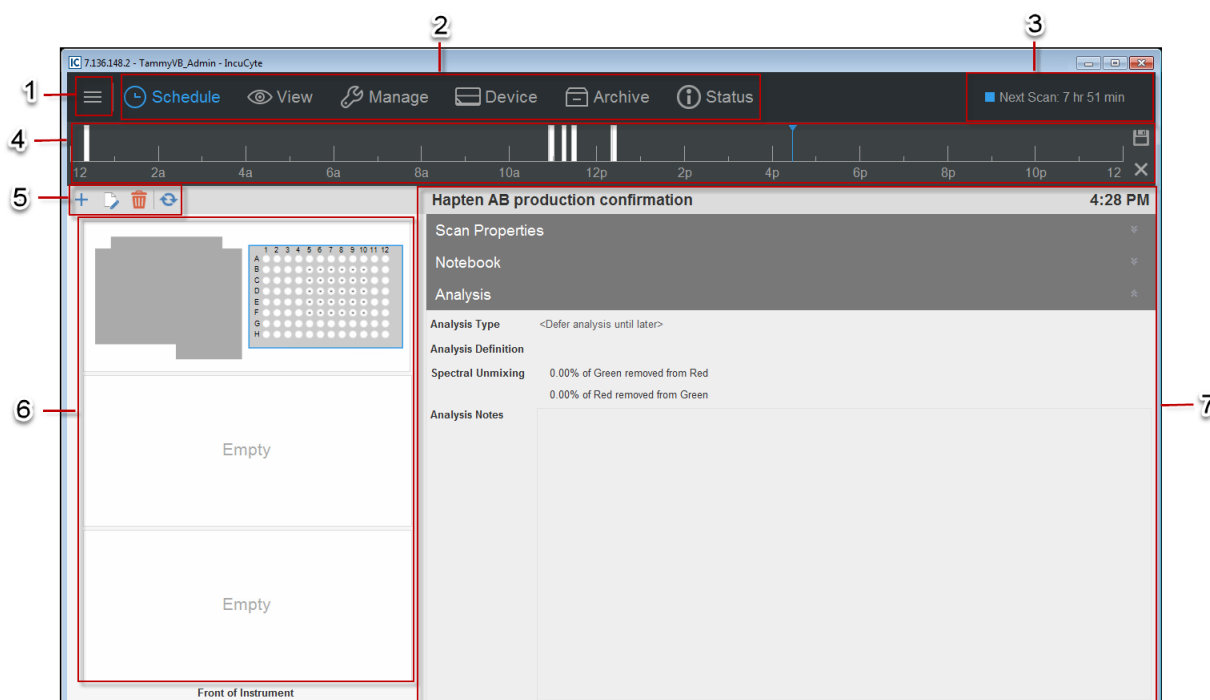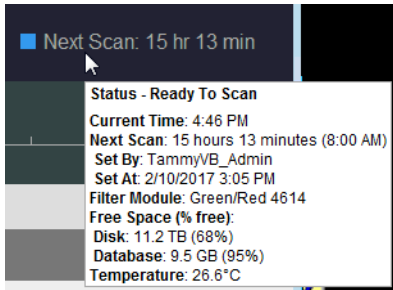
The Acquisition Window

You do all the necessary configuration and scheduling procedures for acquiring images in the Acquisition window. To open the Acquisition window, on the Incucyte main window, click Schedule To Acquire.

Figure 2-1:   Acquisition window

The Acquisition window has the following layout:

	Window component	Description
1	Menu bar	Opens a dropdown menu with the following options:
		• Connections: Provides options for opening (Open) and closing (Close) the connections to your Incucyte system.
		• Tools and Options: Provides options for opening the Plate Map Editor (Plate Map Editor) and specifying a variety of preferences (Preferences) for your Incucyte software installation. See Appendix A, "Plate Map Editor," on page 137.
		• Help: Provides links to a variety of options that offer support for using Incucyte, including the user manual (User Manual), a list of supported vessels (Supported Vessels), and general information about the software installation such as the current GUI version (About).
2	Main menu	The main menu is always displayed at the top of every window in Incucyte. The menu displays all the same options that are available on the Incucyte main window.

|	Window component	Description
3	Scheduling status	Color-coded to indicate the following: • Yellow: Indicates a warning such as the drawer is open, the device is restarting or updating, the disk is running out of space, and so on. • Blue: Indicates the device is idle. The drawer is closed, no error state exists, and the device is not active. The text can vary, based on whether the instrument is processing data, sitting idle until the next scan, or sitting idle with no scans scheduled. If the device is idle waiting for the next scan, then it displays the time to the next scan overall. The selected vessel in the drawer has no relation to the time that is displayed. • Green: Indicates that the device is busy with some activity. Activities for which green is displayed include, but are not limited to, scanning, warming up, self test, and calibration. There is no relation with the selected vessel in the drawer. • Red: Indicates an error condition; however, there is no relation to the selected vessel in the drawer. If no vessels are scheduled for scanning, then the message "No upcoming scans" is displayed. **Note:** You can hold your mouse pointer over the status to open a tooltip that displays general information about the device and when the next scan is to take place. The tooltip has no relation to the selected vessel. *Figure 2-2: Schedule Status tooltip*
4	Vessel Schedule Timeline	Displays the scanning schedules for all the vessels that are shown in the Vessel Drawer Setup pane. The schedule for the vessel that is currently selected in the Vessel Drawer Setup pane is shown in white bars. The schedules for the other vessels are shown in gray bars. A single blue line indicates the current day's time. Options are available for viewing and adjusting the schedule for a selected vessel, combining vessel scanning schedules, scanning vessels on independent schedules (which is referred to as *per vessel scheduling*), saving a vessel scanning schedule, and deleting a vessel scanning schedule. See "The Vessel Schedule Timeline" on page 47.

	Window component	Description
5	Vessel toolbar: Displays icons for carrying out specific tasks in the Acquisition window. From left to right, these icons are the following:	
	➕	Launch Vessel Wizard icon: Opens the Add Vessel wizard. You use the Add Vessel wizard to configure the acquisition settings and the scanning schedule for a vessel. See "Configuring and Scheduling a Vessel Scan" on page 27.
	📝	Edit selected vessel icon: Opens the Add Vessel wizard in Edit mode. You use this mode to edit specific properties for a vessel. See "Editing Vessel Information" on page 51.
	🗑	Remove selected vessel icon: Removes a selected vessel from the scanning schedule. See "Removing a Vessel from the Scanning Schedule" on page 52.
	🔄	Refresh schedule icon: Refreshes the Vessel Schedule Timeline display. If you have scheduled new vessels for scanning, or made changes to an existing vessel scanning schedule, then you should click this icon.
6	Vessel Drawer Setup pane	Indicates the positions in the Incucyte drawer in which you can place a vessel for scanning. The pane depicts the three tray locations in the Incucyte drawer as if you are looking at the drawer from above, which is how you place vessels in the Incucyte system drawer.
		Note: For the Incucyte SX5 and the Incucyte S3, all tray positions are available. For the Incucyte SX1, only the front tray positions are available. All other tray positions are dimmed ("grayed-out").
7	Vessel Information pane	The Vessel Information pane displays different information for the vessel that is currently selected in the Vessel Drawer Setup pane. Up to four different read-only views in the pane are available. Select an option on the pane (Scan Properties, Notebook, Plate Map, or Analysis) to open the corresponding view. • Scan Properties: Displays all the scan properties (Vessel Type, Scan Type, Image Channels, Objective, Scan Duration, and Number of Daily Scans) for the selected vessel. See "To specify the scan settings" on page 31. • Plate Map: Applicable only if the vessel is a microplate and if a plate map has been has defined for the vessel. To open and maximize the plate map view in its own window, click the view once. • Notebook: Displays the Vessel Name, the name of the Vessel Owner, the Cell Type, and the Passage. See "To provide vessel information" on page 37. • Analysis: If an analysis has been defined in the Add Vessel wizard for the selected vessel, then all the corresponding analysis information (analysis type, analysis definition, spectral unmixing percentages, and if present, analysis notes) are displayed for the selected vessel. See "To define the analysis setup" on page 38. See Figure 2-3 on page 26.

Figure 2-3:   Vessel Information pane with Scan Properties view for a selected vessel

Select Plate Map, Notebook, or
Analysis to open the other views.
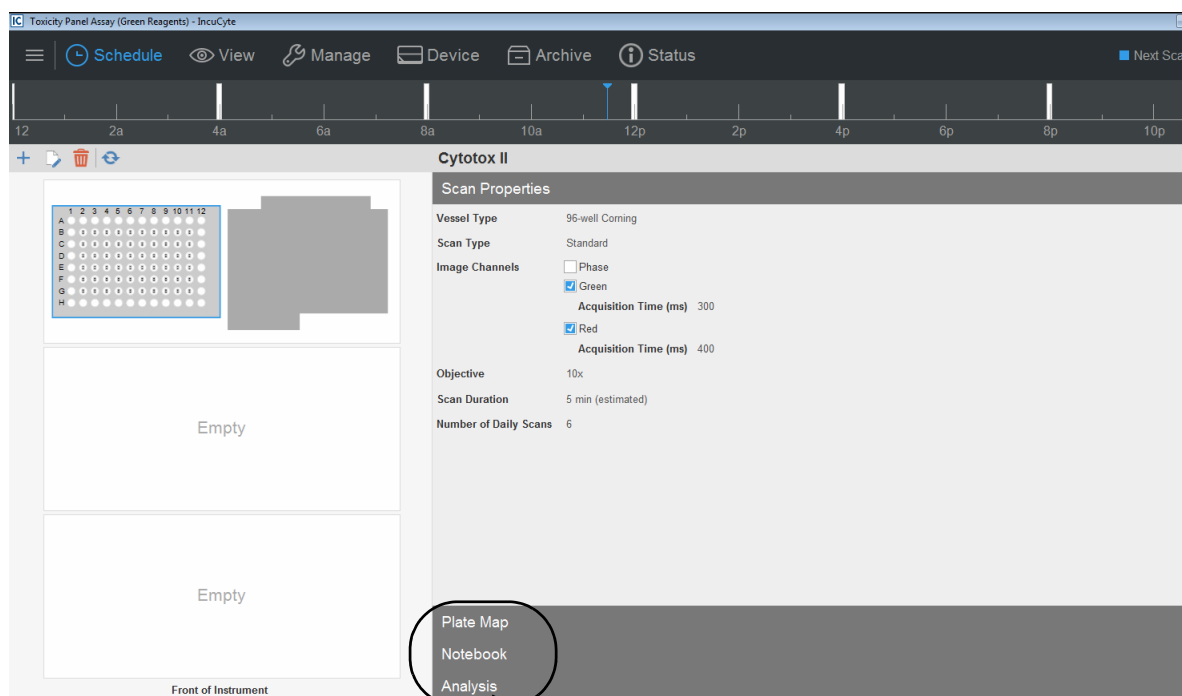
Configuring and Scheduling a Vessel Scan

Acquisition involves configuring the acquisition settings, scheduling the vessel scan, acquiring images in specified vessel locations, and storing vessel data in a database.You use the Add Vessel wizard to configure the acquisition settings and scanning schedule for a single vessel at time.

To demonstrate the full strength of the configuration and scheduling features for a scan in Incucyte, the following procedures describe how to create an entirely new vessel for a Standard scan with a scan schedule that is repeated every 24 hours. Although the steps for configuring and scheduling a Standard scan are different based on whether the vessel is created new, copied, or restored, and whether the scan is a repeating scan or a one time scan, they are still similar enough that the following procedures provide a frame of reference for any approach that you select for configuring and scheduling a vessel scan.

If you are using the Incucyte SX1, then the objective that is currently in use is set as the default objective. If you require a different objective, then you must select it <u>before</u> you configure and schedule the vessel scan. If you configure and schedule a vessel scan for the Incucyte SX1, and then change the objective, the current schedule is immediately cleared and all scans for all vessels currently found in the schedule are immediately ended. See Appendix B, "Changing the Incucyte SX1 Objective," on page 165.

Configuring the acquisition settings and scheduling a vessel scan consists of the following:

- Setting the scanning frequency, which determines the number of times that the vessel is scanned, either on a scheduled 24 hour scan period or a single Scan Now option. See "To set the scanning frequency" on page 28.

- Creating the vessel, which determines how the vessel is created for scanning –as an entirely new vessel, by copying an existing vessel, or by restoring a previously scanned vessel. See "To create the vessel" on page 29.

- Selecting the scan type, which determines the way that the vessel is scanned and the subsequent analysis options. You make your selection based on your assay and application. See "To select the scan type" on page 30.

- Specifying the scan settings, which consist of the image channels, objective, and if applicable, other settings based on the selected Scan Type. See "To specify the scan settings" on page 31.

- Selecting the vessel type that is be scanned, for example, a 96-well microplate or a flask.You select the vessel type, with the available choices pre-filtered based on the objective and scan type that you select for the scan settings. See "To select the vessel type" on page 32.

- Selecting the vessel location, which is the location in the vessel tray in the Incucyte drawer that the vessel is placed in for scanning. See "To select the vessel location" on page 33.

- Setting the scan pattern, which defines the locations in the vessel (wells, sectors, and so on) that you can select for image acquisition based on the selected vessel type. See "To set the scan pattern" on page 35.

- Providing vessel information, which requires that you name the vessel to identify it. You can also provide other information about the vessel such as the cell type that is being analyzed, the cell passage number, and optionally, if applicable, you can also create or import the plate map for your experiment. See "To provide vessel information" on page 37.

- Defining the analysis setup, which defines the analysis that you are carrying out for the vessel images. You can define an analysis that is to launch automatically after the first image acquisition of the vessel, or you can defer the definition. See "To define the analysis setup" on page 38.

- Setting the scanning schedule, which defines the frequency of vessel scanning during a 24-hour period. See "To set the scanning schedule for the Incucyte SX5 or Incucyte S3" on page 39 or "To set the scanning schedule for the Incucyte SX1" on page 40.

- Verifying the acquisition settings, which is a visual confirmation that the acquisition settings and scanning schedule have been correctly set. If so, you can add the vessel to the scanning schedule. See "To verify the vessel acquisition settings and schedule the vessel scan" on page 42.

You can schedule only a single vessel at a time. You must repeat each of these procedures in their entirety for each vessel that you are scheduling.

To set the scanning frequency

1. On the Acquisition window toolbar, click the Launch Add Vessel Wizard icon.

   The Add Vessel wizard opens. The Scanning Frequency page is the open page. Two options are available for scanning frequency: Scan on Schedule, which requires you to set up a scanning schedule for the vessel that is repeated every 24 hours until you end the scan (the default value), or Scan Once Now, which results in the vessel being scanned immediately one time after you specify all the scanning settings.

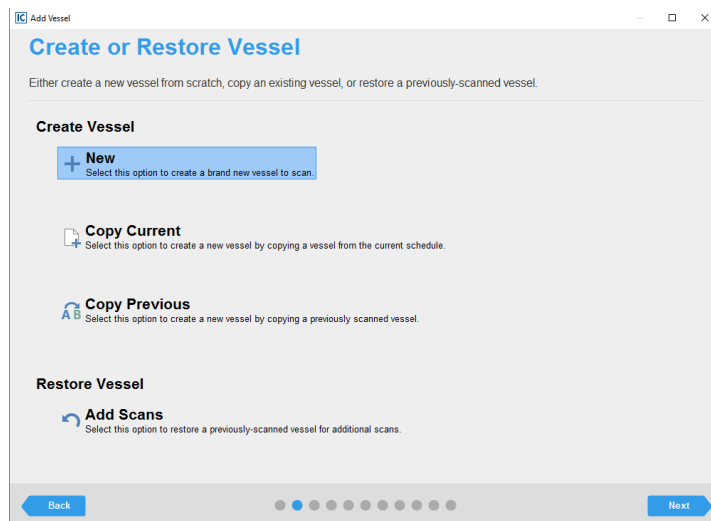*Figure 2-4:   Add Vessel wizard, Scanning Frequency page*
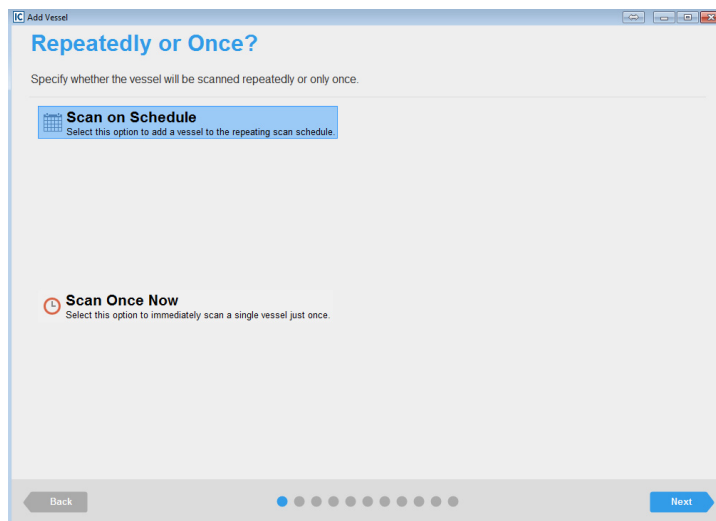
2. Leave Scan on Schedule selected.

3. Click Next.

   The Create Vessel page opens.

4. Continue to "To create the vessel" on page 29.

To create the vessel

*Figure 2-5: Add Vessel wizard, Create or Restore Vessel page*

The Create or Restore Vessel page provides four options for creating the vessel that you are scanning:

Option	Description
Create Vessel	
New	Creates an entirely new vessel to scan.
Copy Current	Creates a new vessel by copying a vessel from the current schedule. The scan settings (objective and channels) and the vessel scan pattern are copied to the new vessel.
Copy Previous	Creates a new vessel by copying a vessel that was previously scanned. The scan settings (objective and channels) and the vessel scan pattern are copied to the new vessel.
Restore Vessel	
Add Scans	Restores a previously scanned vessel for additional scanning. The new scans are added to the data for the previous scans for the vessel.

5. Under Create Vessel, leave New selected.

6. Click Next.

    The Scan Type page opens.

7. Continue to

To select the scan type

Figure 2-6:   Add Vessel wizard, Scan Type page
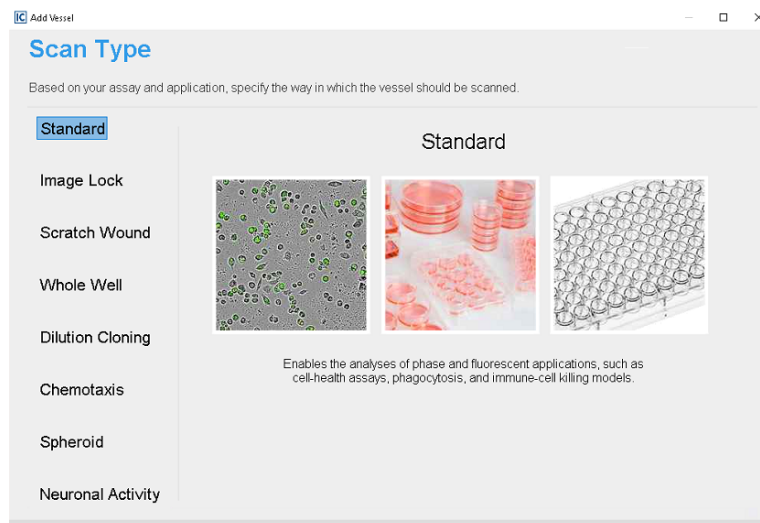
The Scan Type page lists the different ways in which the vessel can be scanned. You make your selection based on your assay and application. Every option other than Chemotaxis, Spheroid, Scratch Wound, and Neuronal Activity are always available and functional. Standard is selected by default. The Chemotaxis, Spheroid, Scratch Wound, and Neuronal Activity modules that are displayed are functional only if you have purchased the appropriate licenses.

If your organization has not licensed the Chemotaxis, Spheroid, Scratch Wound, and/or Neuronal Activity modules, then when you select one of these modules on the Scan Type page, a message opens at the bottom of the page that states that you do not have a license for the selected acquisition type and that you must contact your Essen sales representative for more information.

1. Leave Standard selected.

2. Click Next.

    The Scan Settings page opens.

3. Continue to "To specify the scan settings" on page 31.

To specify the scan settings

Figure 2-7: Add Vessel wizard, Scan Settings page

You specify the image channels, objective, and if applicable, other settings, based on the selected Scan Type on the Scan Settings page.

1. Specify the scan settings.

Option	Description
Channels	Select the correct combination of channels: • Incucyte SX5: Phase, Green, Orange, and NIR. • Incucyte S3: Phase, Green, and Red or Phase, Orange, and NIR. • Incucyte SX1: Phase, Green, and Red. The acquisition time, which is comprised of a series of exposures, is the length of time that the cells are stimulated for fluorescence. The Acquisition Time options are set to the following default values: • Green: 300 ms • Red: 400 ms • Orange: 400 ms • NIR: 400 ms Sartorius has determined that these default values are the optimal values when scanning across a wide variety of fluorescent tags using the Incucyte Live-Cell Analysis Systems. Although you can modify these values, Sartorius does not recommend that you do so, unless you have the following considerations: • If phototoxicity is a problem, then you should lower the acquisition times. • If very faint objects are being imaged on a low background (objects and background together are less than 3 CU), then it might be helpful to increase the acquisition times.
Objective	• Incucyte SX5 and Incucyte S3: The objective is set to a default value of 10x, which is suitable for the majority of vessel types and scan types. Other objectives (4x and 20x) are available for special scan types, for example, the Whole Well and Dilution cloning scan types, which require a 4x objective. • Incucyte SX1: The currently installed objective is set to the default objective. If you require a different objective, see Appendix B, "Changing the Incucyte SX1 Objective," on page 165.

> *After you have scheduled this vessel for acquisition, you can always view a summary of the scan settings. In the Vessel Drawer Setup pane, select the vessel, and then in the Vessel Information pane, select Scan Properties. See "The Acquisition Window" on page 23.*

2. Click Next.

    The Vessel Type Search page opens.

3. Continue to "To select the vessel type" below.

To select the vessel type

The Vessel Type Search page lists all the vessel types that the Incucyte system can support for your assay based on the objective and scan type. When the Vessel Type Search page first opens, the vessels are sorted in ascending order based on the Manufacturer. You can hold your mouse pointer over any column header to display a Filter icon, and you can click once in a column header to display a Sort Order icon.

Figure 2-8:   Add Vessel wizard, Vessel Type Search page

- The direction of the Sort Order icon indicates the sort order, where up indicates that the data is sorted in ascending order based on the data in the column and down indicates that the data is sorted in descending order. To change the sort order of the vessels to a descending sort based on a column, click the Sort Order icon for the column. To return the sort order to ascending order, click the Sort Order icon again.

- To filter the list of vessels to display only a subset of vessels based on a selected property, in the appropriate column header, click the Filter icon, and then select the filter or define a custom filter. For example, to display only those vessel types that Corning manufactures, in the Manufacturer column heading, click the Filter icon, and then on the Filter list, select Corning.

> *You can also right-click the Filter icon to open a context menu with additional filtering options such as sorting, showing/hiding columns, selecting columns, and so on.*

1. Select the appropriate vessel type. If you must search for the vessel type, note the following:

    • To search across all columns, you can enter a search string in the blank search field at the top of the page.

    • To search based on a specific vessel property, such as Manufacturer, you can enter a search string in the blank field below the appropriate column header.

    • The search is limited to the exact order of the characters in the string, but the string is not case-sensitive and it can appear anywhere in the search results. For example, if you search across all columns with **cor** as the search string, then the search results include vessels with a Manufacturer of **Cor**ning or a Vessel Name of 6-well **Cor**ning.
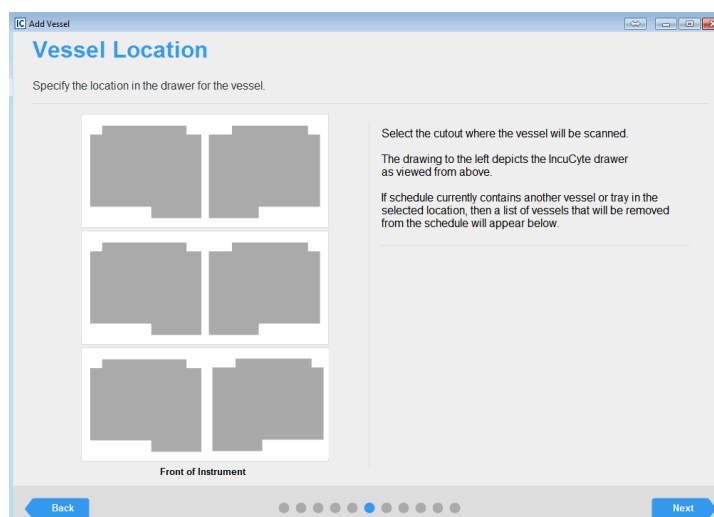
2. Click Next.

    The Vessel Location page opens.

You can also double-click a vessel to select it, and then open the Vessel Location page.

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3. Continue to "To select the vessel location" below.

To select the vessel location

Figure 2-9:   Add Vessel wizard, Vessel Location page for a 96-well microplate

The pattern on the Vessel Location page depicts the Incucyte tray that is associated with your selected vessel type in the system drawer as viewed from above. Each "cutout" represents a location in a tray in which you can potentially place the vessel. For the Incucyte SX5 and the Incucyte S3, all six tray locations are available. For the Incucyte SX1, only the two front tray locations are available.

The shape of the location cutout that is displayed on the page is dependent on the selected vessel type. The number of location cutouts that are available for vessel placement is dependent on your product type (Incucyte SX5 and Incucyte S3, or Incucyte SX1). For example:

• If the selected vessel type is a Corning 96-well microplate, then the cutout is shaped like a 96-well

microplate, and for the Incucyte SX5 and Incucyte S3, the maximum number of vessel locations is six, while for the Incucyte SX1, the maximum number of vessel locations is two.

- If the selected vessel type is a Corning T-25 flask, then the cutout is shaped like a flask and for the Incucyte SX5 and Incucyte S3, the maximum number of vessel locations is increased to 12, while for the Incucyte SX1, the maximum number of vessel locations is increased to four.

1. Click the location in which you are placing the vessel.

   Two results are possible:

   - If any other schedules contain a vessel in the selected location, then a Vessel Schedule table opens. The table displays the following information for each affected vessel: the vessel name, the vessel owner name, and when the first scan for the vessel was acquired. Continue to Step 2.

     > *Before you continue Step 2, if you are scanning the vessel only once now, and another vessel already occupies the selected location, then based on the scanning schedule for the current vessel, you can remove the current vessel, add your vessel and carry out the scan, and then return the original vessel to its location without causing any conflicts with its scan schedule.*

   - If no other schedules contain a vessel in the selected location, then the location cutout is displayed in blue. Continue to Step 3.

Figure 2-10:   Add Vessel wizard, Vessel Location page showing a currently occupied vessel location
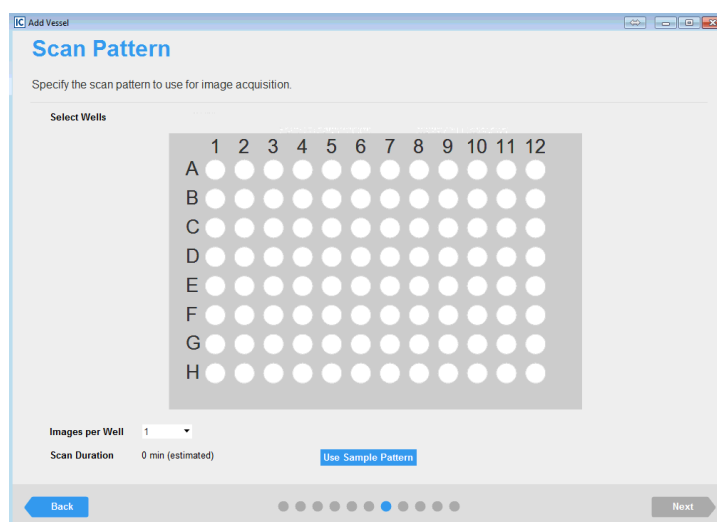

2. Do one of the following:

   - Select a different vessel location.

   - In the Vessel Schedule table, select the vessel or vessels that you are removing from the schedule, and then click "I wish to remove the following vessel(s) from the schedule."

3. Click Next.

   The Scan Pattern page opens.

4. Continue to "To set the scan pattern" on page 35.

To set the scan pattern

The Scan Pattern page displays a vessel map, which shows the locations in the vessel (wells or sectors) that you can select for image acquisition based on the selected vessel type. The scan pattern criteria are dependent on the vessel type. For example, for a 96-well microplate, you would select the *wells* that you are scanning and specify the number of images that you are acquiring in each well. For a flask, you would select the *sectors* that you are scanning and specify the number of images that you are acquiring in each sector. A solid blue rectangle marks each location that is selected for analysis.

Figure 2-11: Add Vessel wizard, Scan Pattern page

1. Do one of the following:

    •   To view and use a sample scan pattern based on the manufacturer and vessel type, click Use Sample Pattern.
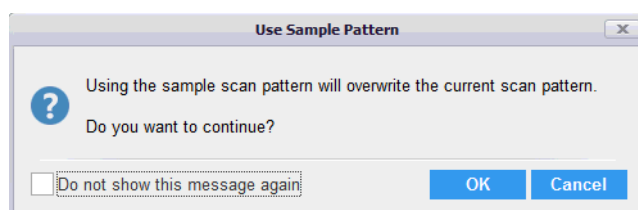

If a scan pattern has already been specified on the vessel map, then selecting the sample scan pattern overwrites the existing pattern.

    •   If the default sample pattern does meet your assay imaging criteria, then to set your own custom scan pattern for the vessel, do any of the following:

When setting your own scan pattern, after you select at least one well or sector, the Use Sample Pattern dialog box opens. To continue setting your scan pattern without having this dialog box continuously opening, even if you close and open the GUI again, click Do not show this message again, and then click Cancel in the dialog box. See Figure 2-12 below.

Figure 2-12: Use Sample Scan Pattern dialog box

- To select a single location in a vessel, for example, a well in a 96-well microplate, click once in the appropriate location in the vessel map.

- To select multiple contiguous locations in a vessel, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.

- To select all the locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Select this Column. Conversely, to clear all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column.

- To select all the locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Select this Row. Conversely, to clear all the selected locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Deselect this Row.

- To select all the locations in a vessel in a single step, right-click any location in the vessel, and on the context menu that opens, select Select All. Conversely, to clear all the selected locations in a column (including multiple contiguous locations), right-click any location in the vessel, and on the context menu that opens, click Deselect All.

To clear all the selected locations in the vessel map, or multiple contiguous locations, you can also press and hold the ALT key, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.

2. Specify the number of images that you are acquiring per vessel location (well, sector, or so on).

   The Scan Duration, which is the estimated amount of time required to scan the microplate, is displayed at the bottom of the Scan Pattern page. The value is based on the number of selected locations as well as the number of images that you are acquiring in each location. If you change one or both of these values, then the Scan Duration is updated accordingly.

If multiple images are acquired per vessel location, then the system considers the images ordered in rows from top to bottom and then, within the rows, from left to right. See Figure 2-13 below.

Figure 2-13: Four images acquired per vessel location

Image 1 — Image 2
Image 3 — Image 4
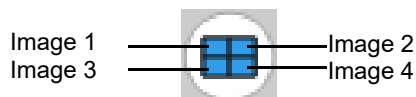
3. Click Next.

   The Notebook page opens.

4. Continue to "To provide vessel information" on page 37.

To provide vessel information

Figure 2-14:   Add Vessel wizard, Notebook page for a microplate vessel type

You use the options on the Notebook page to provide information about the vessel, including its name (required), the cell type being analyzed, and the passage number of cells. Optionally, if applicable, you can also create or import the plate map for your experiment.

1.   Complete the notebook for the vessel. At a minimum, you must enter the vessel name.

If the vessel type is a microplate, then you can use the Plate Map Editor to create a new plate map, or you can import an existing plate map, and then use the Plate Map Editor to edit the plate map as needed. Because a Plate Map is optional, and you are not limited to using the Plate Map Editor within the Add Vessel wizard, its details are not discussed here. Instead, see Appendix A, "Plate Map Editor," on page 137.


After you have scheduled this vessel for acquisition, you can always view a summary of the information that was provided for the vessel, including the plate map that was assigned if applicable. In the Vessel Drawer Setup pane, select the vessel, and then in the Vessel Information pane, select Notebook. See "The Acquisition Window" on page 23.

2.   Click Next.

The Analysis Setup page opens.

3.   Continue to "To define the analysis setup" on page 38.

To define the analysis setup

Figure 2-15:   Add Vessel wizard, Analysis Setup page
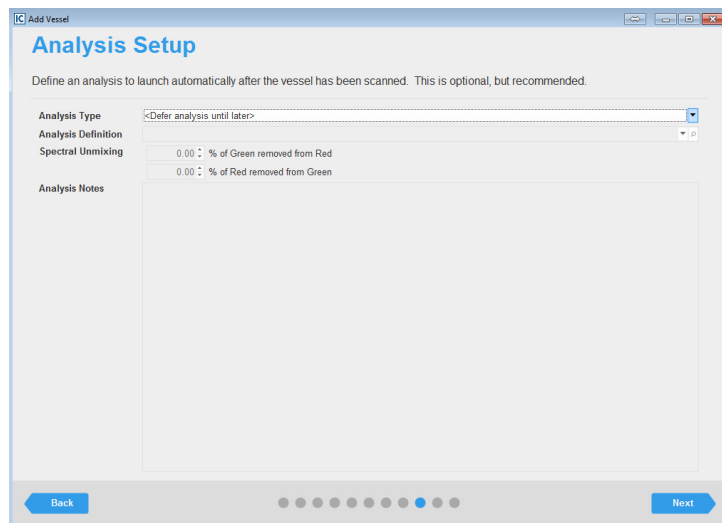
You define the analysis that you are carrying out for the vessel images on the Analysis Setup page. You can define an analysis that is to launch automatically after the first image acquisition of the vessel, or you can defer the definition. Although the analysis definition is ultimately required to obtain the vessel data, you should consider the following *before* you decide to define the analysis within the wizard or at a later date:

- This is the first time that you have analyzed a vessel of this type (microplate, flask, and so on) with this specific setup (objective, cell type, fluorophore, and image channels), so no analysis definition currently exists in the Incucyte system that you can apply to your vessel. Therefore, you would define the analysis at a later date. Leave Analysis Type set to <Defer analysis until later> and go to Step 2.

