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

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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 view the manual online, or print the manual using a single-sided printer, you might see a single blank page at the end of some chapters or appendices, or after a chapter page, appendix page, or section page. These blank pages have been added solely to ensure that the next chapter, appendix, or section begins on an odd-numbered page and in no way indicates that the manual is missing information.
- Screen shots in the manual are currently a combination of Sartorius branding (yellow/black/gray) and Essen BioScience (blue/gray) branding. The differences in branding are solely cosmetic and in no way reflect any differences in or deprecation of the software functions.

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*.

Preface

• 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 25 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 59 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 1, "Selecting a Vessel," on page 61 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 2, "Viewing and Working With Images," on page 71 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 93 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 1, "Defining the Image Analysis," on page 95 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 113 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 141 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 143 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 155 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 157 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 169 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 173 details how to change the optical module for the Incucyte SX5 before you configure and schedule a vessel scan.
 - Appendix D, "Incucyte Security," on page 177 details all the functions that an Incucyte administrator has at their disposal to manage Incucyte users and vessel workgroups.

Preface

Section 1 Scheduling and Acquiring Scans

Section Contents

- "Getting Started With Incucyte" on page 15
- "Managing Scans" on page 25

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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:

- "Starting and Logging In to Incucyte" on page 17.
- "The Incucyte Main Window" on page 19.
- "Filtering and Sorting Data in an Incucyte Window" on page 21.
- "Searching for Data in an Incucyte Window" on page 22.
- "Logging out of Incucyte" on page 23.

Chapter 1 Getting Started With Incucyte

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). When you start Incucyte, you have the option of connecting to a device or opening an Incucyte archive.

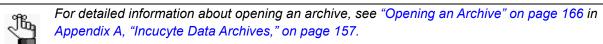


Figure 1-1: Incucyte desktop icon



To start and log in to 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. The drop-down list that is displayed above these two connection options shows a history of device logins.

Figure 1-2: Incucyte Open Connection dialog box



2. On the Incucyte drop-down list, select an instrument identifier, or enter the correct value.



The Incucyte value is the instrument identifier that was assigned to your Incucyte system at installation, for example, IC50014.

3. Click Connect to a Device.

The Login dialog box opens. See Figure 1-3 on page 18.

Figure 1-3: Incucyte Open Connection dialog box



4. Enter your user name and password, and then click Connect.



Sartorius establishes an Administrator user name and password with the purchase of the instrument. If you are the Administrator user, then you can create additional user accounts from the Security main menu option. When you create these additional user accounts, their credentials can be either Incucyte-specific or they can be Microsoft Windows domain credentials. See "Adding a User to Incucyte" on page 179.

The Incucyte main window opens. See "The Incucyte Main Window" on page 19.

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 1-4: 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 title bar of the Incucyte main window 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 Incucyte window.

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 Incucyte window.

Option	Description
Manage	Opens the Manage window, which provides the options for managing critical components (analysis definitions and vessel types) 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 157.
Security	Access the Security functions, which you use for managing all your Incucyte users as well as other security settings. See Appendix D, "Incucyte Security," on page 177.
	Note: Only Administrator users have access to Incucyte security functions.
Status	Opens the Status window, which provides 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.

Filtering and Sorting Data in an Incucyte Window

Many windows or wizard pages in Incucyte display the data in columns, where the data is ordered in ascending order based on the first column. For example, when the Vessel Type Search page in the Add Vessel wizard first opens, the vessels are sorted in ascending order based on the Manufacturer. For such windows or pages, you can hold your mouse pointer over a column header* to display a Filter icon, and you can click once in a column header to display a Sort Order icon.



*With rare exception, the filter and sort functions are available for all the data columns that are displayed for a window or page If these functions are not available for a column, then this is explicitly noted in the manual.

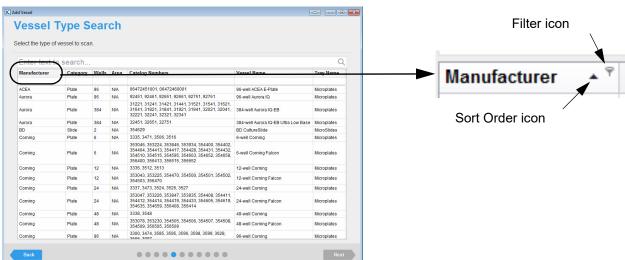


Figure 1-5: Add Vessel wizard, Vessel Type Search page

- 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.
- You can filter the displayed items based on specific property so that only a subset of all available items are displayed. To filter the list based on a selected property, click the Filter icon in the appropriate column header, and then select the filter or define a custom filter. For example, on the Vessel Type Search page, to display only those vessel types that Corning manufactures, click the Filter icon in the Manufacturer column, and then select Corning on the Filter list.



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.

Searching for Data in an Incucyte Window

Many windows or wizard pages in Incucyte display the data in columns, where the data is ordered in ascending order based on the first column. For example, when the Vessel Type Search page in the Add Vessel wizard first opens, the vessels are sorted in ascending order based on the Manufacturer. Many of these windows also have an available Search function to assist you in locating specific data. If you can search the data on an Incucyte window, then a (1) blank Search field that spans all the columns on the window is displayed at the top of the page, with the prompt "Enter text to search" displayed in the field. A (2) blank Search field is also displayed below the header for every column.*



Unless noted otherwise, if a blank Search field is displayed beneath the header for a column, then the column can be searched.

2

IC Add Vesse 👄 🗖 📈 Vessel Type Search Select the type of vessel to scan 1 Enter text to search Manufacture Wells Catalog Number Vessel Nam Cate Area Tray Na ACEA 06472451001 06472460001 Plate 96-well ACEA E-Plat Microplate 92451, 92461, 92651, 92661, 92751, 92761 Aurora Plate 96 N/A 96-well Aurora IQ Microplates 31221, 31241, 31421, 31441, 31521, 31541, 31621, 31641, 31821, 31821, 31941, 31921, 31941, 32021, 32041, 32041, 32221, 32241, 32321, 32341 384 Aurora Plate N/A Microplates Aurora Plate 384 N/A 32451, 32651, 32751 384-well Aurora IQ-EB Ultra Low Base Microplates Slide N/A 354629 BD CultureSlide MicroSlides BD 2 3335, 3471, 3506, 3516 Corning Plate 6 N/A 6-well Corning Microplates 353046, 353224, 353846, 353934, 354400, 354402, 354404, 354413, 354417, 354428, 354431, 354432, 354510, 354515, 354595, 354603, 354652, 354658, Corning Plate 6 N/A 6-well Corning Falcon Microplates 356400, 356413, 356515, 356652 Corning Plate 12 N/A 3336, 3512, 3513 12-well Corning Microplates 12-well Corning 353043, 353225, 354470, 354500, 354501, 354502, 354503, 356470 N/A Plate 12 Corning Microplates Corning Plate 24 N/A 3337, 3473, 3524, 3526, 3527 24-well Corning Microplates 353047, 353226, 353847, 353935, 354408, 354411 Corning Plate 24 N/A 354412, 354414, 354419, 354433, 354605, 354619, 24-well Corning Falcon 354635, 354659, 356408, 356414 Microplates 48 N/A 3338, 3548 Corning Plate 48-well Corning Microplates 353078, 353230, 354505, 354506, 354507, 354508, 354509, 356505, 356509 Corning Plate 48 N/A 48-well Corning Falcon Microplates 3300, 3474, 3585, 3595, 3596, 3598, 3599, 3628, Corning Plate 96 N/A 96-well Cornina Microplates

Figure 1-6: Add Vessel wizard, Vessel Type Search page

When you carry out a search for data in an Incucyte window, note the following:

- To search across all columns, you enter the search string in the blank search field at the top of the page.
- To search based on a specific vessel property, for example, the Manufacturer column on the Vessel Type Search page, you enter the 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 on the Vessel Type Search page 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.

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 (the three vertical lines that are displayed in the top left corner of the window) to open a dropdown menu.
- 2. On the dropdown menu, select Connection.
- 3. On the Connection sub-menu, click Close.

Chapter 1 Getting Started With Incucyte

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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:

- "The Acquisition Window" on page 27.
- "Configuring and Scheduling a Vessel Scan" on page 31.
- "The Vessel Schedule Timeline" on page 52.
- "Editing Vessel Information" on page 56.
- "Removing a Vessel from the Scanning Schedule" on page 57.

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.

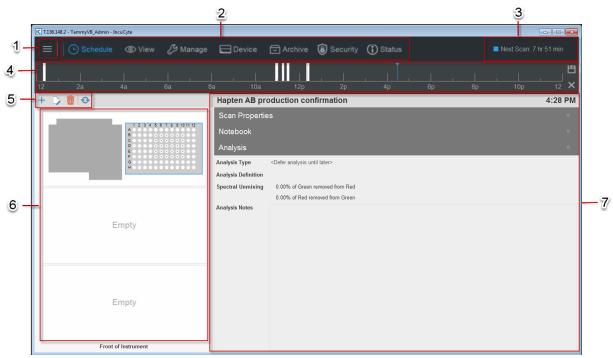


Figure 2-1: Acquisition window

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 143.
		 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	Description 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	
		Next Scan: 15 hr 13 min Status - Ready To Scan Current Time: 4:46 PM Next Scan: 15 hours 13 minutes (8:00 AM) Set By: TammyVB_Admin Set At: 2/10/2017 3:05 PM Filter Module: Green/Red 4614 Free Space (% free): Disk: 11.2 TB (68%) Database: 9.5 GB (95%) Temperature: 26.6°C	
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 <i>per vessel scheduling</i>), saving a vessel scanning schedule, and deleting a vessel scanning schedule. See "The Vessel Schedule Timeline" on page 52.	

	Window component	Description			
5	5 Vessel toolbar: Displays icons for carrying out specific tasks in the Acquisition window. From left 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 31.			
		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 56.			
	Ŵ	Remove selected vessel icon: Removes a selected vessel from the scanning schedule. See "Removing a Vessel from the Scanning Schedule" on page 57.			
	Ð	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 35. 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 			
		 41. 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 43. See Figure 2-3 on page 30. 			

IC Toxicity Panel Assay (Green Reagents) - IncuCyte							
$\equiv igl \bigcirc$ Schedule \textcircled{O} View	🔗 Manage 🛛 🗖 Devi	ce 🚍 Archive	(i) Status			•	Next Sca
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+ 🗅 💼 😔	Cyt	otox II					
1 2 3 4 5 6 7 8 9 10 11 12 A C C C C C C C C C C	Vess Scan	Type Standa e Channels Pha: Cree Acqu Red	se m uisition Time (ms) 300				
Empty		ctive 10x	uisition Time (ms) 400 estimated)				
Empty	No	te Map tebook					
Front of Instrument	Ana	alysis					
				ct Plate I ysis to o			

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

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 a 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 169.

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 32.
- 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 33.
- 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 34.
- 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 35.
- 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 36.
- 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 37.
- 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 39.
- 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 41.

- 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 43.
- 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 44 or "To set the scanning schedule for the Incucyte SX1" on page 45.
- 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 scan" on page 47.

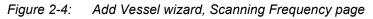


You can schedule only a single vessel at a time. You must repeat each of these procedures in their entirety for each vessel that you are scheduling.

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.



IC Add Vessel		
Repeatedly or Once?	•	
Specify whether the vessel will be scanned rep	peatedly or only once.	
Scan on Schedule Select this option to add a wessel to the repea	ting scan schedule	
• Scan Once Now Select this option to immediately scan a single	e vessel just once.	
Back	••••	Next

- 2. Leave Scan on Schedule selected.
- 3. Click Next.

The Create Vessel page opens.

4. Continue to "To create the vessel" on page 33.

To create the vessel

C Add Vessel		
Create or	Restore Vessel	
Either create a new v	ssel from scratch, copy an existing vessel, or restore a previously-scanned vessel.	
Create Vessel		
+ New Select this	ption to create a brand new vessel to scan.	
Copy C Select this	urrent plan to create a new vessel by copying a vessel from the current schedule.	
R Copy F Select this	revious ption to create a new vessel by copying a previously scanned vessel.	
Restore Vess	al de la constante de la const	
Select this	ans plon to restore a previously-scanned vessel for additional scans.	
Back		Next

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.

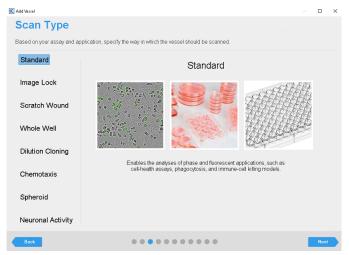
- 5. Under Create Vessel, leave New selected.
- 6. Click Next.

The Scan Type page opens.

7. Continue to "To select the scan type" on page 34.

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.



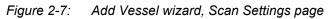
If your organization has not licensed the Chemotaxis, Spheroid, Scratch Wound, and/or Neuronal Activity modules, then when you select one of these modules on the Scan Type page, a message opens at the bottom of the page that states that you do not have a license for the selected acquisition type and that you must contact your Sartorius 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 35.

To specify the scan settings



Add Vessel		
Scan Set	tings	
Specify the image	channels, microscope objective and other settings to use when scanning the vessel.	
Image Channels *	Phase	
	Green	
	Acquisition Time (ms) 300 🗘	
	Red	
	Acquisition Time (ms) 400 ‡	
Objective	10x •	
Back		Next

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 169.



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 27.

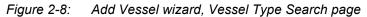
2. Click Next.

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.



/essel Type Search								
Enter text to						C		
Manufacturer	Category	Wells	Area	Catalog Numbers	Vessel Name	Tray Name		
ACEA	Plate	96	N/A	06472451001, 06472460001	96-well ACEA E-Plate	Microplates		
Aurora	Plate	96	N/A	92451, 92461, 92651, 92661, 92751, 92761	96-well Aurora IQ	Microplates		
Aurora	Plate	384	N/A	31221, 31241, 31421, 31441, 31521, 31541, 31621, 31641, 31821, 31841, 31921, 31941, 32021, 32041, 32221, 32241, 32321, 32341	384-well Aurora IQ-EB	Microplates		
Aurora	Plate	384	N/A	32451, 32651, 32751	384-well Aurora IQ-EB Ultra Low Base	Microplates		
BD	Slide	2	N/A	354629	BD CultureSlide	MicroSlides		
Corning	Plate	6	N/A	3335, 3471, 3506, 3516	6-well Corning	Microplates		
Corning	Plate	6	N/A	353046, 353224, 353846, 353934, 354400, 354402, 354404, 354413, 354417, 354428, 354431, 354432, 354510, 354515, 354595, 354603, 354652, 354658, 356400, 356413, 356515, 356652	6-well Corning Falcon	Microplates		
Corning	Plate	12	N/A	3336, 3512, 3513	12-well Corning	Microplates		
Corning	Plate	12	N/A	353043, 353225, 354470, 354500, 354501, 354502, 354503, 356470	12-well Corning Falcon	Microplates		
Corning	Plate	24	N/A	3337, 3473, 3524, 3526, 3527	24-well Corning	Microplates		
Coming	Plate	24	N/A	353047, 353226, 353847, 353935, 354408, 354411, 354412, 354414, 354419, 354433, 354605, 354619, 354635, 354659, 356408, 356414	24-well Corning Falcon	Microplates		
Corning	Plate	48	N/A	3338, 3548	48-well Corning	Microplates		
Corning	Plate	48	N/A	353078, 353230, 354505, 354506, 354507, 354508, 354509, 356505, 356509	48-well Corning Falcon	Microplates		
Corning	Plate	96	N/A	3300, 3474, 3585, 3595, 3596, 3598, 3599, 3628, 2885, 2007	96-well Corning	Microplates		

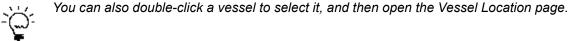
1. Select the appropriate vessel type.



You can filter and sort or search the data on the Vessel Type Search page using the standard Incucyte filter and sort or search functions. See ""Filtering and Sorting Data in an Incucyte Window" on page 21 and "Searching for Data in an Incucyte Window" on page 22.

2. Click Next.

The Vessel Location page opens.



- 3. Continue to "To select the vessel location" on page 37.

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 and the shape of the location cutout are visual indicators of important information.

- 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

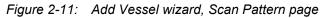
- 2. Do one of the following:
 - Select a different vessel location.
 - In the Vessel Schedule table, select the vessel or vessels that you are removing from the schedule, and then click "I wish to remove the following vessel(s) from the schedule."
- 3. Click Next.

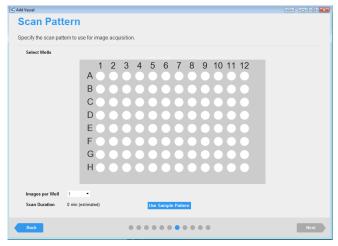
The Scan Pattern page opens.

4. Continue to "To set the scan pattern" on page 39.

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 is selected for analysis. To set a scan pattern, you can use a sample can pattern based on the manufacturer and vessel type, or you can set your own custom pattern.





- 1. Select the appropriate option.
 - To view and use a sample scan pattern, 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.

To set your own custom scan pattern for the vessel, you can do any or all of the following:



For any custom option, after you select at least one well or sector, the Use Sample Pattern dialog box opens. To continue setting your scan pattern without this dialog box always 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 on page 40.

- 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

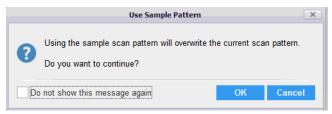
all the selected locations in a vessel column in a single step, right-click a location in the column, and on the context menu that opens, select Deselect this Column.

- 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.

Figure 2-12: Use Sample Pattern dialog box



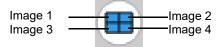
2. Specify the number of images that you are acquiring per vessel location (well, sector, or so on).

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



3. Click Next.

The Notebook page opens.

4. Continue to "To provide vessel information" on page 41.

To provide vessel information

-						
e Everyone						
					+ 🖟 💕	9
1	2	3	4	5	6	_
		No Plate Map S	pecified			
	NA 🕻 🗙 h Everyone	h Everyone	1 Everyone	N Everyone	1 Everyone	1 Everyone + D K

Figure 2-14: Add Vessel wizard, Vessel 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, you can assign a vessel workgroup to the vessel, and also, if applicable, you can 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.
 - A vessel workgroup defines the users who are able to see the data for a vessel. Assigning a workgroup to a vessel is alway optional. By default, the Everyone vessel workgroup is assigned to a vessel, which means that any user can view a vessel and its data. To limit the users who can view a vessel and its data, you must assign one or more vessel workgroups to the vessel. To assign a workgroup to a vessel, do the following:
 - a. At the far right of the Shared with field, click the Edit icon 📑 to open the Edit Vessel Shared Workgroups dialog box.

× *

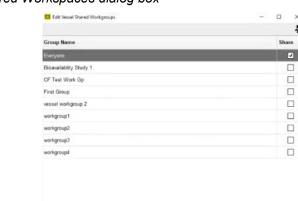


Figure 2-15: Edit Shared Workspaces dialog box

- b. Do one or both of the following:
 - To assign a workgroup to the vessel, click Share for the vessel.



If the appropriate vessel workgroup is not available, contact your Incucyte administrator.

• To remove a workgroup from the vessel, clear the Share selection for the vessel.



If you clear all workgroup assignments for a vessel, then, by default, the assignment is automatically reset to Everyone.

- c. Click Apply to close the Edit Shared Workspaces dialog box and return to the Vessel Notebook page. If applicable, the assigned vessel workgroups are displayed in the Shared with field; otherwise, the **Everyone** vessel workgroup is displayed.
- 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 143.



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 27.

2. Click Next.

The Analysis Setup page opens.

3. Continue to "To define the analysis setup" on page 43.

To define the analysis setup

And an an an abusis to	to send a structure for the state to a send the structure of the large structure to the structure of a d	
venne an analysis to	launch automatically after the vessel has been scanned. This is optional, but recommended.	
Analysis Type	<defer analysis="" later="" until=""></defer>	
Analysis Definition		
Spectral Unmixing	0.00 ² % of Green removed from Red	
	0.00 0 % of Red removed from Green	
Analysis Notes		

Figure 2-16: 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 73.

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 27.

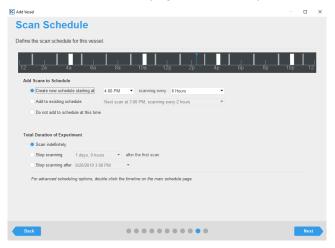
2. Click Next.

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 45.

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

Figure 2-17: 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 52.) 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 52 or "Editing Vessel Information" on page 56.

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 49.

3. Click Next.

The Summary page opens.

4. Continue to "To verify the vessel acquisition settings and schedule the scan" on page 47.

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 2-18 on page 46.
- 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 2-19 on page 46.

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 52.) 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).

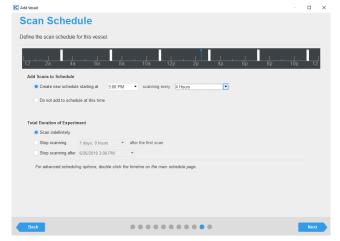


Figure 2-18: Add Vessel wizard, Scan Schedule page for Incucyte SX1 (First scheduled vessel)

Figure 2-19: Add Vessel wizard, Scan Schedule page for Incucyte SX1 (Subsequently scheduled vessel)

IC Add Vessel			×
Scan Schedule			
Define the scan schedule for this vessel.			
12 $2a$ $4a$ $6a$ $8a$ $10a$ $12p$ $2p$ $4p$ $6p$ $8p$	 10p	12	
Add Scans to Schedule			
Add to existing schedule Next scan at 3:00 PM, scanning every 4 hours			
Total Duration of Experiment			
 Scan indefinitely 			
Stop scanning 1 days, 0 hours v after the first scan			
Stop scanning after 6/26/2019 3:00 PM 👻			
For advanced scheduling options, double click the timeline on the main schedule page.			
Back		Next	

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 52 or "Editing Vessel Information" on page 56.

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 49.

3. Click Next.

The Summary page opens.

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

To verify the vessel acquisition settings and schedule the scan

Figure 2-20: Add Vessel wizard, Summary page

erify the information below is correct	before continuing.	
Tray Type	Microplates	
Tray Position	Front	
Vessel Position	Right	
Remove Current Tray	No	
Remove Vessels	No	
Scan Pattern		
Included Wells	15	
Images per Welt	1	
Scan Duration	1 min (estimated)	
/essel Notebook		
Name	delete	
Cell Type	<none specified=""></none>	
Passage	N/A	
Plate Map	No	
Notes	<none specified=""></none>	
Vessel Workgroups	workgroup1, workgroup2	
Analyses		
Analysis Type	<defer analysis="" later="" until=""></defer>	
Scan Schedule		
Add Scans to Schedule	Next scan at 3:21 PM, scanning every 24 hours	
Total Duration of Experiment	Stop scanning 1 Day, 0 Hours after the first scan	

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.

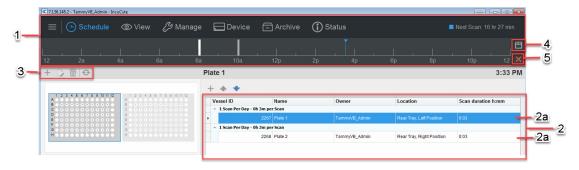


If you did not schedule at least one repeating scan for the vessel, then an exclamation point is displayed on the vessel and when you place your mouse pointer over the vessel, a tooltip opens with a message indicating that no scans are scheduled for the vessel. You can click the Vessel Schedule Timeline 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 52. Optionally, you can edit the vessel. See "Editing Vessel Information" on page 56.

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 2-21: Acquisition window, Advanced scheduling features for a vessel



		Description			
1	at the to indicated is currer schedul	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 52. 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 <i>per vessel scheduling</i> . Vessels with identical daily scanning schedules are organized in the Vessel Schedule table <i>by scan groups</i> .				
2a	Scan groups. Vessels with identical daily scanning schedules are organized in the Vessel Schedule table by <i>scan groups</i> , 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 2-21 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 icc	on: Click this icon to save the changes that you make to an existing vessel schedule.			
5	Cancel i	con: 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 51.

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 2-22: 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 52.
- 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, rightclick 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:

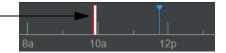
- Delete a single repeating scan from a scan group.
- Delete a single scan from multiple repeating scans for a scan group, or delete all the repeating scans in a scan group.
- Shift the scan time for a single repeating scan in a scan group.
- Shift the scan time for a single scan in a group of repeating 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 56.

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 2-23: 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 2-24: Vessel Schedule Timeline showing schedule for selected vessel

						 	8
12 2a 4a			12p				12 🗙
+ 🗘 💼 😔		Plate 1					2:54 PM
		Scan Properties	3				*
1 2 3 4 5 6 7 8 9 10 11 12 A B B B B B B B B B B B B B B B B B B B	1 2 3 4 5 6 7 8 9 10 11 12 A B • • • • • • • • • • • •	Vessel Type	96-well Corning	(Blk/Wht)			
6	G G G G G G G G G G G G G G G G G G G	Scan Type	Standard				
E = 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	E 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Image Channels	☑ Phase ☑ Green				
н	H • • • • • • • • • • • • • •		Acquisition 1	Fime (ms) 300			

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 2-25: Vessel Schedule Timeline display with a currently scheduled vessel selected in the Vessel Drawer Setup pane

IC 7.136.148.2 - TammyVB_Admin - In	cuCyte								*		
	⊚ View	🔗 Manage		Device	Archive	Security	(i) Status			Next Scan: 1	6 hr 27 min
 122a			8a		0a 12	p2p					12 ×
+			P	late 1							3:33 PM
1 2 3 4 5 6 7 8 9 10 11 12		5 6 7 8 9 10 11 12		+ + •	r -						
A B	A 0 0 0 0 B 0 • • •	0 0 7 8 9 10 11 12		Vessel ID	N	ame	Owner	L	ocation	Scan duratio	on h:mm
C	C			 1 Scan P 	er Day - 0h 3m per S	an					
D 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			•		2267 PI	ate 1	TammyVB_Admin	F	Rear Tray, Left Position	0:03	
F • • • • • • • • • • • • • •					er Day - 0h 3m per S	an					
н					2268 PI	ate 2	TammyVB_Admin	F	Rear Tray, Right Position	0:03	

- 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

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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 2-26 on page 54.
 - 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 2-26: Set Timeline Scan Intervals dialog box

IC Set Timeline Scan Intervals										
Starting At	8:00 AM									
Add Scans Every	4 Hours 🔻									
For a Total of	24 C Hours									
Schedule Repeats Daily										
	ОК									

• 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 **[1]**.

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

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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, then 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 🎝

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

Figure 2-27: Add Vessel wizard in Edit mode, Scan Settings page

dit Vessel		
Scan Se	ttings	
Specify the image	channels, microscope objective and other settings to use when scanning the vessel.	
Image Channels	V Phase	
	Creen	
	Acquisition Time (ms) 300 C	
	✓ Red	
	Acquisition Time (ms) 400 C	
Objective	10x •	
Back	• • • • • •	Next

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 "To verify the vessel acquisition settings and schedule the scan" on page 47.

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.

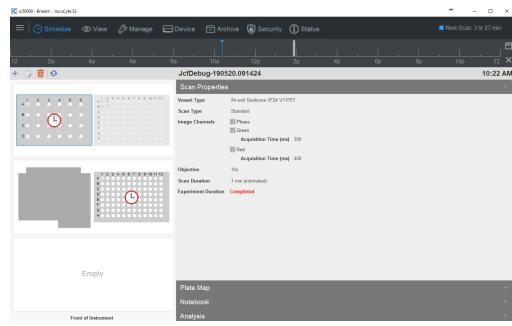


Figure 2-28: Completed scan duration for a vessel

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

- "Selecting a Vessel" on page 61
- "Viewing and Working With Images" on page 71

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Chapter 1 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" on page 63.
- "The Vessels Pane" on page 66.

Chapter 1 Selecting a Vessel

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.

IC ic50017	- LindyO - IncuCyte										x
≡	🕒 Schedule 💿 View 🖉 M	lanage	🗖 Devid	e 🗖 A	rchive	8	Secu	rity	(i) Status		Next Scan: 4 hr 58 min
🔁 📋	Enter text to search								Q		3 4 5 6 7 8 9 10 11 12
Analyses	Vessel Name	Owner	Last Scan 👻	Scan Type	Vessel	Co	Co	Ph	Magnification		
										and the second se	
*	1/19/17 MR Polymer Tox Assay Plate 2	LabUser	1/30/2017 7:48	Standard	58	No	Yes	Yes	20x	and the second s	
>	1/19/17 MR Polymer Tox Assay Plate 1	LabUser	1/30/2017 7:48	Standard	57	No	Yes	Yes	20x		
*	ICC beta tubulin & GFAP	AaronO	1/27/2017 4:43	Standard	60	Yes	Yes	Yes	20x		
>	MB Efferocytosis	MikeB	1/25/2017 9:33	Standard	59	No	Yes	Yes	10x	H 🛛 🗎	
*	1.4.17_AO monoculture kainate	AaronO	1/24/2017 2:14	Standard	54	No	Yes	Yes	20x		
*	1.4.17_AO monoculture glutamate	AaronO	1/24/2017 2:14	Standard	53	No	Yes	Yes	20x	Vessel Name	2/22/17 MR Astrocyte Comparison Study
>	1.4.17_AO coculture kainate	AaronO	1/24/2017 12:3	Standard	56	Yes	Yes	Yes	20x	Scan Type	Standard
*	1.4.17_AO coculture glutamate	AaronO	1/24/2017 12:3	Standard	55	Yes	Yes	Yes	20x	Cell Type	rat forebrain neurons, astrocyte current lot p2
*	Spheroid Assay - SBio ULA Plate	Meagan	1/13/2017 7:35	Spheroid	51	No	Yes	Yes	4x	Image Channels	Phase
	Spheroid Assay - Corning ULA Plate	Meagan	1/13/2017 7:35	Spheroid	50	No	Yes	Yes	4x		Green
	iGLUTA Fluo-4 stain	JohnR	1/5/2017 5:03	Standard	52	Yes	No	Yes	4x		Red
	Neurotrack Red comparison plate	LabUser	1/4/2017 11:43	Standard	49	No	Yes	Yes	20x	Magnification	20x
	iGLUTA Staurosporine	AaronO	1/3/2017 7:00	Standard	48	No	Yes	Yes	20x	Plate Map	Yes
	IGLUTA coculture AMPA	AaronO	1/3/2017 7:00	Standard	47	No	Yes	Yes	20x	Vessel Type	96-well TPP
	iGLUTA coculture glutamate	AaronO	1/3/2017 7:00	Standard	46	No	Yes	Yes	20x	Vessel ID	79
	IGLUTA monoculture AMPA	AaronO	1/3/2017 7:00	Standard	45	No	Yes	Yes	20x	Vessel Notes	
	IGLUTA monoculture glutamate	AaronO	1/3/2017 7:00	Standard	44	No	Yes	Yes	20x		
*	12/7/2016 IGLUTA monoculture Glutamate-inhibitors	LibbyO	12/29/2016 2:1	Standard	36	No	Yes	Yes	20x		
•	12.7.16 IGLUTA monoculture AMPA-inhibitors	LibbyO	12/29/2016 2:1	Standard	34	No	Yes	Yes	20x		
>	IGLUTA Staurosporine Green and Red scan	LabUser	12/29/2016 1:4	Standard	43	Yes	Yes	Yes	20x		

Figure 1-1: 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 143. 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.

Chapter 1 Selecting a Vessel

	Window component	Description
3	Scanned Vessels toolbar: D	Displays two icons for working with vessels in the Scanned Vessels window:
	Ð	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 "Searching for Data in an Incucyte Window" on page 22.
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. <i>Figure 1-2: Schedule Status tooltip</i>

Chapter 1 Selecting a Vessel

	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 66.
		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 1-3 below.