- An analysis definition currently exists in the system with the same analysis settings (objective, cell type, fluorophore, and image channels), but this analysis is not applicable for your vessel type. Therefore, you would define the analysis at a later date. Leave Analysis Type set to <Defer analysis until later> and go to Step 2.

- An analysis job has been created that aligns with your assay scan type, cell type, and magnification. Therefore, you can apply an analysis job at the time of scheduling image acquisition for your vessel. Data is processed following each scan to provide analysis in real-time. Go to Step 1.

1. To define the analysis, do the following:

   a. Select the analysis type.

   The options that are available for Analysis Type depend on the selected vessel type (for example, a 96-well microplate, a flask, and so on), objective, and the modules that are licensed for your Incucyte system.

   b. Select the analysis definition, and then enter the appropriate values for Spectral Unmixing.

   Spectral unmixing might be required to remove fluorophores that produce signal in both color channels. You can also complete spectral unmixing in the Vessel View. See "Spectral Unmixing" in the "The Vessel View Window" on page 67.

c.   Optionally, enter notes for the analysis.

After you have scheduled this vessel for acquisition, you can always view a summary of the analysis settings. In the Vessel Drawer Setup pane, select the vessel, and then in the Vessel Information pane, select Analysis. See "The Acquisition Window" on page 23.

2.   Click Next.

The Scan Schedule page opens.

3.   Continue to one of the following:

•   "To set the scanning schedule for the Incucyte SX5 or Incucyte S3" below.

•   "To set the scanning schedule for the Incucyte SX1" on page 40.

To set the scanning schedule for the Incucyte SX5 or Incucyte S3

Figure 2-16:   Add Vessel wizard, Scan Schedule page for the Incucyte SX5 and Incucyte S3

The Scan Schedule page for the Incucyte SX5 and Incucyte S3 displays the scanning schedules for all the vessels that are currently scheduled for daily scans. The Vessel Schedule timeline that is displayed at the top of the page is identical to the Vessel Schedule Timeline that is displayed at the top of Acquisition window. (See "The Vessel Schedule Timeline" on page 47.) The current time is indicated by a single blue line in the Vessel Schedule Timeline. The scanning schedule for the vessel that you are scheduling is indicated by white bars in the timeline. The scanning schedule for each vessel that is currently scheduled in the vessel tray is shown as gray bars. The width of each schedule bar correlates to the amount of time that is required to scan the corresponding vessel.

1.   To set the scanning schedule for the vessel, select one of the following options :

Option	Description
Create new schedule starting at	Select the starting time for the scan and the scanning interval. White bars in the Vessel Schedule Timeline indicate the schedule.

Option	Description
Add to existing schedule	Select an existing scanning schedule. The available options are determined by the next available start times for the scanning schedules that have already been set for the other vessels in the vessel tray. When you select an existing schedule, the corresponding bars in the Vessel Schedule Timeline are displayed in white instead of gray.
Do not add to the schedule at this time	The scanning location is essentially "reserved." You can use the Vessel Schedule Timeline or edit the vessel to schedule the vessel scan. See "The Vessel Schedule Timeline" on page 47 or "Editing Vessel Information" on page 51.

2. Specify the total duration of the experiment:

Option	Description
Scan indefinitely	The scanning schedule for the vessel is repeated every 24 hours until you manually end the scan.
Stop scanning after the first scan	Select the number of days/hours after the first scan at which the vessel scanning is automatically stopped.
Stop scanning after (date/time)	Select the date/time combination after which the vessel scanning is automatically stopped.

After you schedule a vessel and complete the entire process in the Add Vessel wizard, the option to modify the vessel schedule is available. See "Modifying an Existing Vessel Schedule" on page 44.

3. Click Next.

   The Summary page opens.

4. Continue to "To verify the vessel acquisition settings and schedule the vessel scan" on page 42.

To set the scanning schedule for the Incucyte SX1

The Scan Schedule page that opens for the Incucyte SX1 is different based on whether you are scheduling the first vessel for an experiment, or whether you are scheduling subsequent vessels.

- If you are scheduling the first vessel for an experiment, then the page displays two scheduling options: One option is for creating a new schedule for the vessel and the other option is for not immediately scheduling the vessel. See Figure 2-17 on page 41.

- If you are scheduling a subsequent vessel for an experiment, then the page displays a single option for adding the vessel to an existing schedule. You cannot create a new schedule for the vessel and you cannot delay the scheduling of the vessel. See Figure 2-18 on page 41.

Both pages display the scanning schedules for all the vessels that are currently scheduled for daily scans. The Vessel Schedule timeline that is displayed at the top of the page is identical to the Vessel Schedule Timeline that is displayed at the top of Acquisition window. (See "The Vessel Schedule Timeline" on page 47.) The current time is indicated by a single blue line in the Vessel Schedule Timeline. The scanning schedule for the vessel that you are scheduling is indicated by white bars in the timeline. The width of each schedule bar correlates to the amount of time that is required to scan the corresponding vessel(s).

Figure 2-17:   Add Vessel wizard, Scan Schedule page for Incucyte SX1 (First scheduled vessel)

Figure 2-18:   Add Vessel wizard, Scan Schedule page for Incucyte SX1 (Subsequently scheduled vessel)

1. To set the scanning schedule for the vessel, select one of the following options:

Option	Description
Create new schedule starting at	Displayed only for the first vessel that you are scheduling for an experiment. Select the starting time for the scan and the scanning interval. White bars in the Vessel Schedule Timeline indicate the schedule.
Add to existing schedule	Displayed only for the subsequent vessels that you are scheduling for an experiment. Select an existing scanning schedule. The available options are determined by the next available start times for the scanning schedules that have already been set for the other vessels in the vessel tray. When you select an existing schedule, the corresponding bars in the Vessel Schedule Timeline are displayed in white instead of gray.

Option	Description
Do not add to the schedule at this time	The scanning location is essentially "reserved." You can use the Vessel Schedule Timeline or edit the vessel to schedule the vessel scan. See "The Vessel Schedule Timeline" on page 47 or "Editing Vessel Information" on page 51.

2. Specify the total duration of the experiment:

Option	Description
Scan indefinitely	The scanning schedule for the vessel is repeated every 24 hours until you manually end the scan.
Stop scanning after the first scan	Select the number of days/hours after the first scan at which the vessel scanning is automatically stopped.
Stop scanning after (date/time)	Select the date/time combination after which the vessel scanning is automatically stopped.

After you schedule a vessel and complete the entire process in the Add Vessel wizard, the option to modify the vessel schedule is available. See "Modifying an Existing Vessel Schedule" on page 44.

3. Click Next.

   The Summary page opens.

4. Continue to "To verify the vessel acquisition settings and schedule the vessel scan" below.

To verify the vessel acquisition settings and schedule the vessel scan

Figure 2-19: Add Vessel wizard, Summary page

The Vessel Summary page displays all the selections/settings that you have specified for the vessel, organized by wizard page. Each section heading corresponds to a wizard page, and is a hyperlink to the indicated page. Use the scroll bar at the right side of the page to view all the information on the page.

> *If the scroll bar is not displayed, click once on the far right side of the page to display it. The scroll bar is a thin, gray vertical line.*

1. Review the information for the scheduled vessel, and then do one of the following:

    *   If all the selections/settings are correct for the vessel, then continue to Step 2.

    *   If you must edit any of the selections or settings, then click the appropriate section header/page name to return to the page in the wizard. Edit the selections/settings as appropriate, and then click Next to move through the wizard (making additional edits as required) until you return to the Summary page, and then continue to Step 2.

> *Any edit that you make to the selections/settings for a scheduled vessel might affect downstream selections/settings. For example, if you select a different vessel type, then you might have to edit the scan pattern for the vessel as well. As a result, you always have to move through the remaining pages on the wizard after you edit any selections/settings for the vessel.*

2. Click Add to Schedule.

    A message opens, asking you to wait, and then the message and the Add Vessel wizard close and the Acquisition window opens. The vessel is displayed and selected (outlined in blue) in the Vessel Drawer Setup pane. The Vessel Information window displays the scan properties for the vessel.
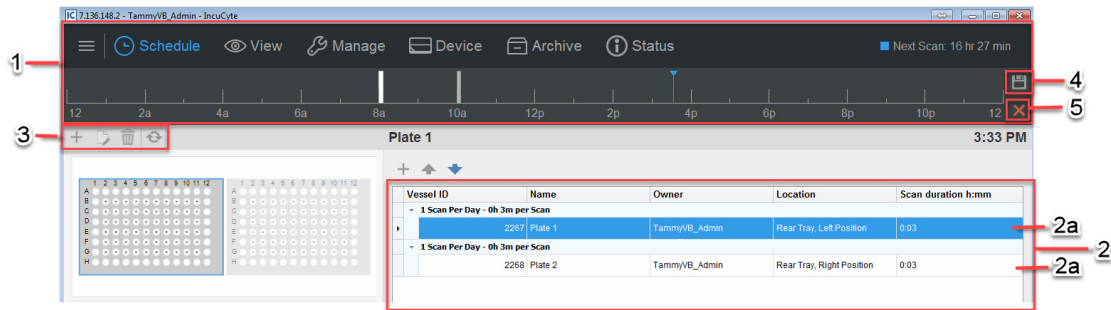
> *If you did not schedule at least one repeating scan for the vessel, then an exclamation point is displayed on the vessel and when you place your mouse pointer over the vessel, a tooltip opens with a message indicating that no scans are scheduled for the vessel. You can click the Vessel Schedule Timeline to move the vessel to an existing scan group that has the appropriate scanning schedule or add a scan and scan intervals. See "The Vessel Schedule Timeline" on page 47. Optionally, you can edit the vessel. See "Editing Vessel Information" on page 51.*

Modifying an Existing Vessel Schedule

After you double-click the Vessel Schedule Timeline on the Acquisition window for a vessel that has been scheduled, the window displays the scanning schedules for all the vessels that are currently scheduled for daily scans. Advanced scheduling options are also available from a context menu.

Figure 2-20:   Acquisition window, Advanced scheduling features for a vessel

	Description
1	The Vessel Schedule Timeline. This timeline is identical to the Vessel Schedule Timeline that is displayed at the top of Acquisition window. See "The Vessel Schedule Timeline" on page 47. The current time is indicated by a single blue line in the Vessel Schedule Timeline. The scanning schedule for the vessel that is currently selected in the Vessel Schedule table is indicated by white bars in the timeline. The scanning schedules for all other vessels are shown as gray bars. The width of each schedule bar correlates to the amount of time that is required to scan the vessels in the corresponding scan group.
2	The Vessel Schedule table. The table displays the daily scan information (Vessel ID, Vessel Name, Vessel Owner, Vessel Location, and Scan Duration in hours:minutes) for each vessel that is currently scheduled in the vessel tray, which is referred to as *per vessel scheduling.* Vessels with identical daily scanning schedules are organized in the Vessel Schedule table *by scan groups.*
2a	Scan groups. Vessels with identical daily scanning schedules are organized in the Vessel Schedule table by *scan groups*, with each scan group having its own unique combination of the number of scans per day and the amount of time (in minutes) required per scan. For example, in Figure 2-20 above, two separate scan groups are displayed in the Vessel Schedule table, with one vessel in each scan group.
3	The Vessel Schedule toolbar. From left to right, the toolbar contains the following icons:
	Create new scan group icon: Applicable only for a multi-vessel scan group.
	Move to previous scan group icon: Applicable only if two or more scan groups are displayed in the Vessel Schedule table.
	Move to next scan group icon: Applicable only if two or more scan groups are displayed in the Vessel Schedule table.
	Reset scan times and scan groups icon: If you make any changes to the scan times and/or scan groups after the Vessel Schedule page first opens, then you can click this icon to immediately return all scan times and scan groups to their initial settings.
4	Save icon: Click this icon to save the changes that you make to an existing vessel schedule.
5	Cancel icon: Click this icon to cancel any changes that you make to an existing vessel schedule.

When the Acquisition window first opens for a scheduled vessel, the window has the following layout:

- If no other vessels are currently scheduled, then the vessel is placed in its own scan group. The scheduling for this new scan group is initially set to 0 scans per day.

- If only a single scan group is available, then the vessel is placed in this scan group.

- If multiple scan groups are available, then the vessel is placed as the last vessel in the last scan group that is displayed in the Vessel Schedule table.

You can do any of the following as appropriate to modify a vessel schedule:

- Schedule the first repeating scan. See "To schedule the first repeating scan for a vessel" below.

- Place a vessel in a different scan group and/or rearrange the order of vessels in a scan group, See "To schedule additional scans for the vessel and/or its scan group" below.

- Schedule additional scans for the vessel and/or its scan group. See "To place a vessel in a different scan group and/or rearrange the order of vessels in a scan group" on page 46.

To schedule the first repeating scan for a vessel

1. If applicable, to schedule the *first* repeating scan for the vessel, in the Vessel Schedule table, select the vessel, and then click once in the location where you are initiating scanning on the Vessel Schedule Timeline.

   A white schedule bar is placed at the selected location. You can hold your mouse pointer over the bar to open a tooltip that displays the current time, the duration of the scheduled scan, and the time for which the scan is scheduled.

Although not required, Sartorius strongly recommends that you schedule at least the first repeating scan for the vessel. If you do not schedule the vessel, options are still available for adding and adjusting the vessel schedule.

Figure 2-21:   Tooltip for a scheduled scan for a vessel
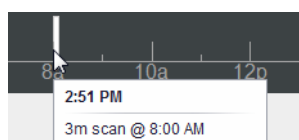
2. Click the Save icon.

   The Acquisition window remains open.

To schedule additional scans for the vessel and/or its scan group

1. To schedule additional scans for the vessel and/or its scan group, adjust scan start times, and/or delete scans, see "The Vessel Schedule Timeline" on page 47.

2. Click the Save icon.

   The Acquisition window remains open.

To place a vessel in a different scan group and/or rearrange the order of vessels in a scan group

1. To place a vessel in a different scan group and/or rearrange the order of vessels in a scan group, right-click the selected vessel, and on the context menu that opens, select the appropriate option.

You can also click the vessel entry in the Vessel Schedule table, and then click the appropriate icon on the Vessel Schedule toolbar.

Option	Description
Create New Scan Group	Places the vessel in a new scan group. The scan group is displayed as the last scan group in the list of scan groups in the Vessel Schedule table, and the initial number of scans for the group is set to 0 scans per day. You must schedule at least one repeating scan for this vessel/scan group.
Move to Previous Scan Group	Moves the vessel to the scan group that is displayed immediately before the vessel's current scan group in the Vessel Schedule table. The vessel is displayed as the last vessel in the scan group. You can select this option as many times as needed to move the vessel to the appropriate scan group.
Move to Next Scan Group	Moves the vessel to the scan group that is displayed immediately after the vessel's current scan group in the Vessel Schedule table. The vessel is displayed as the last vessel in the scan group. You can select this option as many times as needed to move the vessel to the appropriate scan group.
Move Before	Moves the selected vessel up one row at a time in the list of vessels that are displayed for its scan group. You can select this option as many times as needed to move the vessel to the appropriate location in its scan group. The vessels are scanned in the order in which they are displayed in their scan group.
Move After	Moves the selected vessel down one row at a time in the list of vessels that are displayed for its scan group. You can select this option as many times as needed to move the vessel to the appropriate location in its scan group. The vessels are scanned in the order in which they are displayed in their scan group.

2. Click the Save icon.

    The Acquisition window remains open.

The Vessel Schedule Timeline

The Vessel Schedule Timeline that is displayed at the top of the Acquisition window is the tool that you use for advanced scheduling and managing a vessel scan. Using the options on this tool, you can:

• Delete a single repeating scan from a scan group.

• Delete a single scan from multiple repeating scans for a scan group, or delete all the repeating scans in a scan group.

• Shift the scan time for a single repeating scan in a scan group.

• Shift the scan time for a single scan in a group of repeating scans for a scan group, or shift all the scan times for all the repeating scans in a scan group.

• Add scans at set intervals to a scan group.

• Place a vessel in a different scan group and/or rearrange the order of vessels in a scan group.

Multiple scan groups are available for the Incucyte SX5 and Incucyte S3. Only a single scan group for all vessels is available for the Incucyte SX1. If you did not schedule the vessel during the Add Vessel wizard, then for the Incucyte SX5 and Incucyte S3, you can use the options that are available on the Vessel Schedule Timeline to move the vessel to an existing scan group that has the appropriate scanning schedule, or optionally, you can edit the vessel. You can only edit the vessel for the Incucyte SX1. See "Editing Vessel Information" on page 51.

When you are using the Vessel Schedule Timeline to set and/or edit the scanning schedule for a vessel/scan group, note the following:

• If scan times overlap between vessels/scan groups, then a red line is displayed at the overlapping point in the Vessel Schedule Timeline. You must change the time for at least one of the scans before you can save the vessel scanning schedule.

Figure 2-22:   Overlapping scans indicated in the Vessel Schedule Timeline
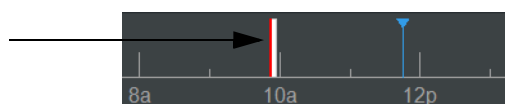
• At any time that you are scheduling vessel scans, you can click the Cancel schedule changes icon ☒ that is displayed to the right of the Vessel Schedule Timeline and undo any and all changes that you have made. The vessel schedules are returned to their starting values, vessels are returned to their original scan groups and listed in their original order within their scan groups, and the Vessel Schedule table closes. The Acquisition window remains open.

To use the Vessel Schedule Timeline

1. In the Vessel Drawer Setup pane, select the appropriate vessel.

   After you select a vessel in the Vessel Drawer Setup pane, the vessel schedule is indicated by white schedule bars on the timeline. The schedule for all other vessels are indicated by gray schedule bars. The width of each schedule bar correlates to the amount of time that is required to scan the vessels in the corresponding scan group. The current time is indicated by a single blue line.

If two or more vessels are members of the same scan group, then when you select any vessel in the group, the color coding on the timeline does not change.

*Figure 2-23:   Vessel Schedule Timeline showing schedule for selected vessel*
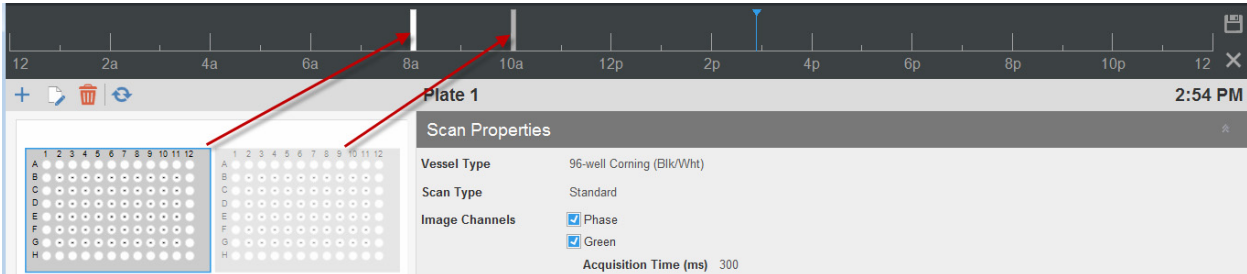
2. In the Vessel Schedule Timeline, right-click any schedule bar for the vessel, and on the context menu that opens, click Edit Schedule.

You can also double-click anywhere in the Vessel Schedule Timeline to open the Vessel Schedule table.

The Vessel Schedule table opens. The table displays all the schedules for all the vessels in the Vessel Drawer Setup pane that are currently scheduled for scanning, organized by scan group. The Delete schedule changes icon is enabled. The entry for the currently selected vessel is highlighted in the table, and white bars in the Vessel Schedule Timeline indicate its corresponding scanning schedule. The Vessel Schedule toolbar is displayed above the Vessel Schedule table.

Figure 2-24:   Vessel Schedule Timeline display with a currently scheduled vessel selected in the Vessel Drawer Setup pane
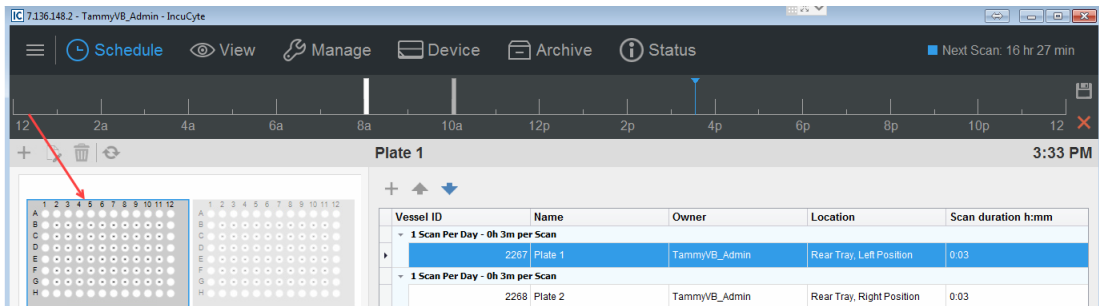
3. For the selected vessel, do any of the following as appropriate to set and/or edit its scanning schedule:

   •   If a *single* repeating scan has been scheduled for the vessel/scan group, or multiple repeating scans have been scheduled and you want to work with only *one* of the repeating scans:

      •   To delete a single repeating scan, right-click the schedule bar, and on the context menu that opens, select Scan > Delete.

      If you delete a *single* repeating scan for the vessel/scan group, then no scan is scheduled for the vessels in the scan group. An exclamation point is displayed on all the affected vessels in the Vessel Drawer Setup pane. You can move the vessel to an existing scan group that has the correct scanning schedule or use the options that are available on the Vessel Schedule Timeline to add a scan and scan intervals.

- To adjust the start time for the repeating scan (start the scan earlier or later than its currently scheduled time), right-click the schedule bar, and on the context menu that opens, select Scan, and then select the appropriate time. For example, to start the scan 5 minutes earlier, select Scan >: 5 minutes.

For a single repeating scan, you can also click and drag the schedule bar to a different location on the Vessel Schedule Timeline.

- If multiple repeating scans have been scheduled for the vessel/scan group, then:

  - To delete *all* the scheduled scans for the vessel, right-click any of the repeating schedule bars, and on the context menu that opens, select Scan Group > Delete All Scans.

  - To adjust the start times for *all* the repeating scans for the vessel (start the scans earlier or later than their currently scheduled times), right-click any of the schedule bars, and on the context menu that opens, select Scan Group, and then select the appropriate time. For example, to start all the repeating scans in the scan group 5 minutes earlier, select Scan Group > –5 minutes.

You can also click and drag any of the schedule bars to a different location on the Vessel Schedule Timeline to adjust the start times for all the repeating scans.

- To add another repeating scan to the vessel's scan group at set intervals, right-click a schedule bar, and on the context menu that opens, select Scan Group > Add Scans at Intervals, and in the Set Timeline Scan Intervals dialog box, set the appropriate values. See Figure 2-25 on page 49.

  - Starting At: Sets the time at which the scan is to start.

  - Add Scans Every: Sets the interval at which the scan is to be repeated.

  - For a Total of: Restricts the number of hours that the scan is to be repeated out of the 24 hour period. If you are not placing any restrictions on the scan, then leave the value set to the default value of 24 hours.

Figure 2-25: Set Timeline Scan Intervals dialog box
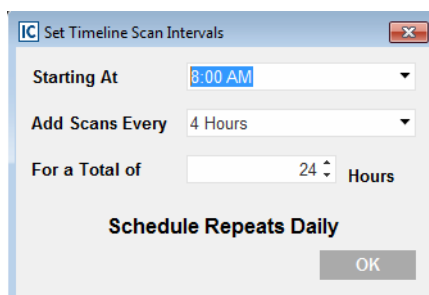
- To place a vessel in a different scan group and/or rearrange the order of vessels in a scan group, right-click the selected vessel, and on the context menu that opens, select the appropriate option.

You can also select the vessel entry in the Vessel Schedule table, and then click the appropriate icon on the Vessel Schedule toolbar.

Option	Description
Create New Scan Group	Places the vessel in a new scan group. The scan group is displayed as the last scan group in the list of scan groups in the Vessel Schedule table, and the initial number of scans for the group is set to 0 scans per day. You must schedule at least one repeating scan for this vessel/scan group.
Move to Previous Scan Group	Moves the vessel to the scan group that is displayed immediately before the vessel's current scan group in the Vessel Schedule table. The vessel is displayed as the last vessel in the scan group. You can select this option as many times as needed to move the vessel to the appropriate scan group.
Move to Next Scan Group	Moves the vessel to the scan group that is displayed immediately after the vessel's current scan group in the Vessel Schedule table. The vessel is displayed as the last vessel in the scan group. You can select this option as many times as needed to move the vessel to the appropriate scan group.
Move Before	Moves the selected vessel up one row at a time in the list of vessels that are displayed for its scan group. You can select this option as many times as needed to move the vessel to the appropriate location in its scan group. The vessels are scanned in the order in which they are displayed in their scan group.
Move After	Moves the selected vessel down one row at a time in the list of vessels that are displayed for its scan group. You can select this option as many times as needed to move the vessel to the appropriate location in its scan group. The vessels are scanned in the order in which they are displayed in their scan group.

4. After you have successfully scheduled all the appropriate vessels, on the Vessel Schedule Timeline, click the Save schedule changes icon 💾.

The Vessel Schedule table closes. The Acquisition window remains open.

Editing Vessel Information

As long as the first scan has *not* started for a vessel, then you can edit any and all of the following information for the vessel: the scan settings, the scan pattern, the vessel information (the vessel notebook), the analysis definition, and/or the scan schedule. If scanning has already started for a vessel, then you can still edit the scanning schedule; however, you cannot edit any other vessel information. Also, if you did not schedule at least one repeating scan for the vessel while configuring the vessel in the Add Vessel wizard, you can edit the vessel to set its scanning schedule.

To prevent the selected vessel type from being invalidated, you cannot change the objective.

To edit vessel information

1. In the Vessel Drawer Setup pane, select the vessel that you are editing, and then on the Vessel

    toolbar, click the Edit selected vessel icon [icon].

    The Add Vessel wizard opens in Edit mode. The Scan Settings page is the open page.

Figure 2-26: Add Vessel wizard in Edit mode, Scan Settings page
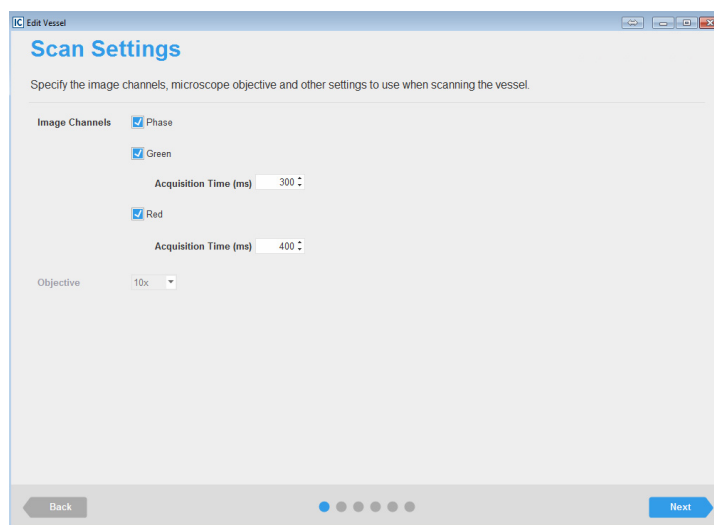
2. Edit the selections/settings as appropriate, and then click Next to move through the wizard (making additional edits as required) until the Summary page opens.

The Summary page here is almost identical to the Summary page in the Add Vessel wizard, and you can interact with it the same way. See

3. After you have made all the necessary edits for the vessel, click Save Changes.

    The Add Vessel wizard closes. The Acquisition window remains open with the vessel selected in the Vessel Drawer Setup pane.

Removing a Vessel from the Scanning Schedule

When you remove a scheduled vessel, the vessel is no longer displayed in the Vessel Drawer Setup pane. You are not deleting the vessel and/or its associated data from the database. You can remove any scheduled vessel, even if scans have already been acquired for the vessel. If the scanning duration for a vessel is complete, then a Clock icon is displayed on the vessel image on the Acquisition page. if you select the vessel image, then "Completed" is displayed in the scan properties for the vessel.

Figure 2-27:   Completed scan duration for a vessel
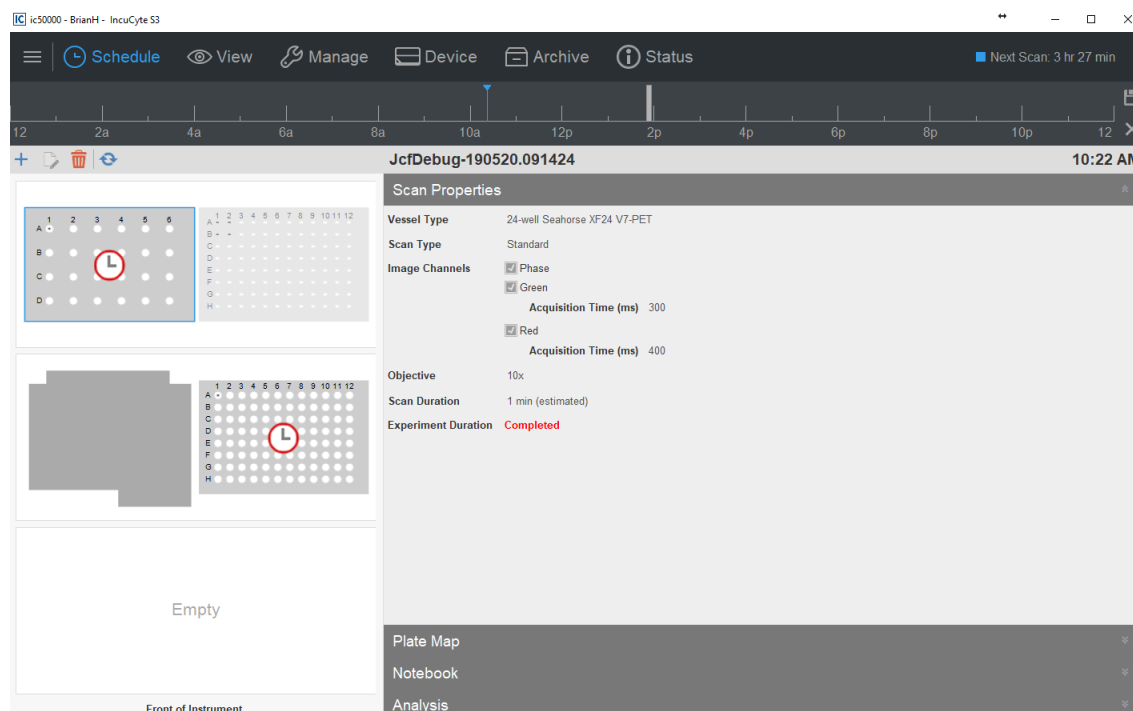
If you remove a *scheduled* vessel, note the following:

• You can remove only a single vessel at a time.

• If the vessel is the only vessel in its scan group, then its scanning schedule is also removed from the Vessel Schedule Timeline.

• If the vessel is part of a scan group, then the scanning schedule for the scan group remains displayed on the Vessel Schedule Timeline.

• Removing a vessel does not impact any scans that have already been acquired for the vessel. Going forward, scans are simply not acquired for the vessel.

To remove a vessel from the scanning schedule, in the Vessel Drawer Setup pane, select the vessel, and then on the Vessel toolbar, click the Remove selected vessel icon 🗑 .

> *To delete a vessel from the database, use the functions under the Manage menu (Manage > Vessel Delete).*

Section 2
Viewing Images

Section Contents

Incucyte Live-Cell Analysis Systems User Manual

Chapter 2
Selecting a Vessel

The first step in analyzing images is to select the vessel in the database that contains the images that you are analyzing. This chapter details the Scanned Vessels window, which displays all the vessels that have ever been scanned on your Incucyte system by any user, as well as all the options that are available on the window for selecting the correct vessel.

This chapter covers the following topics:

The Scanned Vessels Window

The Scanned Vessels window displays all the vessels that have ever been scanned on your Incucyte system by any user. To open the Scanned Vessels window, on the Incucyte main window, click View.