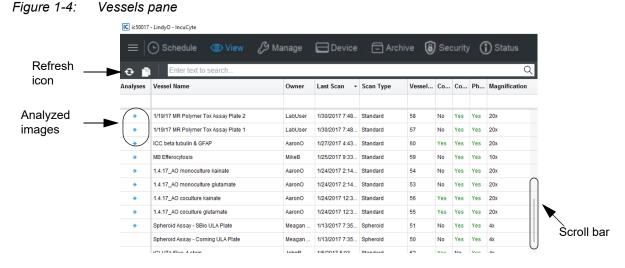
Figure 1-3: Vessel Information pane

IC ic50005 -	LindyO - IncuCyte										-	σ×
=	🕒 Schedule 🛛 💿 View	🖋 Mar	age 🗲	Device	🚍 Ar	chiv	e	6	Security	(i) Status	Next Scan: 5 mi	n
🔁 🗋	Enter text to search									Q		
Analyses	Vessel Name	Owner	Last Scan -	Scan Type	Vessel ID	Col	Col	Pha	. Magnification	Vessel Type		
•	QC2 GFP-HUVEC 717210-BT2-29346 NHDF 717210-A-29345	5 LibbyO	2/12/2017 4:46 AM	Standard	2368	Yes	No	Yes	4x	96-well Corning	D	
•	Anglogenesis PrimeKit	LibbyO	2/12/2017 4:46 AM	Standard	2367	Yes	No	Yes	4x	96-well Corning	E0000000000000	
	2/7/17 MR Viral Titer	MeaganR	2/9/2017 5:56 AM	Standard	2372	Yes	Yes	Yes	10x	24-well Corning	F • • • • • • • • • • • • • •	
•	2/7/17 Libby VDCG	LabUser	2/7/2017 11:36 AM	Whole Well	2371	Yes	No	Yes	4x	24-well Corning	G • • • • • • • • • • • • • •	
*	2/7/17 MR VDCG	LabUser	2/7/2017 11:36 AM	Whole Well	2370	Yes	No	Yes	4x	24-well Corning	НОССССССССССССССССССССССССССССССССССССС	
•	2/7/17 Katie VDG	LabUser	2/7/2017 11:22 AM	Whole Well	2369	Yes	No	Yes	4x	24-well Corning		
*	1/19/17 MR SBio HT-1080 NLR Spheroids 10X	Nevine	2/3/2017 10:10 AM	Spheroid	2366	Yes	Yes	Yes	10x	[10x/4x INTERNAL]	Vessel Name Non-adherent T Cell Killing	
*	1/19/17 Corning HT-1080 NLR Spheroids 10X	Nevine	2/3/2017 10:10 AM	Spheroid	2365	Yes	Yes	Yes	10x	96-well Coming Ro	Scan Type Standard	
*	1/19/17 MR SBIo HT-1080 NLR Spheroids	Nevine	1/27/2017 8:27 AM	Spheroid	2364	Yes	Yes	Yes	4x	[10x/4x INTERNAL]	Cell Type WIL2-NS NucLight Red, PBMCs	
*	1/19/17 Corning HT-1080 NLR Spheroids	Nevine MikeB	1/27/2017 8:27 AM	Spheroid Standard	2363 2362	Yes	Yes	Yes	4x 10x	95-well Corning Ro 6-well Corning	Image Channels 🔤 Phase	
	MB HepG2 Spheroid Infection 2 MB HepG2 Spheroid Infection 1	MikeB	1/20/2017 10:00 AM		2362	No No	No	Yes	10x	6-well Corning	Green	
	Non-adherent T Cell Killing	MeaganR	1/19/2017 1:00 PM		2358	Yes	Yes	Yes	4x	96-well Corning	E Red	
											Magnification 4x	
	alysis Definition Name Analysis Type	e	Creator	Date Completed		nalysis	ID	Analy	ysis Notes		Plate Map	
Nor	n-adherent T Cell Killing (WILS-2NS NLR) Basic Analyzer		MeaganR	1/19/2017 1:02 PM	2	210					Vessel Type 96-well Coming	
	1/19/17 MR SBio HT-1080 NLR Spheroids	LabUser	1/19/2017 11:58 AM		2360	No	Yes		4x	[10x/4x INTERNAL]	Vessel ID 2358 Vessel Note	
	1/19/17 Corning HT-1080 NLR Spheroids	LabUser	1/19/2017 11:58 AM		2359	No	Yes	Yes	4x	96-well Corning Ro	Vessel Note	
*	Adherent T Cell Killing Assay	MeaganR	1/13/2017 7:53 AM	Standard	2353	Yes	Yes	Yes	10x	96-well Corning		
*	Chemotaxis - Low Contrast Adherent Non-Adherent T Cell Killing Assay	LabUser	1/11/2017 8:01 AM	Chemotaxis (Top/Bot)	2357	No	No	Yes	10x	96-well Essen Cle 96-well Coming		
*	bubble ch	MeaganR	1/11/2017 7:53 AM	Standard	2354	Yes	Yes	Yes	4x	96-well Coming	- • ×	
	Non-Adhe 1 2	3	4 5	6	7				9	10 11	12	
	Adherent											
	Adherent *											
	scan failu											
	20 uL, po: *	PLO coating 0.03% (1.2-NS NucLight Red (1) 10K / PDMCs (1) 100K / well 1.200 Annexis V	wit .									
	c	PLO coating 0.02% (8.2-NE NucLight Red (1) 10K / PBMCs (1) 100K / well	vel									
		1:200 Annula V CD3/8-2										
	0											
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	6											
											· · · ·	

Chapter 1 Selecting a Vessel

The Vessels Pane

The list of scanned vessels on the Scanned Vessels window is displayed in the Vessels pane. All vessels that have been assigned to the **Everyone** vessel workgroup and all the vessels that are contained in the vessel workgroups to which you have been assigned are displayed in the 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.



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 **Sec.**
- 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 filter the list of vessels to display only a subset of vessels based on a selected property and you can change the sort order of the vessels in the pane and. See "To filter and/or sort the Vessels pane display" on page 67.
 - 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 67.
 - 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 67.

 You can open the Vessel View window for selected vessel. See "To open the Vessel View window" on page 69.

To filter and/or sort 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 1-5: Sort order icon and Filter icon for a column in the Vessels pane

Analyses	Vessel Name	Owner	Last Scan	Scan Type	Vessel ID
>	121515 MR HT01080 Mix Invasion	LabUser	12/21/2015 8:20 AM	Scratch Wound	1039
>	120315 MR A549 NLR Apoptosis	LabUser	12/7/2015 11:21 AM	Standard	1022
•	120315 MR A549 NLR Apoptosis	LabUser	12/7/2015 11:21 AM	Standard	1021
				Filter icon	
	Vessel Name			Sort icon	
				Contribon	

You can filter and sort the data on the Search page using the standard Incucyte filter and sort functions. See "Filtering and Sorting Data in an Incucyte Window" on page 21.

To search for a vessel or vessels in the Vessels pane

With the exception of the Analyses column, you can use the standard Incucyte search function to 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. See "Searching for Data in an Incucyte Window" on page 22.

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.

Annexin V NeuroHealth Movie	М	ikeB	5/20/2016 4:47 PM		Standard (with lock)	2201	
Analysis Definition Name Analysis Type Creator		Creator	Date Completed		Analysis ID	Analysis Notes	
movie annexin neurotrack		JohnR		:19 PM			

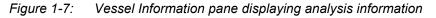


Although the Analysis pane is shown with columns, no filter functions are available.

Chapter 1 Selecting a Vessel



To update the Vessel Information pane display to include the information for an analysis definition, select the analysis in the Analysis pane. 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 1-7 below.



	🕒 Schedule 🛛 💿 View	19 Manao	ne 🖂	Device 🗐	Archive 🔒	Security	A	Stat					Next Scan: 5 m
		Gr			•								
P	Enter text to search										Q		1 2 3 4 5 6 7 8 9 10 11 12
												A	
es	Vessel Name		Owner	Last Scan -	Scan Type	Vessel ID	Col	Col	Pha	Magnification	Vessel Type	E	
												C	
	QC2 GFP-HUVEC 717210-BT2-29346 NHE	F 717210-A-29345	LibbyO	2/12/2017 4:46 AM	Standard	2368	Yes	No	Yes	4x	96-well Coming	0	
	Angiogenesis PrimeKit		LibbyO	2/12/2017 4:46 AM	Standard	2367	Yes	No	Yes	4x	96-well Corning	E	
	2/7/17 MR Viral Titer		MeaganR	2/9/2017 5:56 AM	Standard	2372	Yes	Yes	Yes	10x	24-well Corning	F	
	2/7/17 Libby VDCG		LabUser	2/7/2017 11:36 AM	Whole Well	2371	Yes	No	Yes	4x	24-well Corning	G	
	2/7/17 MR VDCG		LabUser	2/7/2017 11:36 AM	Whole Well	2370	Yes	No	Yes	4x	24-well Corning	F	
	2/7/17 Katie VDG		LabUser	2/7/2017 11:22 AM	Whole Well	2369	Yes	No	Yes	4x	24-well Corning		
	1/19/17 MR SBio HT-1080 NLR Spheroids	10X	Nevine	2/3/2017 10:10 AM	Spheroid	2366	Yes	Yes	Yes	10x	[10x/4x INTERNAL]	Vessel Name	Non-adherent T Cell Killing
	1/19/17 Corning HT-1080 NLR Spheroids 1	0X	Nevine	2/3/2017 10:10 AM	Spheroid	2365	Yes	Yes	Yes	10x	96-well Corning Ro	Scan Type	Standard
	1/19/17 MR SBIO HT-1080 NLR Spherolds		Nevine	1/27/2017 8:27 AM	Spheroid	2364	Yes	Yes	Yes	4x	[10x/4x INTERNAL]	Cell Type	WIL2-NS NucLight Red, PBMCs
	1/19/17 Coming HT-1080 NLR Spheroids		Nevine	1/27/2017 8:27 AM	Spheroid	2363	Yes	Yes	Yes	4x	96-well Corning Ro	Image Channels	🖾 Phase
	MB HepG2 Spheroid Infection 2		MikeB	1/20/2017 10:00 AM	Standard	2362	No	No	Yes	10x	6-well Corning	1	🖾 Green
	MB HepG2 Spheroid Infection 1		MikeB	1/20/2017 10:00 AM	Standard	2361	No	No	Yes	10x	6-well Corning	1	I Red
	Non-adherent T Cell Killing		MeaganR	1/19/2017 1:00 PM	Standard	2358	Yes	Yes	Yes	4x	96-well Corning	Magnification	4x
	ysis Definition Name	Analysis Type		Creator	Date Completed		Analysis	ID	Analy	sis Notes		Plate Map	Yes
	adherent T Cell Killing (WILS-2NS NLR)	Basic Analyzer		MeaganR	1/19/2017 1:02 PM		2210		Analy	11 110/03		1	
-011	Fadilerent i Cell Killing (WILS-2NS NLR)	Basic Analyzer		meaganic	1/19/2017 1.02 PM		2210					Vessel Type	96-well Coming
	1/19/17 MR SBio HT-1080 NLR Spheroids		LabUser	1/19/2017 11:58 AM	Spheroid	2360	No	Yes	Yes	4x	[10x/4x INTERNAL]	Vessel ID	2358
	1/19/17 Corning HT-1080 NLR Spheroids		LabUser	1/19/2017 11:58 AM	Spheroid	2359	No	Yes	Yes	4x	96-well Corning Ro	Vessel Notes	
	Adherent T Cell Killing Assay		MeaganR	1/13/2017 7:53 AM	Standard	2353	Yes	Yes	Yes	10x	96-well Corning		
	Chemotaxis - Low Contrast Adherent		LabUser	1/11/2017 8:01 AM	Chemotaxis (Top/Bot)	2357	No	No	Yes	10x	96-well Essen Cle		
	Non-Adherent T Cell Killing Assay		MeaganR	1/11/2017 7:53 AM	Standard	2354	Yes	Yes	Yes	4x	96-well Coming		
	bubble check HUVEC CHEMO v&v		LabUser	1/6/2017 11:42 AM	Chemotaxis (Top/Bot)	2355	No	No	Yes	10x	96-well Essen Cle		
	Non-Adherent T Cell Killing		MeaganR	1/6/2017 10:35 AM	Standard	2352	Yes	Yes	Yes	4x	96-well Corning		
	Adherent T Cell Killing Assay		MeaganR	1/6/2017 10:35 AM	Standard	2351	Yes	Yes	Yes	10x	96-well Corning		
	Adherent T Cell Killing Assay		MeaganR	1/6/2017 9:57 AM	Standard	2350	Yes	Yes	Yes	10x	96-well Corning		
	scan failure test HUVEC		MariaT	1/5/2017 1:13 PM	Chemotaxis (Top/Bot)	2348	No	No	Yes	10x	96-well Essen Cle		
	20 uL, post AE target reduction		JohnS	1/5/2017 1:13 PM	Chemotaxis (Top/Bot)	22.40	No	No	Yes	10x	96-well Essen Cle		

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 115.						
	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 116.						
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 1-9 on page 69.						

Option	Description							
Export Analysis Definition	Opens the Export Analysis Definition dialog box, which provides the options for exporting the full definition for the selected analysis.							
	Figure 1-8: Export Analysis Definition dialog box							
	C Export Analysis Definition							
	Name movie annexin neurotrack							
	Filename C:\Users\Spectrum Writing\Desktop\movie annexin neurotrack.icalysdef Browse Export							
	Tip: This option is particularly useful if your organization has 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 1-9: Analysis Details window

IC Analysis Definition Name: Cytotox Green							
Analysis De	tails				Analysis Definition		
Analysis Type	Basic Analyzer	Analysis Version	1.0				
Created	1/3/2017 1:43:01 PM	Analysis ID	3		Phase *		
Completed	1/3/2017 1:52:55 PM	Images Analyzed	1105		Object Name HT-1080 Parameters		
Od 0h 0m Od 2h 53m Od 5h 53m Od 5h 53m Od 1h 53m Od 11h 53m Od 12h 53m Od 17h 53m Od 12h 53m 1d 0h 0m 1d 2h 53m 1d 5h 53m 1d 5h 53m 1d 11h 53m Od 11h 53m	I A B C C D C C D C C C C C C C C C C C C C	2 3 4 5 6 0	7 8 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	9 10 11 12 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	Segmentation Adjustment: 2 Background Cells Cleanup Hole Fill (µm ⁴) 0.0000 Adjust Size (pixels) 0 Filters Area (µm ⁶): I min 50.000 Eccentricity: min 0.0000 I max 0.0000 I		
					Green ¥		
					Red ¥		
					Whole Well (Unused) ¥		
					Арріу		

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 73.

Chapter 1 Selecting a Vessel

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Chapter 2 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:

- "The Vessel View Window" on page 73.
- "Exporting Images" on page 81.

Chapter 2 Viewing and Working With Images

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.

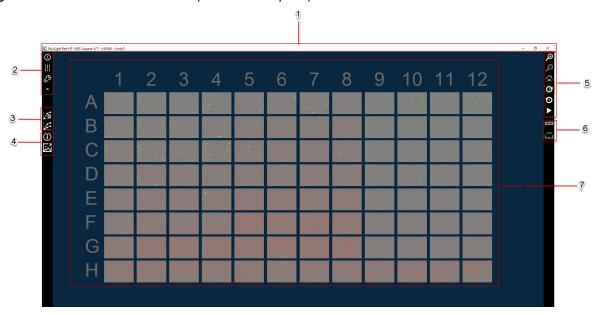


Figure 2-1: Vessel View window (96-well microplate)

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 76.
		 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 Analy	rsis toolbar contains two icons.
	6	Launch Analysis icon: Opens the Launch Analysis wizard, which you use to define the analysis for the images in the scanned vessel. See Chapter 1, "Defining the Image Analysis," on page 95.
		Graph Metrics icon: Opens the Graph Metrics window, which provides options for graphing the <i>scan</i> metrics for the vessel. See Chapter 2, "The Graph Metrics Window," on page 116.
4	Visualization toolbar: The V	/isualization toolbar contains two icons.
	\bigcirc	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 75.
		Export Images and Movie icon: Launches the Image Export wizard. See "Exporting Images" on page 81.
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 79.
6	Measure toolbar: The Meas	sure 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.
	µm L	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 2-2 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 76.) 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 2-3 below.
		Figure 2-2: Comparing the number of images in a vessel location $ \begin{array}{c} 1 \\ $
		Figure 2-3: 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 29.) 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 143.) 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.

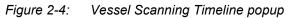
The Image toolbar

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.

lcon	Description
Q	Scans icon: Opens the Vessel Scanning Timeline popup. See "To select a scan time for the Vessel View display" below.
i!!	Layers icon: Opens the Image Layers popup. See "To work with the image channels" on page 77.
C?	Tools icon: Opens the Tools popup. See "To work with the Vessel View display" on page 79.

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.





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 79.

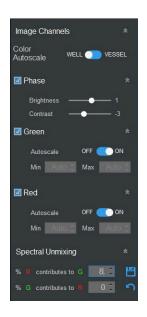
After you select a scan time, it is displayed in white and in a larger font than the other scan times in the Vessel Scanning Timeline popup. The Vessel View display is dynamically updated based on the selected scan time and the selected scan time is temporarily shown at the top of the display.

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 78.

Figure 2-5: 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 79.

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 2-6: Spectral unmixing percentages edited



Figure 2-7 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.

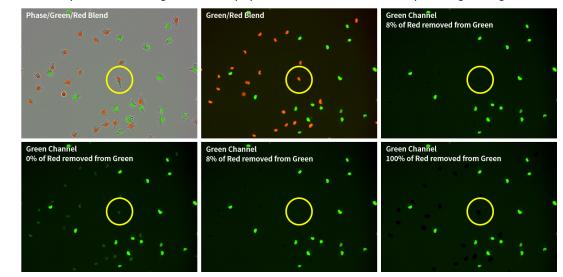


Figure 2-7: Spectral unmixing of a mixed population of HT-1080 cells expressing Nuclight Green or Red

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	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 <i>current</i> 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 81.
Export Image as Stored	See "To export images as stored" in "Exporting Images" on page 81.

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.

lcon	Description
Ð	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.

lcon	Description
D	Zoom Out icon: Available only after at zooming in on the Vessel View display. Click as needed to zoom out the Vessel View display.
you	can also use the scroll button on your mouse to zoom in on and zoom out the Vessel View display and can also click and hold the mouse pointer anywhere on the Vessel View display, and then drag the lay 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.)
ବ	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.
0	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 87.



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 69.
- 2. On the Visualization toolbar, click the Export Images and Movie icon 🖾

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

Figure 2-8: Image Export wizard, Export Type page

IC Image Export			×
Export Type			
Export movies and images a	is displayed to the screen or export raw images.		
As Displayed	Export data as currently displayed on the screen, layering image channels and masks to create a customized view. Cropping, scaling and overlay customization as also differed. Exports of this		
As Stored	type can be in the form of movies or series of individual image files. Common Uses: Presentations, Reports, Ernail		
Back	• • • • •	Next	

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.

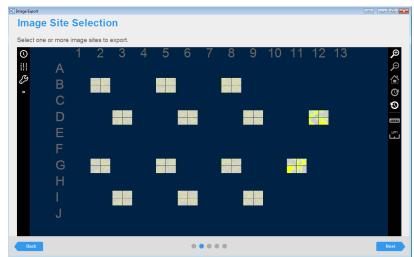


Figure 2-9: Image Export wizard, Image Site Selection page



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 73.

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 2-10 on page 83.

Sectex the proof of sequence to export (a single movie or a series of individual images) and the set of scars.	Image Export		
Select Sequence Type Select Scans Series of Images Od 0h Orn Od 2h Orn Od 2h Orn Od 8h Orn Od 8h Orn Od 1h Orn Od 1 D Orn O	Sequence Type an	d Scans	
Single Movie Control Control Contro Contro Control Control Control	Select the type of sequence to export (a s	ingle movie or a series of individual images) and the set of scans.	
Output Name Oct 0th Om Series of Images Oct 2th Om Oct 0th Om Oct 0th Om Oct 0th Om Oct 0th Om Oct 0th Om Oct 0th Om Oct 10th Om Oct 10th Om Oct 14th Om Oct 14th Om Oct 14th Om Oct 14th Om	Select Sequence Type		
States of Images Od 2h 0m Od 4h 0m Od 4h 0m Od 4h 0m Od 4h 0m Od 4h 0m Od 4h 0m Od 4 0h 0m Od 10h 0m Od 10h 0m Od 10h 0m Od 11h 0m Od 14h 0m Od 14h 0m Od 14h 0m	Single Movie		
	Series of Images	0 d 2h 0m 0 d 4h 0m 0 d 6h 0m 0 d 8h 0m 0 d 10h 0m 0 d 12h 0m 0 d 14h 0m 0 d 14h 0m	

Figure 2-10: 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.

- 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 Figure 2-11 on page 84.



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.

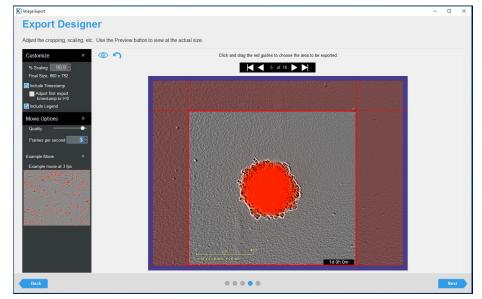


Figure 2-11: Image Export wizard, Export Designer page

- 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 (1) 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 (1).
 - 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 2-12 and Figure 2-13 on page 85.

C Image Export			
File Inforn	nation		
0.111.0.111.111.1	and the second first second first second		
Set the output folder, r	aming convention, and file format.		
Target Folder	Click Browse button to set folder	Browse	
File Format	MPEG-4 Video	•	
File Name Prefix	VID2197		
Example File Name	VID2197_C7_1.mp4		

Figure 2-12: Image Export wizard, File Information page (Single movie)

Figure 2-13: Image Export wizard, File Information page (Individual image files)

sge Export			
ile Inform	ation		
et the output folder, na	aming convention, and file format.		
Target Folder	Click Browse button to set folder	Browse	
File Format	JPEG	•	
File Name Prefix	VID2197		
File Name Timestamp	Use Calendar Time		
Example File Name	VID2197_C7_1_00d00h00m.jpg		
Back			Epport

11. Specify the file information.

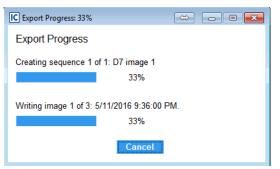
Option	Description
Target Folder	The folder in which the exported images are stored. Click Browse to open the Select a folder to export to dialog box, and then browse to and select the appropriate folder.
File Format	The default value for a single movie is MPEG-4 Video and for individual files is JPEG.
File Name Prefix	 The exported file is automatically named according to the following: For a single movie: <filename prefix="">_<site id="" well="">_<image number=""/>_<file format="">, for example, VID2197_D7_1mp4.</file></site></filename> For a series of individual images: <filename prefix="">_<site id="" well="">_<image number=""/><date timestamp=""><file format="">, where the File Name Timestamp option determines the timestamp. See "File Name Timestamp" on page 86.</file></date></site></filename>
	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_100d00h00m.jpg

12. Click Export.

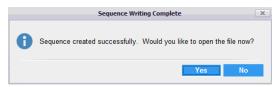
The Image Export wizard closes. You return to the Vessel View window. An Export Progress dialog box opens on top of the Vessel View window.

Figure 2-14: Export Progress dialog box

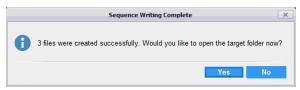


After the export progress is 100%, a Sequence Writing Complete dialog box opens.

- 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.
- Figure 2-15: Sequence Writing Complete dialog box



- 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 2-16: 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 "To open the Vessel View window" on page 69.
- 2. On the Visualization toolbar, click the Export Images and Movie icon

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

Figure 2-17: Image Export wizard, Export Type page

Image Export		
Export Type		
Export movies and images as display	ad to the screen or export raw images.	
As Displayed	Export data as currently displayed on the screen, layering image channels and masks to create a customized view. Crooping, scaling and overlay-customization is also softend. Exports of this	
As Stored	type can be in the form of movies or series of individual image files. Common Uses: Presentations, Reports, Ernail	
Back	• • • • •	Next

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 2-18: Image Export wizard, Image Type page*

IC Image Export		
Image Type Select	tion	
Select an image type to export.		
Select Image Type Phase original Green calibrated Represented Represented Red calibrated	Raw 8-bit image	
Back		Next
Batx		next

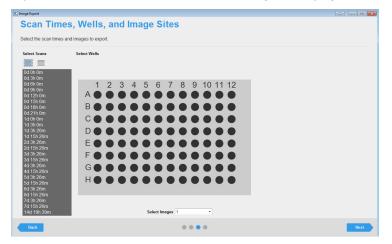
4. Select the image type that you are exporting.

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 2-19: 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 <i>not</i> 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. See Figure 2-13 on page 40.

7. Click Next.

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

Figure 2-20: Image Export wizard, File Information page

nage Export			
File Inform	nation		
Set the output folder, na	aming convention, and file format.		
Target Folder	Click Browse button to set folder	Browse	
File Format	JPEG	-	
File Name Prefix	VID2197		
File Name Timestamp	Use Calendar Time		
Example File Name	VID2197_C7_1_00d00h00m.jpg		
Back		••••	

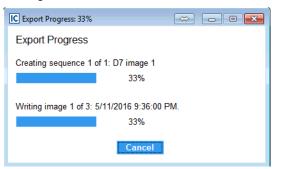
8. Specify the file information.

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 id="" well="">_<image number=""/><date timestamp=""><file format="">, where the File Name Timestamp option determines the timestamp. See "File Name Timestamp" below.</file></date></site></filename
	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_100d00h00m.tif

9. Click Export.

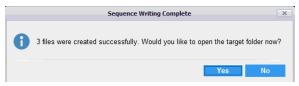
The Image Export wizard closes. You return to the Vessel View window. An Export Progress dialog box opens on top of the Vessel View window.

Figure 2-21: 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 2-22: Sequence Writing Complete dialog box



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

- "Defining the Image Analysis" on page 95
- "Visualizing the Analysis Results" on page 113

SVIFCTSV3

Chapter 1 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:

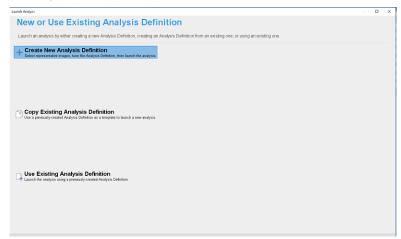
- "Launch Analysis Wizard Overview" on page 97.
- "Defining a New Analysis" on page 98.
- "Copying an Existing Analysis Definition" on page 109.
- "Using an Existing Analysis Definition" on page 111.

Chapter 1 Defining the Image Analysis

Launch Analysis Wizard Overview

You use the Launch Analysis wizard to analyze vessel images.

Figure 1-1: 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 98.
- To copy and modify an existing analysis definition. See "Copying an Existing Analysis Definition" on page 109.
- To use an existing analysis definition, see "Using an Existing Analysis Definition" on page 111.

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 109.

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 98.
- Selecting the analysis type, which is dependent on the scan type. See "To select the Analysis Type" on page 99.
- Selecting the image channels that you are analyzing. See "To select the image channels for analysis" on page 100.
- 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 101.
- Refining the analysis parameters for each of the image channels as appropriate. See "To define the analysis parameters" on page 102.
- Selecting the scan times and images that you are analyzing. See "To select the scan times and wells/ image sites" on page 104.
- Save and apply the analysis definition. See "To save and apply the analysis definition" on page 106.
- Confirming the analysis definition, and then launching the analysis. See "To review the analysis summary information" on page 107.



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 69.

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

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

Figure 1-2: Launch Analysis wizard, New or Use Existing Analysis Definition page

aunch Analysis	- 1
New or Use Existing Analysis Definition	
Launch an analysis by either creating a new Analysis Definition, creating an Analysis Definition	on from an existing one, or using an existing one.
+ Create New Analysis Definition Select representative images, loss the Analysis Definition, then learch the analysis.	
Copy Existing Analysis Definition Use a predoxity-created Analysis Definition as a template to lounch a new analysis.	
Use Existing Analysis Definition	

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 1-3: Launch Analysis wizard, Analysis Type page

Launch Analysis		-		×
Analysis Type				
Select the type of analysis to run on your images.				
Basic Analyzer	Basic Analyzer			
	Toticity Panel Assay			
Angiogenesis				
	Quantily phase and fluorescent object metrics in real time.			
NeuroTrack				
Back			Next	

1. Select the analysis type (In this example, Basic Analyzer is selected).

Chapter 1 Defining the Image Analysis

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.



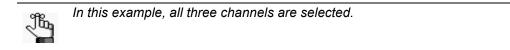
Figure

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

1-4:	Launch Analysis wizard, Image Channels page		
	Launch Analysis	-	
	the end of end of a		

Launch Analysis			-	U	×
Image Ch	annels				
The Basic Analyze	requires at least one channel to be selected for analysis.				
Image Channel	C Phase				
intage chamier	S Green				
	V Red				
	Overlap				
Back		•••••		Next	

- 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.



2. Click Next.

The Image Set Selection page opens.

3. Continue to "To select the images for analysis" on page 101.

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 "The Vessel View Window" on page 73.
- 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 1-5: 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.

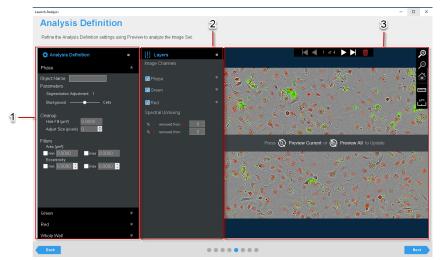
The Analysis Definition page opens.

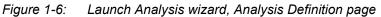
3. Continue to "To define the analysis parameters" on page 102.

Chapter 1 Defining the Image Analysis

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.





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 1-6 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.

Chapter 1 Defining the Image Analysis

	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 1-6 on page 102, 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.</n>
		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 104.

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 1-7: Scan Times and Wells page

Launch Analysis															
Scan Times And	d Wells														
Select the scan times and images	s to analyze along	with the	option to	analyze	future sc	ans.									
Select Scans	Select Wells														
Od 0h 0m			1	2	3	4	5	6	7	8	9	10	11	12	
0d 2h 53m 0d 5h 53m 0d 8h 53m		Α													
0d 11h 53m 0d 14h 53m 0d 17h 53m		В									8	•		•	
0d 20h 53m 1d 0h 0m 1d 2h 53m		С									8	8		•	
1d 5h 53m 1d 8h 53m 1d 11h 53m		D					•				8	•		•	
1d 14h 53m 1d 17h 53m 1d 20h 53m		Е									8	•	•	•	
2d 0h 0m 2d 2h 53m 2d 5h 53m 2d 8h 53m		F									8			•	
2d on 55m 2d 11h 53m 2d 14h 53m 2d 14h 53m 2d 17h 53m		G									8			•	
2d 20h 53m 3d 0h 0m		Н													
Back							• • •	•							Next

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" on page 106.

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 1-8: Launch Analysis wizard, Save and Apply Analysis Definition page

Launch Analysis		
Save a	nd Apply Analysis Definition	
Assign the Ana	lysis Definition a name and, if desired, add notes.	
Definition Name •		
Analysis Notes		
Back	• • • • • • •	Next

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 1-6 on page 67.