Figure 2-1: View window
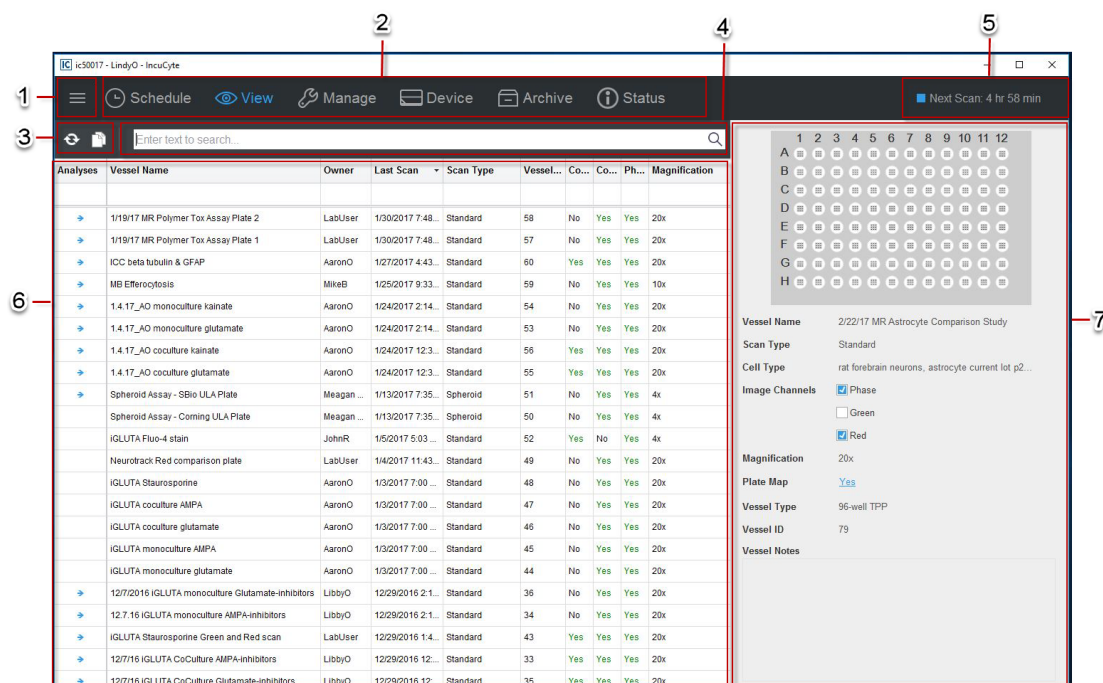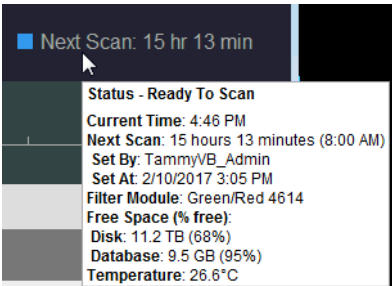
The Scanned Vessels window has the following layout:

	Window component	Description
1	Menu bar	Opens a dropdown menu with the following options: • Connections: Provides options for opening (Open) and closing (Close) the connections to your Incucyte Live-Cell Analysis Systems. • Tools and Options: Provides options for opening the Plate Map Editor (Plate Map Editor) and specifying a variety of preferences (Preferences) for your Incucyte software installation. See Appendix A, "Plate Map Editor," on page 137. • Help: Provides links to a variety of options that offer support for using the Incucyte software including the user manual (User Manual), a list of supported vessels (Supported Vessels), and general information about the software installation such as the current GUI version (About).
2	Main menu	The main menu is always displayed at the top of every window in the Incucyte software. The menu displays all the same options that are available on the Incucyte main window.

	Window component	Description
3	Scanned Vessels toolbar: Displays two icons for working with vessels in the Scanned Vessels window:	
		Refresh Vessels icon: Refreshes the display of the vessels in the Vessels pane.
		Copy Grid icon: Copies all the information that is currently displayed in the Vessels pane to your client's clipboard. You can then use the standard Paste commands to paste the information in to a third-party application such as Microsoft Excel.
4	Search field	To search for a specific vessel or vessels across all columns, enter your search criteria in this field. See "To search for a vessel or vessels in the Vessels pane" on page 61.
5	Scheduling status	Color-coded to indicate the following: • Yellow: Indicates a warning such as the drawer is open, the device is restarting or updating, the disk is running out of space, and so on. • Blue: Indicates the device is idle. The drawer is closed, no error state exists, and the device is not active. The text can vary, based on whether the instrument is processing data, sitting idle until the next scan, or sitting idle with no scans scheduled. If the device is idle waiting for the next scan, then it displays the time to the next scan overall. The selected vessel in the drawer has no relation to the time that is displayed. • Green: Indicates that the device is busy with some activity. Activities for which green is displayed include, but are not limited to, scanning, warming up, self test, and calibration. There is no relation with the selected vessel in the drawer. • Red: Indicates an error condition; however, there is no relation to the selected vessel in the drawer. If no vessels are scheduled for scanning, then the message "No upcoming scans" is displayed. **Note:** You can hold your mouse pointer over the status to open a tooltip that displays general information about the device and when the next scan is to take place. It has no relation to the selected vessel. *Figure 2-2: Schedule Status tooltip*

	Window component	Description
6	Vessels pane	Displays all the vessels that have ever been scanned on the instrument by any user. When the Scanned Vessels window first opens, the vessels are sorted in the pane in reverse chronological based on the scan date/timestamp. An Expand/Collapse icon in the Analysis column indicates that the images that were acquired for the vessel have been analyzed. See "The Vessels Pane" on page 60. **Note:** The Vessels pane is not dynamically updated after a vessel is scanned. You must click the Refresh Vessel icon to update the display after a vessel is scanned.
7	Vessel Information pane	Displays the vessel acquisition map (the vessel locations for which images were acquired as well as the number of images that were acquired per vessel location) and all the information that was defined in the Add Vessel wizard for a selected vessel. If the vessel is a microplate and a plate map was defined for the vessel, then next to the plate map entry, you can click Yes to open a read-only graphic of the map. See Figure 2-3 below.

Figure 2-3: *Vessel Information pane*

The Vessels Pane

The list of scanned vessels on the Scanned Vessels window is displayed in the Vessels pane. When the window first opens, the vessels are sorted in the pane in reverse chronological order based on the scan date/timestamp. By default, the first vessel that is displayed in the list of vessels is selected. An Expand/Collapse icon in the Analysis column indicates that the images that were acquired for the vessel have been analyzed.

Figure 2-4:   *Vessels pane*
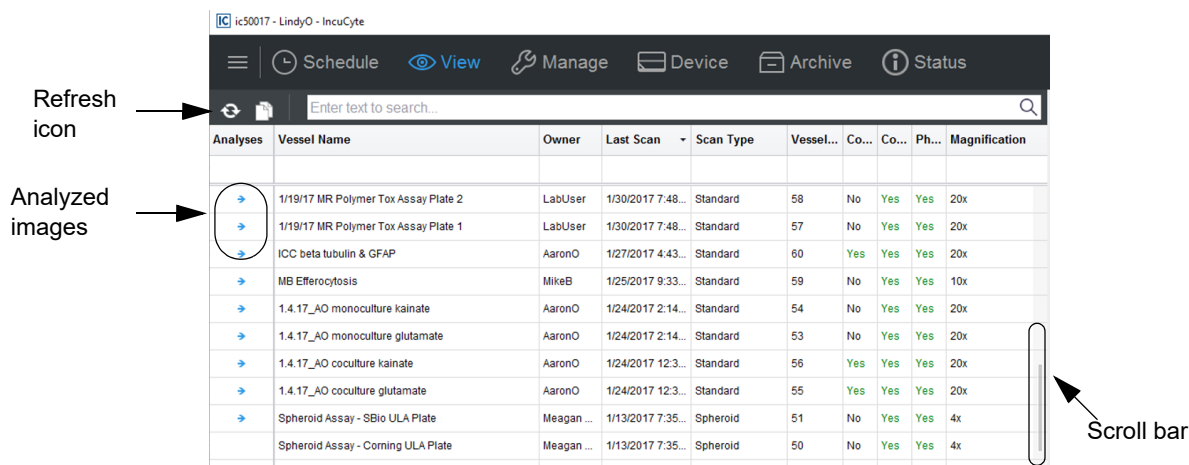
Note the following about the Vessels pane:

- The Vessels pane is not dynamically refreshed after a vessel is scanned. To ensure that all scanned vessels are being displayed in the pane, you should periodically click the Refresh Vessels icon.

- You can use the Up and Down arrow keys on your client keyboard to scroll through the display, or you can use the Scroll bar on the right side of the pane.

If the scroll bar is not displayed, click once on the far right side of the pane to display it. The scroll bar is a thin, gray vertical line.

- The following actions are available for the Vessels pane:

  - You can change the sort order of the vessels in the pane and you can filter the list of vessels to display only a subset of vessels based on a selected property. See "To sort and/or filter the Vessels pane display" on page 61.

  - You can search for a specific vessel or vessels in the pane. See "To search for a vessel or vessels in the Vessels pane" on page 61.

  - You can view the analysis definitions that are associated with a scanned vessel. See "To view the analysis definitions for a scanned vessel" on page 62.

  - You can open the Vessel View window for selected vessel. See "To open the Vessel View window" on page 64.

To sort and/or filter the Vessels pane display

With the exception of the Analyses column, you can hold your mouse pointer over any column header in the Vessels pane to display a Filter icon, and you can click once in a column header to display a Sort Order icon.

Figure 2-5:   Sort order icon and Filter icon for a column in the Vessels pane

- The direction of the Sort Order icon indicates the sort order, where up indicates that the data is sorted in ascending order based on the data in the column and down indicates that the data is sorted in descending order. To change the sort order of the vessels to a descending sort based on a column, click the Sort Order icon for the column. To return the sort order to ascending order, click the Sort Order icon again.

- To filter the list of vessels to display only a subset of vessels based on a selected property, in the appropriate column header, click the Filter icon, and then select the filter or define a custom filter. For example, to display only those scanned vessels that a specific user owns, in the Owner column heading, click the Filter icon, and then on the Filter list, select the owner.

You can also right-click the Filter icon to open a context menu with additional filtering options such as sorting, showing/hiding columns, selecting columns, and so on.

To search for a vessel or vessels in the Vessels pane

With the exception of the Analyses column, you can search for a specific vessel or vessels across all columns (vessel properties) in the Vessels pane, or you can search based on a specific vessel property.

- To search across all columns, you can enter a search string in the blank search field at the top of the page.

- To search based on a specific vessel property, such as Owner, you can enter a search string in the blank field below the appropriate column header.

For either type of search, the search is limited to the exact order of the characters in the string, but the string is not case-sensitive and it can appear anywhere in the search results. For example, if you search across all columns with **sph** as the search string, then the search results include vessels with a Vessel Name of 384 **Spheroid** HT 1080 or a Scan Type of **Spheroid**.

To view the analysis definitions for a scanned vessel

If the images have been analyzed for a scanned vessel, then to view the associated analysis (definition, type, and so on), click the Analysis arrow. The Analysis arrow is now displayed vertically, and an Analysis pane that displays the associated analysis information opens. Multiple analysis definitions for a single vessel are allowed.

Figure 2-6:   Analysis definitions associated with a scanned vessel
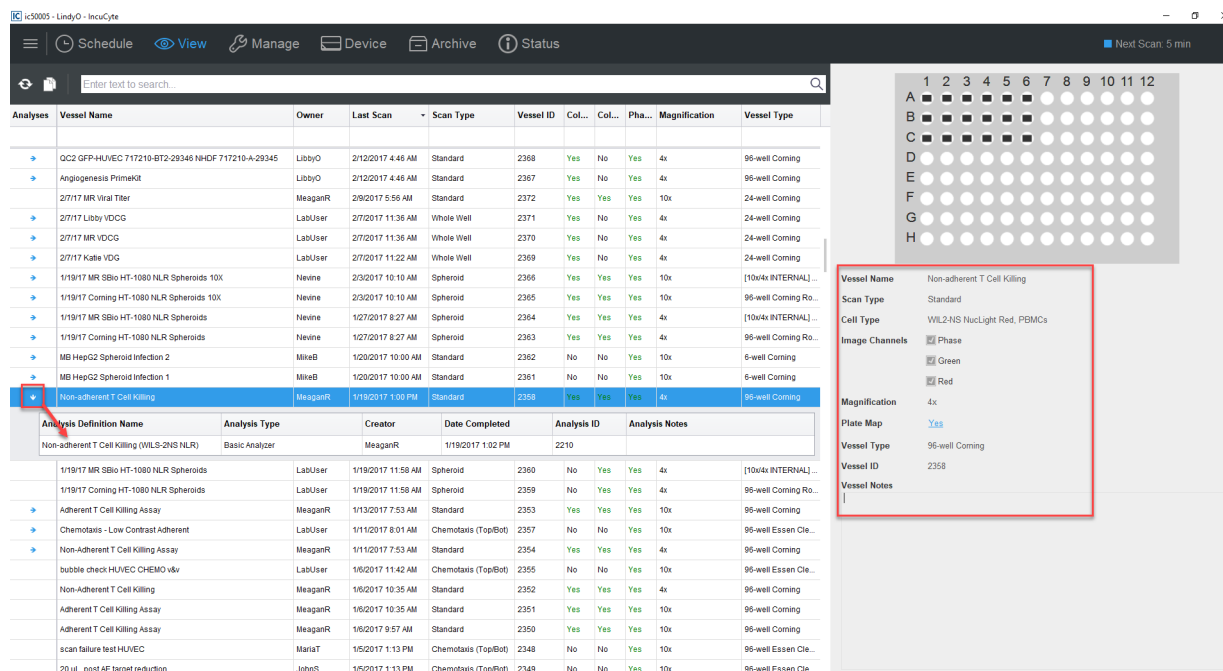
Although the Analysis pane is shown with columns, no filter functions are available.

To update the Vessel Information pane display to include the information for an analysis definition, in the Analysis pane, select the analysis. The vessel graphic at the top of the Vessel Information pane is updated to show only those vessel locations for which the selected analysis is applicable. See Figure 2-7 below.

Figure 2-7:   Vessel Information pane displaying analysis information

You can right-click any analysis for a selected vessel to open a context menu with the following analysis options:

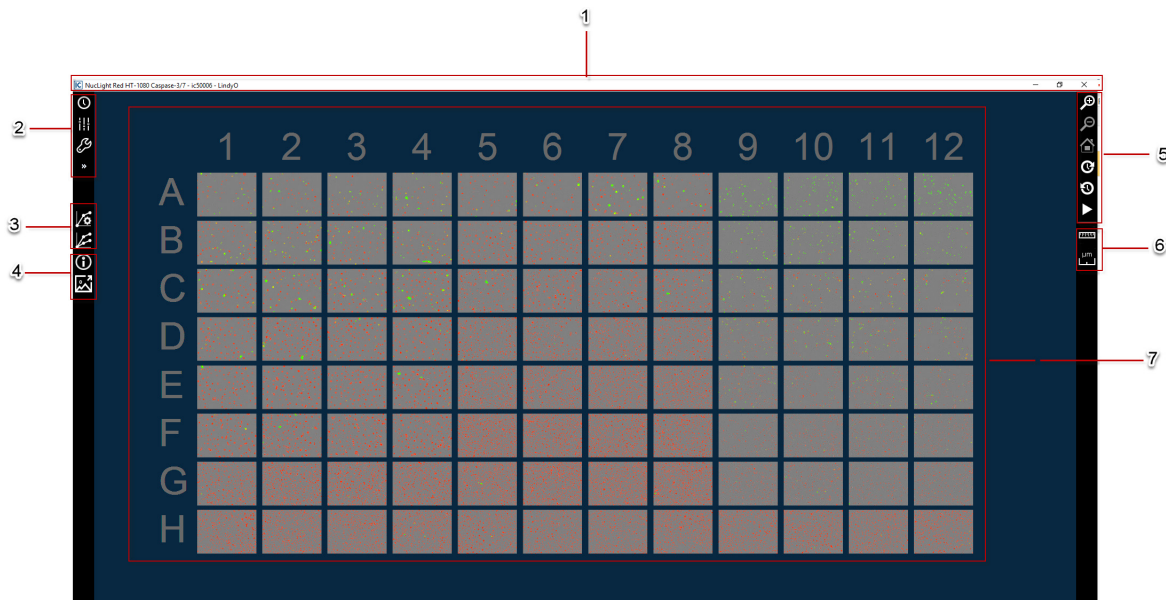| Option	Description
Open Analysis	Opens the Vessel View window, which now displays the associated analysis masks that were applied to the images. See "Viewing the Analysis Masks" on page 109. **Tip:** You can also double-click an analysis to open the Vessel View window and view the analysis masks.
Graph Analysis Metrics	Opens the Graph Metrics window, which provides options for graphing the metrics that are associated with the analysis without having to open the vessel. See "The Graph Metrics Window" on page 110.
View Analysis Details	Opens the Analysis Details window, which details all the parameters that were specified in the Launch Analysis wizard for analyzing the images in the vessel. The parameters that are displayed depend on the analysis type, for example, Basic Analyzer, that was selected for the vessel. See Figure 2-9 on page 64.
Export Analysis Definition	Opens the Export Analysis Definition dialog box, which provides the options for exporting the full definition for the selected analysis. *Figure 2-8: Export Analysis Definition dialog box* **Tip:** This option is particularly useful if your organization two or more Incucyte systems. Instead of setting up the same definition on each of the instruments, you can set up the definition one time on one instrument, and then export this definition to all your other instruments.
Delete Analysis	Deletes the selected analysis for the vessel. **Warning:** If you delete a selected analysis for a scanned vessel, then only the analysis data is deleted. Neither the vessel nor its images are deleted from the database.

Figure 2-9:   Analysis Details window
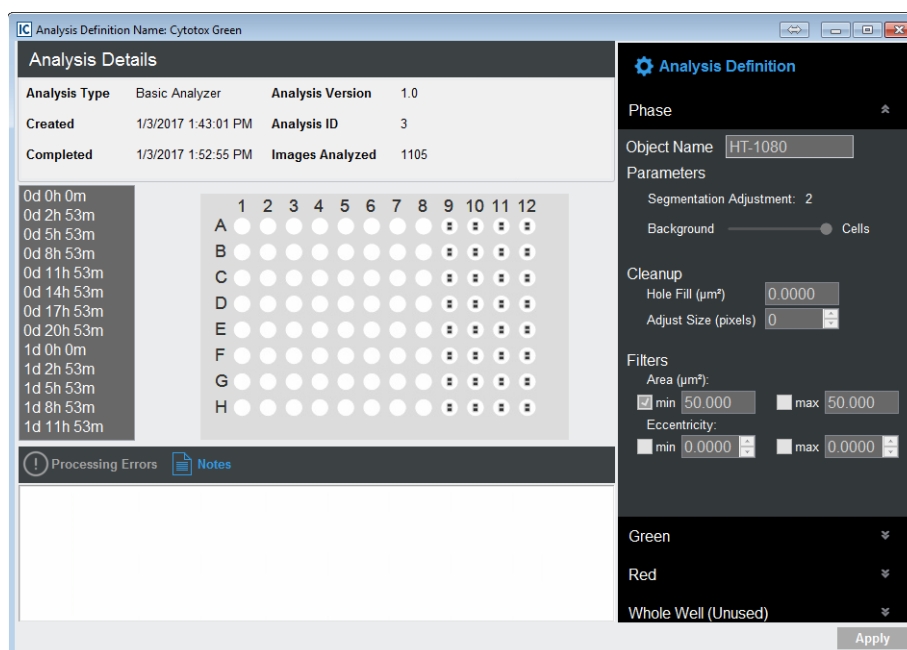

To open the Vessel View window

The Vessel View window displays the scanned vessel that has been selected for image analysis. The window provides the options for viewing, comparing, and ultimately selecting the images that are to be analyzed. To open the Vessel View window for a scanned vessel, do one of the following in the Vessels pane in the Scanned Vessels window:

- Right-click the vessel, and on the context menu that opens, click Open.

- Double-click the vessel.

See "The Vessel View Window" on page 67.

Chapter 3
Viewing and Working With Images

After you have selected the vessel in the database that contains the images that you are analyzing, you can then view the images and adjust the image properties in a variety of ways to examine and compare the images. For example, you can set a range of minimum and maximum intensities to help you determine the areas of the image that have the highest intensities, you can turn channels on and off, and you can remove signal in a given channel (spectral unmixing). Based on all the information that you collect from this examination and comparison, you can then decide which vessel locations are appropriate for image analysis, and how you are analyzing the images. This chapter details the Vessel View window, which displays the scanned vessel that has been selected for image analysis, and all the options that are available on the window for viewing, comparing, and ultimately selecting the images that you are analyzing.

This chapter covers the following topics:

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The Vessel View Window

The Vessel View window displays the scanned vessel that has been selected for image analysis. The window provides the options for viewing, comparing, and ultimately selecting the images in the vessel that you are analyzing. To open the Vessel View window for a scanned vessel, do one of the following in the Vessels pane:

• Right-click the vessel, and on the context menu that opens, click Open.

• Double-click the vessel.

Figure 3-1:   Vessel View window (96-well microplate)
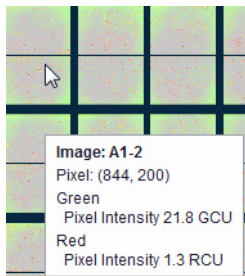
The Vessel View window has the following layout:

Window component		Description
1	Title bar	Displays the Vessel Name for the selected vessel, the ID for the Incucyte system on which the vessel was scanned, and the name of the logged in user. The title bar also displays the standard Minimize, Maximize, and Close window icons.
2	Image toolbar	The Image toolbar contains options for viewing and working with the images that have been acquired for the scanned vessel. See "The Image toolbar" on page 70. **Note:** An Expand/Collapse icon is displayed next to many options on the Image toolbar. If a toolbar option is collapsed, then the icon is displayed as an inverted double arrow ⯆. To expand the option, click the icon. The icon is now displayed as an upright double arrow ⯅. To collapse the toolbar option, click the icon again.

	Window component	Description
3	Analysis toolbar: The Analysis toolbar contains two icons.	
		Launch Analysis icon: Opens the Launch Analysis wizard, which you use to define the analysis for the images in the scanned vessel. See Chapter 2, "Defining the Image Analysis," on page 89.
		Graph Metrics icon: Opens the Graph Metrics window, which provides options for graphing the *scan* metrics for the vessel. See Chapter 2, "The Graph Metrics Window," on page 110.
4	Visualization toolbar: The Visualization toolbar contains two icons.	
		Vessel Information icon: Opens the Vessel Information window, which displays different information about the vessel. See "Vessel Information window (from the Vessel View window)" on page 69.
		Export Images and Movie icon: Launches the Image Export wizard. See "Exporting Images" on page 75.
5	Navigation toolbar	The Navigation toolbar contains options for navigating the Vessel View display, including zooming in, zooming out, and moving through the scans for the vessel. See "The Navigation toolbar" on page 73.
6	Measure toolbar: The Measure toolbar contains two icons:	
		Measure image features icon: Enables the measurement of an object to determine its size. The unit of measure that is available, for example, microns, depends on the measured size of the image feature. To use the Measure image features function, click the Measure image features icon, and then drag your mouse pointer to measure the length or width of the object. The value is displayed in the lower right corner of the image.
		Tip: After you measure an object, you can click the Measure image features icon again to clear the measurement information that is displayed on the image.
		Note: When defining analysis parameters for vessel images, if Top-Hat color processing is selected, then you must use this feature to set the radius parameter. See "Top Hat" on page 146.
		Legend icon: Toggles the display of a legend for the measurement scale. The unit of measurement depends on the zoom level.

	Window component	Description
7	Vessel View display	Displays the acquisition map for the scanned vessel. The number of images that have been acquired for each vessel location (as specified in the Add Vessel wizard) is indicated by the number of individual squares in the vessel locations. For example, in Figure 3-2 below, one image per vessel location is indicated for Figure A while four images per vessel location is indicated for Figure B. The display is dynamically updated based on the scan time that is selected on the Vessel Scanning Timeline. (See "To select a scan time for the Vessel View display" on page 70.) You can hold your mouse pointer over a vessel location to open a tooltip that displays information about the image in the location. See Figure 3-3 below.

Figure 3-2:   *Comparing the number of images in a vessel location*

Figure A.   Figure B.

Figure 3-3:   *Image tooltip in the Vessel View window*

Vessel Information window (from the Vessel View window)

The Vessel Information window has up to three different information views: Scan Properties, Plate Map, and Notebook. When the window first opens, if the vessel is a microplate, then the Plate Map view is the open view; otherwise, the Notebook view is the open view. The Scan Properties view and the Notebook view are identical to the Scan Properties view and Notebook view in the Vessel Information pane in the Acquisition window. (See"Vessel Information pane" on page 25.) Unlike the Plate Map view from the Acquisition window, however, the Plate Map view from the Vessel View window is not read-only. Instead, all the functionality that is associated with the Plate Map Editor is available. (See Appendix A, "Plate Map Editor," on page 137.) You can do the following in the Vessel Information window:

- To open and maximize the Plate Map view in its own window, click the view once.

- To edit the vessel name, cell type and/or passage, open the Notebook view, and then edit the information as appropriate.

- To add notes for the vessel, open the Notebook view, and then click the Add Note icon ✛ to open the Add Notes dialog box, enter the notes where indicated in the dialog box, and then click OK.

The Image toolbar

The Image toolbar on the Vessel View window contains options for viewing and working with the images that have been acquired for the scanned vessel. Click an icon on the toolbar to access the associated functions.

Icon	Description
🕐	Scans icon: Opens the Vessel Scanning Timeline popup. See "To select a scan time for the Vessel View display" below.
⋮⋮⋮	Layers icon: Opens the Image Layers popup. See "To work with the image channels" on page 71.
🔧	Tools icon: Opens the Tools popup. See "To work with the Vessel View display" on page 73.

To select a scan time for the Vessel View display

The Vessel Scanning Timeline popup displays all the time points at which the vessel was scanned (xd xh xm), with the scan times listed in ascending order, with the first scan time (0d 0h 0m) always at the top of the list.

Figure 3-4:   Vessel Scanning Timeline popup
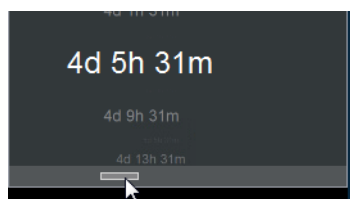
When the Vessel View window *first* opens, the results of the most recent scan for the vessel are displayed. To select a different scan time, do any of the following:

• Click the scan time in the Vessel Scanning Timeline popup.

• Click at the bottom of the Vessel Scanning Timeline popup to display a horizontal gray scroll bar, and then click and drag the scroll bar to move through and select a scan time.

• Click any scan time in the Vessel Scanning Timeline, and then use the Up and Down arrows on your client keyboard, or use your mouse scroll wheel.

• Use the Next Scan and/or Previous Scan icons on the Navigation toolbar. See "The Navigation toolbar" on page 73.

After you select a scan time, it is displayed in white and in a larger font than the other scan times in the Vessel Scanning Timeline popup. The Vessel View display is dynamically updated based on the selected scan time and the selected scan time is temporarily shown at the top of the display.

To work with the image channels

The Image Layers popup provides options for working with the channels that were used when acquiring the vessel images, including auto-scaling, turning on and/or turning off channels, adjusting the brightness and contrast for the Phase channel (if applicable), and spectral unmixing. See:

- "Color Autoscale" below.

- "Spectral Unmixing" on page 72.

Figure 3-5:   Image Layers popup
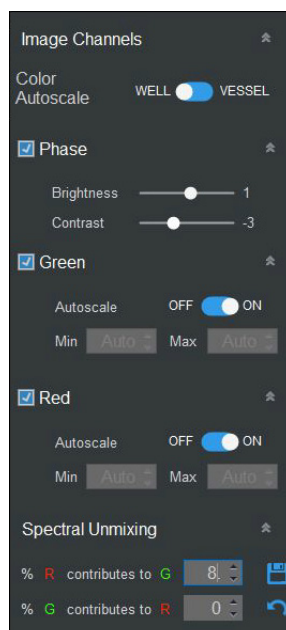
Color Autoscale

Color Autoscale automatically sets a range of minimum and maximum intensities to account for changes in biological samples across different samples and time. When viewing a vessel in the Vessel View window:

- If Vessel (the default value) is selected, then an intensity range is automatically applied across the entire vessel based on the minimum and maximum brightness of the entire vessel. If you turn off Autoscale for a channel with Vessel selected, then you can manually adjust the intensity range for the channel.

- If Well is selected, then an intensity range is automatically applied *to each vessel location*. If you turn off Autoscale for a channel with Well selected, then the intensity range for the channel is automatically set for the entire vessel based on the intensity range for the image that is closest to the viewport. For example, if the vessel view is not zoomed for a 96-well microplate, then the intensity range for the channel would be set for the entire microplate based on the intensity range for the image that is in Well D6. If the vessel view is zoomed in for a 96-well microplate to Well A1, then the intensity range for the channel would be set for the entire microplate based on the intensity range for the image that is in Well A1.

For information about navigating the Vessel View display, including zooming in and zooming out, see "The Navigation toolbar" on page 73.

Spectral Unmixing

The Spectral Unmixing option is displayed only if more than one fluorescent channel has been used for image acquisition. For example, if a fluorophore was used that produces signal in both the Red and Green channels, then spectral unmixing might be necessary to account for signal that has been contributed from one of the given channels. In this example, green fluorophores typically do not require any spectral unmixing while red fluorophores require a small amount of adjustment (2% - 8%) to account for contribution in to the green channel. You must apply spectral unmixing values before you run an analysis job. To ensure the most appropriate values, you should make adjustments in small increments. After you change one or more spectral unmixing values, two icons are displayed on the Image Channel popup - a Save icon and an Undo icon. To save the changes that you make to the spectral unmixing values, you must always click the Save icon. At any time after you make an edit, but before you click Save, you can click the Undo icon to return the spectral unmixing values to their last *saved* values.

Figure 3-6:   *Spectral unmixing percentages edited*
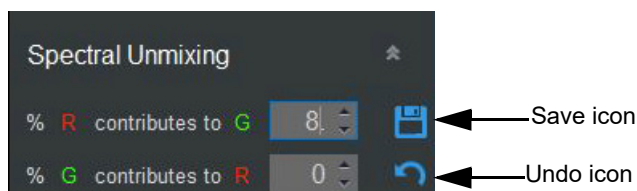
Figure 3-7 below is an example of correct and incorrect spectral unmixing. In this figure, the top row shows blended images of a mixed population of HT-1080 Nuclight Red and Nuclight Green cells. The bottom row demonstrates optimized spectral unmixing. Using the HT-1080 Nuclight Red cell that is circled in yellow as a guide, the red fluorophore is visible in the green channel with an average Green calibrated Unit (GCU) of 8.2 and an average Red Calibrated Unit (RCU) of 29.2. Optimization of the red fluorophore in Incucyte showed a spectral unmixing adjustment for 8% of the red contributing to the green was required (GCU - (RCU x 0.08) = the amount of red signal that contributed to the green channel). If the amount of red signal contributing to the green channel is set too high, then over corrected pixels develop, giving the appearance of "pits" or "holes" in the images, which can affect assay metrics.

Figure 3-7:   *Spectral unmixing of a mixed population of HT-1080 cells expressing Nuclight Green or Red*
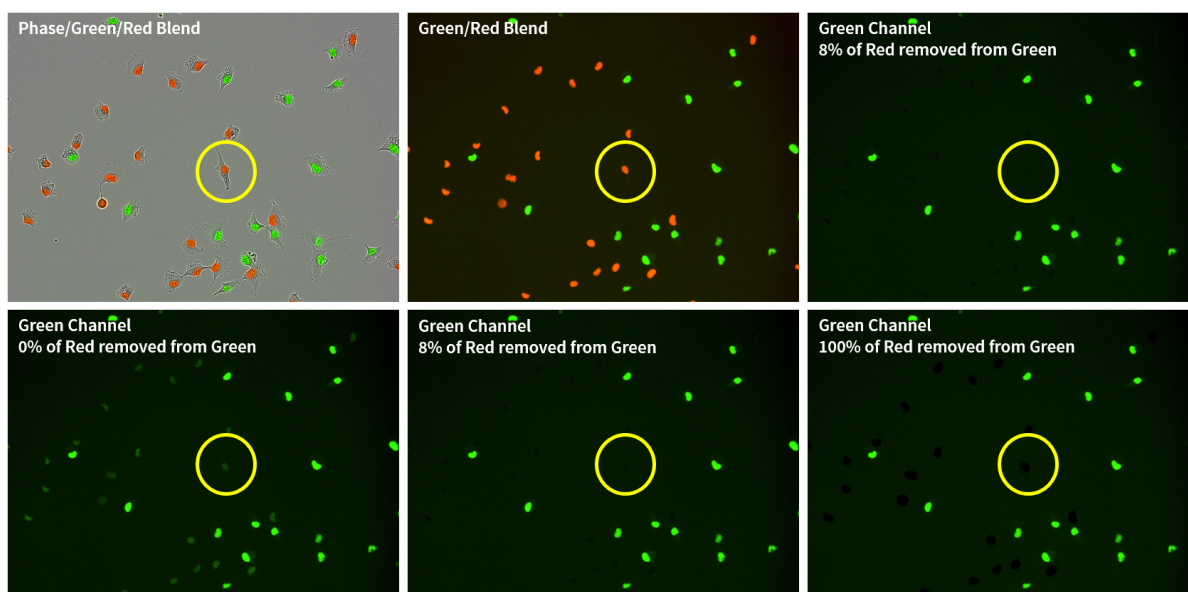
To work with the Vessel View display

The Tools popup contains options for working with and changing the information that is shown for the Vessel View display.

Option	Description
Labels	Selected by default. Shows the vessel locations (for example, A1 for a well in a 96-well microplate) while panning and zooming the Vessel View display.
Image Metrics	Shows a snapshot of the image data for the vessel at the selected scan time. The value for the selected metric is displayed on each image. The data is dynamically updated as you select a different time in the Vessel Scanning Timeline. For a vessel that has not been analyzed, only Scan metrics are available. For an analyzed vessel, analysis-specific metrics are also available. Use the Transparency slider bar to adjust the opacity of the selected metric. **Note:** The RMS Row Shift and RMS Column Shift scan metrics are primarily for diagnostic use for the microscope functionality. Unlike the other scan metrics (Focus Position, Phase Exposure Time, and so on), they have no "biology-related" use. **Tip:** To change the color of the metric that is displayed, click the solid white circle that is displayed to the right of the Transparency slider bar to open the standard Windows Color dialog box, and then select a basic color, or define a custom color for the metric.
Packed View	Selected by default. Removes the dead space between vessel locations to optimize the viewing area. **Note:** Sartorius recommends that you leave the Packed View option selected for the Vessel View window.