- 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 107.

To review the analysis summary information

Figure 1-9: Launch Analysis wizard, Summary page

aunch Analysis		
Summary		
	on below is correct before launching the Analysis.	
Analysis		
Туре	Basic Analyzer	
Analyze Future Scans	Yes	
Images to Analyze	8400 (* Initial value only. This is an 'Analyze Future Scans' analysis.)	
Notes		
Vessel Info		
Name	Toxicity Panel Assay (Green Reagents)	
Туре	96-well Corning	
ID	1013	
Analysis Definition		
Name	Test	
Objective	10x	
Filter Module	Green/Red 4614	
Channels	Phase, Green, Red	
Back		Finish

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.

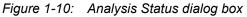
- 1. Review the information for the planned analysis, and then do one of the following:
 - If all the selections/settings are correct for the analysis, then continue to Step 2.
 - If you must edit any of the selections or settings, then click the appropriate section header/page name to return to the 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.



ic50002 Analysis Status				- 0	×
Image: The second sec			Q		
Analysis Definition	Analysis Type	Creator	Analysis ID	Remaining Tasks	
HT-1080 caspase and nuclei count	Basic Analyzer	Lindy0	29	6176	

Chapter 1 Defining the Image Analysis

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 1-11: Status page

ic50002 - LindyO - Incu	yte									– a ×
$\equiv \odot Sche$	dule 💿 View	🔑 Manage	Device	Archive	(i) Status					
System						Temperature				
Filter Module Gree	n/Red 4614					36300				
Hard Disk										History 24 hours 💌
Capacity 16.4 Used Space 1.9	TB (12%)							Idle 📕 Active		
Free Space 14.5	TB (88%)					1				
						0.8				
						0.0 000 0.0 000 0.0 000 0.0 000 0.0 000				
						50 0.4				
						a :				
						0 0.1 0.2	0.3 0.4	0.5 0 Date / Time	1.6 0.7	0.8 0.9 1
								Date / Time		
Archive						Analysis				
Archive Name	User	Destination	Status	Rema	ining Tasks	Analysis Definition	Analysis Type	Creator	Analysis ID	Remaining Tasks
						HT-1080 caspase and nuclei count	Basic Analyzer	Lindy0	29	6176
		No Archive In	Progress						l≥	

• 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 1-12: Analysis pane showing all the analyses (in progress and completed) for a selected vessel

IC ic50	002 -	LindyO - IncuCyte											×		
=		🕒 Schedule		🔗 Manage	De ^v	vice	; = A	rchi∨	e 🛈 Statu	5					
Ð	1	Enter text to se	arch												Q
Analys	es	Vessel Name			Owner		Last Scan	•	Scan Type	Vessel ID	Colo	Colo	Phas	Magnification	
										1013					
¥		Toxicity Panel Assay (Gre	en Reagents)		MeaganR		1/3/2017 10:06	АМ	Standard	1013	Yes	Yes	Yes	1 0x	
	Ana	ulysis Definition Name		Analysis Type		Сге	ator	Date	Completed	Analysis ID	Anal	/sis Note:	5		
(HT-	1080 caspase and nuclei (count	Basic Analyzer	L		LindyO			29)				
	Cytotox Green Basic Analyza		Basic Analyzer		MeaganR		1/3/20	17 1:52 PM	3						
	Caspase 3/7		Basic Analyzer		MeaganR 1/3/2017 1:41 PM		17 1:41 PM	2							
	Annexin Green Bas		Basic Analyzer		Mea	ganR	1/3/20	17 1:31 PM	1						

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 98.

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 69.
- 2. On the Analysis toolbar, click the Launch Analysis icon K.

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

Figure 1-13: Launch Analysis wizard, New or Use Existing Analysis Definition page

Launch Analysis		×
New or Use Existing Analysis Definition		
Launch an analysis by either creating a new Analysis Definition, creating an Analysis Definition from an existing one, or using an existing one.		
Create New Analysis Definition Select representative maper, tase the Analysis Definition, then lauch the analysis.		
Copy Existing Analysis Definition Use a previously-created Analysis Definition a a template to launch a new analysis.		
Use Existing Analysis Definition		

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 1-14 on page 110.

Chapter 1 Defining the Image Analysis

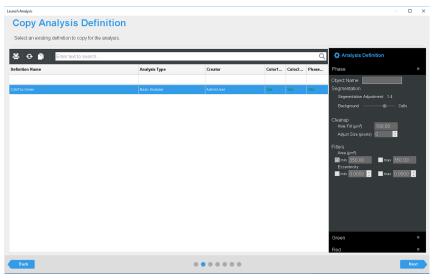


Figure 1-14: Launch Analysis wizard, Copy Analysis Definition page

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 Copy Analysis Definition page has the standard Incucyte filter and sort functions that you have encountered on other wizard pages, for example, on the Vessel Type Search page in the Add Vessel wizard. You can use these features to help you quickly search for and select the appropriate analysis. See "Filtering and Sorting Data in an Incucyte Window" on page 21.

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 101.

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 69.
- 2. On the Analysis toolbar, click the Launch Analysis icon 1/6.

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

Figure 1-15: Launch Analysis wizard, New or Use Existing Analysis Definition page

Launch Analysis		
New or Use Existing Analysis Defin	nition	
Launch an analysis by either creating a new Analysis Definition, creating an A	Analysis Definition from an existing one, or using an existing one.	
+ Create New Analysis Definition Select representative images, tune the Analysis Definition, then launch the analysis.		
Copy Existing Analysis Definition Use a previously-created Analysis Definition as a template to launch a new analysis.		
Use Existing Analysis Definition		
Back		Next

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 1-16 on page 112.

Chapter 1 Defining the Image Analysis

Launch Analysi п **Analysis Definition** Select an Color1 Data Color2 Phas Basic Analyze Basic Analyze Yes Yes Cytotox Gr Yes Yes Caspase 3/7 Basic Analyze Basic Analyze Yes

Figure 1-16: 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 standard Incucyte filter and sort functions that you have encountered on other wizard pages, for example, on the Vessel Type Search page in the Add Vessel wizard. You can use these features to help you quickly search for and select the appropriate analysis. See "Filtering and Sorting Data in an Incucyte Window" on page 21.

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 104.

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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 115.
- "The Graph Metrics Window" on page 116.
- "Graphing the Analysis Results for a Microplate" on page 120.
- "Graphing the Analysis Results for Any Vessel Type" on page 124.
- "Graphing Analysis Results as a Histogram" on page 129.
- "Graphing Analysis Results as a Concentration Response Curve" on page 131.
- "Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve" on page 134.
- "Exporting Graph Data" on page 138.

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 68.) To view the analysis masks, open the Image Channels popup (see "The Image toolbar" on page 76), and then in the Analysis Masks pane, toggle on and off the analysis masks as appropriate.

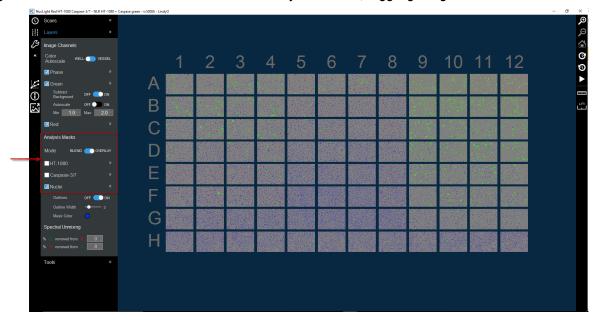


Figure 2-1: Vessel View with the associated analysis window, toggling image masks on and off

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 2. See "Viewing the Analysis Masks" on page 115.
- 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 67.

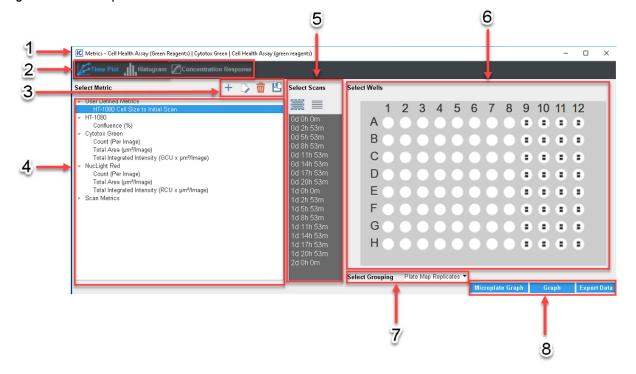


Figure 2-2: Graph Metrics window

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: Displa	ys 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 118.		
	D	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.		
	ŵ	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.		
	ß	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 74.		
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.		

Chapter 2 Visualizing the Analysis Results

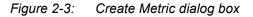
	Window component	Description
7	Select Grouping dropdown list	Displays the options for specifying how to group the data points on the Y axis. The default values are the following:
		 If the vessel is a microplate and a plate map has been specified, then the data is grouped by Plate Map replicates.
		 If the vessel is a microplate and a plate map has <i>not</i> 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 138.
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.



IC Create Metric						-		×
	Channel		Metric	1	Value			
Metric	Phase	•	Area 👻		Confluence			•
Normalized to (by division)	None	Ŧ	Ţ					٣
Normalize to Scan (by division)	<none></none>	•						
Display as	Percent	•						
Default Name	Phase Area Confluence (%)							
Name Override								
							0	ĸ

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 120.
 - "Graphing the Analysis Results for Any Vessel Type" on page 124.
 - "Graphing Analysis Results as a Histogram" on page 129.

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.

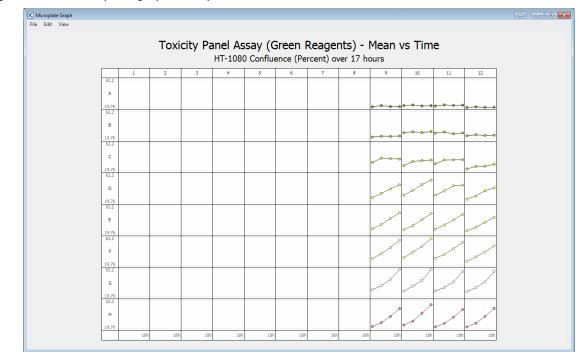


Figure 2-4: Microplate graph example



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 116.
- 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 row, 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 Select 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:

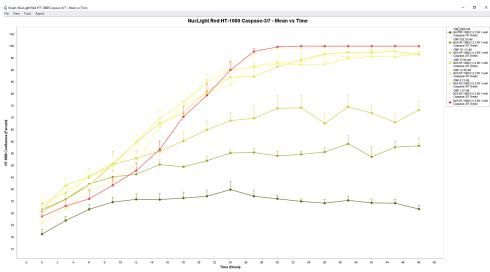
Option	Description			
Edit				
Titles and Subtitles	Opens the Edit Titles dialog box. You use the options on this dialog box to edit the default title for the graph and/or add your own custom subtitles. You can also change the maximum font size of the title and subtitles.			
	Figure 2-5: Edit Titles dialog box			
	IC Edit Titles			
	Toxicity Panel Assay (Green Reagents) - Mean vs Time			
	HT-1080 Confluence (Percent) over 17 hours			
	Fonts can grow to 22 ⁺ points. OK			
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. <i>Figure 2-6: Edit Axes dialog box</i>			
	IC Edit Axes			
	Minimum 19.7568190493164 C From 12/30/2016 -			
	Maximum 62.1961402893066 C To 12/31/2016 -			
	Reset values to original Reset time range to original 42.44 over 17 hours OK			
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			
	IC Save Graph as Image			
	Image Type BMP: High Quality, Large File Size 🔹			
	Size Small (600x400) Good for email			
	Folder C:\Users\Spectrum Writing\Desktop Browse			
	File Documentation Image			
	Save			
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.





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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 "The Graph Metrics Window" on page 116.
- 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 126.
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 row, 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 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. 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). In this example, we assume that Plate Map Replicates is selected.

6. Click Graph.

The Graph is generated and displayed onscreen in its own 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. See Figure 2-9 on page 126.

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 134 and/or "Exporting Graph Data" on page 138.

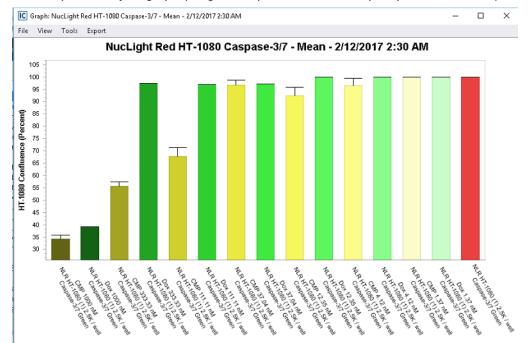


Figure 2-9: Endpoint analysis graph (Single time point and Plate Map Replicates selected)

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 124.
- 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 below shows two individual metric graphs brought to the front of the screen display.

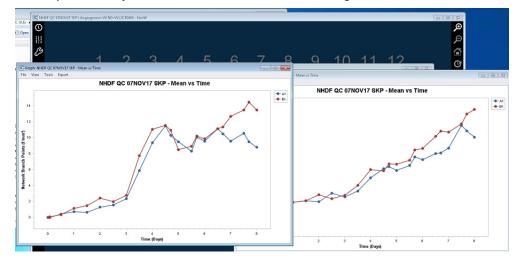
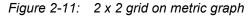
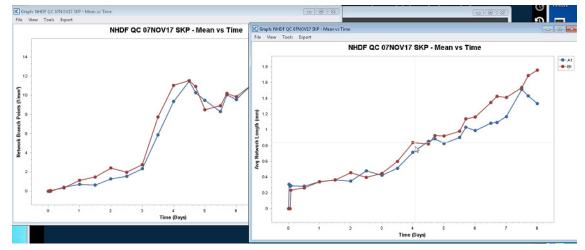


Figure 2-10: Graphs of analysis results for individual metrics brought to the front of the screen display

- 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.

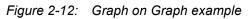


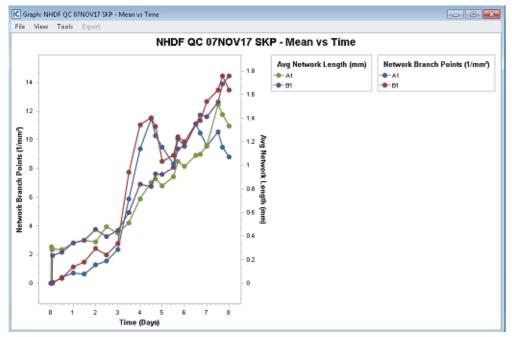


b. Click and hold the right mouse button on the individual metric graph, drag the graph onto the destination graph, and then release the mouse button.

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

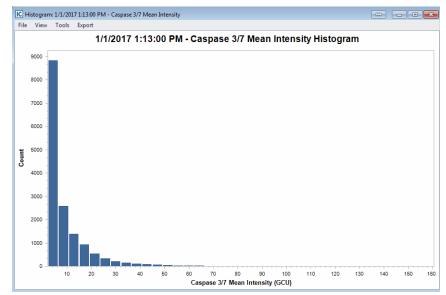
- The graph window is set to a default size. You must use standard Windows functionality to manually resize the graph window. The destination graph size is adjusted accordingly.
- By default, the individual graph legends are displayed at the top of the graph window and to the right and outside of the graph. You can manually adjust the position of each graph legend. See "To customize the graph display (Non-default properties)" on page 136.
- 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 126, 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 on page 128, 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 136.





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?





To graph analysis results as a histogram

- 1. Open the Graph Metrics window. See "The Graph Metrics Window" on page 116.
- 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 134 and/or "Exporting Graph Data" on page 138.

Graphing Analysis Results as a Concentration Response Curve

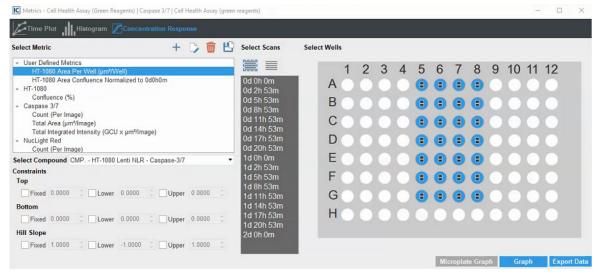
In addition to the common time plot and histogram, you can also graph your analysis results as a concentration response curve. Typically, you graph your analysis results as a concentration response curve if you are interested in determining the EC_{50} value of your compound or treatment. To graph analysis 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 143.

To graph analysis results as a concentration response curve

- 1. Open the Graph Metrics window. See "The Graph Metrics Window" on page 116.
- 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 EC₅₀, 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.

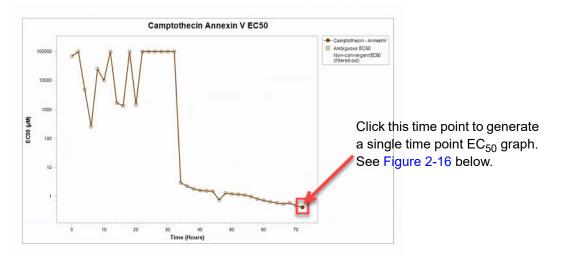
The Concentration Response Curve is generated and displayed onscreen in its own window. An r Sq value and an EC_{50} value are generated and displayed for the graph. A menu (File, Edit, View) is displayed at the top of the graph window and a default title is displayed above the graph.

- For a sample kinetic EC₅₀ graph, see Figure 2-15 on page 133.
- For a sample single time point EC₅₀ graph, see Figure 2-16 on page 133.



Figure 2-16 on page 133 was generated using the above mentioned shortcut by clicking the single time point indicated in Figure 2-15 on page 133.

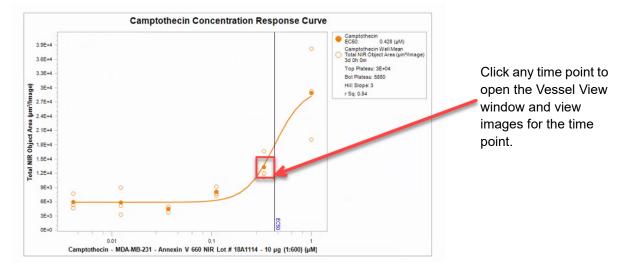
Figure 2-15: Kinetic EC₅₀ graph





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 EC₅₀ graph



- 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 134 and/or "Exporting Graph Data" on page 138.

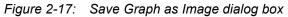
Customizing a Time Plot Graph, Histogram, or a Concentration Response Curve

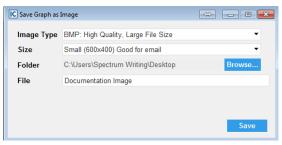
Optionally, after you generate a time plot graph, a histogram, or a concentration response curve for your analysis data, options are available for working with the graph, customizing the graph display and/or exporting the graph data. See:

- "To save the graph as an image file and/or print the graph" below.
- "To edit the default graph display" on page 134.
- "To save graph as an image to a third-party application or the raw data to a document" on page 135.
- "To customize the graph display (Non-default properties)" on page 136.

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.





• 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 136.

1. On the Graph window menu, click View.

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. 		

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

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.

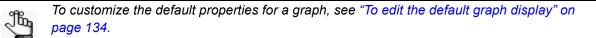
- 1. Make sure that the correct third-party application is open.
- 2. On the Graph window menu, click View.
- 3. Under View, select Drag and Drop, and then select one of the following:
 - Image to Document. (The default value.)
 - Raw Data to Document.
- 4. Right-click the image, and then while holding 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 is placed in 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.

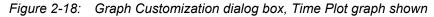
To customize the graph display (Non-default properties)

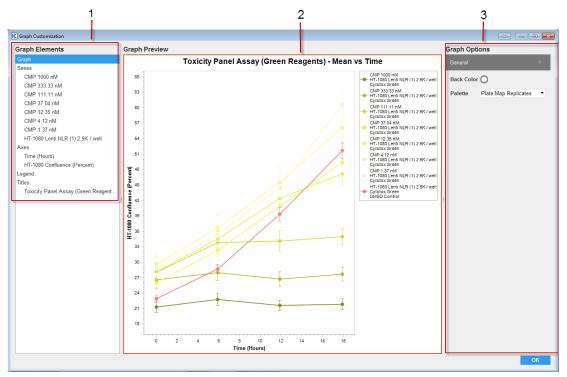
You use the Graph Customization tool to customize the non-default properties of the graph display.



- 1. On the Graph window menu, click Tools.
- 2. Under Tools, click Customize Graph.

The Graph Customization dialog box opens.





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.

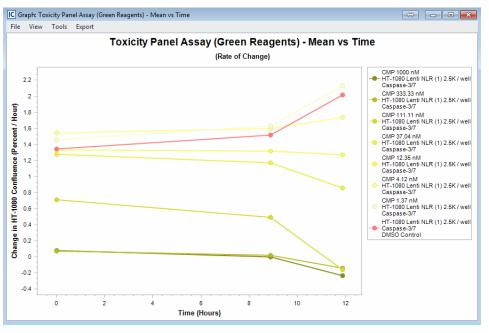
To generate a Rate of Change graph

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.







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 134.

Exporting Graph Data

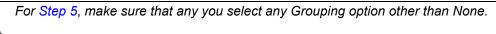
You can export the data that was used to generate a graph to either a text file or your client clipboard. If you export the data to your client clipboard, you can then import this data in to a third-party spreadsheet program such as Microsoft Excel. When you export the data, by default, each scan is 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

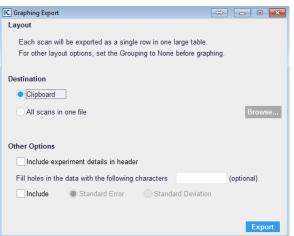
1. Do Step 2 through Step 5 of "To graph the analysis results for any vessel type (Single metric)" on page 124.



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

The Graphing Export dialog box opens.

Figure 2-20: Graphing Export dialog box

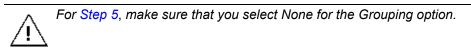


- 3. Do one of the following to select the export destination:
 - To export the data to your client's clipboard, leave the default value of Clipboard selected.
 - To export the data to a single file, select All scans in one file, and then click Browse to open the Export Metrics As dialog box, and then 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 124.



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

The Graphing Export dialog box opens.

Figure 2-21: Graphing Export dialog box

IC Graphing Export			\$ - •	×
Layout				
Show each scan as a single row in one large table. Column by column: A1, B1, C1,, A2, B2, C2,				
Row by row: A1, A2, A3, B1, B2, B3,				
Organize the metrics by replicate wells per the Plate Map				
	scan as its own table (co w and column labels	lumns: 1, 2 rows	s: A, B)	
Destination				
Clipboard				
All scans in one file C.\Users\Spectrum Writing\Desktop\Image Files Browse Each scan in a separate file				
Folder			Browse.	
Folder File Prefix			Blowse.	
Preview	(enter a file prefix)			
Other Options				
Include experiment details in header				
Fill holes in the data with the following characters (optional)				
Include	Standard Error	Standard Devia	tion	
Break data down into individual images				
			Export	

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

• "Plate Map Editor" on page 143

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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 145.
- "Plate Map Editor Layout" on page 146.
- "Defining a Plate Map with the Plate Map Editor" on page 149.
- "Editing an Existing Plate Map" on page 153.

Appendix A Plate Map Editor

Opening the Plate Map Editor

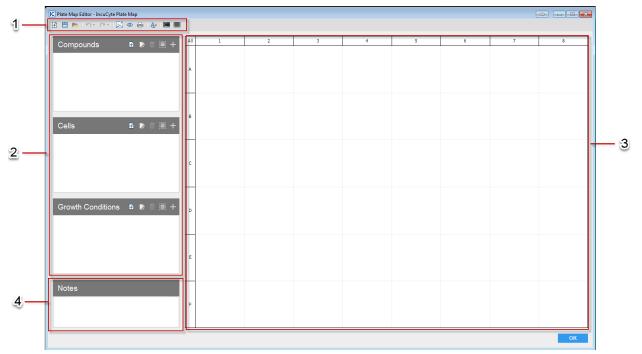
When you install the Incucyte software, the Plate Map Editor is also installed as separate, standalone application. A Plate Map Editor shortcut icon is automatically placed on the client desktop. Although the Plate Map Editor is installed as a standalone application, it is still accessible from within 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 27.
- From the Notebook page in the Add Vessel wizard. See "To provide vessel information" on page 41.
- 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 75.
- From the Plate Map Editor desktop icon

Continue to "Plate Map Editor Layout" on page 146.

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 147.
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 148.
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

lcon	Description		
4	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.		
2	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.		
5	Undo Last Action icon: Undoes the last action or "n" number of actions that were carried out for the plate map.		
č	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			
	IC Plate Map Image Export		
	Image Type PNG: High Quality, Small File Size		
	Image Size Current size in editor (1006x730) ▼ Note: The editor's font will be used. To increase the		
	text size in the exported image, increase the size in the editor.		
	ОК		
	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.		
0	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.		

Appendix A Plate Map Editor

lcon	Description
A	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

lcon	Description
Ŧ	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).</well></well>
	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).</well></well>
ŵ	Delete Selected <well item=""> icon: Deletes the selected well item from all affected wells in the plate map.</well>
_	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.</well>
÷	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.</well>

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 solely to provide a frame of reference. You can select whichever approach 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 153.

To define a plate map with the Plate Map Editor

- 1. Open the Plate Map Editor. See "Opening the Plate Map Editor" on page 145.
- 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.

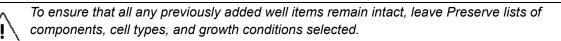
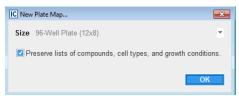


Figure A-3: New Plate Map dialog box



- 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.



IC Add/Edit Compound	×
Short Name	(for display in plate map)
Description	
Color	
	ок
	ON

Appendix A Plate Map Editor

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. 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 th 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 minimum 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 ____. The well item determines the next step.
 - If the well item is a compound, then the Add Compound Or Dilution dialog box opens. (SeeFigure A-5 on page 151.) Go to Step 6.
 - If the well item is a cell type, then the Adding <Cells> to plate dialog box opens. (See Figure A-6 on page 152.) 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.

IC Add Compound Concentration or Dilution			
Add 100.00 ¢ mg/ml ▼ of CMP to th	e selected wells.		
Create Dilution			
Dilution Type			
By Division			
Dilution Factor 2.00 🗘			
O By Subtraction		CMP 100 mg/ml	- 100 - 100 - 11 - 11 - 12 - 12 - 12 - 12 - 12
🕙 By Manual Steps			
Dilution Direction			
🔿 📱 Top to Bottom			
🔿 📱 Bottom to Top			
East Left to Right			
O BBB Right to Left			
Replicate Direction			
Vertical			
🔿 🚥 Horizontal			
			ОК

Figure A-5: Add Compound Concentration or Dilution dialog box

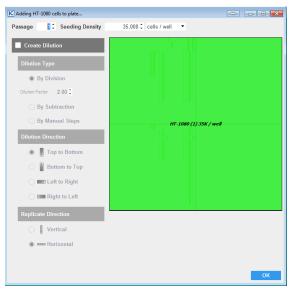
- 6. In the Add Compound Concentration or Dilution dialog box, do the following:
 - a. Indicate the concentration of the compound that you are adding to the selected wells.
 - b. If the compound is a dilution series, then select Create Dilution, and then specify the appropriate dilution parameters (Dilution 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.

Appendix A Plate Map Editor

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.

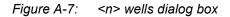
2. In the Open Plate Map dialog box, browse to and select the appropriate plate map file.

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 149), and then right-click anywhere in the selection.

An <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.

<u>6 wells</u>
(Enter custom label)
Compounds
🗙 СМР
Growth Conditions
× BSA
Color for Selected Wells
СМР
Clear Everything



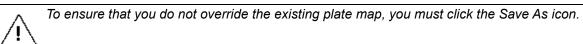
4. Edit the selected wells as needed, and then click Close.

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 149.

Appendix A Plate Map Editor

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.





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 Data Archives" on page 157
- "Changing the Incucyte SX1 Objective" on page 169
- "Changing the Incucyte SX5 Optical Module" on page 173
- "Incucyte Security" on page 177

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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 159.
- "Creating a New Archive" on page 161.
- "Viewing a List of the Archives Attached to Incucyte" on page 165.
- "Opening an Archive" on page 166.

Appendix A Incucyte Data Archives

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.

Appendix A Incucyte Data Archives

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 73.

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.

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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

ic50006 - LindyO - Incul	Cyte				– a ×
$\equiv \bigcirc$ Sche	edule 💿 View 🖉 Manage 🚍 Device 🚍 Archive 🔞 Security 🚺 Status				
Create New Arch	nive View Archives Attached to IncuCyte				
	🛃 😔 👔 🛛 Enter text to search				C
Select	Vessel Name	Owner	Last Scan	Scan Type	Vessel ID
	3.17.17 Uhler iNeuron 10x phase BrainPhys	Aaron 0	3/20/2017 9.39 AM	Standard	2323
	3.17.17 Uhler iNeuron 10x phase 3N media	Aaron O	3/20/2017 9:26 AM	Standard	2321
	Uhler cells 4X green	JohnR	3/18/2017 11:17 AM	Standard	2320
	Uhler iNeurons 20x green scan clusters	Aaron O	3/10/2017 11:15 AM	Standard	2308
	Uhler iNeurons 20x green scan	Aaron 0	3/10/2017 10:59 AM	Standard	2306
	IC13 plate on IC6 _2nd attent	LabUser	2/27/2017 4:47 PM	Standard	2303
	IC13 plate scan on IC8	LabUser	2/27/2017 4:39 PM	Standard	2301
	022317_Transient_HEK_single FP_greens	YongC	3/10/2017 1:00 PM	Standard	2300

- 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.

Appendix A Incucyte Data Archives

- 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

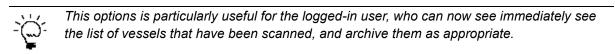
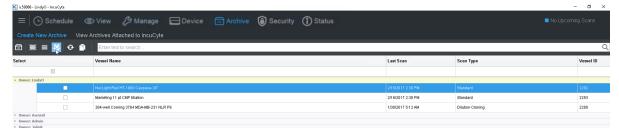


Figure A-2: Archive page, Vessels grouped by owner

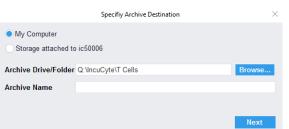


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The columns on the Archive page contain the standard options for sorting and filtering the data that you have encountered in other areas of the application, for example, the Add Vessel wizard. Refer to one of these sections in the manual if you need a refresher about sorting and/or filtering the data.

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. See Figure A-4 below.

Figure A-4: Incucyte-attached Storage message

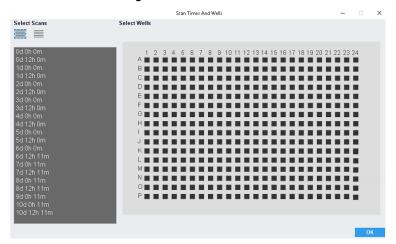


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- 6. Enter a name for the archive.
- 7. Click Next.

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 for 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 Step 10.





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. See Figure A-7 on page 164.

Appendix A Incucyte Data Archives

Figure A-7: Estimating Archive dialog box

	Archive Estimation Completed	>	<
Estimated 1 vessel.			
Archive Size Estimate	36.14 MB		
Archive Time Estimate	approximately < 10 minutes @ 10-20 MB/s		
		Create Archive	
		CICULE AICHIVE	

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 166.

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 161.

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. Note the following about the window:

- The standard Incucyte filter and sort and search functions are available for searching for a specific archive. See "Filtering and Sorting Data in an Incucyte Window" on page 21 and "Searching for Data in an Incucyte Window" on page 22.
- 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.