You can also right-click any vessel location in the Vessel View display to open a context menu with the following options:

Option	Description
Copy Viewing Area to Clipboard	Copies the *current* Vessel View display to your client's clipboard. You can then use the standard Paste commands to paste the copied display in to a third-party application such as Microsoft PowerPoint.
Export Image Via Designer	See "To export images as displayed" in "Exporting Images" on page 75.
Export Image as Stored	See "To export images as stored" in "Exporting Images" on page 75.

The Navigation toolbar

The Navigation toolbar contains options for navigating the Vessel View display, including zooming in, zooming out, and moving through the scans for the vessel. When the Vessel View window first opens, the zoom level of the display is set to a starting, or *Home*, value of 100%, which means that the entire vessel is shown in the Vessel View display.

Icon	Description
🔍⊕	Zoom In icon: Click as needed to zoom in on the Vessel View display. **Tip:** To zoom in to the maximum extent possible on a vessel location, you can double-click the location.

Icon	Description
	Zoom Out icon: Available only after at zooming in on the Vessel View display. Click as needed to zoom out the Vessel View display.
Tip:	You can also use the scroll button on your mouse to zoom in on and zoom out the Vessel View display and you can also click and hold the mouse pointer anywhere on the Vessel View display, and then drag the display to move it to a different location.
	Home icon: Available only after zooming in on or zooming out the Vessel View display. Click once to return the Vessel View display to its starting, or Home, value of 100%. (The entire vessel is shown in the Vessel View display.)
	Next Scan icon: Updates the Vessel View display to the next scan in the Vessel Scanning Timeline. Click as needed to move forward through the scans for the vessel.
	Previous Scan icon: Updates the Vessel View display to the previous scan in the Vessel Scanning Timeline. Click as needed to move backward through the scans for the vessel.
	Start/Stop Slide Show icon: Creates a self-running presentation of all the scans for the vessel. As the presentation runs, the corresponding date/timestamp for each scan is temporarily shown at the top of the Vessel View display. To stop the slide show at any time, click the Stop Slide Show icon.

Exporting Images

After you have acquired images in a vessel, you can export the images from one or more image sites at a time. You use the Image Export wizard to export the image. Two choices are available for exporting an image:

- As Displayed: Exports the data as it is currently displayed in the Vessel View window.You can layer image channels and masks to customize the image view, you can crop and scale the image, and you can apply an overlay. You typically select As Displayed if you are intending to use the image in reports or presentations, and/or sharing the image data, such as in an email. When you export the image as displayed, you can export the image as a series of individual files, or you can collate the images and export them as a single movie. See "To export images as displayed" below.

- As Stored: Exports the image and mask data as stored on the Incucyte system *prior* to displaying in the Vessel View window. This is typically referred to as the "raw" data. User-specified settings are not reflected in this type of export and the image components are exported to individual files. You typically select As Stored when you are importing the image in to third-party analysis tools or you are sending the image as part of an Incucyte support request. See "To export images as stored" on page 81.

The Launch Analysis wizard uses the current display (zoom level, focus, and scan time) of the vessel in the Vessel View window. Before you open the wizard, optionally, you can set the zoom level of the Vessel View window to its Home value (100%) and you can confirm that the preferred scan time is selected on the Vessel Scanning Timeline.

To export images as displayed

1. Open the vessel in the Vessel View window. See "To open the Vessel View window" on page 64.

2. On the Visualization toolbar, click the Export Images and Movie icon ![icon].

   The Image Export wizard opens. The Export Type page is the open page. As Displayed is selected.
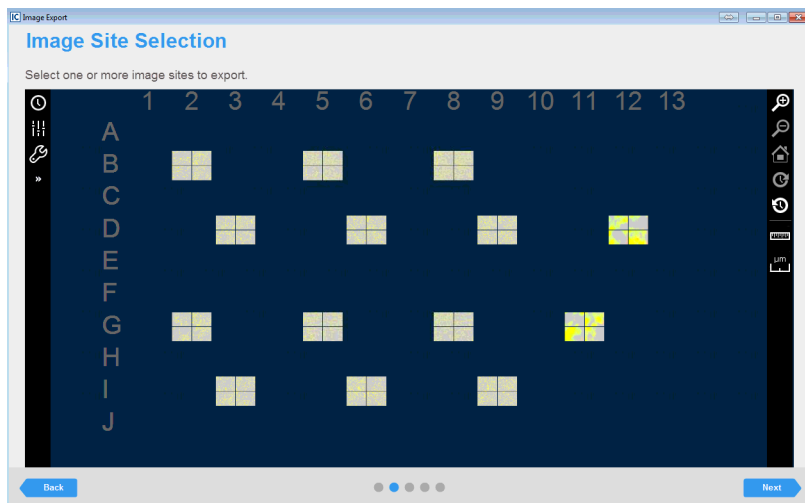
Figure 3-8:   Image Export wizard, Export Type page
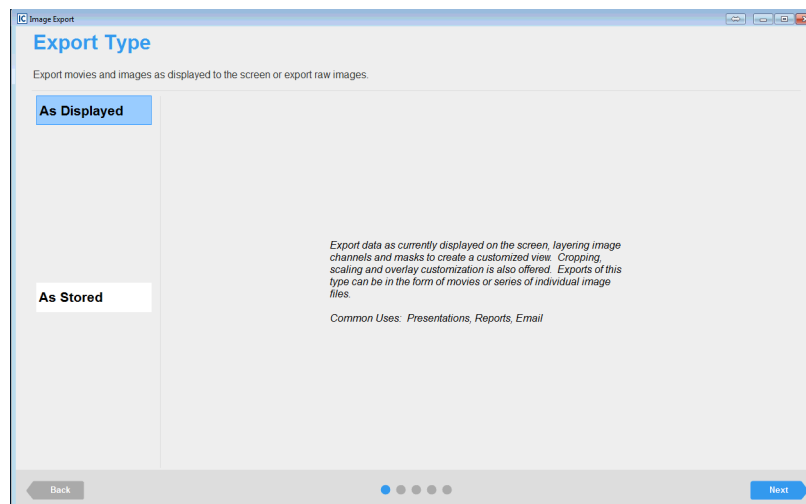
3. Leave As Displayed selected, and then click Next.

The Image Site Selection page opens. The page shows the vessel as it was displayed (zoom level, focus, and scan time) in the Vessel View window at the time that you opened the wizard.

Figure 3-9:   Image Export wizard, Image Site Selection page

All the options that are available on the Image Site Selection selection page are identical to that on the Vessel View page. With the exception of the spectral unmixing option, you can use all the options to help you select the images that you are exporting. For example, if you did not have the correct scan time selected in the Vessel View window before you opened the Image Export wizard, then you can open the Vessel Scanning Timeline for the selected vessel and scroll through and select a different scan time, you can use the options on the Navigation toolbar to zoom in on the selected vessel location to confirm that it shows the biology of interest, and so on. See "The Vessel View Window" on page 67.

4. For each image site that you are exporting, click the site. (To clear an image selection, click it again.)

As you hover over a site, the mouse pointer changes to a blue plus sign. After you click a site to select it, the pointer changes to a red X and the selected site/well is filled with a solid light blue color.

If multiple images have been acquired from a site or well, then you must select each image that you are exporting.

5. Click Next.

The Sequence Type and Scans page opens. You use the options on this page to select the sequence type that you are exporting (a single movie or a series of individual images) and the specific scans for which you are exporting the images. The page displays the Vessel Scanning Timeline for the vessel. By default, single movie is selected. See Figure 3-10 on page 77.

Figure 3-10:   Image Export wizard, Sequence Type and Scans page
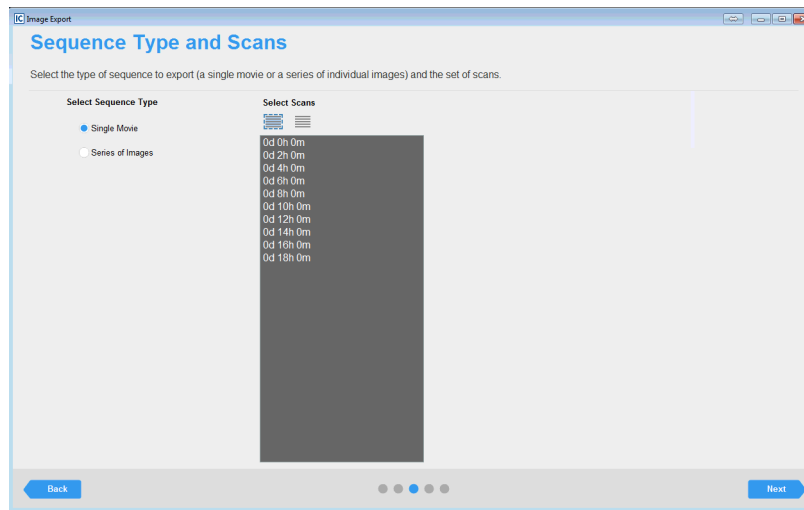
6. To export the images as a movie, leave Single Movie selected; otherwise, to export the images as a series of individual files, select Series of Images.

Although the option is "Series of Images," you can export a single image scan type.

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7. Select the scans for the images that you are exporting. You can do any of the following:

   • To select all scans in a single step, under Select Scans, click the Select All Scans icon ☰. To clear an individual scan, click the scan again.

   • In the Vessel Scanning Timeline, do either one or both of the following:

      • To select an individual scan, click the scan. You can select multiple, individual scans. To clear an individual scan, click it again.

      • To select a range of scans, click the first scan in the series of scans, press and hold the Shift key, and then click the last scan in the series of scans.

   • To clear all selected scans in a single step, under Select Scans, click the Clear All Scans icon ☰.

8. Click Next.

   The Export Designer page opens. You use the options on this page to customize the movie/image files before exporting, including cropping the image, adding a legend, and so on. When the page first opens, default values are set for many of the options. For either export sequence, the Final Size is the final export size in pixels. See

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If you selected Single Movie, then depending on the image size, a series of messages about Automatic Scaling and/or Export Size might open before the Export Design page, indicating that to automatically reduce the size of the exported movie, automatic scaling has been applied. The messages also prompt you to consider additional scaling and/or cropping to further reduce the size. Click OK to close these messages and continue to the Export Designer page.

Figure 3-11:   Image Export wizard, Export Designer page
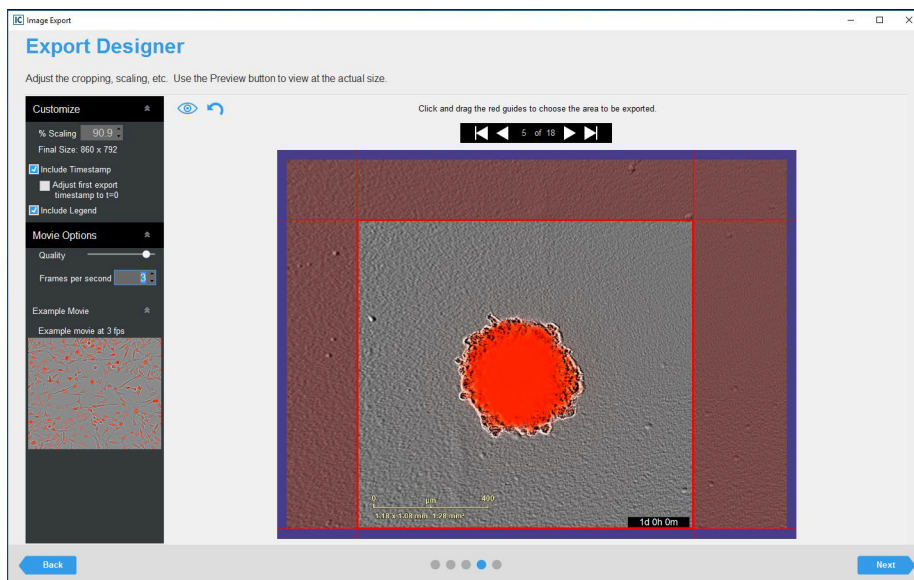
9. Modify any of the design options as needed, including cropping and scaling the image. Note the following about this page:

    •   After you make any edits, the display on the Export Designer page is dynamically updated.

    •   You can click the Full Screen Preview icon ◉ to view a full, onscreen preview of the movie or image file that you are exporting at any time. To reset all values to their default values in a single step, click the Reset icon ↰.

    •   The image quality is set to approximately the highest value. Although not recommended, you can use the Quality slider bar to reduce the image quality, which also reduces the final file size.

    Although not common, you might reduce the quality, for example, in the event that you simply want to share the perspective of what is occurring in a selected image site with a colleague and you want to reduce the file size to make it easier to share via email.

    •   If you are exporting a single movie, then you can increase the Frames per second value to play the movie at a faster rate. After you adjust the value, you can hold your mouse pointer over the Example Movie display to preview a *stock movie* that is played at the specified rate.

    It is important to note that the preview is that of a stock movie display. It is not built from your selected image site.

10. Click Next.

    The File Information page opens. You specify the export settings such as the target folder on this page. The options on this page are different for a single movie and individual image files. See Figure 3-12 and Figure 3-13 on page 79.

Figure 3-12:   Image Export wizard, File Information page (Single movie)

Figure 3-13:   Image Export wizard, File Information page (Individual image files)

11. Specify the file information.

Option	Description
Target Folder	The folder in which the exported images are stored. Click Browse to open the Select a folder to export to dialog box, and then browse to and select the appropriate folder.
File Format	The default value for a single movie is MPEG-4 Video and for individual files is JPEG.
File Name Prefix	The exported file is automatically named according to the following: • For a single movie: <FileName Prefix>_<Site/Well ID>_<Image Number>_<File Format>, for example, VID2197_D7_1_.mp4. • For a series of individual images: <FileName Prefix>_<Site/Well ID>_<Image Number><Date/Timestamp><File Format>, where the File Name Timestamp option determines the timestamp. See "File Name Timestamp" on page 80. **Note:** The default value for the file name prefix is the Vessel ID for the selected vessel. You can leave this value as-is, or you can edit it as necessary.

Option	Description

File Name Timestamp	Applicable only for individual image files.
	• Select Use Calendar Time to append the file name with the date and timestamp that the selected image was acquired, for example,
	    VID2197_D7_1_2016y05m11d_21h36m.jpg
	• Clear Use Calendar to append the filename with the relative date and timestamp that the selected image was acquired, for example,
	    VID2197_D7_1_.00d00h00m.jpg

12. Click Export.

The Image Export wizard closes. You return to the Vessel View window. An Export Progress dialog box opens on top of the Vessel View window.

Figure 3-14:   Export Progress dialog box
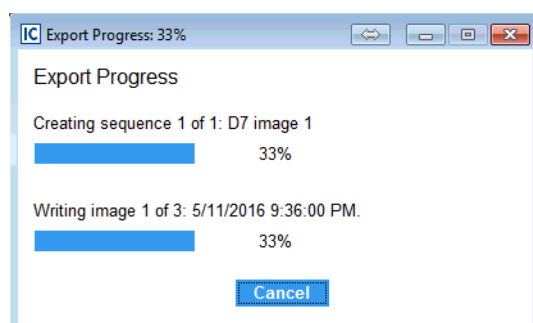
After the export progress is 100%, a Sequence Writing Complete dialog box opens.

• If you exported a single movie, then the dialog box indicates that the sequence was successfully created, and asks you if you want to open the file.

• If you exported individual image files, then the dialog box indicates the number of files that were created successfully and asks you if you would like to open the target folder.

Figure 3-15:   Sequence Writing Complete dialog box
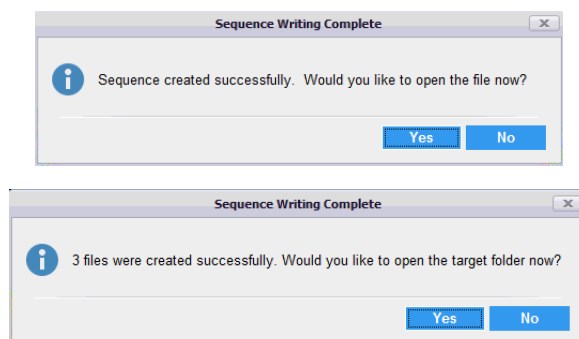
13. Do one of the following:

- To close the wizard, return to the Vessel View page, and open the file or target folder, click Yes.

- To close the wizard and return to the Vessel View page, click No.

To export images as stored

1. Open the vessel in the Vessel View window. See .

2. On the Visualization toolbar, click the Export Images and Movie icon ![icon].

    The Image Export wizard opens. The Export Type page is the open page. As Displayed is selected.

Figure 3-16: Image Export wizard, Export Type page
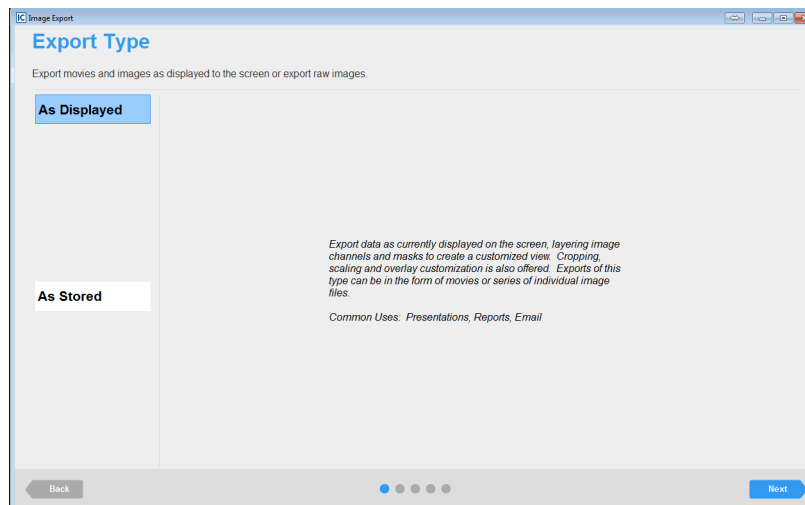
3. Select As Stored, and then click Next.

    The Image Type Selection page opens. You specify the image type that are exporting on this page.

Figure 3-17: Image Export wizard, Image Type page
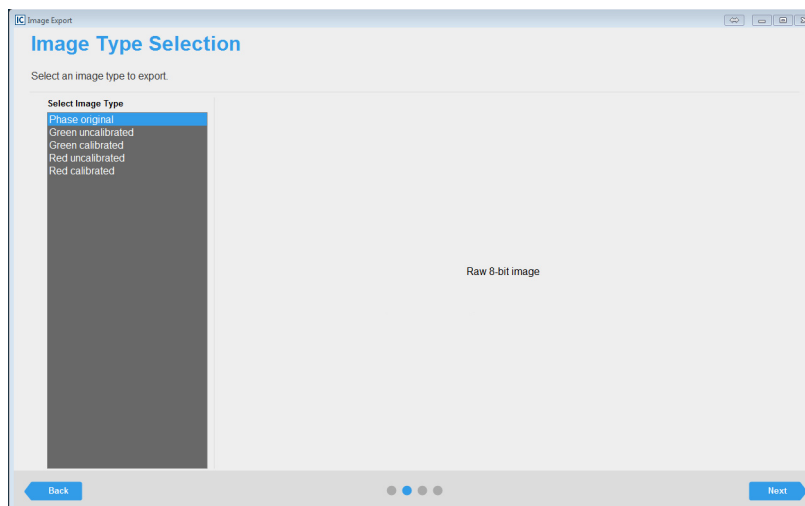
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Viewing and Working With Images

4. Select the image type that you are exporting.

Image Type	Description
Phase original	Raw 8-bit image
Green uncalibrated	Raw 16-bit image without calibration
Green calibrated	32-bit floating point image in calibrated units
Red uncalibrated	Raw 16-bit image without calibration
Red calibrated	32-bit floating point image in calibrated units

5. Click Next.

The Scan Times, Wells, and Image Sites page opens. The page displays the Vessel Scanning Timeline for the vessel and the vessel acquisition map. You use the options on this page to select the vessel locations, the number of images, and the scans for which you are exporting the raw image data.

Figure 3-18:   Image Export wizard, Scan Times, Wells, and Image Sites page
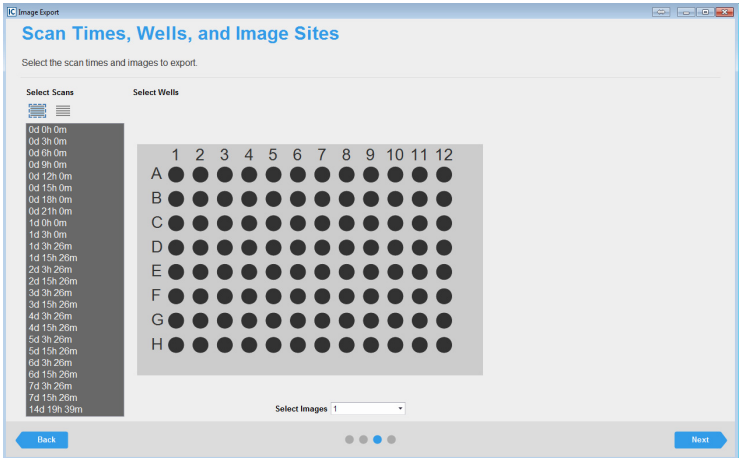
6. Do the following as indicated to select the scan times, image sites, and number of images per site for which you are exporting the raw data:

Option	Description

To select scan times	• To select all scans in a single step, under Select Scans, click the Select All Scans icon ▤. To clear an individual scan, click it.
	• In the Vessel Scanning Timeline, do either one or both of the following:
	  • To select an individual scan, click the scan. You can select multiple, individual scans. To clear an individual scan, click it again.
	  • To select a range of scans, click the first scan in the series of scans, press and hold the Shift key, and then click the last scan in the series of scans.
	• To clear all selected scans in a single step, under Select Scans, click the Clear All Scans icon ▤.

Option	Description
To select wells/image sites	• To select a single location in a vessel, for example, a well in a 96-well microplate, click once in the appropriate location in the vessel map. • To select multiple contiguous locations in a vessel, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them. • To select all the locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Select this Column. Conversely, to clear all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column. • To select all the locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Select this Row. Conversely, to clear all the selected locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Deselect this Row. **Tip:** To clear all the selected locations in the vessel map, or multiple contiguous locations, you can also press and hold the ALT key, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.
To select which images to export	Applicable only if more than a single image has been acquired per vessel location. By default, all images are selected for export. To select different images to export, open the Select Images dropdown list, clear the selections for the images that you are *not* exporting, and then click OK. **Note:** Remember, if multiple images were acquired for image sites, then the system considers the images ordered in rows from top to bottom, and then, within the rows, from left to right. Figure 2-13 on page 36.

7. Click Next.

The File Information page opens. You specify the export settings such as the target folder on this page.

*Figure 3-19:   Image Export wizard, File Information page*

8. Specify the file information.

Option	Description
Target Folder	The folder in which the exported images are stored. Click Browse to open the Select a folder to export to dialog box, and then browse to and select the appropriate folder.
File Format	The default value is dependent on the image type that is selected for export.
File Name Prefix	The exported file is automatically named according to the following: <FileName Prefix>_<Site/Well ID>_<Image Number><Date/Timestamp><File Format>, where the File Name Timestamp option determines the timestamp. See "File Name Timestamp" below. **Note:** The default value for the file name prefix is the Vessel ID for the selected vessel. You can leave this value as-is, or you can edit it as necessary.
File Name Timestamp	• To append the file name with the date and timestamp that the selected image was acquired, select Use Calendar Time. For example: VID2197_D7_1_2016y05m11d_21h36m.tif • To append the filename with the relative date and timestamp that the selected image was acquired, which is always 00d00h00m, clear Use Calendar Time. For example: VID2197_D7_1_.00d00h00m.tif

9. Click Export.

The Image Export wizard closes. You return to the Vessel View window. An Export Progress dialog box opens on top of the Vessel View window.

Figure 3-20:   Export Progress dialog box
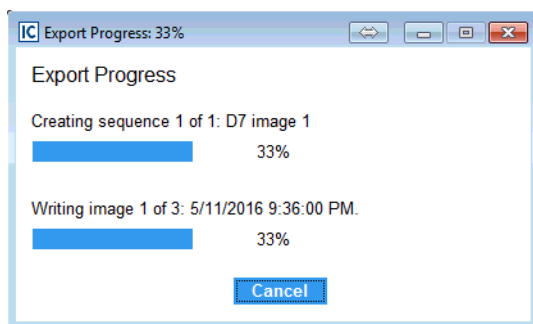
After the export progress is 100%, a Sequence Writing Complete dialog box opens. The dialog box indicates the number of files that were created successfully and asks you if you would like to open the target folder.

Figure 3-21:   Sequence Writing Complete dialog box

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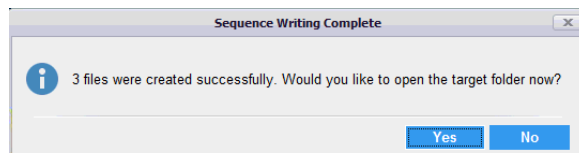
10. Do one of the following:

    • To close the wizard and return to the Vessel View page, click No.

    • To close the wizard and return to the Vessel View page and open the target folder, click Yes.

Section 3
Analyzing Images and Visualizing Results

Section Contents

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Chapter 2
Defining the Image Analysis

After viewing and accessing your vessel images, your next step is to select the images that are representative of the biology that is occurring in your vessel and define the analysis parameters. The parameters that you define based on these representative images are then applied to analyze all images within in the vessel. This chapter details the Launch Analysis wizard, which you use to define or select your image analysis parameters, and then launch the analysis of the images.

This chapter covers the following topics:

Launch Analysis Wizard Overview

You use the Launch Analysis wizard to analyze vessel images.

Figure 2-1:   Launch Analysis wizard
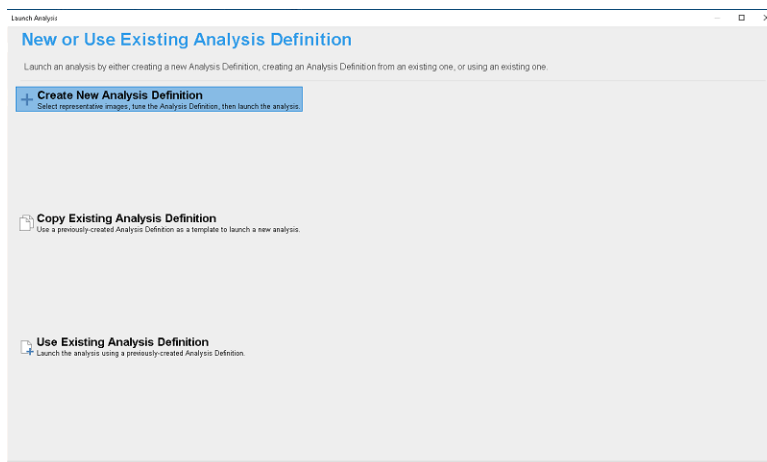
Three options are available in the wizard for analyzing vessel images: You can create a new analysis definition for analyzing vessel images, you can copy and modify an existing definition, or you can use an existing analysis definition as-is.

• To create a new analysis definition (the default value), see"Defining a New Analysis" on page 92.

• To copy and modify an existing analysis definition. See "Copying an Existing Analysis Definition" on page 103.

• To use an existing analysis definition, see "Using an Existing Analysis Definition" on page 105.

Defining a New Analysis

The following procedure provides high-level information for defining a completely new analysis for images. For defining a new analysis by copying and then modifying an existing analysis, see "Copying an Existing Analysis Definition" on page 103.

Defining a new analysis consists of the following:

- Indicating whether to create a new analysis definition, or use an existing definition. See "To create a new analysis definition" on page 92.

- Selecting the analysis type, which is dependent on the scan type. See "To select the Analysis Type" on page 93.

- Selecting the image channels that you are analyzing. See "To select the image channels for analysis" on page 94.

- Selecting the images that best represent the biology of interest that is occurring in the vessel. See "To select the images for analysis" on page 95.

- Refining the analysis parameters for each of the image channels as appropriate. See "To define the analysis parameters" on page 96.

- Selecting the scan times and images that you are analyzing. See "To select the scan times and wells/image sites" on page 98.

- Save and apply the analysis definition. See "To save and apply the analysis definition" on page 100.

- Confirming the analysis definition, and then launching the analysis. See "To review the analysis summary information" on page 101.

For the following procedures, the vessel is assumed to be a 96-well microplate and the analysis type is Basic Analyzer. The majority of information, however, is "vessel-agnostic" and offers a frame of reference for defining a new analysis for any scan type with any analysis type.

The Launch Analysis wizard uses the current display (zoom level, focus, and scan time) of the vessel in the Vessel View window. Before you open the wizard, optionally, you can set the zoom level of the Vessel View window to its Home value (100%) and you can confirm that the preferred scan time is selected on the Vessel Scanning Timeline.

To create a new analysis definition

1. Open the appropriate vessel in the Vessel View window. See "To open the Vessel View window" on page 64.

2. On the Analysis toolbar, click the Launch Analysis icon .

The Launch Analysis wizard opens. The New or Use Existing Analysis Definition page is open. Create New Analysis Definition is selected.

Figure 2-2: *Launch Analysis wizard, New or Use Existing Analysis Definition page*
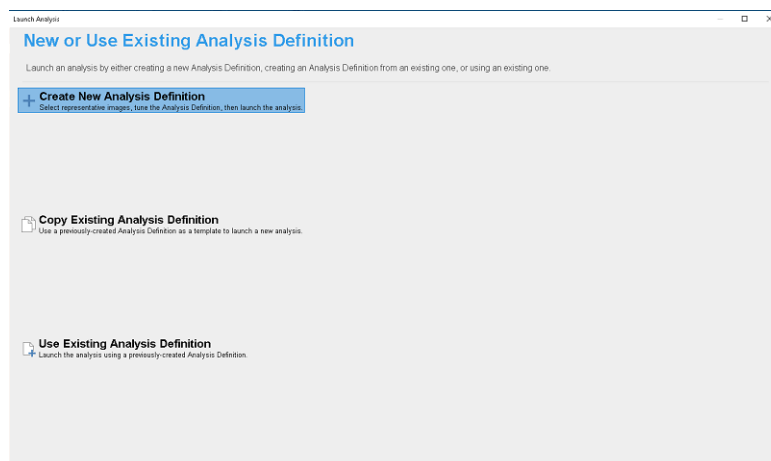
3. Leave Create New Analysis Definition selected, and then click Next.

The Analysis Type page opens.

4. Continue to "To select the Analysis Type" below.

To select the Analysis Type

The Analysis Type page displays the analysis types that are available are based on the scan type and the image channels that were collected during scanning. Basic Analyzer is always available.

Figure 2-3: *Launch Analysis wizard, Analysis Type page*
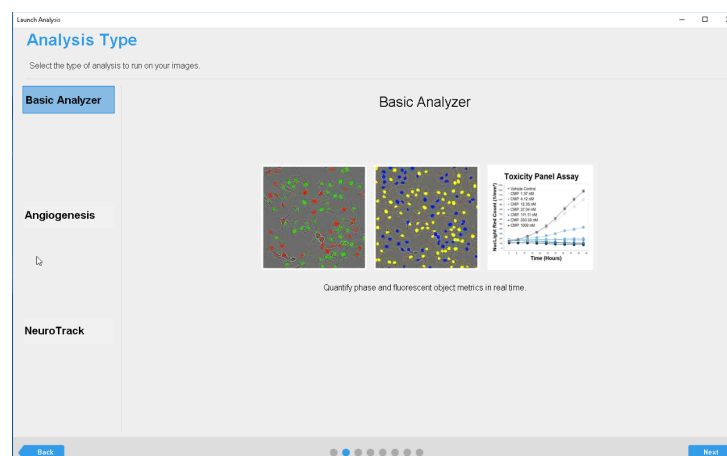
1. Select the analysis type (In this example, Basic Analyzer is selected).

2. Click Next.

The Image Channels page opens.

3. Continue to "To select the image channels for analysis" below.

To select the image channels for analysis

The Image Channels page displays all the possible image channels (Phase, Red, and Green) for the analysis. By default, all the channels for which images were acquired are selected.

Overlap is applicable for co-incident object analysis (red and green objects overlap) and is not discussed here.

Figure 2-4:   Launch Analysis wizard, Image Channels page
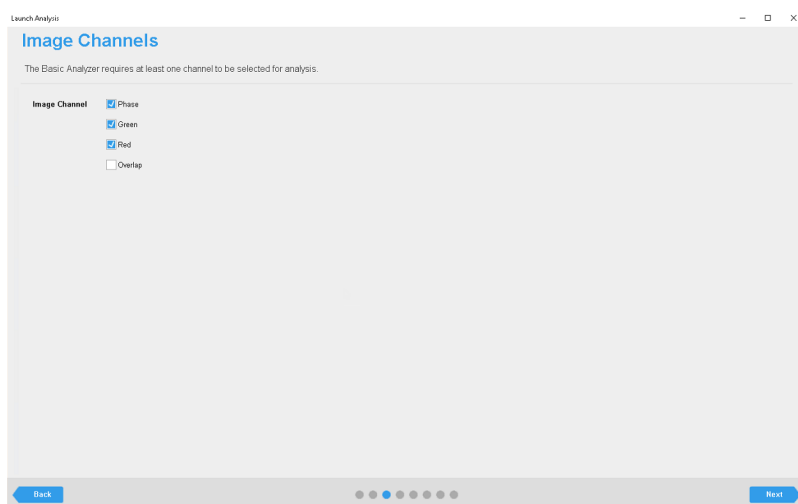
1. Select the channels for analysis. When selecting the channels, note the following:

    • To limit your analysis to a green object and/or red object count, leave Green and/or Red selected as appropriate, and clear Phase.