Appendix A Incucyte Data Archives

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. You have two options for opening an archive: You can open an archive during the start up of Incucyte, or you can open an archive with a connection after you have opened and logged in to Incucyte.



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.

To open an archive

- 1. Do one of the following:
 - To open an archive when you start Incucyte, double-click the desktop icon to launch Incucyte, or select the option from the Start menu.
 - To open an archive with a connection, do the following:
 - a. From any window with a Menu bar in Incucyte, click the Menu bar to open a dropdown menu.
 - b. On the dropdown menu, select Connection.

The Incucyte Open Connection dialog box opens.

Figure A-10: Incucyte Open Connection dialog box



2. Click Open an Archive.

An Instructions for Opening Archives message opens. The message details how to access an Incucyte archive.

Figure A-11: Instructions for Opening Archives message



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To keep the Instructions for Opening an Archive message from opening again for this and all future instances of Incucyte, click Do not show this message again.

3. Optionally, read the message to ensure that you understand how to access an archive, and then click OK.

The message closes and the Select Folder dialog box opens.

4. 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 Incucyte filter and sort and search functions are available for searching for a specific archive. See "Filtering and Sorting Data in an Incucyte Window" on page 21 and "Searching for Data in an Incucyte Window" on page 22.
- 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 to update the display with the most recently archived 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-12: Open Archive window

Open Arch	ive			
Browse From R:\IC6\V&V Archives				
😔 🖹 Enter text to search				
Vessels	Name	Created At	Creator	
Vessels	Name	Created At	Creator	
÷				
•	Chemotaxis (5)	5/25/2017 10:59:32 AM	MeaganR	
•	Dil Cloning (2)	5/24/2017 11:31:51 AM	MeaganR	
•	NeuroTrack VV (3)	5/24/2017 11:32:40 AM	MeaganR	
•	T Cell Killing	5/24/2017 11:31:51 AM	MeaganR	



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 73.

Appendix A Incucyte Data Archives

SVIPCTRVS

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" on page 171.

Appendix B Changing the Incucyte SX1 Objective

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 1-4 on page 19.

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

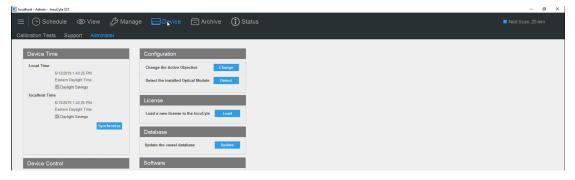


You can also click Device in the menu that is displayed at the top of every window in Incucyte.

2. Click Administer.

The Administer tab opens.

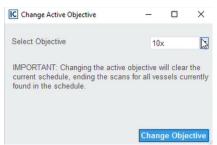
Figure B-1: Incucyte SX1 Administer tab



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



Appendix B Changing the Incucyte SX1 Objective

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 31.

SVIPCTRA3

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:

• "Changing the Incucyte SX5 Optical Module" on page 175.

Appendix C Changing the Incucyte SX5 Optical Module

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 1-4 on page 19.

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

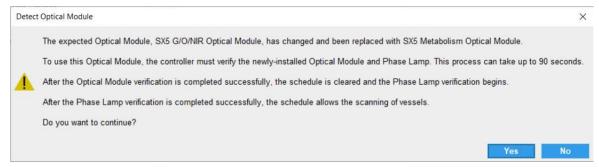
Device Time	Configuration
ocal Time 3/27/2020 3:36:25 PM Eastern Daylight Time I Daylight Savings	Detect the installed Optical Module Detect
ocalhost Time	License
3/27/2020 3:36:25 PM Eastern Daylight Time Daylight Savings	Load a new license to the IncuCyte Load
Synchronize	Database
	Update the vessel database Update
evice Control	Software

Appendix C Changing the Incucyte SX5 Optical Module

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.

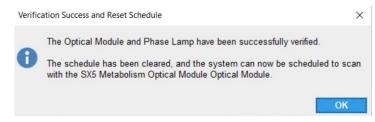
Figure C-2: Detect Optical Module message



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 31.

SVIPCTRV3

Appendix D Incucyte Security

Users are the people who log into Incucyte, whether they are adding and reviewing content, or just using Incucyte in a read-only capacity. Remember, when Incucyte is first installed, it is installed with a default Administrator user. As the Incucyte administrator, you must manage all the necessary users for your Incucyte installations, which includes adding new users, and editing users, including editing user permissions and inactivating users.

This appendix covers the following topics:

- "Adding a User to Incucyte" on page 179.
- "Specifying User Settings for an Incucyte" on page 185.
- "Editing a User for an Incucyte" on page 187.
- "Managing Vessel Workgroups" on page 190.

Appendix D Incucyte Security

Adding a User to Incucyte

When you add a user, you must specify the *user type* and the *user permission level*. The user type is either User or Domain User, where:

- User requires a user to log in to Incucyte with credentials (user name and password) that are specific for the instrument. This type of user is referred to as a *local user*.
- Domain User allows a user to log in to Incucyte with his/her Microsoft Windows credentials (user name and password). These credentials, therefore, are not specific to the instrument. As the Incucyte administrator, you can make it optional for a domain user to log in using domain credentials, or you can make it required.

The permission level determines the access that a user has to Incucyte and the actions that a user can therefore carry out, such as read-only access which means that the user cannot carry out any actions in Incucyte, or Access to Schedule, which, at a minimum level, means that the user can create a new vessel to scan on schedule. Table D-1 below details the user permission levels in Incucyte, organized by UI location.

		Permission Level			
UI	Action/Access	Admin	Standard	Limited	Guest
Launch	Access to Schedule	Х	Х		
	Access to View	Х	Х	Х	Х
	Access to Manage	Х	Х	Х	
	Access to Device	Х	Х	Х	
	Access to Archive	Х	Х	Х	
	Access to Security	Х			
	Access to Status	Х	Х	Х	Х
Application Menu	Change Password	Х	Х	Х	
Schedule	Create New Vessel to Scan	Х	Х		
	Edit Any Unscanned Vessel	Х	Х		
	Edit Experiment End For Any Vessel	Х	Х		
	Remove Any Vessel from Repeating Schedule	X	Х		
	Create New Scan Group for Any Vessel	Х	Х		
	Modify Any Scan Group	Х	Х		
View	Open Any Vessel	Х	Х	Х	Х
	Open Any Analysis	Х	Х	Х	Х
	Graph Metrics for Any Analysis	Х	Х	Х	Х
	View Details for Any Analysis	Х	Х	Х	Х
	Export Definition for Any Analysis	Х	Х	Х	
	Delete Analysis Created by You	Х	Х	Х	

Table D-1: User Permission Levels

Appendix D Incucyte Security

UI	Action/Access	Permission Level				
		Admin	Standard	Limited	Guest	
	Delete Any Analysis	Х				
Manage	Edit Analysis Definition Created by You	Х	Х	Х		
	Edit Any Analysis Definition	Х				
	Rename Analysis Definition Created by You	Х	Х	х		
	Rename Any Analysis Definition	Х				
	Create Copy of Any Analysis Definition	Х	Х	Х		
	Create Protected Copy of Any Analysis Definition	Х	Х	х		
	Import or Export Analysis Definition	Х	Х	Х		
	Delete Analysis Definition Created by You	Х	Х	Х		
	Delete Any Analysis Definition	Х				
	Delete Any Vessel Created by You	Х	Х			
	Delete Any Vessel	Х				
Device	Perform and View Calibrations	Х				
	Export Setup Files or Logs	Х	Х	Х		
	Export Databases	Х	Х	Х		
	Export Scan Diagnostics	Х	Х	Х		
	View Administrator Page	Х	Х	Х		
	Synchronize Device Time	Х	Х	Х		
	Shut Down or Restart Incucyte	Х	Х	Х		
	Perform Hardware or Software Changes	Х				
Archive	View Create Archive Page	Х	Х	Х		
	Create Local Archive for Any Vessel	Х	Х	Х		
	Create Device-Attached Archive for Any Vessel	Х	Х	х		
	View Archives Attached to Incucyte Page	Х	Х	Х		
	Delete Device-Attached Archive Created by You	Х	Х	х		
	Delete Any Device-Attached Archive	Х				
Security	View, create or edit users	Х				
	Change Local User Password	Х				
	Change login settings	Х				
Status	View Status Page	Х	Х	Х	Х	
	Delete In Progress Archive Created by You	Х	Х	Х		
	Delete Any In Progress Archive	Х				

Table D-1: User Permission Levels (Continued)

			Permissi	on Level	
UI	Action/Access	Admin	Standard	Limited	Guest
	Delete In Progress Analysis Created by You	х	Х	Х	
	Delete Any In Progress Analysis	Х			
	Add Service Log Entry	Х	Х	Х	
	Export Log Entries	Х	х	Х	
Vessel View	View Any Scan Time	Х	Х	Х	Х
	Modify Image Channel Options (On/Off, Scaling, Brightness, Contrast)	Х	Х	Х	Х
	Modify Spectral Unmixing Values for Any Vessel	х	Х	Х	
	Launch New Analysis for Any Vessel	Х	Х	Х	
	Go to Scan Metrics	Х	Х	Х	Х
	Go to Scan Metrics with All Diagnostic Metrics	Х			
	Go to Vessel Information	Х	Х	Х	Х
	Launch Image and Movie Export	Х	Х	Х	
Launch Analysis	Create Analysis	Х	Х	Х	
Analysis View	Go to Edit User-Defined Metrics for any Analysis	Х	Х	Х	
	Launch Classification for Any Cell-by-Cell Analysis	х	Х	Х	
Metrics	View Time Plot Metrics for Any Analysis	Х	Х	Х	Х
	Create or Edit User-Defined Time Plot Metric for Any Analysis	х	Х	Х	
	Delete Any User-Defined Time Plot Metric for Any Analysis	х	Х	Х	
	Apply Any User-Defined Time Plot Metrics to Analysis Definition Created by You	Х	X	Х	
	Apply Any User-Defined Time Plot Metrics to Any Analysis Definition	х			
	Save or Load Generated Graphs to or from Disk	Х	Х	Х	
	Export Metric Data	Х	Х	Х	
Vessel/Analysis Information	Create, Edit, or Delete Plate Map for Any Vessel	х	х	Х	
	Import or Export Plate Map for Any Vessel	Х	Х	Х	
	Create or Modify Details/Notes for Any Vessel	х	Х	Х	

Table D-1: User Permission Levels (Continued)

Table D-1: User Permission Levels (Continued)

		Permission Level			
UI	Action/Access	Admin	Standard	Limited	Guest
	Create or Modify Details/Notes for Any Analysis	Х	Х	Х	

To add a local user to Incucyte

1. On the Incucyte main menu, click Security.

The Users window opens. The window lists all the users (local and Domain) that have been added to the instrument.

Figure D-1: Users window

E IC50000 - sartorius\a.wilson - Incuc	yte SX5 - SX5 G/O/NIR Optical Module		-		×
$\equiv \bigcirc$ Schedule	⊚ View 🔑 Manage	Device Archive 🙆 Security 🛈 Status	Scanning (9	8%)	
LUsers	User Name	Permission Level	User Type A	ctive •	
💄 Add User	Admin1	Admin	Local User	4	
💄 Add Domain User	AdminHarvey	Admin	Local User	4	
Refresh	AdminSteve	Admin	Local User	4	
Vessel Workgroups	AdminUser	Admin	Local User	2	

2. Click Add User.

The window is updated with an Add User pane.

Figure D-2: Users window with Add User pane



3. Enter the information for the new user.

Option	Description
User name	User names for local user accounts cannot contain the following characters: a space, or any of the following: $\langle \rangle $

Option	Description
Password	 Passwords are alphanumeric and must have the following characteristics: Contain at least 8 characters. Contain at least one upper case character. Contain at least one lower case character. Contain at least one number.
	Caution: No option is available for recovering a user's password if the user loses/ forgets it. Make sure that the user is aware that the password should be stored in a safe and recoverable location; otherwise, the only option that is available is to reset (edit) the user's password. See "Editing a User for an Incucyte" on page 187,
Confirm Password	You must enter the password in this field exactly as you did in the Password field.
	Note: This field is protected. You cannot copy the password from the Password field, and then paste the password into this field.
Permission Level	Select the appropriate permission level for the user. See Table D-1 on page 179 for a detailed list of the access/actions provided to a permission level.
Vessel Workgroups	Optional. Select the vessel workgroups to assign to the user. The user can view all vessels and their data in the assigned workgroups. The user cannot view any vessels and their data in the vessel workgroups that you do not assign to him/her.

4. Click Save.

The local user is added by default as an Active user.



After you add a new user, click Refresh to refresh the display and ensure that the user was added successfully.

To add a Domain user to Incucyte

Even though a Domain user uses Windows domain credentials to log in to Incucyte, you must still add the user to Incucyte before they can log in to the instrument. If a Domain user is to have access to multiple Incucytes within a domain, then you must add the Domain user to every applicable Incucyte.

1. On the Incucyte main menu, click Security.

The Users window opens. The window lists all the users (local and Domain) that have been added to the instrument.

ure D-3: Users w	indow		
E IC50000 - sartorius\a.wilson - Incucyte SIC	5 - SX5 G/O/NIR Optical Module		
$\equiv \bigcirc Schedule @$	View 🔑 Manage 🗖 Device	Archive Security (1) Status	Scanning (
Users	User Name	Permission Level	User Type
💄 Add User	Admin1	Admin	Local User
💄 Add Domain User	AdminHarvey	Admin	Local User
Refresh	AdminSteve	Admin	Local User
Vessel Workgroups	AdminUser	Admin	Local User
Settings	AdminUser_PreDemo	Admin	Local User

4 4 1 ... 1 J ...

2. Click Domain User.

The window is updated with an Add Domain User pane.

Figure D-4: Users window with a Domain User pane

LUsers	Add Domain User
💄 Add User 💄 Add Domain User	User Name
↔ Refresh	Domain:
Settings	Permission Level
	Admin Guest Limited User
	Standard User
	Save Close

3. Enter the user name for the domain user, making sure to specify the correct domain in the correct format; for example, sartorius\john.smith.



If you enter the user name correctly, then a "Valid" indicator is displayed next to the name. See Figure D-5 below.

Figure D-5: Valid indicator for a Domain User name

User Name	
sartorius\john.smith	📀 Valid
Domain: sartorius	

- 4. Select the correct permission level for the user.
- 5. Click Save.

The domain user is added by default as an Active user.



After you add a new user, click Refresh to refresh the display and ensure that the user was added successfully.



If you add Domain users to Incucyte, and they do not have local user accounts, then you must enable at least one of the available Windows Authentication settings, otherwise, your users are not able to log in to Incucyte. See "Specifying User Settings for an Incucyte" on page 185.

Specifying User Settings for an Incucyte

You have the option of specifying two settings—User Inactivity Timer and Automatic Logoff Countdown that all apply to all users (local and Domain) that have been added to an instrument. You can also specify values for Windows Authentication settings that apply only to Domain Users for the instrument.

Option	Description
Windows Authentication - These values apply only to	the Domain users that have been added to an instrument.
 Enable associated Windows domain accounts to login to the Incucyte using their Windows domain user name and passwords 	• By default, turned Off. If turned On, then users can use their Windows domain credentials to log in to Incucyte, but they are not required to do so. As a result, if the user has a local user account, then the user can still log in to Incucyte.
Require users to log in with Window domain user names when connecting to the device	 If enabled, then a Domain user can use only Domain credentials to log in to Incucyte, even if the user has a local account.
User Inactivity Timer - This value applies to all users (I	ocal and Domain) that have been added to an instrument.
Specify how long a user may be Inactive before warning of automatic logoff	The default value is None. The value that you specify determines how long a logged in user can be Inactive on an instrument before a User Inactivity message opens, indicating the amount of time that remains before the user is automatically logged off the instrument. Allowed inactivity values range from a low of 1 minute to a high of 5 hours, or Never. See Figure D-6 below.
Automatic Logoff Countdown - This value applies to al instrument.	l users (local and Domain) that have been added to an
Specify how long an inactive user has to respond before being automatically logged off.	The default value is None. Works in conjunction with the User Inactivity Timer value. The dynamic countdown value that is displayed in the User Inactivity message indicates how long a user has to respond to the message before being automatically logged off. Countdown values range from a low of 1 minute to a high of 5 hours, or Never.