    • To include the Phase metric in your analysis, leave Phase selected.

In this example, all three channels are selected.

2. Click Next.

    The Image Set Selection page opens.

3. Continue to "To select the images for analysis" on page 95.

To select the images for analysis

You use the options on the Image Set Selection page to select the images that best represent the biology of interest that is occurring in the vessel so that you can preview and refine the analysis before applying it to all the images that you are analyzing. When selecting these representative images, note the following:

- All the options that are available on the Image Set Selection selection page are identical to that on the Vessel View page. With the exception of the spectral unmixing option, you can use all the options to help you select the images that you are analyzing. For example, if you did not have the correct scan time selected in the Vessel View window before you opened the Launch Analysis wizard, then you can open the Vessel Scanning Timeline for the selected vessel and scroll through and select a different scan time, you can use the options on the Navigation toolbar to zoom in on the selected vessel location to confirm that it shows the biology of interest, and so on. See

- Typically, two to four images are adequate for defining the analysis; however, if the biology is significantly different across samples, then you can select more images. Ideally, regardless of the number of images, one of these images should be a control image.

- The images need not have been acquired at the same scan time. You can select images that have been acquired at different scan times. Select the appropriate scan time on the Vessel Scanning Timeline popup to update the vessel display with the images that were acquired at the selected time.

Figure 2-5:   Launch Analysis wizard, Image Set Selection page
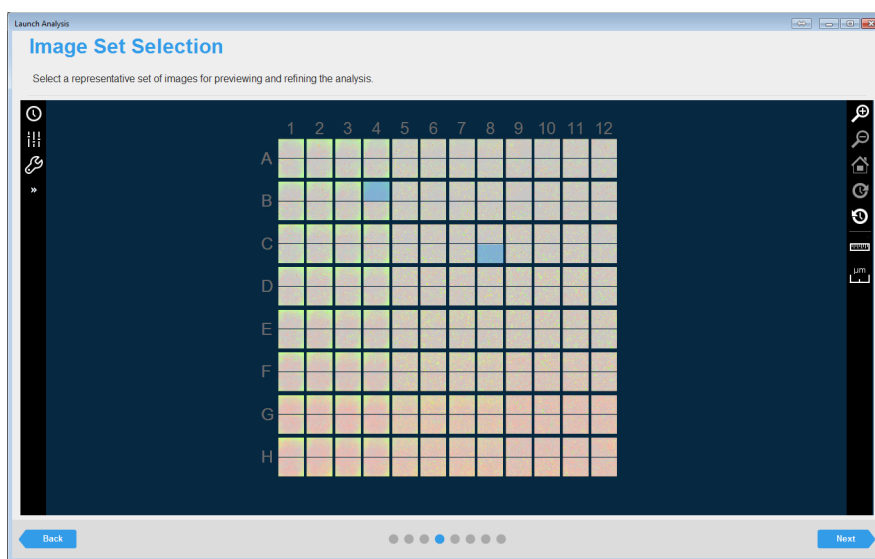
1. To select an image site for analysis, click the site.

   As you hover over a site, the mouse pointer changes to a blue plus sign. After you click a site to select it, the pointer changes to a red X and the selected site is filled with a solid light blue color. To clear an image selection, click it again.

2. Click Next.

   The Analysis Definition page opens.

3. Continue to

To define the analysis parameters

You use the options on the Analysis Definition page to initially define the analysis parameters based on the images that you just selected, and then iteratively, preview and refine the analysis parameters until you are satisfied that the definition can be applied to all the images that you select for analysis.

Figure 2-6:   Launch Analysis wizard, Analysis Definition page
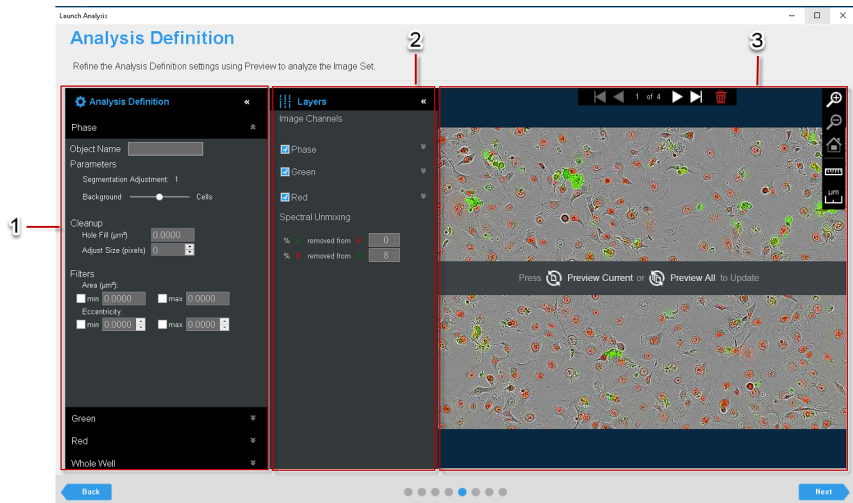
The Analysis Definition page has the following layout:

	Window component	Description
1	Analysis Definition pane	Displays the image channels that will be used to test the analysis definition. For example, in Figure 2-6 above, all three image channels (Phase, Red, and Green) were selected for analysis.
		Note: If the analysis options for a channel are collapsed, then the Expand/ Collapse icon is displayed as an inverted double arrow ⊻. To expand the option, click the icon. The icon is now displayed as an upright double arrow ⌃. To collapse the toolbar option, click the icon again.
		Note: Whole Well is always displayed but it is applicable only for whole well imaging, dilution cloning, and chemotaxis.
2	Layers pane	Displays the image channel layers, which can be turned on or off to help define the analysis parameters.
		Note: The spectral unmixing values that are displayed in the Layers pane are for informational purposes only. You cannot adjust spectral unmixing values in the Launch Analysis wizard.
		Note: Changes made in the image channel, for example, autoscale or contrast aid in evaluating the Analysis Mask. Changing these values does not affect the analysis definition.

	Window component	Description
3	Image pane	Displays the images that were selected for creating the analysis definition. A navigation bar at the top of the pane indicates 1 out of <n> total images. For example, in Figure 2-6 on page 96, four total images were selected for the analysis definition, so the navigation bar shows 1 of 4. You use the Preview options that are displayed in the Image pane to preview the image that is currently selected (as shown in the Navigation bar), and to Preview All selected images, which updates the analysis parameters and filters across all the image channels for all the images that were selected for testing the analysis definition.
		Tip: At any time, you can hold your mouse pointer over any object in an image to open a tooltip that displays information about the object.

When refining the analysis definitions for an image channel:

- You should define the analysis parameters for the image channels in the following order: Phase, Green, and Red.

- Prior to changing any parameters, you use the default values that are provided for the analysis definition.

- Ideally, you should define only a single analysis parameter at a time.

1. Leave the first image that you selected for defining the analysis parameters displayed in the Image pane, or use the Navigation toolbar at the top of the pane to select a different image.

2. Refine the analysis definition settings for each of the image channels as appropriate based on your selected analysis type.

3. Click Preview All.

   The analysis parameters are applied to all the images that you selected.

4. Use the Navigation toolbar at the top of the Image pane to move to each image and then toggle your masks on and off to confirm that the analysis parameters that you have defined are appropriately mapping your objects of interest in each image. Make adjustments where necessary. If you make any adjustments, then you can preview a single image or preview all images as appropriate. When previewing your images, note the following:

   - If you decide that you do not want to include the image during the analysis definition, you can click the Remove Image icon 🗑 at the top of the Image pane to remove the image.

   - If you decide that you must include more or different images during the analysis definition, click Back once to return to the Image Set Selection page, select the additional image or images, and then click Next to return to the Analysis Definition page.

5. Click Next.

⚠️ *Next is available only after you have previewed all your selected images.*

The Scan Times and Wells page opens. Continue to "To select the scan times and wells/image sites" on page 98.

To select the scan times and wells/image sites

You use the options on the Scan Times and Wells page to select the scan times and images that you are analyzing. You also indicate whether to analyze futures scans for the vessel according to the currently defined parameters.

Figure 2-7:   Scan Times and Wells page
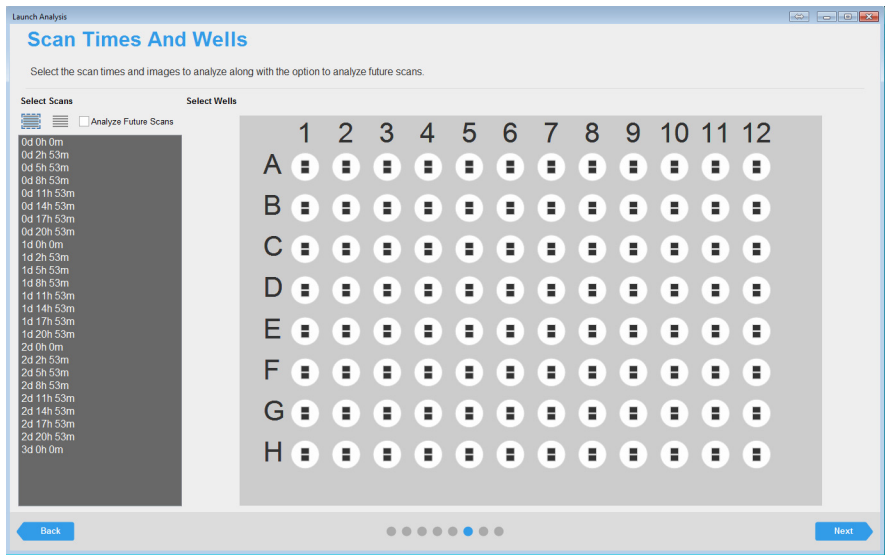
1. Select the scan times and image sites for which you are analyzing the data.

Option	Description
To select scan times	• To select all scans in a single step, under Select Scans, click the Select All Scans icon (the left icon that is displayed in blue). To clear an individual scan, click it. • In the Vessel Scanning Timeline, do either one or both of the following: 　• To select an individual scan, click the scan. You can select multiple, individual scans. To clear an individual scan, click it again. 　• To select a range of scans, click the first scan in the series of scans, press and hold the Shift key, and then click the last scan in the series of scans. • To clear all selected scans in a single step, under Select Scans, click the Clear All Scans icon (the right icon that is displayed in gray).

Option	Description
To select wells/image sites	• To select a single location in a vessel, for example, a well in a 96-well microplate, click once in the appropriate location in the vessel map. • To select multiple contiguous locations in a vessel, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them. • To select all the locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Select this Column. Conversely, to clear all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column. • To select all the locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Select this Row. Conversely, to clear all the selected locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Deselect this Row. **Tip:** To clear all the selected locations in the vessel map, or multiple contiguous locations, you can also press and hold the ALT key, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.

2. Optionally, to run the defined analysis on all future scans of this vessel (the images are analyzed in real time), select Analyze Future Scans.

If you select Analyze Future Scans, then the vessel can remain in the Incucyte instrument. If the vessel has been removed, then you can use the Restore function to scan it at a later time.

3. Click Next.

   The Save and Apply Analysis Definition page opens.

4. Continue to

To save and apply the analysis definition

If you are following this procedure when working with an existing analysis definition, then the Analysis Notes page replaces the Save and Apply Analysis Definition page. The Definition Name is read-only. You can enter only analysis notes on this page.

Figure 2-8:   Launch Analysis wizard, Save and Apply Analysis Definition page

1. In the Definition Name field, enter the name for the analysis definition.

Enter a name that is descriptive for the experiment/assay that you are carrying out, for example, HT-1080 Apoptosis The name does not need to include any user identification (the name of the user who is defining the assay). The user name is automatically associated with the assay and is displayed in the Analysis tooltip in the Vessel View window. See Figure 2-6 on page 62.

2. Optionally, in the Analysis Notes field, enter pertinent notes about the analysis.

3. Click Next.

    The Summary page opens.

4. Continue to "To review the analysis summary information" on page 101.

To review the analysis summary information

Figure 2-9:   Launch Analysis wizard, Summary page

The Summary page displays all the selections/settings that you have specified for the image analysis, organized by wizard page. Each section heading corresponds to a wizard page, and is a hyperlink to the indicated page.

1. Review the information for the planned analysis, and then do one of the following:

    *   If all the selections/settings are correct for the analysis, then continue to Step 2.

    *   If you must edit any of the selections or settings, then click the appropriate section header/page name to return to the page in the wizard. Edit the selections/settings as appropriate, and then click Next to move through the wizard (making additional edits as required) until you return to the Summary page, and then continue to Step 2.

> *Any edit that you make to the selections/settings for a scheduled vessel might affect downstream selections/settings. For example, if you select a different analysis type, then you must edit the analysis parameters for an image channel. As a result, you always have to move through the remaining pages on the wizard after you edit any selections/settings for the vessel.*

2. Click Finish.

    The analysis is launched and an Analysis Status dialog box opens. The dialog box displays all the analyses that are currently taking place in your Incucyte system in reverse chronological order based on their launch dates and times.

Figure 2-10:   Analysis Status dialog box

You can now view information about the analysis in two locations in the software:

*   You can view the information that is displayed in the Analysis Status dialog box on the Status page. On any page menu, click Status and view this information in the Analysis pane (lower right corner) on the Status page.

Figure 2-11:   Status page
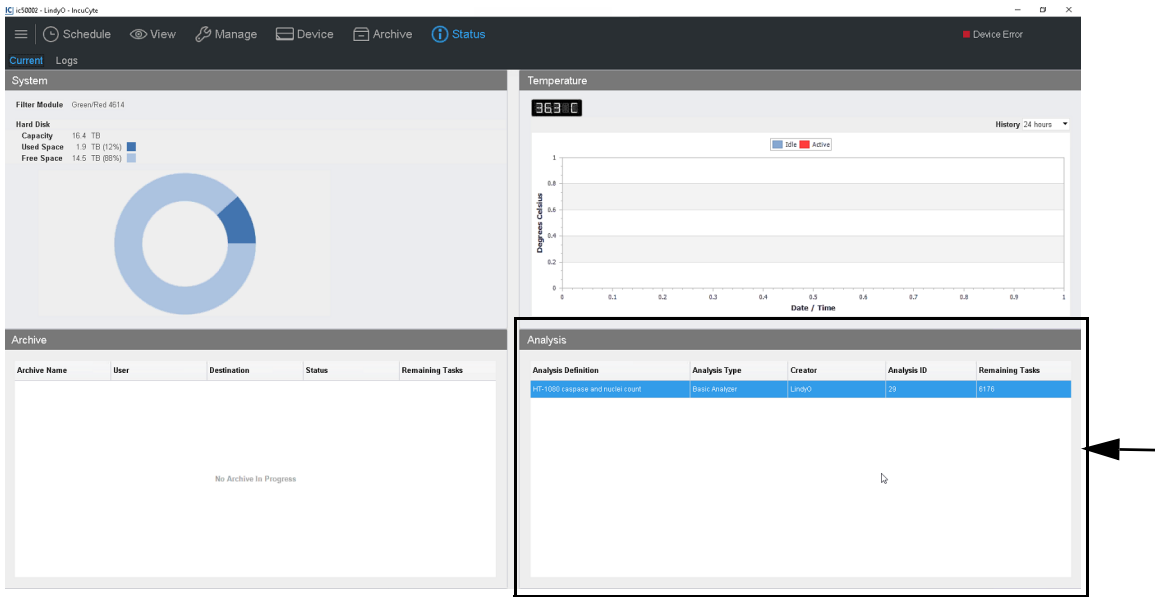
*   You can open the Scanned Vessels window, and then open the Analysis pane for the vessel. While the analysis is in progress, the pane displays the analysis without a Date Completed timestamp. After the analysis is completed, if you refresh the Analysis pane, a Date Completed timestamp is displayed.

Figure 2-12:   Analysis pane showing all the analyses (in progress and completed) for a selected vessel
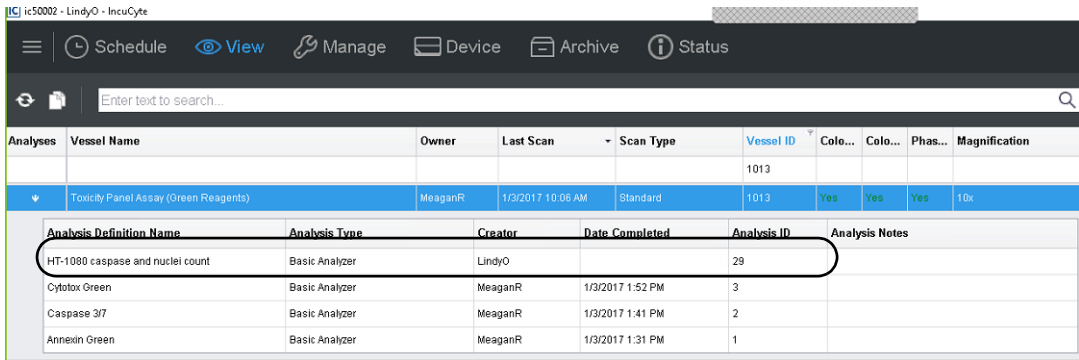
Copying an Existing Analysis Definition

If an existing analysis definition is similar to the new definition that you are creating, then you can copy the existing definition, which is considered to be a template, and then edit it as appropriate to create a new definition. When you select an existing analysis definition to copy, you must select one that has the required channels and objective. (To add or remove channels in the analysis, you must create an entirely new definition and because the objective is set during acquisition, you cannot select an objective in the launch analysis workflow.)

For detailed information about creating a completely new analysis definition, see "Defining a New Analysis" on page 92.

To copy an existing analysis definition

1. Open the appropriate vessel in the Vessel View window. See "To open the Vessel View window" on page 64.

2. On the Analysis toolbar, click the Launch Analysis icon .

   The Launch Analysis wizard opens. The New or Use Existing Analysis Definition page is open. Create New Analysis Definition is selected.

Figure 2-13:   Launch Analysis wizard, New or Use Existing Analysis Definition page
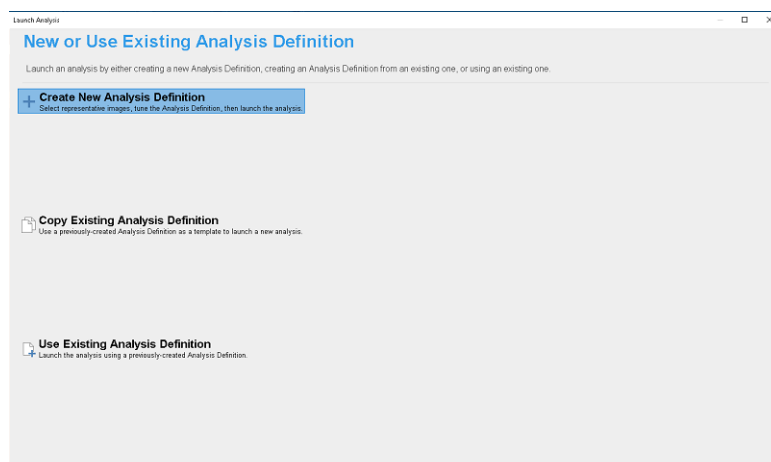
3. Select Copy Existing Analysis Definition, and then click Next.

   The Copy Analysis Definition page opens. The page lists all the analysis definitions that have been saved to the database and that can be used as a template to create a new definition. See Figure 2-14 on page 104.

Figure 2-14:   Launch Analysis wizard, Copy Analysis Definition page
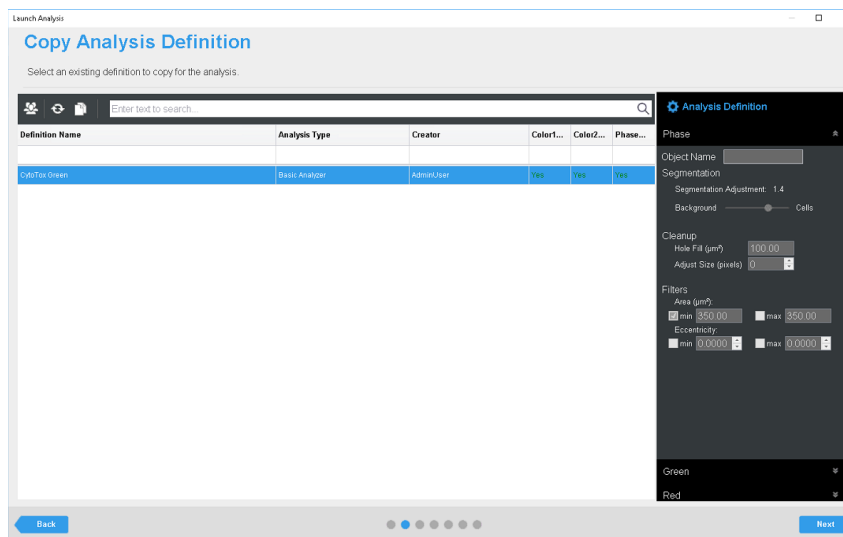
4. Select the analysis definition that you are using as your template.

All the analysis parameters for the selected definition are displayed in read-only mode in the Analysis Definition (right) pane on the page. Before continuing, make sure to confirm that the selected analysis definition has the correct objective and image channels.

The Analysis Definition page has the same search and filter features that you have encountered on other wizard pages, for example, on the Vessel Type Search page in the Add Vessel wizard. (See "To select the vessel type" on page 32.) You can use these features to help you quickly search for and select the appropriate analysis.

5. Click Next.

    The Image Set Selection window opens. With the following exceptions, the remainder of the procedure is now identical to that when creating a new analysis definition.

    •   On the Save and Apply Analysis Definition page, the Definition Name field is pre-populated with the name of the copied analysis definition appended with the word "Copy," for example, CytoToxGreen_Copy. You can edit this value.

    •   On the Summary page, the first heading is Copy Existing Analysis Definition.

6. Continue to "To select the images for analysis" on page 95.

Using an Existing Analysis Definition

Because you cannot edit any of the analysis parameters for an existing analysis definition, you must use one that is defined for your scan type, vessel type, objective, and all the color channels and phase that you used for your analysis.

To use an existing analysis definition

1. Open the appropriate vessel in the Vessel View window. See "To open the Vessel View window" on page 64.

2. On the Analysis toolbar, click the Launch Analysis icon .

   The Launch Analysis wizard opens. The New or Use Existing Analysis Definition page is open.

Figure 2-15:   Launch Analysis wizard, New or Use Existing Analysis Definition page
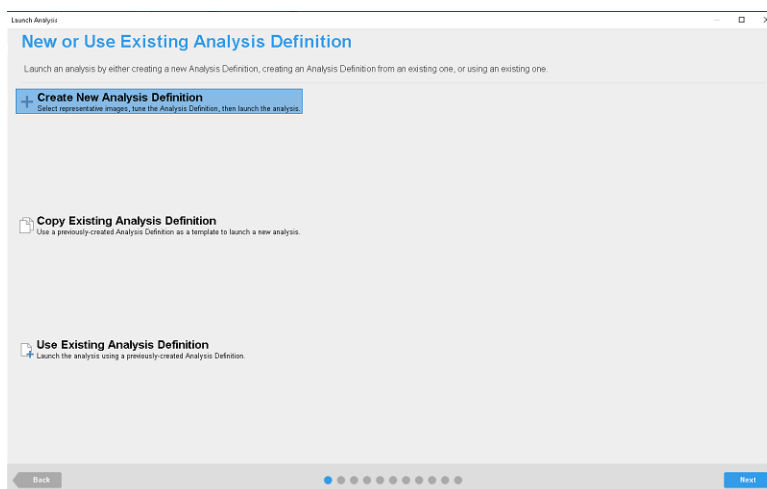
3. Select Use Existing Analysis Definition.

   The Analysis Definition page opens. The page lists all the current analysis definitions for your scan type and vessel type (microplate, flask, or so on) that use the same objective and at least one of the same channels for image acquisition. See Figure 2-16 on page 106.

Figure 2-16:   Launch Analysis wizard, Analysis Definition page
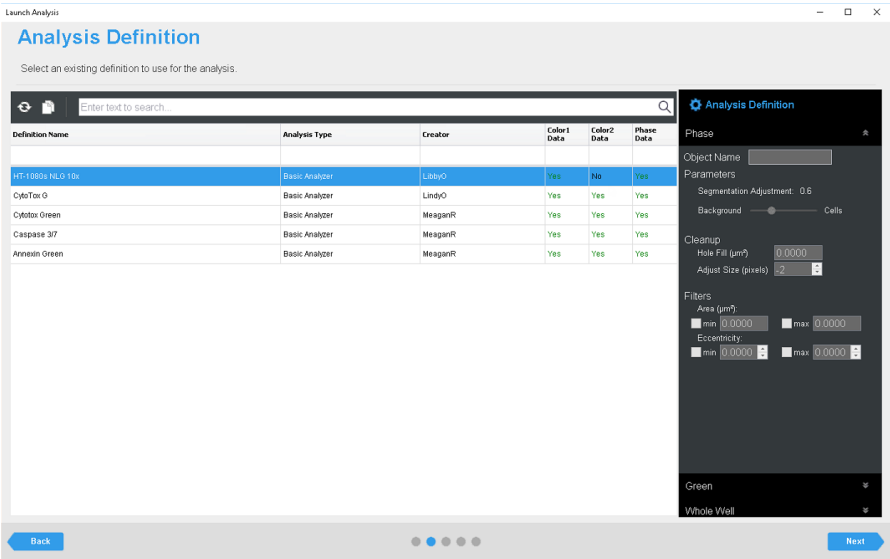
4.   Select the correct analysis definition.

All the analysis parameters for the image channels are displayed in read-only mode in the Analysis Definition (right) pane on the page. Before you select an analysis definition, make sure to view the relevant information for each image channel to confirm that values are appropriate for your analysis.

The Analysis Definition page has the same search and filter features that you have encountered on other wizard pages, for example, on the Vessel Type Search page in the Add Vessel wizard. (See "To select the vessel type" on page 32.) You can use these features to help you quickly search for and select the appropriate analysis.

5.   Click Next.

The Scan Times and Wells page opens. With one exception, the remainder of the procedure is now identical to that when creating a new analysis definition.

The Analysis Notes page replaces the Save and Apply Analysis Definition page. The Definition Name is read-only. You can enter only analysis notes on this page.

6.   Continue to "To select the scan times and wells/image sites" on page 98.

Chapter 2
Visualizing the Analysis Results

After your analysis is complete, the Incucyte software provides several ways for visualization and investigation of your results, including the Vessel View with Analysis window and the graphing of your analysis results. This chapter details the various ways that you can visualize your analysis results so that you can access and interpret your data and identify the keys areas of your assay that worked well as well as the areas that need attention or improvement.

This chapter covers the following topics:

- "Viewing the Analysis Masks" on page 109.

- "The Graph Metrics Window" on page 110.

- "Graphing the Analysis Results for a Microplate" on page 114.

- "Graphing the Analysis Results for Any Vessel Type" on page 118.

- "Graphing Analysis Results as a Histogram" on page 123.

- "Graphing Analysis Results as a Concentration Response Curve" on page 125.

- "Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve" on page 128.

- "Exporting Graph Data" on page 132.

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Viewing the Analysis Masks

After your vessel data has been analyzed, you can open the Vessel View window, which now displays the associated analysis masks that were applied to the images. (See "Open Analysis" on page 63.) To view the analysis masks, open the Image Channels popup (see "The Image toolbar" on page 70), and then in the Analysis Masks pane, toggle on and off the analysis masks as appropriate.

Figure 2-1: *Vessel View with the associated analysis window, toggling image masks on and off*
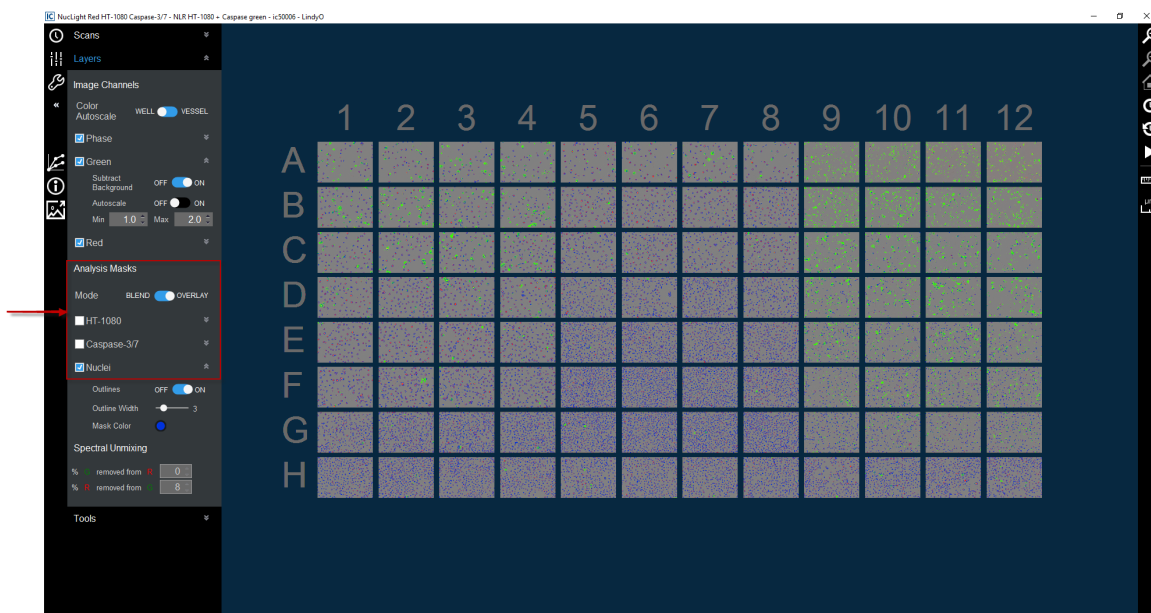
The Graph Metrics Window

The Graph Metrics window is the starting point for graphing your analysis results. The window provides all the functionality for graphing the analysis results for a microplate (Microplate Graph) or vessel (Vessel Graph), and for exporting the analysis results (Export Data). You can graph the results for a single metric at a time, where the metric is a pre-defined metric or a user-defined metric. To open the Graph Metric window, do one of the following:

- On the Vessel View window that shows the analysis masks, click the Graph Metrics icon ⬚. See "Viewing the Analysis Masks" on page 109.

- In the Vessel View window, right-click the analysis and on the context menu that opens, click Graph Analysis Metrics. See "To view the analysis definitions for a scanned vessel" on page 62.

Figure 2-2:   Graph Metrics window
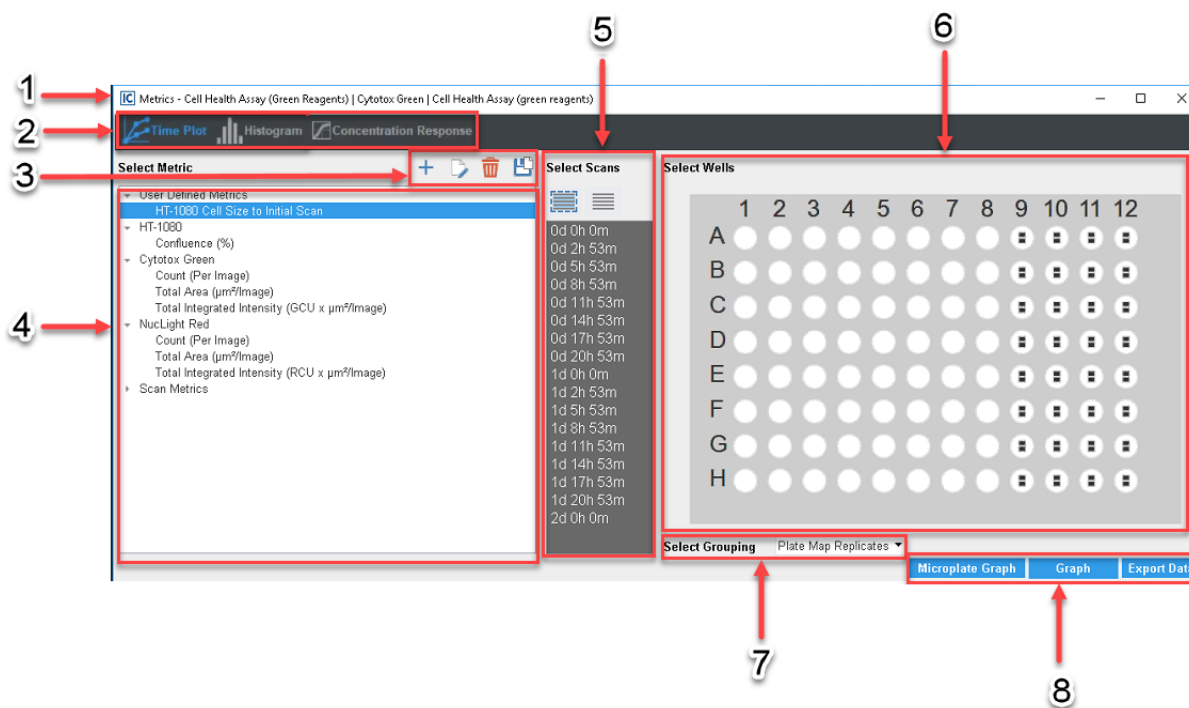
The Graph Metric window has the following layout:

	Window component	Description
1	Title bar	Displays the name of the currently selected analysis.
2	Graph menu bar	Displays the three types of graphs that you can plot for analysis results: a Time Plot (the default value), a Histogram, or a Concentration Response curve.

	Window component	Description
3	Metric menu bar: Displays options for working with a user-defined metric:	
	+	Create Metric: Opens the Create Metric dialog box, which contains all the options for defining a new metric for the analysis. See "To define user metrics" on page 112.
		Edit Metric: Opens the Edit Metric dialog box, which displays all the values for the currently selected user-defined metric. You can edit these values as needed and save the edited metric. Note: This action does not change any data for the currently selected analysis. If the metric was linked to the analysis definition, this action also does not affect the data for any other analysis that used the same analysis definition.
	🗑	Delete Metric: Deletes the currently selected user-defined metric from the analysis. Note: This action does not delete any data for the current analysis. If the metric was linked to the analysis definition, this action also does not affect the data for any other analysis that used the same analysis definition. The metric is simply not available going forward for any other analyses.
		Apply Metric: Saves and links the currently selected user-defined metric to the analysis definition that was used to generate the current data. This user-defined metric will now always be available when you use the same analysis definition for any other vessel.
4	Metrics pane	Always displays the pre-defined Analysis and Scan metrics that can be plotted for your analysis. Scan metrics are always displayed for any vessel. The analysis metrics that are available for graphing are specific to the channels that were used to acquire the images. The analysis metrics are grouped by image channel (click the Expand/Collapse icon next to a grouping to view the metrics in the grouping), and the name for each analysis metric is preceded by the object name that was specified in the analysis definition for the channel. If any user variables have been defined for the analysis, then these metrics are displayed under the heading of User Defined Metrics. Note: If you opened the Graphic Metrics window for a vessel from the Vessel View window (no analysis), then only the Scan metrics for the vessel are available for graphing. See "Analysis toolbar: The Analysis toolbar contains two icons." on page 68.
5	Scan pane	Displays the options for selecting the scans for which you are graphing the metrics.
6	Vessel pane	Displays the acquisition map for the scanned vessel. The number of images that have been acquired for each vessel location (as specified in the Add Vessel wizard) is indicated by the number of individual squares in the vessel locations.

	Window component	Description
7	Select Grouping dropdown list	Displays the options for specifying how to group the data points on the Y axis. The default values are the following: • If the vessel is a microplate and a plate map has been specified, then the data is grouped by Plate Map replicates. • If the vessel is a microplate and a plate map has *not* been specified, then the default grouping is "None." • If the vessel is any vessel other than a microplate, then the default grouping is "None." Other options are All, Columns, and Rows. **Note:** For information about exporting graph data, "Exporting Graph Data" on page 132.
8	Window options	• Microplate Graph: Option to graph the analysis results specifically for a microplate. • Vessel Graph: Option to graph the analysis results for any vessel type, including a microplate. • Export Data: Option to export selected analysis results to your client clipboard or to a text file.

To define user metrics

Basic Analyzer, Cell-by-Cell, and Cell-by-Cell Classification analyses support the creation of user-defined metrics for the purpose of normalizing data. You can define user metrics for these analyses that are based on division calculations to normalize data to another metric, to a scan time, or to both. For example, for an apoptotic event, you might normalize to another metric, where the metric Green/Area/Confluence is normalized to the metric Phase/Area/Confluence. To account for changes in seeding density, you might normalize to a scan time, where the scan time that the data is normalized to is the starting scan time.

1. On the Metric menu bar, click the Create Metric icon.

    The Create Metric dialog box opens.

Figure 2-3:   Create Metric dialog box

2. Enter the values—Channel, Metric, and Value—for the metric that you are normalizing.

The selected Metric value determines the available options for Value.

3. Do one of the following:

    • To normalize to another metric, enter the metric value—Channel, Metric, and Value—for Normalized to (by division).

    • To normalize to a scan time, on the Normalize to Scan dropdown list, select the scan time.

    • To normalize to both another metric and a scan time, enter the metric value—Channel, Metric, and Value—for Normalized to (by division) *and* on the Normalize to Scan dropdown list, select the scan time.

4. For Display as, leave Ratio selected (the default value), or select Percent.

5. The default name for the metric indicates the division calculation that is being carried out, for example, when normalizing to a metric, Green Area/Phase Area. You can leave the default name as-is, or optionally, in the Name Override field, enter a different name for the metric.

6. Click OK.

   The Create Metric dialog box closes. The metric is displayed in the Metrics pane under the heading User Defined Metrics.

7. Optionally, to save and link the user-defined metric to the analysis definition that was used to generate the current data, on the Metric menu bar, click the Apply Metric icon.

   This user-defined metric is now always be available when you use the same analysis definition for any other vessel.

8. You can now graph this user-defined metric. Continue to one of the following:

    • "Graphing the Analysis Results for a Microplate" on page 114.

    • "Graphing the Analysis Results for Any Vessel Type" on page 118.

    • "Graphing Analysis Results as a Histogram" on page 123.

Graphing the Analysis Results for a Microplate

You use the Microplate Graph option to graph the analysis results specifically for a microplate. The graph that is produced is displayed in the same layout as the selected microplate, with an individual data point plotted for each scan time that was selected for graphing in every well that was selected for graphing. If a plate map was specified, then the colors that are displayed in the graph correspond to the colors that were defined in the plate map. After the default graph is generated and displayed, options are available for customizing the graph, including options that are required for sharing the graph in presentations or reports.

Figure 2-4:   Microplate graph example
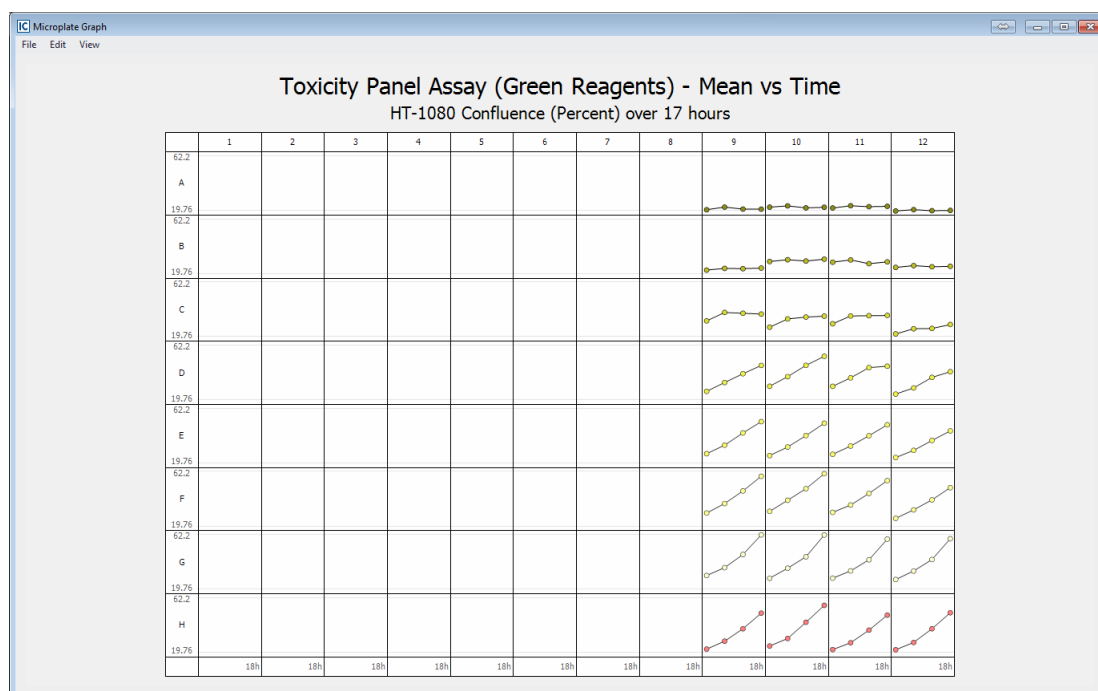
In this example, we assume a 96-well plate for which a plate map was specified. Four scan times were selected for graphing and image data in Wells 9 through 10 in Rows 1 through 8 was selected for graphing.

To graph the analysis results for a microplate

1. Open the Graph Metrics window. See "The Graph Metrics Window" on page 110.

2. On the Menu bar, leave Time Plot selected.

3. In the Metrics pane, select the metric for which you are graphing the analysis results.

You might have to click the Expand/Collapse icon next to a grouping to expand the grouping and view the available metrics.

4. Select the scan times and images that you are graphing.

Option	Description
To select scan times	• To select all scans in a single step, under Select Scans, click the Select All Scans icon (the left icon that is displayed in blue). To clear an individual scan, click it. • In the Vessel Scanning Timeline, do either one or both of the following: • To select an individual scan, click the scan. You can select multiple, individual scans. To clear an individual scan, click it again. • To select a range of scans, click the first scan in the series of scans, press and hold the Shift key, and then click the last scan in the series of scans. • To clear all selected scans in a single step, under Select Scans, click the Clear All Scans icon (the right icon that is displayed in gray).
To select wells/image sites	• To select a single location in a vessel, for example, a well in a 96-well microplate, click once in the appropriate location in the vessel map. • To select multiple contiguous locations in a vessel, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them. • To select all the locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Select this Column. Conversely, to clear all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column. • To select all the locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Select this Row. Conversely, to clear all the selected locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Deselect this Row. **Tip:** To clear all the selected locations in the vessel map, or multiple contiguous locations, you can also press and hold the ALT key, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.

5. On the Select Grouping dropdown list, select the grouping option.

6. Click Microplate Graph.

    The Microplate Graph is generated and displayed onscreen in its own window. A menu (File, Edit, View) is displayed at the top of the graph window and a default title is displayed above the graph. The display is color-coded according to the colors that were specified in the plate map.
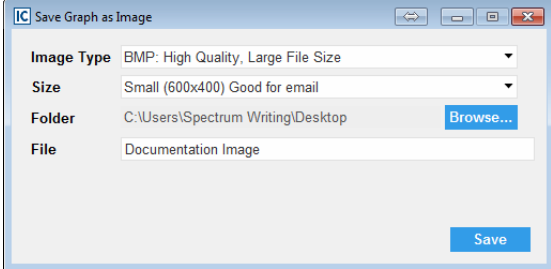
7. Optionally, after the microplate graph is generated, do any of the following to customize the graph display:

Option	Description
Edit	
Titles and Subtitles	Opens the Edit Titles dialog box. You use the options on this dialog box to edit the default title for the graph and/or add your own custom subtitles. You can also change the maximum font size of the title and subtitles. *Figure 2-5:* *Edit Titles dialog box*
Reset Axis Scales	Opens the Edit Axes dialog box. You use the options on this dialog box to edit the system-generated values for the X and Y axes. You can always click Reset values to original and/or Reset time range to original to reset the values to their system- generated values. *Figure 2-6:* *Edit Axes dialog box*
View	
Logarithmic Y Axis	Click to toggle the display of the Y axis between a decimal scale and a logarithmic scale.
Show Time Axis	Selected by default. Click to toggle the display of the X axis on and off.
Show Plate Map Colors	Available only for a microplate for which a Plate Map is specified. Selected by default. The data points are plotted in the colors as specified in the Plate Map Editor. Click to toggle the color display on and off. If you toggle this option off, then all the data points are displayed in blue.
Marker Size	The size of the data points in the graph. Available options are Small, Medium (the default value), and Large. As you select a different size, the graph display is dynamically updated.

8. Optionally, open the File menu, and then do any or all of the following as appropriate:

Option	Description
Save Graph as Image	Opens the Save Graph as Image dialog box. You use the options on this dialog box to save the displayed graph as image in a specific format (BMP, JPEG, PNG, or TIFF) and with a specific size. To save the file, you must select the location in which to save the image file, and you must name the file. *Figure 2-7:* *Save Graph as Image dialog box*
Print	Opens the standard Windows Print dialog box for printing the graph.
Print Preview	Opens a Print Preview dialog box in which you can view what the graph looks like before it is printed, and make adjustments to the graph (such as zooming in on an area, or changing the number of pages across which you are printing the graph) before printing the graph.

Graphing the Analysis Results for Any Vessel Type

You use the Graph option to graph the analysis results for any vessel type, including a microplate for which a plate map was specified. The graph that is generated is a standard time plot graph, with the selected scan time (Time) on the X axis and the selected metric on the Y axis. If the range of scan times is less than or equal to 96 hours, then, by default, the time is displayed in hours. If the range of scan times is greater than 96 hours, then, by default, the time is displayed in days. After the default graph is generated and displayed, options are available for customizing the graph, including options that are appropriate for sharing the graph in presentations or reports. You can graph a single metric for the analysis results for any vessel type, or you can graph multiple single metrics for the analysis results for any vessel type, and then combine the individual metric graphs in to a single multi-metric graph, which is referred to as the Graph on Graph option.

Figure 2-8:   Graph example
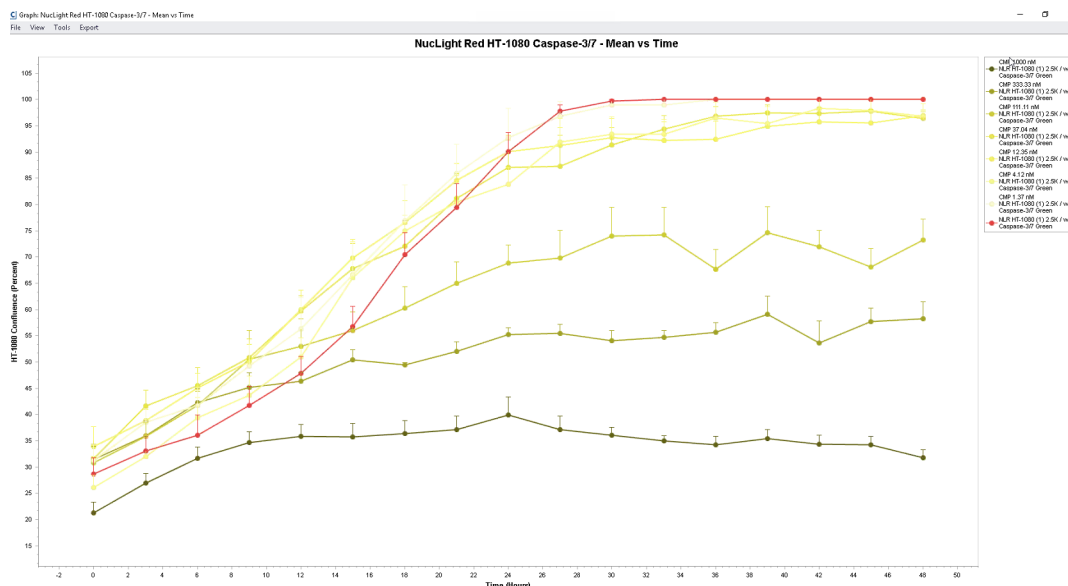
> The following procedure assumes a 96-well plate for which a plate map was specified. The majority of information, however, is "vessel-agnostic" and offers a frame of reference for graphing data for any vessel type.

To graph the analysis results for any vessel type (Single metric)

1. Open the Graph Metrics window. See

2. On the Menu bar, leave Time Plot selected.

3. In the Metrics pane, select the metric for which you are graphing the analysis results.

You might have to click the Expand/Collapse icon next to a grouping to expand the grouping and view the available metrics.

4. Select the scan times and images that you are graphing.

Option	Description

To select scan times	• To select all scans in a single step, under Select Scans, click the Select All Scans icon (the left icon that is displayed in blue). To clear an individual scan, click it.
	 • In the Vessel Scanning Timeline, do either one or both of the following:
	   • To select an individual scan, click the scan. You can select multiple, individual scans. To clear an individual scan, click it again.
	   • To select a range of scans, click the first scan in the series of scans, press and hold the Shift key, and then click the last scan in the series of scans.
	 • To clear all selected scans in a single step, under Select Scans, click the Clear All Scans icon (the right icon that is displayed in gray).
	 Note: For an endpoint analysis, select a single time point to generate the corresponding bar graph. See Figure 2-9 on page 120.

To select wells/image sites	• To select a single location in a vessel, for example, a well in a 96-well microplate, click once in the appropriate location in the vessel map. • To select multiple contiguous locations in a vessel, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them. • To select all the locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Select this Column. Conversely, to clear all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column. • To select all the locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Select this Row. Conversely, to clear all the selected locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Deselect this Row. **Tip:** To clear all the selected locations in the vessel map, or multiple contiguous locations, you can also press and hold the ALT key, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.

5. On the Select Grouping dropdown list, select the option for grouping the data.

If the vessel is a microplate and a plate map has been specified, then by default, Plate Map Replicates is selected and this is the recommended value; otherwise, if a plate map has not been specified or the vessel is any other vessel type, then None is the default value and you can select another value (All, Rows or Columns) as appropriate. In this example, we assume that Plate Map Replicates is selected.

6. Click Graph.

   The Graph is generated and displayed onscreen in its own window (the Graph window). If the vessel is a microplate and a plate map has been specified, then information from the plate map (colors, concentrations, and so on) is carried over for the legend. You can hold your mouse pointer over any data point in the graph to open a tooltip that displays information about the data point.

7. Optionally, after the time plot graph is generated, options are available for customizing the graph display and exporting the graph data. See "Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve" on page 128 and/or "Exporting Graph Data" on page 132.

Figure 2-9:   Endpoint analysis graph (Single time point and Plate Map Replicates selected)
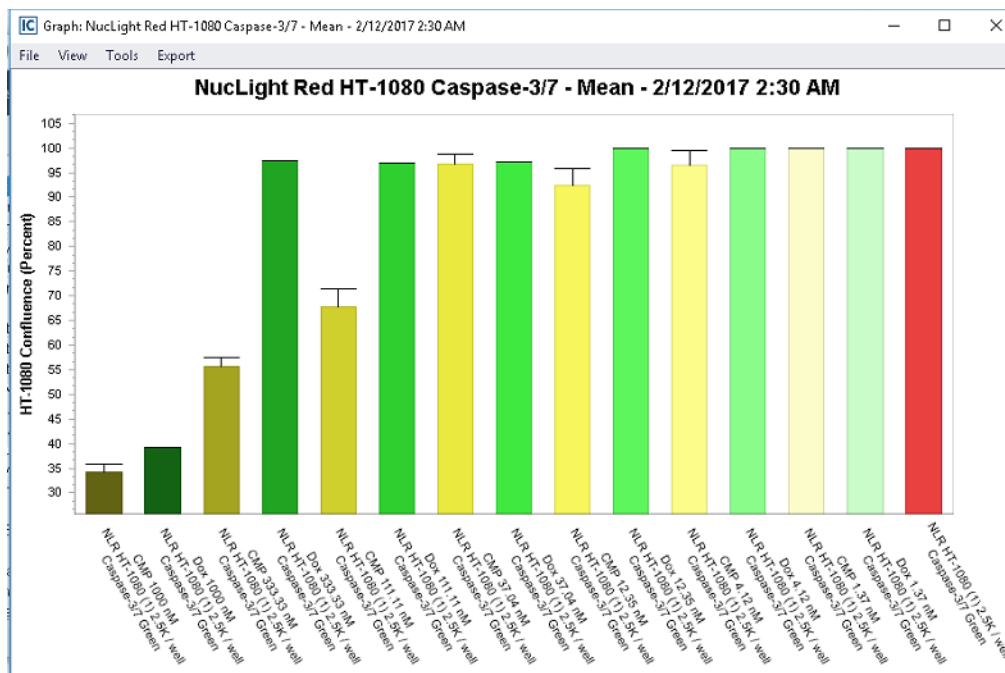
To combine multiple metrics into a single graph (Graph on Graph)

1. For *each* metric that you are including in the multi-metric graph, graph the individual analysis results. See "To graph the analysis results for any vessel type (Single metric)" on page 118.

2. After you have graphed all the individual analysis results for each metric that you are including in the multi-metric graph, use standard Windows functions to bring all the individual metric graphs to the front of the screen display. For example, Figure 2-10 on page 121 shows two individual metric graphs brought to the front of the screen display.

Figure 2-10:   Graphs of analysis results for individual metrics brought to the front of the screen display
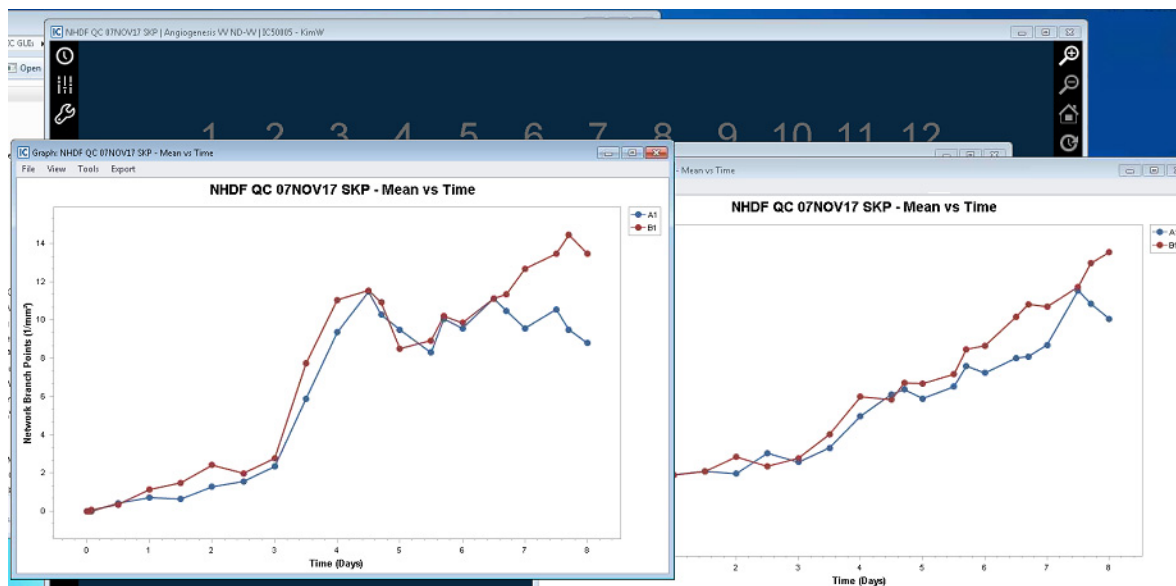
3. Determine which of the individual metric graphs should be the *destination* graph. The destination graph is the graph onto which you are dropping the other individual metric graphs.

4. For *each* individual metric graph that you are dropping onto the destination graph, do the following:

    a. On the graph's main menu, click View > Drag and Drop > Graph on Graph.

        A 2 x 2 grid is displayed on the individual metric graph.

Figure 2-11:   2 x 2 grid on metric graph
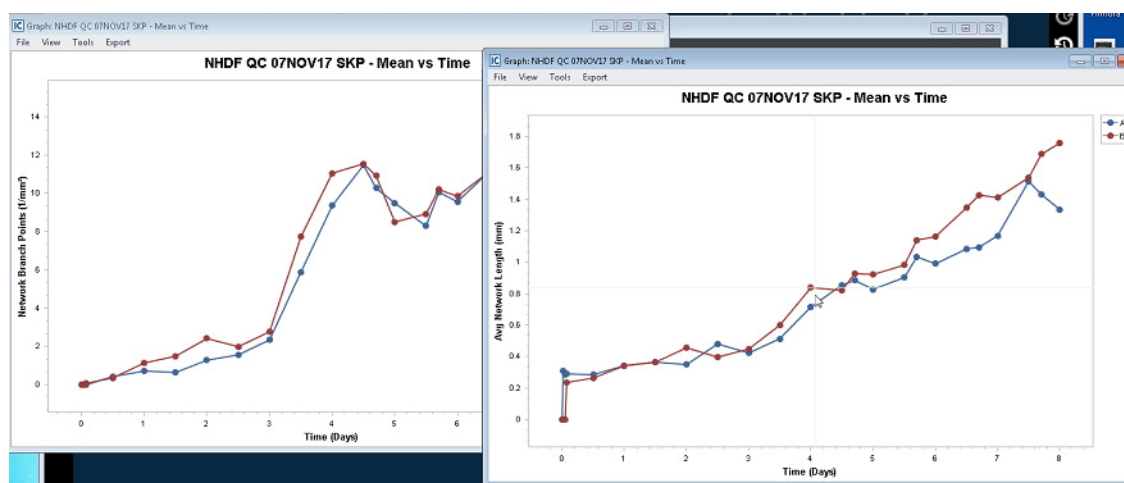
b. Click and hold the right mouse button on the individual metric graph, drag the graph onto the destination graph, and then release the mouse button.

The two individual metric graphs are combined into a single metric graph with the following characteristics:

•   The graph window is set to a default size. You must use standard Windows functionality to manually resize the graph window. The destination graph size is adjusted accordingly.

•   By default, the individual graph legends are displayed at the top of the graph window and to the right and outside of the graph. You can manually adjust the position of each graph legend. See "To customize the graph display (Non-default properties)" on page 130.

•   If two more or more graphs use the same legend colors, then when the graphs are combined, the software automatically modifies the colors for one of the graphs so that the different data points can be distinguished from each other. For example, as shown in Figure 2-10 on page 121, both individual metric graphs used the colors blue and red for their legends. After the graphs were combined into a single graph, as shown in Figure 2-12 below, the software changed the legend for one of the graphs from blue and red to green and purple. You can manually adjust the options (visibility, back color, font, and/or color) for any legend. See "To customize the graph display (Non-default properties)" on page 130.

Figure 2-12:   Graph on Graph example
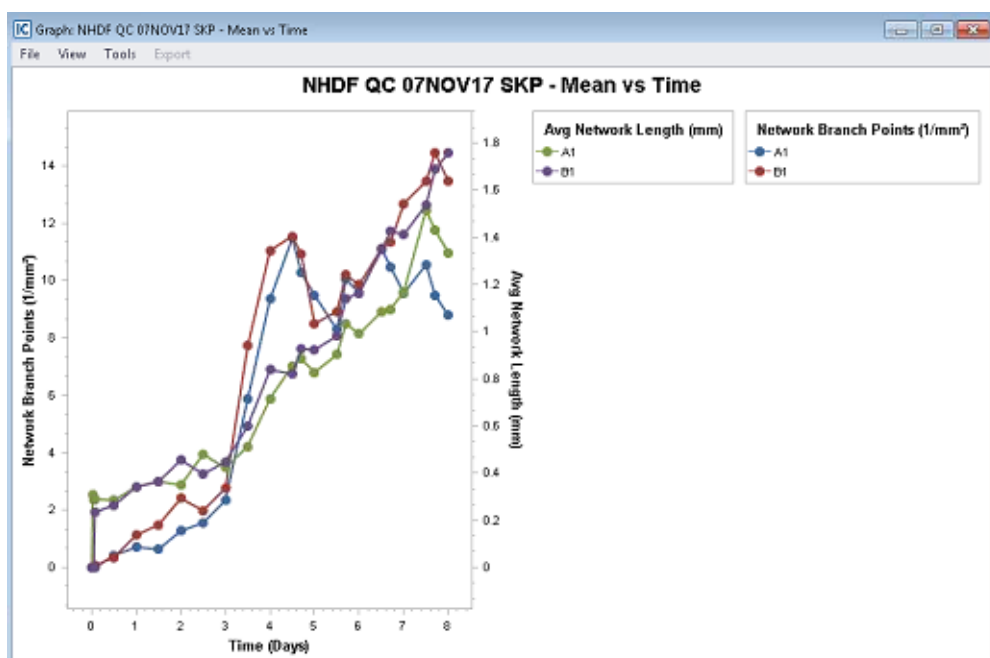
Graphing Analysis Results as a Histogram

In addition to the common time plot, you can also graph your analysis results as a histogram. Typically, you graph your analysis results as a histogram if you are interested in determining the intensity of a fluorophore. For example, for a transfection, how consistent is the expression level based on your selected fluorophore?

Figure 2-13:   Histogram example
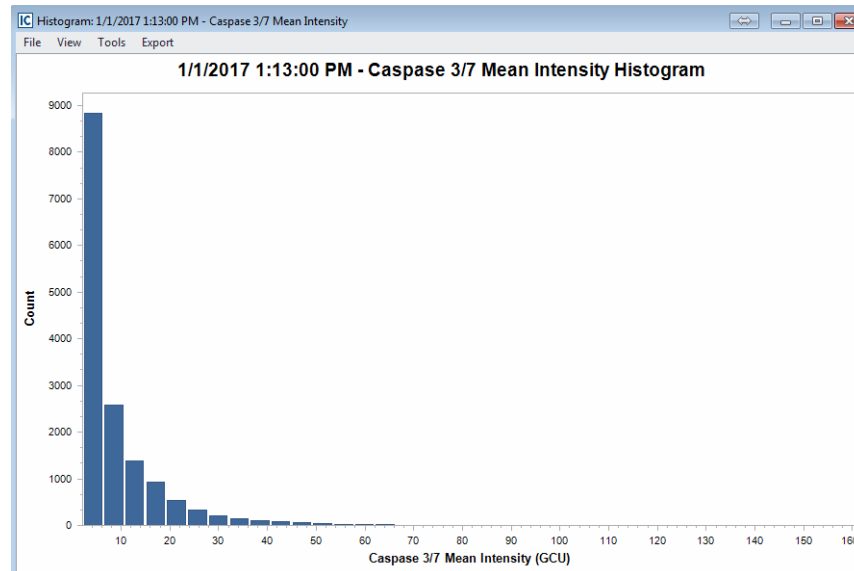
To graph analysis results as a histogram

1. Open the Graph Metrics window. See "The Graph Metrics Window" on page 110.

2. On the Menu bar, click Histogram.

3. In the Metrics pane, select the metric for which you are graphing the analysis results.

> *You might have to click the Expand/Collapse icon next to a grouping to expand the grouping and view the available metrics.*

4. To select an individual scan, click the scan. To clear an individual scan, click it again.

> *You can select only a single scan time for a histogram. You cannot select a range of scans and the Select All and Deselect All icons are not enabled.*

5. Select the wells/image sites.

    •   To select a single location in a vessel, for example, a well in a 96-well microplate, click once in the appropriate location in the vessel map.

    •   To select multiple contiguous locations in a vessel, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around

the locations as you are selecting them.

- To select all the locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Select this Column. Conversely, to clear all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column.

- To select all the locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Select this Row. Conversely, to clear all the selected locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Deselect this Row.

To clear all the selected locations in the vessel map, or multiple contiguous locations, you can also press and hold the ALT key, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.

6. Leave AutoScale set to On, or optionally, turn AutoScale off, and then manually adjust the values for Min, Max and/or Bins.

After you select the wells/image sites that you are analyzing, the values for Min, Max and Bins are automatically generated. Although you can manually adjust these values, Sartorius recommends that you at least first graph the data according to these system-generated values.

For finer resolution of the data, you can increase the number of bins.

7. Optionally, after the histogram is generated, options are available for customizing the graph display and exporting the graph data. See "Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve" on page 128 and/or "Exporting Graph Data" on page 132.

Graphing Analysis Results as a Concentration Response Curve

In addition to the common time plot and histogram, you can also graph your analysis results as a concentration response curve. Typically, you graph your analysis results as a concentration response curve if you are interested in determining the EC_{50} value of your compound or treatment. To graph analysis results as a concentration response curve, your plate must have an associated Plate Map that consists of at least one treatment at three different concentrations. See Appendix A, "Plate Map Editor," on page 137.

To graph analysis results as a concentration response curve

1. Open the Graph Metrics window. See "The Graph Metrics Window" on page 110.

2. On the Menu bar, click Concentration Response.

    The Graph Metrics window is refreshed with a Select Compound dropdown list and a Constraints panel.

Figure 2-14:   Graph Metrics window for an EC_{50} analysis
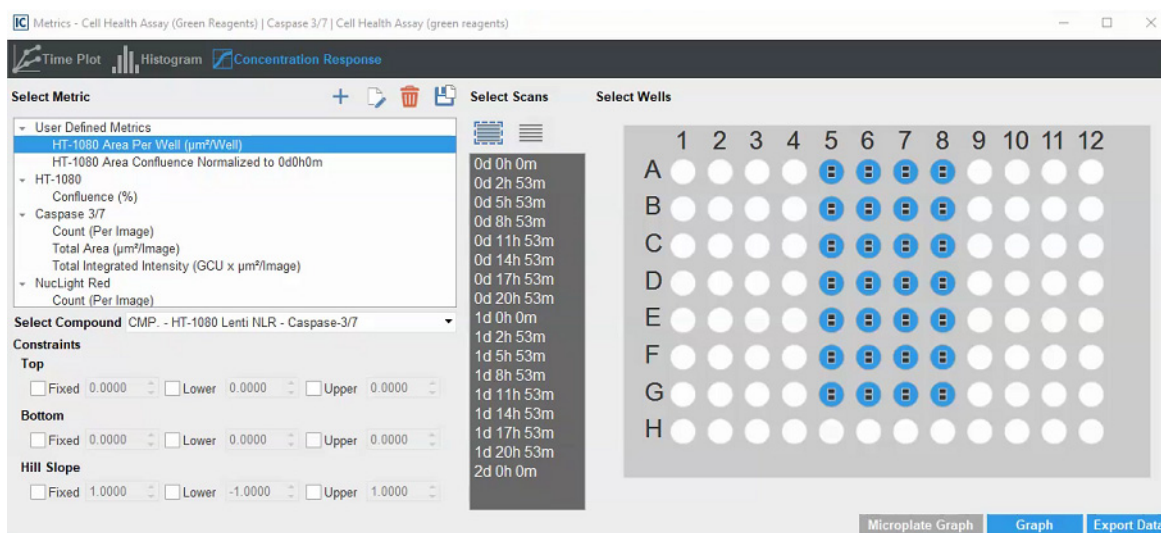
3. In the Metrics pane, select the metric for which you are graphing the analysis results.

You might have to click the Expand/Collapse icon next to a grouping to expand the grouping and view the available metrics.

4. On the Select Compound dropdown list, select the compound for which you are generating the concentration response curve.

   After you select the compound, all the wells that can be graphed are automatically selected based on the corresponding Plate Map. You can leave all the wells selected, or you can deselect wells of your choosing, for example, outliers.

   • To clear a single well, click the well.

   • To clear all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column.

   • To clear all the selected locations in a vessel row in a single step, right-click a location in the row, and on the context menu that opens, select Deselect this Row.

To clear all the selected locations in the vessel map, or multiple contiguous locations, you can also press and hold the ALT key, click and hold the left mouse button, and then drag the mouse pointer over the appropriate locations in the vessel map. A box is formed around the locations as you are selecting them.

5. Select the scan time or times that you are graphing.

   • For a kinetic EC_{50}, select the full time course or a subset of time points.

   • For a single time point EC_{50}, select the specific acquisition time.

For a shortcut to generate a single EC_{50} time point graph, you can first generate the kinetic EC_{50} graph, and then click a point on the kinetic graph to generate the corresponding single time point graph.

6. Click Graph.

   The Concentration Response Curve is generated and displayed onscreen in its own window. An r Sq value and an EC_{50} value are generated and displayed for the graph. A menu (File, Edit, View) is displayed at the top of the graph window and a default title is displayed above the graph.

   • For a sample kinetic EC_{50} graph, see Figure 2-15 on page 127.

   • For a sample single time point EC_{50} graph, see Figure 2-15 on page 127.

Figure 2-16 on page 127 was generated using the above mentioned shortcut by clicking the single time point indicated in Figure 2-15 on page 127.

Figure 2-15: *Kinetic EC$_{50}$ graph*

Click this time point to generate a single time point EC$_{50}$ graph. See Figure 2-16 below.
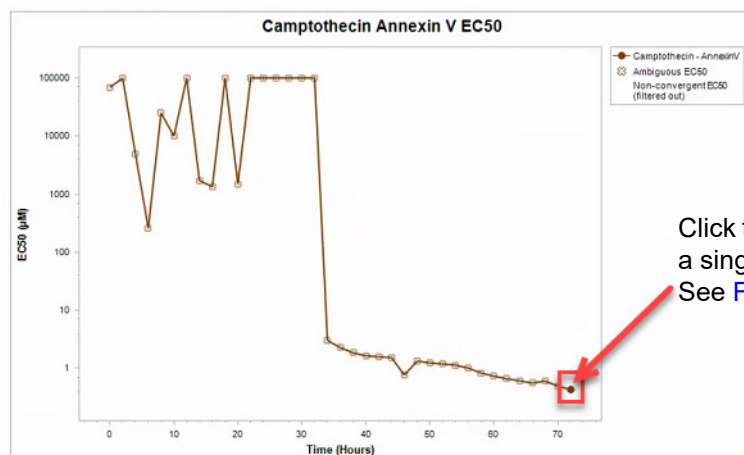
In a kinetic EC$_{50}$ graph, ambiguous data is marked with an open "x." Non-convergent data is filtered and is not displayed on the graph.

Figure 2-16: *Single time point EC$_{50}$ graph*

Click any time point to open the Vessel View window and view images for the time point.
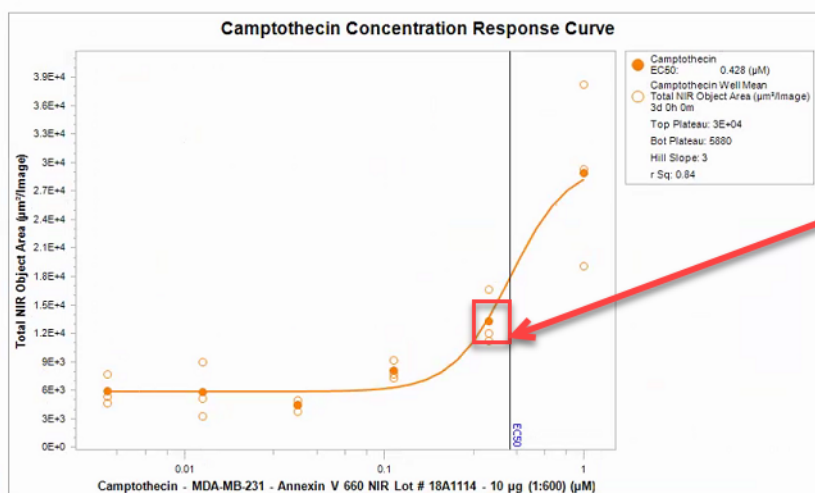
7. Optionally, after the concentration response curve is generated:

 • If the response does not have the expected fit, then based on the data expectations, you can apply constraints to modify the fit, and then generate the graph again.

 • If applicable, you can customize the graph display and/or export the graph data. See "Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve" on page 128 and/or "Exporting Graph Data" on page 132.

Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve

Optionally, after you generate a time plot graph, a histogram, or a concentration response curve for your analysis data, options are available for working with the graph, customizing the graph display and/or exporting the graph data. See:

- "To save the graph as an image file and/or print the graph" below.

- "To edit the default graph display" on page 128.

- "To save graph as an image to a third-party application or the raw data to a document" on page 129.

- "To customize the graph display (Non-default properties)" on page 130.

To save the graph as an image file and/or print the graph

1. On the Graph window menu, click File.

2. Under File, click one of the following:

   - Save as Image. Opens the Save Graph as Image dialog box. You use the options on this dialog box to save the displayed graph as image in a specific format (BMP, JPEG, PNG, or TIFF) and with a specific size. To save the file, you must select the location in which to save the image file, and you must name the file.

Figure 2-17:   Save Graph as Image dialog box
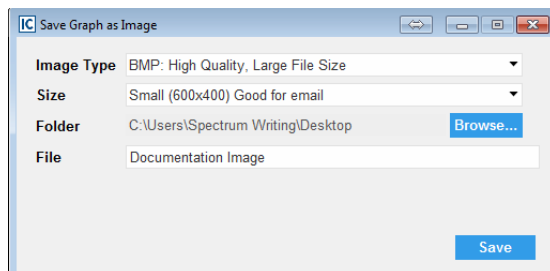
   - Print. Opens the standard Windows printing dialog box for printing the graph.

To edit the default graph display

As you edit the default display for the graph in the Graph window, the graph is dynamically updated based on the edits that you make.

To customize the non-default properties for a graph, see "To customize the graph display (Non-default properties)" on page 130.

1. On the Graph window menu, click View.

2. Under View, select any of the following to edit the corresponding display:

Option	Description
Time Plot graph	
Error Bars	• No Error Bars: By default, errors bars are displayed on a metric graph. Select No Error Bars to remove the error bars from the graph display.
	• Standard Error/Standard Deviation: If Error Bars are turned on, then by default, the standard error is displayed for the error bars. Optionally, you can elect to display the standard deviation instead.
	• Full Bars/Top Bars Only/Bottom Bars Only: If Error Bars are turned on, then by default, full bars are displayed. Optionally, you can elect to display either top bars only or bottom bars only.
Logarithmic Y-axis	Use a base-10 log scale for the Y axis.
Show X-Axis in Hours	If the range of scan times is less than or equal to 96 hours, then, by default, the time is displayed in hours. Clear this option to display the time in days. If the range of scan times is greater than 96 hours, then, by default, the time is displayed in days and this option is cleared.
Histogram	
Y Axis Display	• Count: The default value. The total number of objects that are contained within a bin.
	• Percent: The percentage of all objects that are contained within a bin.

To save graph as an image to a third-party application or the raw data to a document

If you are intending to use the graph in a presentation or a report, then you can save the graph as an image to a third-party application such as Microsoft Word or PowerPoint. If you are archiving the raw data outside Incucyte, or carrying out a statistical analysis of the raw data outside Incucyte, then you can save the raw data to a third-party application such as Microsoft Excel.

1. Make sure that the correct third-party application is open.

2. On the Graph window menu, click View.

3. Under View, select Drag and Drop, and then select one of the following:

   • Image to Document. (The default value.)

   • Raw Data to Document.

4. Right-click the image, and then while holding down the right mouse key, drag the image into the open document.

   A context menu opens with the following options: Move Here and Copy Here.

5. On the context menu, select the appropriate option.

   • Move Here. A one-time "copy" of the selected image/data to the opened document. To copy the image again, you must repeat Step 3 through Step 5.

   • Copy Here. The information is copied to your client clipboard, which allows for pasting the copied information an unlimited number of times in to one or more open documents.

To customize the graph display (Non-default properties)

You use the Graph Customization tool to customize the non-default properties of the graph display.

To customize the default properties for a graph, see "To edit the default graph display" on page 128.

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1. On the Graph window menu, click Tools.

2. Under Tools, click Customize Graph.

   The Graph Customization dialog box opens.

Figure 2-18:   Graph Customization dialog box, Time Plot graph shown
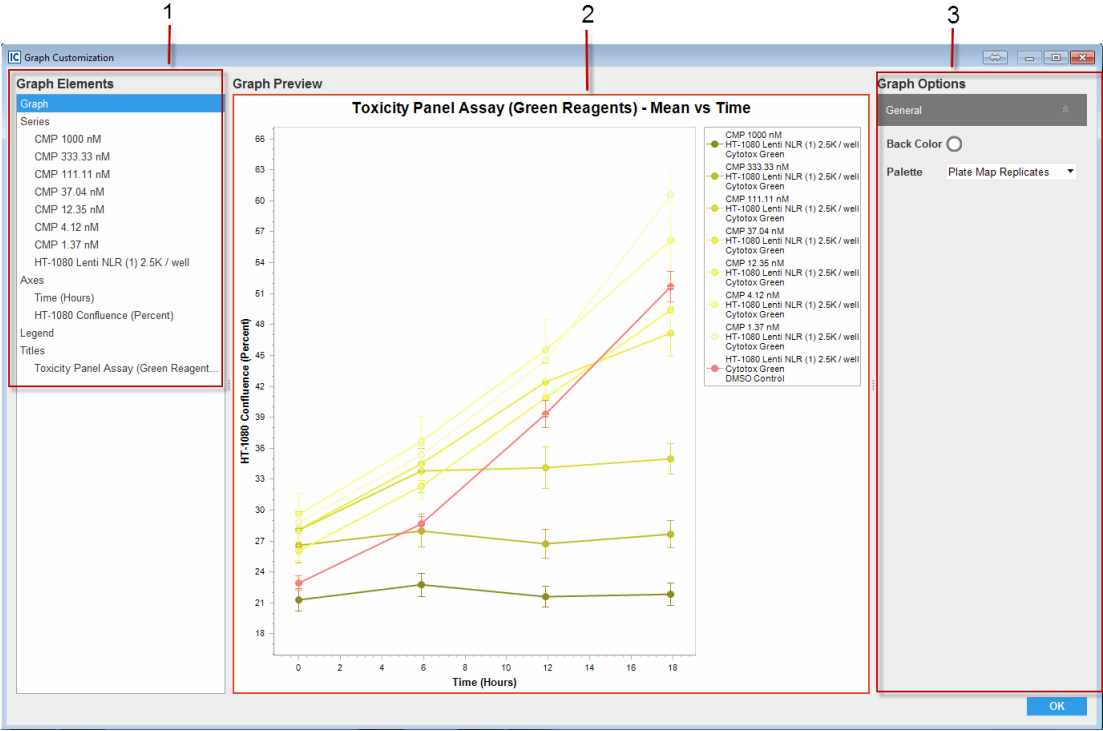
The Graph Customization dialog box has the following layout:

	Window component	Description
1	Graph Elements pane	Displays all the graph elements for which you can customize the properties.
2	Graph Preview pane	Displays the graph and the graph legend according to the values that are currently set for its elements.
3	Graph Options pane	Displays the values that you can edit when customizing a selected graph element. As you customize a graph element, the graph and graph legend display are dynamically updated in the Graph Preview pane.

3. For each graph element that you are customizing, do the following:

   • In the Graph Elements pane, select the element that you are customizing.

     The Graph Options pane displays the values that you can edit for the selected graph element.

   • In the Graph Options pane, edit the values for the selected element accordingly.

4. After you have edited all the necessary graph elements, click OK.

   The Graph Customization dialog box closes. The Graph window remain open and the display is dynamically updated according to the edits that you made.

To generate a Rate of Change graph

After you generate a time plot graph, you have the option of use the Rate of Change tool to generate a corresponding Rate of Change graph, which provides an estimated rate of change for each point on the graph.

1. On the Graph window menu, click Tools.

2. Under Tools, click Estimate Rate of Change.

   The Rate of Change graph is automatically generated.

Figure 2-19:   Rate of Change graph
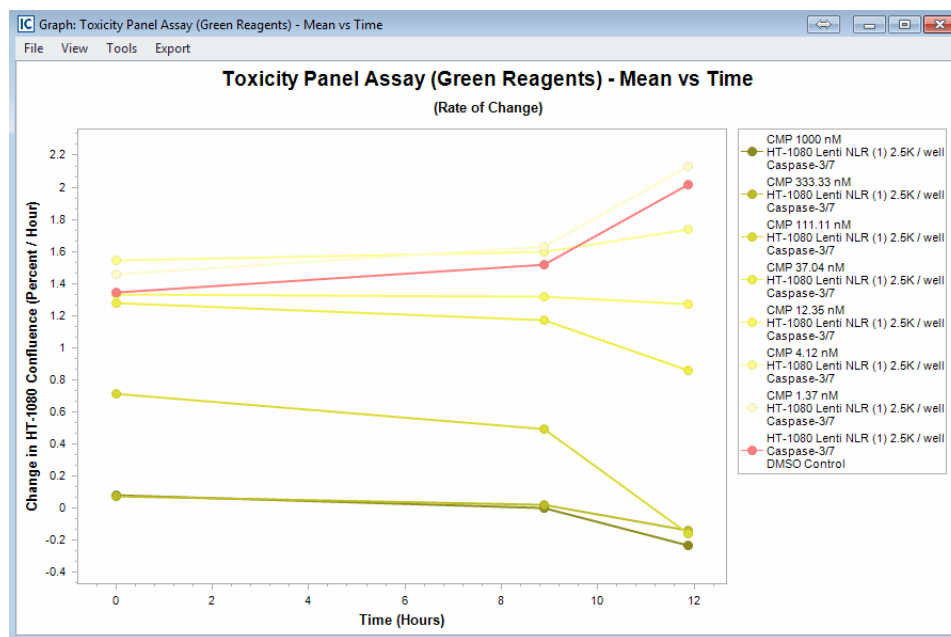
The same options that are available for working with and customizing a time plot graph are also available for the Rate of Change graph. See *"Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve" on page 128.*

Exporting Graph Data

You can export the data that was used to generate a graph to either a text file or your client clipboard. If you export the data to your client clipboard, you can then import this data in to a third-party spreadsheet program such as Microsoft Excel. When you export the data, by default, each scan is exported as a single row and all the rows are contained in a single table for any Grouping option other than None. To access options by which to customize the layout of the exported data, you must select None as the Grouping option.

Exporting the data to your client clipboard gives you the option of archiving your data and/or carrying out additional statistical analysis of your data outside the Incucyte software.

To export graph data for any grouping option other than None

1. Do Step 2 through Step 5 of "To graph the analysis results for any vessel type (Single metric)" on page 118.

For Step 5, make sure that any you select any Grouping option other than None.

2. On the Graph Metrics window, click Export Data.

    The Graphing Export dialog box opens.

Figure 2-20: Graphing Export dialog box
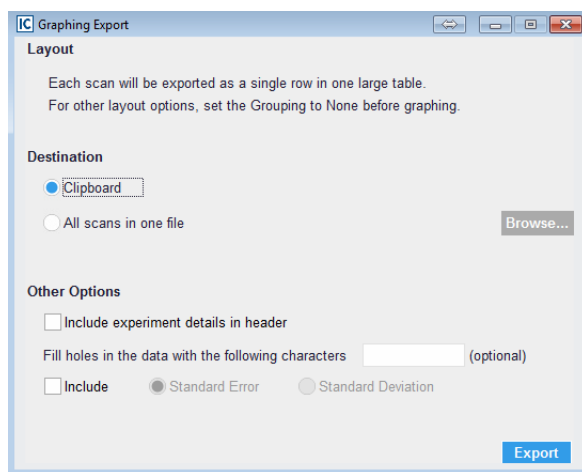
3. Do one of the following to select the export destination:

    *   To export the data to your client's clipboard, leave the default value of Clipboard selected.

    *   To export the data to a single file, select All scans in one file, and then click Browse to open the Export Metrics As dialog box, and then browse to and select the destination folder. You must name the file that you are exporting. (The file type is text and you cannot change this.)

4. Optionally, under Other Options, indicate how you are modifying the data before exporting it.

5. Click Export.

    •   If you exported the graph data to your client clipboard, then you can now open a spreadsheet program such as Microsoft Excel and paste the data.

    •   If you exported the graph data to a file, then you can use any text editor such as Notepad to open the file and work with the data in the file.

To export graph data for the None grouping option

1. Do Step 2 through Step 5 of "To graph the analysis results for any vessel type (Single metric)" on page 118.

⚠ *For Step 5, make sure that you select None for the Grouping option.*

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2. On the Graph Metrics window, click Export Data.

    The Graphing Export dialog box opens.

Figure 2-21:   Graphing Export dialog box
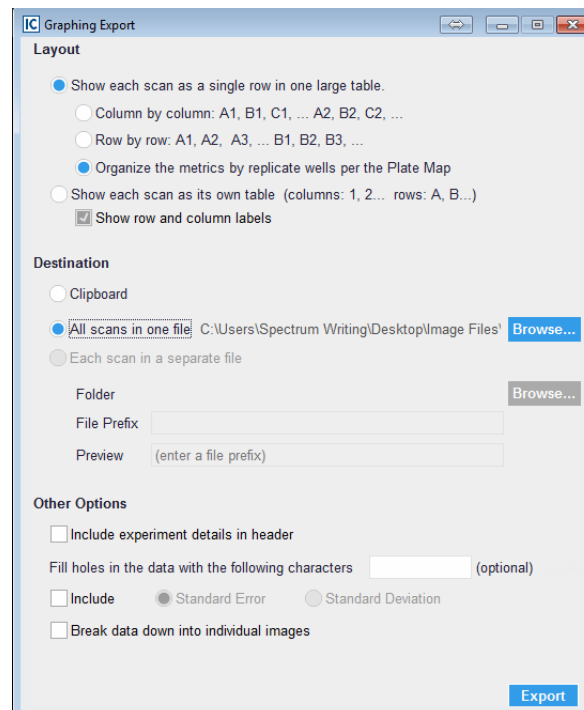
3. Under Layout, select the layout for the exported data.

   By default, Show each scan as a single row in a single table is selected. Also, if the vessel is a microplate for which a plate map has been specified, then, by default, Organize the metrics by replicate wells per the Plate Map is selected for the single table scan and this is the recommended value. If you are storing each scan in its own separate file, then you must select Show each scan as its own table.

4. Do one of the following to select the export destination:

   • To export the data to your client's clipboard, select Clipboard.

   • To export each scan to its own separate file, select Each scan in a separate file, and then click Browse to open the Export Metrics As dialog box, and then browse to and select the destination folder. You must name the file that you are exporting. (The file type is text and you cannot change this.) Go to Step 5.

   • To export the data to a single file, leave the default value of All scans in one file selected, and then click Browse to open the Export Metrics As dialog box, and then browse to and select the destination folder. You must name the file that you are exporting. (The file type is text and you cannot change this.) Go to Step 6.

Each scan in a separate file is available only if you select Show each scan as its own table for the export layout.

5. Optionally, in the File Prefix field, enter the prefix that you are adding to each individual file name.

   As you enter the prefix, an example file name is displayed in the Preview field. Each individual file is named according to the following format: <Prefix>_<yyyy>y<mm>m<dd>d<scan time>.txt.

6. Optionally, under Other Options, indicate how you would like to modify the data before it is exported.

Remember, you can acquire one or more images per vessel location. By default, multiple images are exported as a single data point. If you select Break down data into individual images, then every image is exported as an individual data point.

7. Click Export.

   • If you exported the graph data to your client clipboard, then you can now open a spreadsheet program such as Microsoft Excel and paste the data.

   • If you exported the graph data to a file or files, then you can use any text editor such as Notepad to open a file and work with the data in the file.

Section 4
Supporting Information

Section Contents

Incucyte Live-Cell Analysis Systems User Manual

Appendix A
Plate Map Editor

To assist in experimental design and data analysis, the Incucyte software includes a Plate Map Editor. You use the Plate Map Editor to define a custom plate map for any microplate-based experiment, ranging from 6-well to 384-well formats. You can define and save a plate map at any point before or during an experiment, or after the experiment has been completed. This appendix details the use of the Plate Map Editor for defining a custom plate map.

This appendix covers the following topics:

- "Opening the Plate Map Editor" on page 139.

- "Plate Map Editor Layout" on page 140.

- "Defining a Plate Map with the Plate Map Editor" on page 143.

- "Editing an Existing Plate Map" on page 147.

Appendix A
Plate Map Editor

Opening the Plate Map Editor

When you install the Incucyte software, the Plate Map Editor is also installed as separate, standalone application. A Plate Map Editor shortcut icon is automatically placed on the client desktop. Although the Plate Map Editor is installed as a standalone application, it is still accessible from within the Incucyte software. As a result, four different access points are available for the Plate Map Editor:

- From the Menu bar: Tools and Options > Plate Map Editor. See "Menu bar" on page 23.

- From the Notebook page in the Add Vessel wizard. See "To provide vessel information" on page 37.

- From the Vessel Information window that is opened by clicking the Vessel Information icon on the Visualization toolbar for the Vessel View window. See "Vessel Information window (from the Vessel View window)" on page 69.

- From the Plate Map Editor desktop icon .

Plate Map Editor Layout
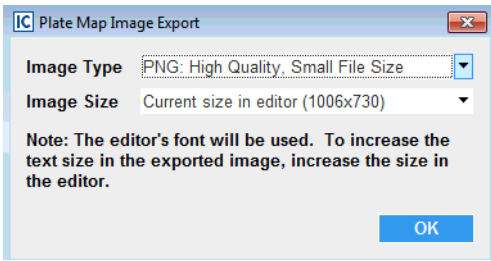
Figure A-1:   Plate Map Editor
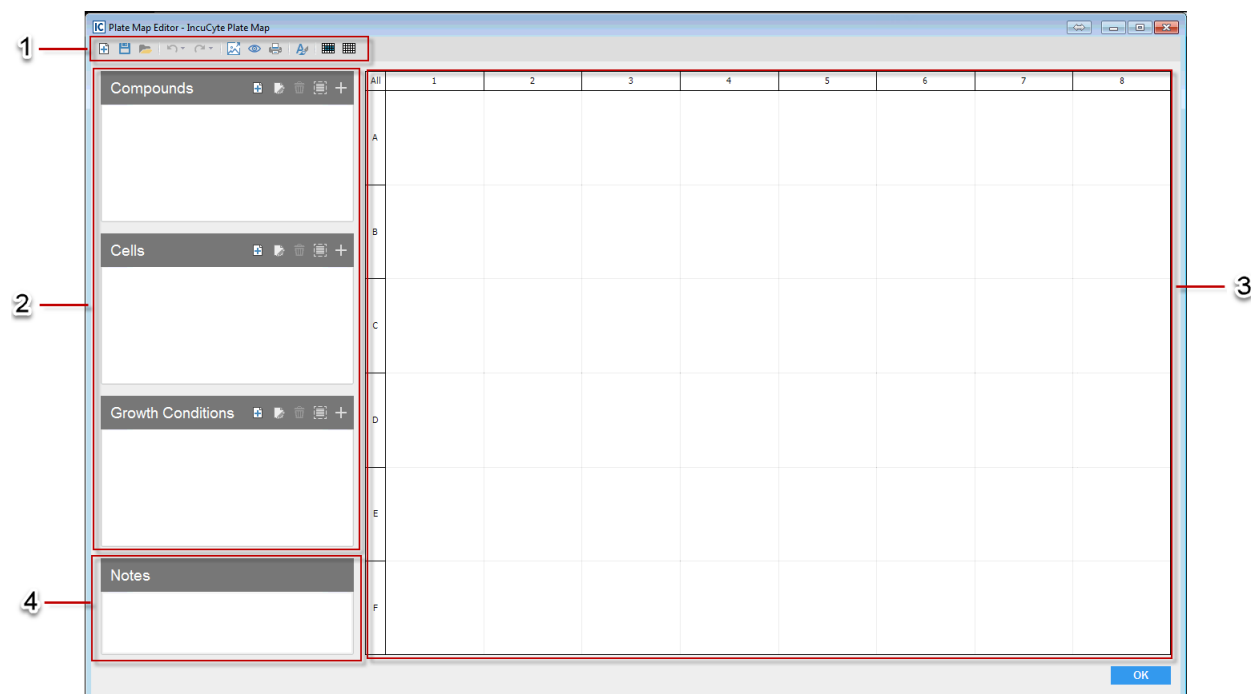
The Plate Map Editor has the following layout:

	Window component	Description
1	Menu bar	Displays the icons that you use to access specific Plate Map Editor functions. See "Plate Map Editor menu bar icons" on page 141.
2	Well Items panes	Before you can specify the experimental settings for any well on a plate, you must first define the appropriate items that can be added to the plate wells: • Compounds: Any treatment to a well that requires either concentration or dilution to be specified. • Cells: Specifies the type, passage, and seeding density for the cells that are present in the plate wells. • Growth Conditions: Any addition such as growth medium where the concentration of a reagent does not vary or is not required to be specified on the plate map. See "Well Items panes icons" on page 142.
3	Microplate pane	Displays the format for the selected microplate, and ultimately, your custom plate map for the microplate. When the Plate Map Editor first opens, by default, the format is set to that of a 96-well plate. You can use the New Plate Map icon to select a different format. You use the pane options to select the wells when defining your custom plate map.
4	Notes pane	Optional. Enter any additional information that you are associating with the plate map.

Plate Map Editor menu bar icons

Icon	Description
	New Plate map icon: Readies the Microplate pane for adding a new plate map with a format other than a 96-well plate.
	Save Plate Map to File icon: Opens the Save Plate Map As dialog box, which provides the options for saving the currently displayed plate map to a file. You must specify the name of the file and the location in which to save the file. The file is saved with the type of .PlateMap and you cannot change this.
	Save As icon: Saves the currently displayed plate map with a different name and/or in a different location. **Tip:** Useful if you simply need to edit an existing plate map to create a new plate map and you do not want to override the existing plate map.
	Open From File icon: Opens the Open Plate Map dialog box in which you can browse to and select an existing plate map file (*.PlateMap) to load in to the Plate Map Editor.
	Undo Last Action icon: Undoes the last action or "n" number of actions that were carried out for the plate map.
	Redo Last Action icon: Enabled only if one or more actions have been undone for the current session of the Plate Map Editor. Redoes the last action or "n" number of actions that were carried out for the plate map.
	Export as Image icon: Opens the Plate Map Image Export dialog box, which provides the options for exporting the currently displayed plate map as a read-only image. You must specify the image format and the image size before you can export the image. *Figure A-2: Plate Map Image Export dialog box* **Note:** When you export the plate map as an image, the font that is displayed on the plate map is exported as shown (family, size, and style). To change any properties of the font before you export the image, you must use the font editor that is embedded in the Plate Map Editor. See the Set Font icon.
	Print Preview icon: Opens a Print Preview dialog box in which you can view what the plate map looks like before it is printed, and make adjustments to the map (such as changing the number of pages across which you are printing the graph) before printing the map.
	Print icon: Opens the standard Windows Print dialog box for printing the currently displayed plate map.

Icon	Description
	Set Font icon: Opens the standard Windows Font dialog box, in which you can adjust the font for any text that is displayed on the plate map. The selected font (family, size, and style) is also the font that is used when printing the plate map or exporting the plate map to an image.
	Keep Current Selection icon: Select the appropriate well or wells, and then click this icon to apply all subsequent actions that you carry out in the Plate Map Editor only to the selected well or wells. **Note:** This icon is a toggle. You can click it as needed to it on or off.
	Deselect All Wells icon: Clears all currently selected wells in a single step.

Well Items panes icons

Icon	Description
	Add New <Well Item> icon: Opens the Add/Edit <Well Item> dialog box, in which you can add the indicated new well item (Compound, Cell Type, or Growth Condition).
	Edit Selected <Well Item> icon: Opens the Add/Edit <Well Item> dialog box, in which you can edit the indicated well item (Compound, Cell Type, or Growth Condition).
	Delete Selected <Well Item> icon: Deletes the selected well item from all affected wells in the plate map. **Tip:** This deletion affects only the currently displayed plate map. It does not affect any other plate maps that might have used the same well item.
	Select All Wells that contain currently selected <Well Item> icon: Selects all wells in the plate map to which the currently selected well item has been added.
	Add <Well Item> to Plate icon: Enabled only after selecting one or more wells in the plate map. Adds the currently selected well item to all the wells that are selected in the plate map.

Defining a Plate Map with the Plate Map Editor

The following procedure describes, at a high level, how to define a new plate map with the Plate Map Editor. Note that multiple approaches are appropriate when you use the Plate Map Editor to define a plate map. The following procedure is for example purposes only and is designed to provide a frame of reference for a best practice approach. You can select whichever approach or combination of approaches best suits your working needs.

> *The following procedure is written for defining a new plate map. To define a plate map by editing an existing map, see "Editing an Existing Plate Map" on page 147.*

To define a plate map with the Plate Map Editor

1. Open the Plate Map Editor. See "Opening the Plate Map Editor" on page 139.

2. Do one of the following:

   • If the plate map is for a 96-well plate, then continue to Step 3.

   > *Remember, when the Plate Map Editor first opens, the format is set to that of a 96-well plate*

   • If the plate map is for any plate other than a 96-well plate, then on the main menu bar, click the New Plate Map icon, and then on the New Plate Map dialog box, select a different microplate size, and click OK. Continue to Step 3.

   > ⚠ *To ensure that all any previously added well items remain intact, leave Preserve lists of components, cell types, and growth conditions selected.*

Figure A-3:   New Plate Map dialog box
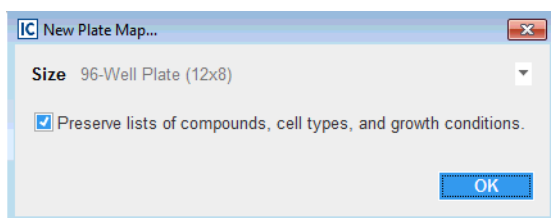
3. Define the needed well items. To add a well item, do the following:

    a. In the Well Item pane, click the Create <Well Item> icon. For example, to add a new compound, in the Compounds Well Item pane, click the Create New Compound icon.

        The Add/Edit <Well Item> dialog box opens.

Figure A-4:   Add/Edit Compound dialog box
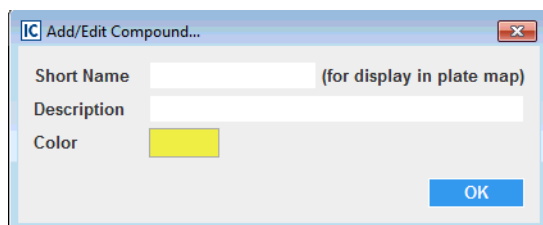
    b. Enter the information for the new well item.

Option	Description
Short Name	Required. A short, descriptive name for the well item, for example, for a new compound, CMP. This is the name that is displayed on the selected wells in the plate map.
Description	Optional. Additional information about the added well item. This information is not displayed on the plate map.
Color	Indicates the color in which to display the wells in the plate map that contain the added well item. You can leave the automatically selected color as-is, or you can click the color icon to open the Choose a Color dialog box and select a different color.

    c. Click OK.

        The Add/Edit <Well Item> dialog box closes. The well item is displayed in the <Well Item> pane.

4. In the Microplate pane, select the wells to which you are adding a well item.

    •   To select a single well, click the well. To clear the selection, click the well again.

    •   To select multiple contiguous wells, click and hold the left mouse button, and then drag the mouse pointer over the appropriate wells. A box is formed around the wells as you select them. To clear all the wells in the selection, repeat this step. To clear individual wells in the selected area, click the wells.

    •   To select all wells in the microplate a single step, in the upper left corner of the Microplate pane, click All. To clear all selected wells, click All again.

> *To clear all selected wells in a single step, you can also click the Deselect All Wells icon ▦ on the menu bar of the Plate Map Editor.*

    •   To select all wells in a single row or column in a single step, click the appropriate row or column header. To clear the selection, click the header again. To clear individual wells in the row or column, click the wells.

5. To add a well item to the selected wells, do the following:

    a. In the appropriate Well Items pane, select the well item.

b. Click the Add <Well Item> icon ➕ .

- If the well item is a compound, then the Add Compound Or Dilution dialog box opens. Go to Step 6.

- If the well item is a cell type, then the Adding <Cells> to plate dialog box opens. Go to Step 7.

- If the well item is a growth condition, then the item is added to the selected wells and the plate map is dynamically updated with the well item and associated information. Go to Step 8.

Figure A-5:   Add Compound Concentration or Dilution dialog box
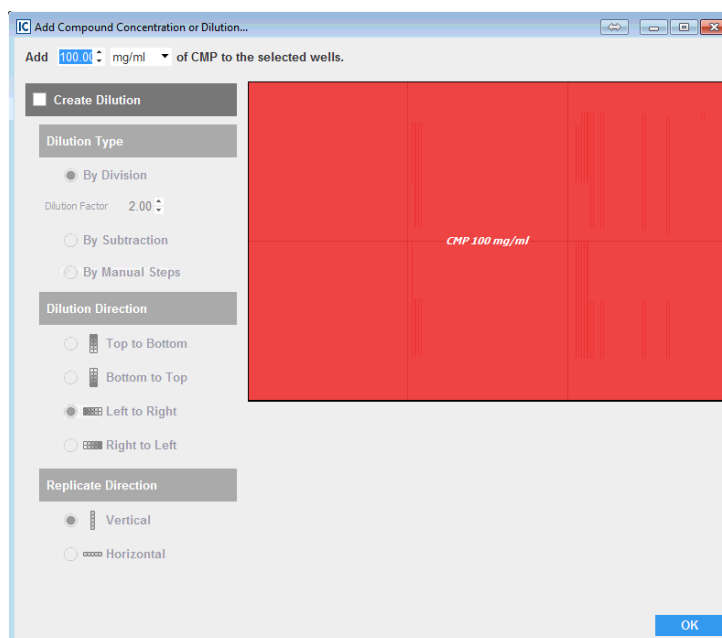
6. In the Add Compound Concentration dialog box, do the following:

a. Indicate the concentration of the compound that you are adding to the selected wells.

b. If the compound is a dilution series, then select Create Dilution, and then specify the appropriate dilution parameters (Dilution Type, Dilution Direction, and Dilution Replicate Direction).

c. Click OK to close the dialog box.

The plate map is dynamically updated with the well item and associated information.

d. Continue to Step 8.

Figure A-6:   Adding <Cells> to plate dialog box
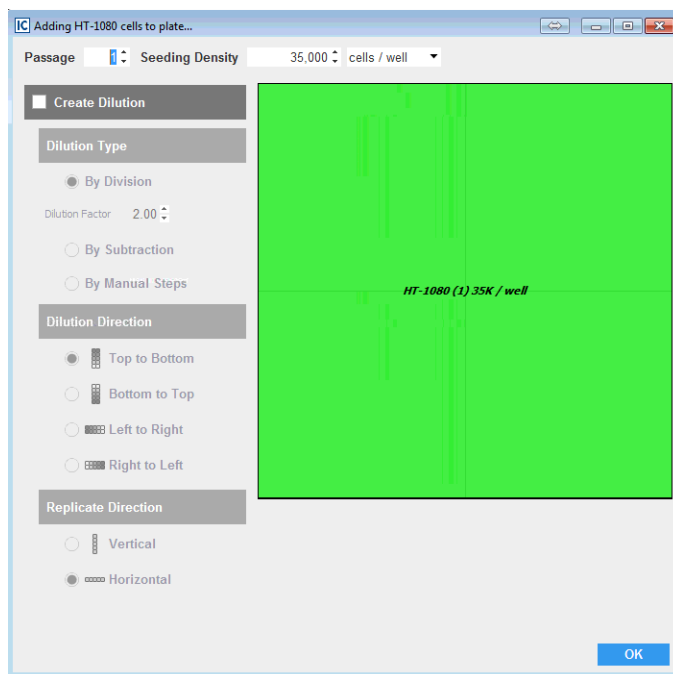
7. In the Adding <Cells> to plate dialog box, do the following:

    a. Indicate the passage and seeding density of the cells that you added to the selected wells.

    b. If the cells are a dilution series, then select Create Dilution, and then specify the appropriate dilution parameters (Dilution Type, Dilution Direction, and Dilution Replicate Direction).

    c. Click OK to close the dialog box.

        The plate map is dynamically updated with the well item and associated information.

    d. Continue to Step 8.

8. Repeat Step 3 through Step 5 as needed to add all the necessary well items and define the plate map.

9. After you have defined the necessary plate map, on the Plate Map menu bar, click the Save to File icon 💾 to save the plate map.

> *You must specify the name of the file and the location in which to save the file. The file is saved with the type of .PlateMap and you cannot change this.*

Editing an Existing Plate Map

Instead of defining an entirely new plate, you can define a new plate map by editing an existing plate map. When you edit an existing plate map, you have the option of changing the label for a selected well or group of wells, changing the color for a selected well or group of wells, or clearing all the information for a selected well or group of wells, and then defining all the well options (well item, label, and color) again.

To edit an existing plate map

1. On the Plate Map Editor menu bar, click the Open from File icon.

   The Open Plate Map dialog box opens.

2. In the Open Plate Map dialog box, browse to and select the appropriate plate map file.

   The selected plate map is loaded in to the Plate Map Editor. All the information for the selected plate map (well items, dilutions, concentrations, colors, and so on) are displayed.

3. To edit the plate map, select the well or group of wells that you are editing (see Step 4 of "To define a plate map with the Plate Map Editor" on page 143), and then right-click anywhere in the selection.

   A <n> wells dialog box opens. The dialog box indicates the number of wells that have been selected for editing, the well items that can be edited for the selected wells, and the current color for the selected wells.

Figure A-7:   <n> wells dialog box

4. Edit the selected wells as needed, and then click Close.

   You can enter a custom label for the wells, remove one or more items from the selected wells, change the color of the selected wells, or click Clear Everything to clear all current well specifications for the selected wells. The Plate Map Editor display is dynamically updated as you make your edits.

   After you close the dialog box, if you removed one or more well items from the selected wells, or cleared everything, then optionally, you can define new information for the wells, including adding new well items. See "Defining a Plate Map with the Plate Map Editor" on page 143.

5. After you have defined the necessary plate map, on the Plate Map menu bar, click the Save As icon to save the new plate map.

To ensure that you do not override the existing plate map, you must click the Save As icon.

You must specify the name of the file and the location in which to save the file. The file is saved with the type of .PlateMap and you cannot change this.

Section 5
Incucyte Management and Administration
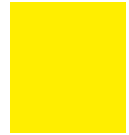
Section Contents

Incucyte Live-Cell Analysis Systems User Manual
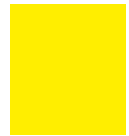
Appendix A
Incucyte Data Archives

Vessel image data, plate maps, analyses, and all associated vessel data are stored on the Incucyte controller. Although the associated hard drive is approximately 16 TB, eventually, you will have to clear space to continue acquiring and processing vessel data. To clear space while still maintaining historical records of all your vessel data, you can archive your data. With the Archive data management function that is available in Incucyte, you can save historical vessel data to a location other than the hard drive on the Incucyte controller. After you have archived this information, you can then delete the vessel and its associated data from the controller's hard drive, which frees up the space that you need to continue acquiring and processing new vessel data. This appendix details Incucyte's Archive function.

This appendix covers the following topics:

- "Archiving Overview" on page 153.

- "Creating a New Archive" on page 155.

- "Viewing a List of the Archives Attached to Incucyte" on page 160.

- "Opening an Archive" on page 161.

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Archiving Overview

An archive contains vessel images and their associated analyses. A single archive can consist of images and the associated analyses for one or more vessels. When you archive vessel data, you must specify the location in which to store the archive. Two locations are available for archiving data in the Incucyte system: My Computer and Storage Attached to Incucyte Controller.

- My Computer: If you select "My Computer" as your archiving location, then you can save the archives to either a local drive on the Incucyte client, or to a network drive. If continuous access to the archives is anticipated, then local archives are especially advantageous for you; however, you must be aware of the following:

    - Archiving to My Computer can be significantly slower than archiving to Storage Attached to Incucyte Controller.

    - Archiving to My Computer is more susceptible to interruptions (for example, system reboots) than archiving to Storage Attached to Incucyte Controller.

    - Archiving to My Computer requires that your Incucyte client be on for the duration of the archiving process.

    - Archiving to My Computer is, overall, a generally less efficient process than archiving to Storage Attached to Incucyte Controller.

- Storage Attached to Incucyte Controller: If you select "Storage Attached to Incucyte Controller" as your archiving location, then the archives are stored on a dedicated storage device that is directly attached to the Incucyte controller either through an eSATA (the fastest and preferred method) or through a USB port. Incucyte-attached archives are immune to network failures, computer reboots, or logoffs; however, you can access Incucyte-attached archives only if you remove the storage from the Incucyte controller and connect it directly to a local computer. As a result, if continuous access to the archives is anticipated, then you should archive to My Computer.

An optimized Incucyte Archive drive is available for purchase from Sartorius.

Because archiving is for historical data management purposes, immediate access to the data is typically not a priority. As a result, if you select "Storage Attached to Incucyte Controller" as your archiving location, then note the following:

- You cannot open the archives while the storage device is attached to the Incucyte controller.

- Sartorius recommends that you detach and reattach the external storage device only when absolutely necessary. Ideally, the storage device should remain connected until it is completely full.

- If you must detach the storage device, then before doing so, you must open the Status page and verify that archives are not currently in the queue or are being processed.

- You must not power off or unplug the storage device during the archiving process.

Although regular archiving is important for data management, before you archive any vessel data, note the following:

- Although you can analyze image data that is contained within an archive, Sartorius strongly recommends that you do *not* archive the data for vessels that are being actively evaluated. Instead, the

vessels should remain on the controller until all evaluation is complete and only then should you archive the data.

- After you archive the data for a vessel, and then delete the vessel from the Incucyte controller, you cannot retrieve the vessel.

- Because of the volume of data that can be involved (the number of scans, for example, scans every hour, the number of channels scanned, the associated analysis, for example, Basic or Angiogenesis, and so on), archives are more likely to be written successfully when the archive size is kept small. Moreover, not only are smaller archives more easily transported and shared, but also they are less likely to be interrupted by system issues (system reboots, logoffs, Windows updates and so on). As a result, Sartorius recommends that you archive data on at least on a monthly basis, if not more frequently.

Creating a New Archive

You use the Archive function to create a new vessel archive. You can archive any vessel that has ever been scanned by any user on your Incucyte instrument. You are not limited to archiving the vessels that only you own. When you create an Incucyte archive, it runs as an independent task, which means that, if applicable, you can exit the Incucyte software during the archiving process.

> ⚠️ *If you elect to archive to My Computer, you can still exit the Incucyte software, but remember, the Incucyte client must remain on for the duration of the archiving process.*

> 💡 *Because of the volume of data that is involved in an archive, before you create a new archive, you should review the vessels that you are archiving and their associated analyses, and delete any unnecessary data. See "The Vessel View Window" on page 67.*

To create a new archive

1. On the Incucyte menu, click Archive.

   The Archive window opens. By default, Create New Archive is selected. The window displays all the vessels that have ever been scanned by any user on your Incucyte instrument.

> 💡 *Because the Archive window displays the vessels as they are scanned in real-time, you should periodically click the Refresh icon ⟳ to update the display with the most recently scanned vessels.*

> 💡 *At any time, you can click the Copy icon 📋 to copy the information that is currently displayed in the Archive window to your client clipboard, and then you can paste this information in to a third-party application such as Microsoft Excel.*

Figure A-1:   Archive window, Create New Archive selected

2. Select the vessels that you are archiving. When selecting the vessels that you are archiving, note the following options that are available for searching for and selecting the appropriate vessels:

    *   To select all displayed vessels for archiving, click the Select all icon ▦.

    *   To select one or more vessel at a time for archiving, in the Selected column, click the blank check box for the vessel. If the vessel is not to be archived, then click the check box again to clear the selection.

    *   To clear all currently selected vessels in a single step, click the Clear All icon ▤.

    *   To group the vessels by the owner (user), click the Group by User icon 👥.

> *This options is particularly useful for the logged-in user, who can now see immediately see the list of vessels that he/she has scanned, and archive them as appropriate.*

Figure A-2:   Archive page, Vessels grouped by owner
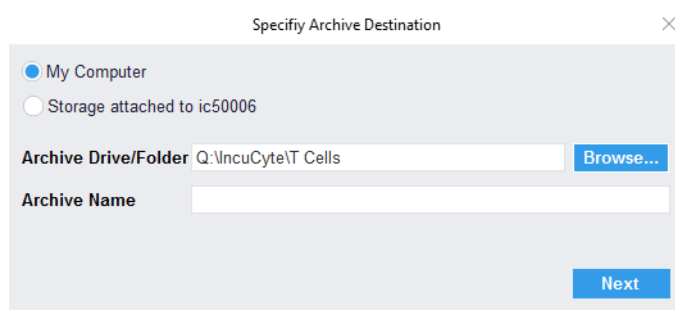
> *The columns on the Archive page contain the standard options for sorting and filtering the data that you have encountered in other areas of the application, for example, the Add Vessel wizard. Refer to one of these sections in the manual if you need a refresher about sorting and/or filtering the data.*

3. After you have selected all the vessels that you are archiving, click the Archive Vessel icon ▣.

    The Specify Archive Destination dialog box opens. By default, My Computer is selected.

Figure A-3:   Specify Archive Destination dialog box

4. Leave My Computer selected, or select Storage Attached to Incucyte.

5. Click Browse to open the Select a folder dialog box, and then browse to and select the appropriate drive and folder for the archive.

> 🖐 *If you selected Storage Attached to Incucyte, then an Incucyte-attached Storage message opens with a list of caveats about selecting this option. You must click OK to close this message before you can continue to the next step.*

Figure A-4:   Incucyte-attached Storage message
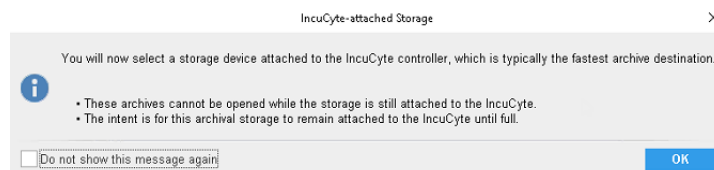
6. Enter a name for the archive.

7. Click Next.

    Two results are possible:

    •   If you selected only a single vessel for archiving, then the Scan Times and Wells dialog box opens. Go to Step 8.

Figure A-5:   Scan Times and Wells dialog box
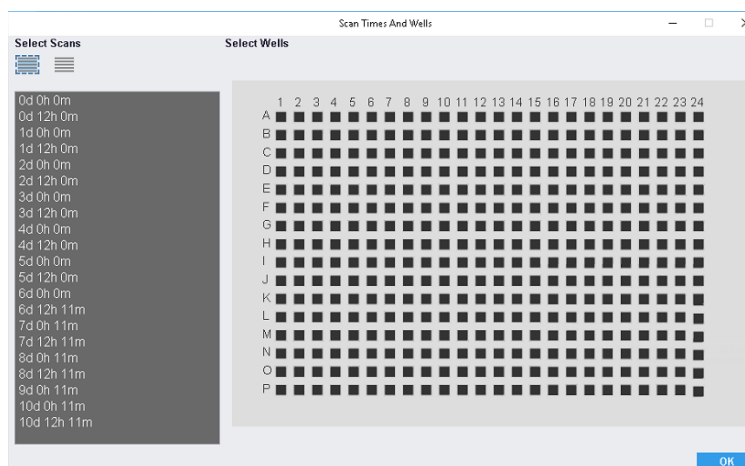
    •   If you selected more than one vessel for archiving, then the Estimating Archive dialog box opens. The dialog box displays the estimates of both the archive size and the time required to archive the selected data. After the estimates are completed, Create Archive is enabled on the dialog box. Go to

Figure A-6:   Estimating Archive dialog box
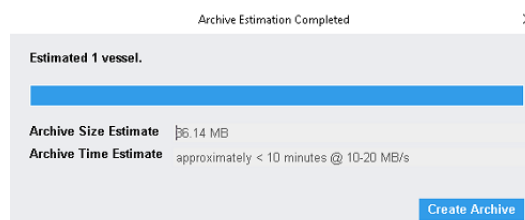
8. Use the options on the Scan Times and Wells dialog box to select the scan times and vessel locations that you are archiving.

The Scan Times and Wells dialog box contains the same standard options for selecting scan times and vessel locations that you have encountered previously in the Incucyte software, such as when you define an analysis or export an image. Refer to one of these sections in the manual if you need a refresher about selecting scan times and/or vessel locations.

9. Click OK.

The Scan Times and Wells dialog box closes. The Estimating Archive dialog box opens. The dialog box displays the estimates of both the archive size and the time required to archive the selected data. After the estimates are completed, Create Archive is enabled on the dialog box.

Figure A-7: Estimating Archive dialog box
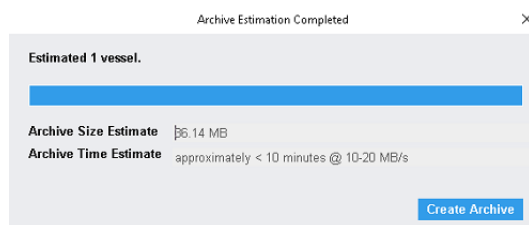
10. Click Create Archive.

A Preparing Archive message opens. The message informs you that the archive job runs in a new window and you can exit the Incucyte software. A separate Archiving status message also opens, indicating the status of archiving the selected data.

Figure A-8: Preparing Archive message
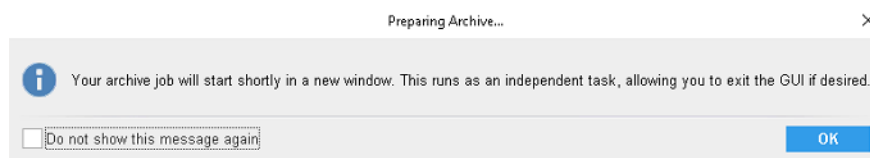
To keep the Preparing Archive message from opening again for this and all future instances of Incucyte, click Do not show this message again.

Figure A-9: Archiving status message
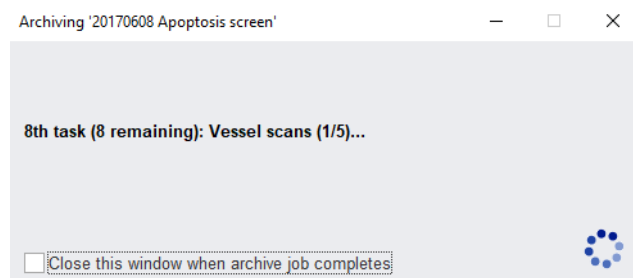
11. In the Preparing Archive message, click OK.

    The Preparing Archive message box closes.

12. Optionally, in the Archiving status message, click Close this window when archive job completes, or wait until the message indicates that the archive job is complete, and then in the upper right corner of the message, click the "X" to close it.

Viewing a List of the Archives Attached to Incucyte

Because you cannot open archives while the storage is attached to the Incucyte controller and you should detach and reattach the external storage device only when absolutely necessary, a View Archives Attached to Incucyte function is provided in the Incucyte software. This function allows you to view a list of all the archives that are currently stored on the dedicated device that is attached to the Incucyte controller without having to detach the device. You can view the archives that are attached to your Incucyte controller at any time.

To open an archive that is attached to the Incucyte storage device, see "Opening an Archive" on page 161.

To view a list of the archives attached to Incucyte

1. On the Incucyte menu, click Archive.

    The Archive window opens. By default, Create New Archive is selected. See Figure A-1 on page 155.

2. Click View Archives Attached to Incucyte.

    The Archive window is refreshed with a list of archives, sorted by archive name, that are stored on the dedicated device that is attached to your Incucyte controller. The standard Search, Sort, and Filter features are available for searching for a specific archive.

Because the Archive window displays the vessels as they are scanned in real-time, you should periodically click the Refresh icon to update the display with the most recently scanned vessels.

At any time, you can click the Copy icon to copy the information that is currently displayed in the Archive window to your client clipboard, and then you can paste this information in to a third-party application such as Microsoft Excel.

Opening an Archive

An archive is saved with a file extension of icarch. You can open and view any archive that has been created for your Incucyte system, whether the archive is saved to My Computer or Storage Attached to Incucyte. If you elect to open an archive that has been saved to Storage Attached to Incucyte, then be aware of the following:

To view an archive that has been saved to Storage Attached to Incucyte, you must first detach the storage device from your Incucyte system, and then attach the device to your Incucyte client so that the Incucyte software can recognize the device.

You have two options for opening an archive: You can open an archive during the launch of Incucyte, or you can open an archive with a connection after you have launched Incucyte.

To open an archive when you launch Incucyte

1. Double-click the desktop icon to launch Incucyte, or select the option from the Start menu.

    The Incucyte Open Connection dialog box opens. By default, Connect to Incucyte is selected. See

2. Select Open an Archive.

    The Connection option is updated to display Open.

Figure A-10:   Incucyte Open Connection dialog box, Open an Archive selected
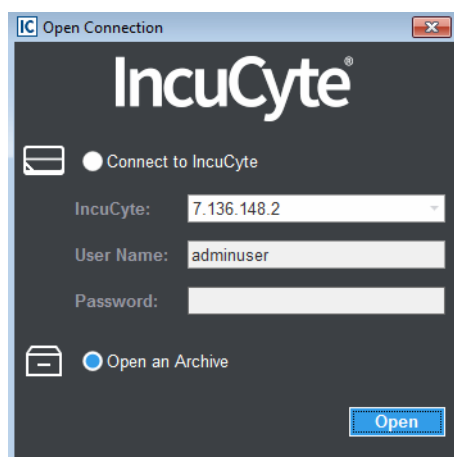
3. Continue to

To open an archive with a connection

1. From any window with a Menu bar in Incucyte, click the Menu bar to open a dropdown menu.

2. On the dropdown menu, select Connection.

3. On the Connection sub-menu, click Open.

   The Incucyte Open Connection dialog box opens. By default, Connect to Incucyte is selected. See

4. Select Open an Archive.

   The Connection option is updated to display Open.

Figure A-11:   Incucyte Open Connection dialog box, Open an Archive selected
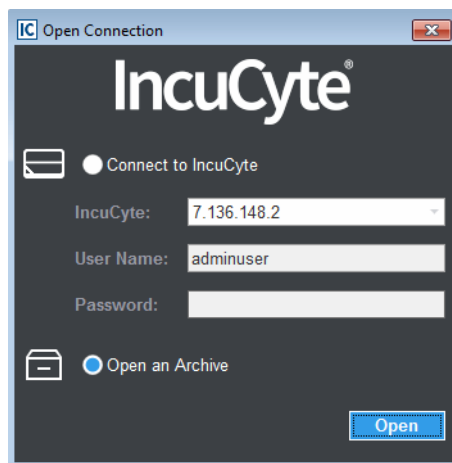
5. Continue

To open an archive

1. Click Open.

   An Instructions for Opening Archives message opens. The message indicates that you must select a folder where Incucyte archives have been written and that folder and all its sub-folders are automatically searched for archives.

Figure A-12:   Instructions for Opening Archives message
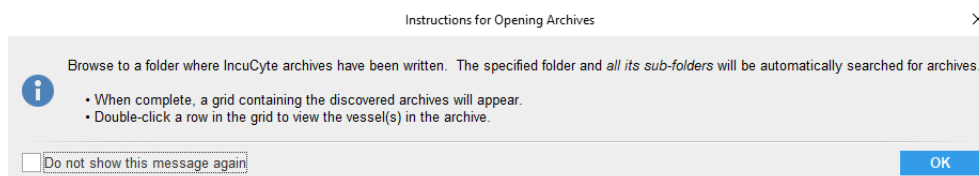
> *To keep the Preparing Archive message from opening again for this and all future instances of Incucyte, click Do not show this message again.*

2. Click OK.

   The message closes and the Select Folder dialog box opens.

3. In the Select Folder dialog box, browse to and select the appropriate folder.

    The Open Archive window opens. The window displays a list of all the archives that are contained in the selected folder. Note the following about the window:

    - The standard Search, Sort, and Filter features are available for searching for a specific archive.

    - You can click the Expand/Collapse icon that is displayed in the Vessels column for an archive to expand the archive and view a list of all the vessels that are contained in the archive.

    - Because the Archive window displays the vessels as they are archived in real-time, you should periodically click the Refresh icon ![icon] to update the display with the most recently archived vessels.

    - At any time, you can click the Copy icon ![icon] to copy the information that is currently displayed in the Archive window to your client clipboard, and then you can paste this information in to a third-party application such as Microsoft Excel.

Figure A-13:   Open Archive window
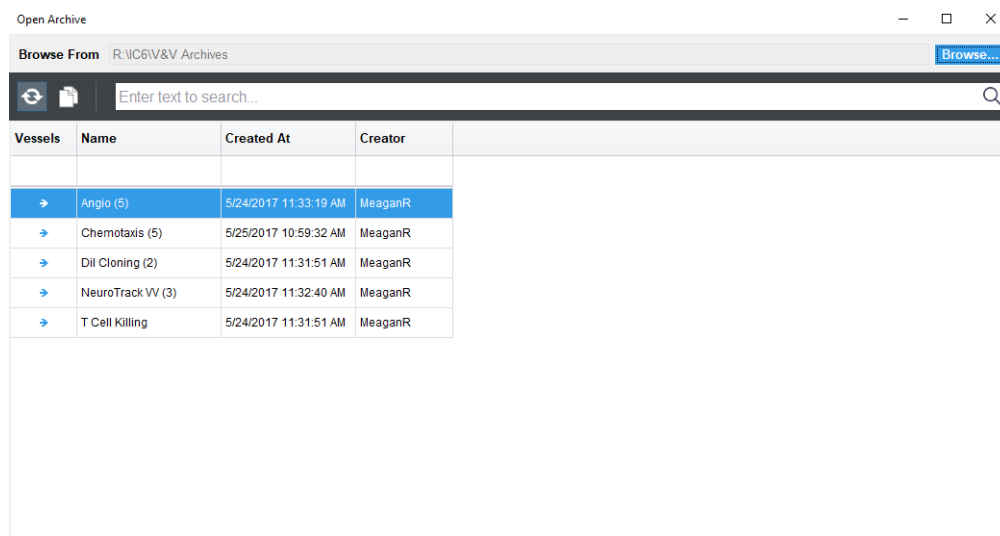
![hand icon] *After you open an archived vessel, the Vessel View window, which has the exact same features as the Vessel View window for an unarchived vessel, opens. For full details about this window and its available functions, see "The Vessel View Window" on page 67.*

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Appendix B
Changing the Incucyte SX1 Objective

If you are using the Incucyte SX1, then when you are specifying the settings for a vessel scan, the currently installed objective is set as the default objective. If you require a different objective, then you must select it *before* you configure and schedule a vessel scan. You must have Administrator privileges to change the objective.

> ⚠️ *If you configure and schedule a vessel scan for the Incucyte SX1, and then change the objective, the current schedule is immediately cleared and all scans for all vessels currently found in the schedule are immediately ended.*

This appendix covers the following topics:

-

Changing the Incucyte SX1 Objective

If you are using the Incucyte SX1, then when you are specifying the settings for a vessel scan, the currently installed objective is set as the default objective. If you require a different objective, then you must select it *before* you configure and schedule a vessel scan. You must have Administrator privileges to change the objective.

> ⚠ *If you configure and schedule a vessel scan for the Incucyte SX1, and then change the objective, the current schedule is immediately cleared and all scans for all vessels currently found in the schedule are immediately ended.*

To select the objective for the Incucyte SX1

1. On the Incucyte main window, click Device. See Figure 1-3 on page 18.

   The Device menu opens. The Calibration Tests tab is the open tab.

   > 💡 *You can also click Device in the menu that is displayed at the top of every window in Incucyte.*

2. Click Administer.

   The Administer tab opens.

Figure B-1:   Incucyte SX1 Administer tab
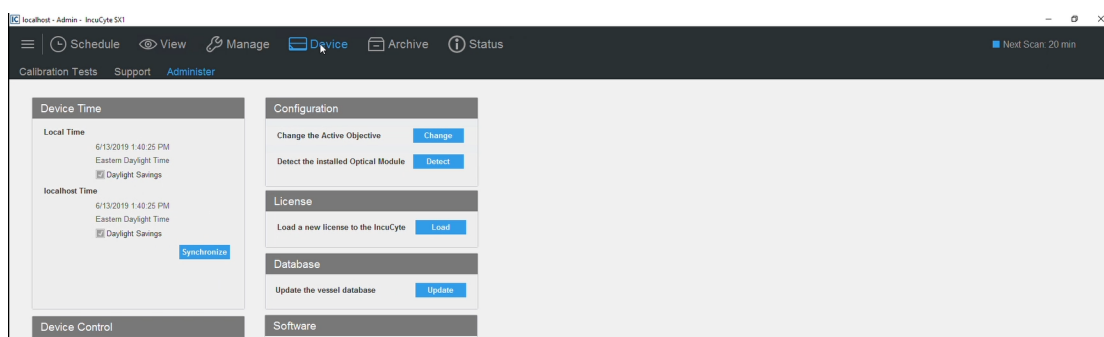
3. Under Configuration, select Change the Active Objective, and then click Change.

   The Change Active Objective dialog box opens.

Figure B-2:   Change Active Objective dialog box
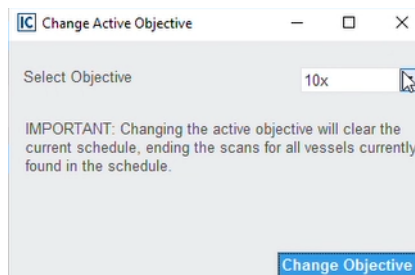
4. On the Select Objective dropdown list, select the new objective, and then click change Objective.

    A Change Active Objective message opens on top of the Change Active Objective dialog box. The message states that the objective has been changed, and that the schedule has been cleared.

Figure B-3:   Change Active Objective message
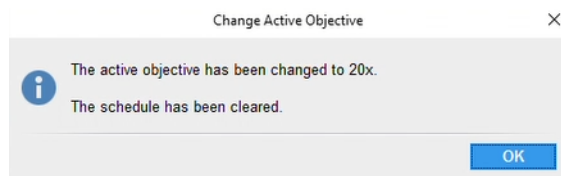
5. Click OK.

    Both the Change Active Objective dialog box and message close. You can now configure and schedule a vessel scan. See "Configuring and Scheduling a Vessel Scan" on page 27.

Appendix C

Changing the Incucyte SX5 Optical Module

If you are using the Incucyte SX5, and you require a different optical module, then you must select it *before* you configure and schedule a vessel scan. You must have Administrator privileges to change the optical module.

⚠️ *If you configure and schedule a vessel scan for the Incucyte SX5, and then change the optical module, the current schedule is immediately cleared and all scans for all vessels currently found in the schedule are immediately ended.*

This appendix covers the following topics:

Appendix C
Changing the Incucyte SX5 Optical Module

Incucyte Live-Cell Analysis Systems User Manual

Changing the Incucyte SX5 Optical Module

If you are using the Incucyte SX5, and you require a different optical module, then you must select it *before* you configure and schedule a vessel scan. You must have Administrator privileges to change the optical module.

> ⚠ *If you configure and schedule a vessel scan for the Incucyte SX5, and then change the optical module, the current schedule is immediately cleared and all scans for all vessels currently found in the schedule are immediately ended.*

To change the Incucyte SX5 optical module

1. On the Incucyte main window, click Device. See Figure 1-3 on page 18.

   The Device menu opens. The Calibration Tests tab is the open tab.

> 💡 *You can also click Device in the menu that is displayed at the top of every window in Incucyte.*

2. Click Administer.

   The Administer tab opens.

Figure C-1:   Incucyte SX5 Administer tab
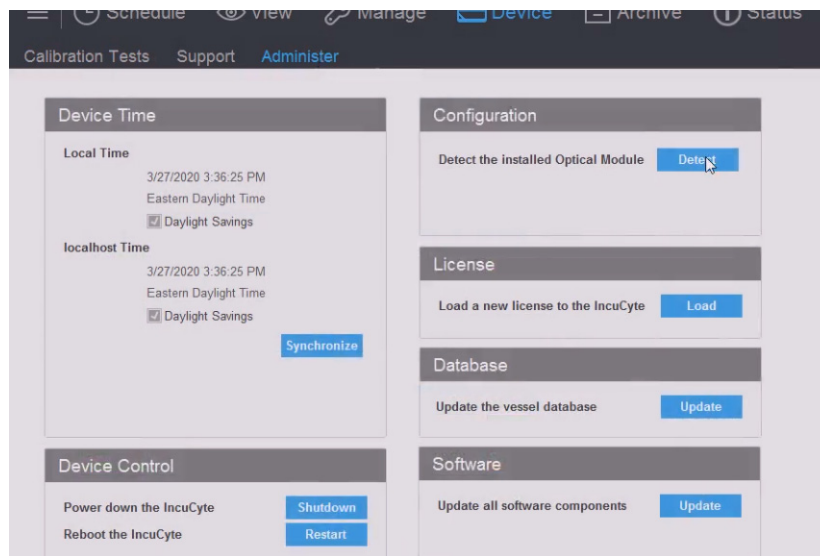
3. For the Configuration option, click Detect.

    A Detect Optical Module message opens, informing you that detection verification of the optical module and phase lamp is going to take place, and asking you if you want to continue.

Figure C-2: Detect Optical Module message
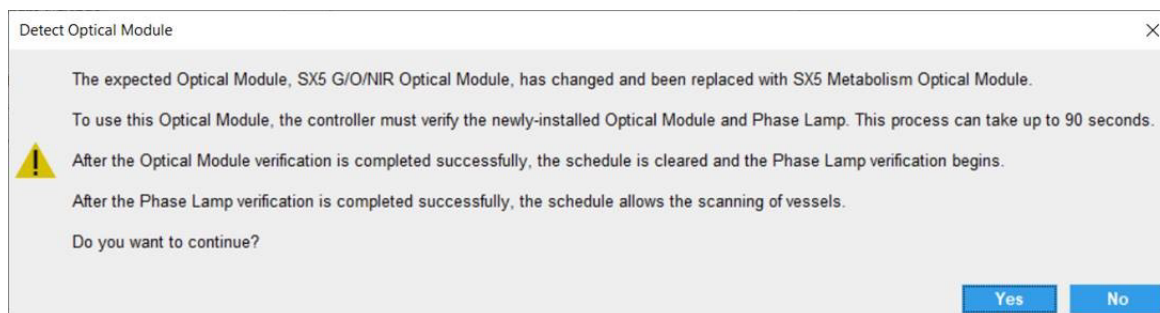
4. Click Yes.

    A status message opens, indicating that the detection verification process has begun. After the process has successfully completed, the status message closes and a Verification Success and Reset Schedule message opens.

Figure C-3: Verification Success and Reset Schedule message
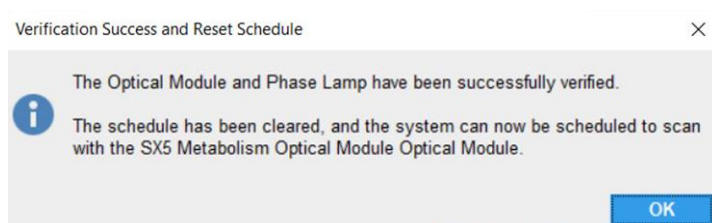
5. Click OK.

    You can now configure and schedule a vessel scan. See "Configuring and Scheduling a Vessel Scan" on page 27.

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