Figure D-6: User Inactivity message



To specify user settings for an Incucyte

To ensure that at least one user always has access to the Security page, the following caveats are applicable before you can turn on the Require users to log in with Domain names setting:

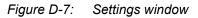
- A Domain user with Admin permissions must already exist. See "To add a Domain user to Incucyte" on page 183.
- The Require users to log in with a Domain name setting is enabled only *after* you turn on and save the Enable associated Windows domain accounts setting.
- 1. On the Incucyte main menu, click Security.

The Users window opens. The window lists all the users (local and Domain) that have been added to the instrument. See Figure D-1 on page 182.

2. Click Settings.

The Settings window opens. The window displays the following user settings:

- Windows Authentication. These values apply only to the Domain users that have been added to the instrument. By default, these values are turned off.
- User Inactivity Timer and Automatic Logoff Countdown. and their current values. By default, the values are initially set to None.





- 3. Do any or all of the following:
 - Turn on or turn off Enable associated Windows domain accounts.
 - Turn on or turn off Require users to log in with Domain names.



If you turn off Require users to log in with Domain names, Enable associated Windows domain accounts remains turned on.

- Specify the values as appropriate for the User Inactivity Timer and/or Automatic Logoff Countdown.
- 4. Click Save.

Editing a User for an Incucyte

You can edit the user permission level, the vessel workgroups, and the user status (Active or Inactive) for a local user or a Domain user for an Incucyte. Because a status edit is instrument specific, if a Domain user has access to multiple Incucytes within a domain, and the user is to have an Inactive status across all instruments in the Domain, you must edit the status on every applicable Incucyte. You can also edit the password for a local user.



Because scans and analyses are tied to a specific owner and the user name is displayed for the owner, you cannot edit the user name for a user. To edit a user name, you must set up an entirely new user. See "Adding a User to Incucyte" on page 179.

To edit a user for an Incucyte

1. On the Incucyte main menu, click Security.

The Users window opens. The window lists all the users (local and Domain) that have been added to the instrument.

Figure D-8: Users window

$\equiv \bigcirc$ Schedule	💿 View 🤔 Manage 🗖 De	evice Archive 🖲 Security 🚯 Status	Scanning (98%)	
Users	User Name	Permission Level	User Type Active -	
💄 Add User	Admin1	Admin	Local User	•
💄 Add Domain User	AdminHarvey	Admin	Local User 🗸	
Refresh	AdminSteve	Admin	Local User	
Vessel Workgroups	AdminUser	Admin	Local User	
Settings	AdminUser PreDemo	Admin	Local User	



The standard Incucyte filter and sort functions are available on the Users window to assist you in searching for a specific user. See "Filtering and Sorting Data in an Incucyte Window" on page 21.

2. To the far right of the entry for the applicable user, click the Ellipsis (...) icon.

A drop-down menu opens with two options: Edit and Change Password.

Figure D-9: User drop-down menu

😑 IC50000 - Ash Wilson - Incucyte SX5	- SX5 G/O/NIR Optical Module					-		\times	
$\equiv \bigcirc$ Schedule	⊚ View 🔑 Man	age 🔚 Device	- Archive	Status	No Up	oming So			
Users	User Name		▲ Per	rmission Level	User Type	Active	•		
💄 Add User	Brian		Adr	nin	Local User	~	Þ	E	dit
Add Domain User	BrianH		Adr	min	Local User	1	•		Change Password
Refresh	Admin1		Adr	nin	Local User	1	•		
🙅 Vessel Workgroups	Administration		Ade		Local Days				

- 3. Continue to one of the following:
 - "To edit the permission level, vessel workgroups, or user status" on page 188.
 - "To edit the password for a local user" on page 189.

To edit the permission level, vessel workgroups, or user status

1. Click Edit.

The Users window is refreshed with an Edit User pane. The current information for the selected user (name, permission level, and status) is displayed in the pane.

Figure D-10: Edit User pane

E C Schedule	🛇 View 🦪 Manage 🗖	Device 🖃 Archive <u>③</u>	Security	(i) Status		No Upcoming Scans	
Users	User Name	Permission Level	User Type	Active +	Edit User		
💄 Add User	Admin1	Admin	Local User	1	User Name		
💄 Add Domain User	AdminHarvey	Admin	Local User	4	Brian	-	
😔 Refresh	AdminSteve	Admin	Local User	1	Permission	a Level	
Vessel Workgroups	AdminUser	Admin	Local User	4	Admin		
	AdminUser_PreDemo	Admin	Local User	4	Guest Limited Us	er	
Settings	BasicSteve	Standard User	Local User	4	Standard U	lser	
	BMurray	Standard User	Local User	1			
	BreeP	Admin	Local User	4			
	Brian	Admin	Local User	2			
	BMurray	Standard User	Local User	4			
	BreeP	Admin	Local User	•	 Active 		
					Vessel Wo	orkgroups (Optional)	
					Select	Group Name	
						CF Test Work Gp	
						First Group	
						vessel workgroup 2	
						workgroup1	
						workgroup2	
						workgroup3	
					Save	Close	

2. Edit any or all of the current information for the user.

Option	Description
Permission Level	Select the appropriate permission level for the user. See Table D-1 on page 179 for a detailed list of the access/actions provided to a permission level.
Vessel Workgroup	Optional. To assign a vessel workgroup to the user, click Select for the workgroup. You can assign multiple vessel workgroups to a user. To remove a vessel workgroup assignment, clear the Select option for the workgroup.
Status	By default, a user is added as an Active user. To inactivate a user, clear the Active checkbox. Conversely, to active an inactive user, select Active.
	Tip: Remember, If a Domain user has access to multiple Incucyte instruments within a domain, and the user is to have an Inactive status across all instruments in the Domain, then you must edit the status on every applicable Incucyte.

3. Click Save.

The Edit User pane closes. The Users window remains open.



After you edit a user, click Refresh to refresh the display and ensure that your changes have been saved.

To edit the password for a local user

1. Click Change Password.

The User window is refreshed with a Change User Password pane. The password displays the current user name for the selected user, but you cannot edit the value here.

Figure D-11: Edit User Password pane

ne	Permission Level	User Type	Active -	Change User Password
	Standard User	Local User		
	Standard User	Local User		User AaronO
	Standard User	Local User		New Password
	Admin	Local User		New Password
	Admin	Local User		Repeat Password
	Standard User	Local User		
er -	Guest	Local User		Save Close
	Standard User	Local User		Passwords must be at least 8 characters long.
	Standard User			Passwords must contain at least one upper-case character Passwords must contain at least one lower-case character
	Admin	Local User		Passwords must contain at least one digit. The password fields must match.
	Standard User	Local User		ne password nerds must match.

2. Edit the user's password.

Option	Description
Password	Passwords are alphanumeric and must have the following characteristics:
	Contain at least 8 characters.
	Contain at least one upper case character.
	Contain at least one lower case character.
	Contain at least one number.
Confirm Password	You must enter the password in this field exactly as you did in the Password field.
	Note: This field is protected. You cannot copy the password from the
	Password field, and then paste the password into this field.

3. Click Save.

The Change User Password pane closes. The Users window remains open.

Managing Vessel Workgroups

A vessel workgroup defines the users who are able to view specific vessels and the vessels' data. You create and organize a vessel workgroup based on common vessel characteristics that you determine such as your organizational structure (for example, the name of the lab that uses the vessels) or the name of the study that uses the vessels (for example, CAR-T assay). After you create and name a vessel workgroup, you assign the necessary vessels to the workgroup, and then you assign users to the workgroup. Only those users who are assigned to the vessel workgroup can view the vessels and the vessels' data in the vessel workgroup. A user can be assigned to one or more vessel workgroups. The permission levels for the user determine the actions that the user can carry out for a vessel and its data in any vessel workgroup to which the user is assigned. Only Administrator users can manage vessel workgroups. Managing vessel workgroups consists of creating vessel workgroups, editing vessel workgroups, and deleting vessel workgroups.



Optionally, you can assign a vessel workgroup to a vessel at the time that you are configuring and scheduling a vessel scan. See "To provide vessel information" on page 41.

To create a vessel workgroup

1. On the Incucyte main menu, click Security.

The Users window opens.

E IC50000 - sartorius\a.wilson - Incucyte	sX5 - SX5 G/O/NIR Optical Module				- 0) X
$\equiv $ \bigcirc Schedule	💿 View 🔑 Manage	Device 🗖 Arch	ive (i) Security (i) Status	Scar	nning (98%)	
Users	User Name		Permission Level	User Type	Active -	
💄 Add User	Admin1		Admin	Local User	4	
💄 Add Domain User	AdminHarvey		Admin	Local User	4	
Refresh	AdminSteve		Admin	Local User	4	
Vessel Workgroups	AdminUser		Admin	Local User	4	
Settings	AdminUser_PreDemo		Admin	Local User	4	

2. Click Vessel Workgroups.

The Vessel Workgroups window opens. The window is divided into two panes. The Group Name (left) pane lists all the vessel workgroups that are currently defined for the Incucyte. After you select a workgroup in the left pane, the right pane lists all the users who have been assigned to the selected vessel workgroup. If no users have been assigned to the selected vessel workgroup, then the message "No users are associated with the selected vessel workgroup" is displayed.

Figure D-13:	Vessel	Workaroups	window
riguic D io.	100001	workgroups.	win aow

E C50000 - sartorius\arwiison - Incucyte SX		Device	- Arch	ive 🚯 Security 🚯 St	atus	– □
Q Users	Group Name			User Name	Permission Level	User Type
Vessel Workgroups	CF Test Work Gp			Bryan	Admin	Local User
+ Add Workgroup	Dr. Hunter's Lab			Charishma	Admin	Local User
Refresh				David	Standard User	Local User
				Jake	Standard User	Local User
🗘 Settings				Jasper	Admin	Local User

3. Click Add Workgroup.

The Add Workgroup window opens. The right pane contains the options for adding a new vessel workgroup. The Group Name field is blank. The Group Name (right) pane lists all the vessel workgroups that are currently defined for the Incucyte.

Figure D-14: Add Workgroup window

IC50000 - sartorius\a.wilson - Incucyte S	\$X5 - \$X5 6/0/1	NIR Optical Module			- 0
$\equiv \bigcirc$ Schedule	⊚ View	🖋 Manage 📘	Device 🚍 Archive	Security (i) Statu	IS Next Scan: 5 min
Q Users	Add Worl	kgroup			Group Name
Vessel Workgroups	Group Na				CF Test Work Gp
+ Add Workgroup	Group Na	ine			Dr. Hunter's Lab
Refresh	Individua	al Users			
🗘 Settings	Select	User Name	Permission Level	User Type	
er Gettings		AdminSteve	Admin	Local User	
		AdminUser	Admin	Local User	
		AdminUser_PreDemo	Admin	Local User	
•		BMurray	Standard User	Local User	
		BreeP	Admin	Local User	

- 4. In the Group Name field, enter the name for the new vessel workgroup.
- 5. To add users to the vessel workgroup, click Select for each user that you are adding to the workgroup.

The standard Incucyte filter and sort functions are available to assist you in searching for the users whom you are adding to the vessel workgroup. See "Filtering and Sorting Data in an Incucyte Window" on page 21.

6. Click Save.

A Success message opens, indicating that the workgroup was created successfully.

Figure D-15: Success message



7. Click OK.

The Success message closes. The newly added vessel workgroup is displayed in the Group Name pane.



After you add a vessel workgroup, click Vessel Workgroups, and then click Refresh to refresh the display on the Vessels Workgroup page and ensure that your changes have been saved.

To edit a vessel workgroup

You can edit the name of a vessel workgroup and you can edit the users (add or remove) for a workgroup.

1. On the Incucyte main menu, click Security.

The Users window opens.

Figure D-16: Users window

IC50000 - sartorius\a.wilson - Incucyte S	X5 - SX5 G/O/NIR Optical Module				- 0)
$\equiv $ \bigcirc Schedule \bigcirc	🔊 View 🔗 Manage	Device Archiv	e Security Status	Sca	nning (98%)	
Users	User Name	• P	ermission Level	User Type	Active +	
💄 Add User	Admin1	A	dmin	Local User	4	
💄 Add Domain User	AdminHarvey	A	dmin	Local User	1	
Refresh	AdminSteve	A	dmin	Local User		
Vessel Workgroups	AdminUser	A	dmin	Local User	7	
Settings	AdminUser_PreDemo	A	dmin	Local User	7	

2. Click Vessel Workgroups.

The Vessel Workgroups window opens.

Figure D-17: Vessel Workgroups window

E IC50000 - sartorius\a.wilson - Incucyte SX	5 - SX5 G/O/NIR Optical Module				- 🗆 ×
≡ C Schedule @	View 🔑 Manage 🗖 Device (- Archi	ive Security (i) Status	Next	Scan: 5 min
Lusers	Group Name		User Name	Permission Level	User Type
Vessel Workgroups	CF Test Work Gp		Bryan	Admin	Local User
+ Add Workgroup	Dr. Hunter's Lab		Charishma	Admin	Local User
Refresh			David	Standard User	Local User
C Halloon			Jake	Standard User	Local User
Settings			Jasper	Admin	Local User



The standard Incucyte filter and sort functions are available to assist you in searching for the vessel workgroup that you are editing. See "Filtering and Sorting Data in an Incucyte Window" on page 21.

3. To the far right of the entry for the workgroup that you are editing, click the Ellipsis (...) icon.

A drop-down menu opens with two options: Edit and Delete.

Figure D-18: Vessel Workgroups window with drop-down menu for selected vessel workgroup

IC50000 - sartorius\a.wilson - Incucyte S	X5 - SX5 G/O/NIR Optical Module			
$\equiv \bigcirc$ Schedule	🔊 View 🥬 Manage 🗖 De	vice 🚍 Arch	ive 🚯 Security 👔	Status
👤 Users	Group Name		User Name	Permission
Vessel Workgroups	Bioavailabity Study 1		Bryan	Admin
+ Add Workgroup	CF Test Work Gp		Charishma	Admin
Refresh	Dr. Hunter's Lab		David	Standard Us
*			Edit	Standard Us
Settings	First Group		Delete	Admin

4. Click Edit.

The Edit Workgroup window opens. The Group Name (left) pane lists all the vessel workgroups for the Incucyte. The workgroup that you are currently editing is selected in the pane. The right pane displays the all the users who are currently assigned for the workgroup (a check mark is displayed in the Selected column) first and then all the users that are available for adding to the group.

Figure D-19: Edit Workgroup window

oups	Bioavailablity Study 1						
	CF Test Work Gp	Group Na					
	Dr. Hunter's Lab		Dr Hunter's Lab Individual Users				
	First Group vessel workgroup 2	Select	User Name	Permission Level			
	Wilson Research Team		Bryan	Admin			
	workgroup1 workgroup2	4	Charishma	Admin			
		4	David	Standard User			
				a			

5. Do any or all of the following:

- Edit the group name.
- To add a user to the group, click Select for the user.
- To remove a user from the group, clear the check mark for the user.



If you delete a user from a vessel workgroup, the user is deleted only for the selected workgroup. The user is not deleted from any other workgroups to which they are assigned.

6. Click Save.

A Success message opens, indicating that the workgroup was updated successfully.

Figure D-20: Success message



7. Click OK.

The Success message closes. The newly edited vessel workgroup remains selected in the Group Name pane.

To delete a vessel workgroup

You can delete a vessel workgroup with or without assigned users.

1. On the Incucyte main menu, click Security.

The Users window opens.

Figure D-21: Users window

EI IC50000 - sartorius\a.wilson - Incucyt	e SX5 - SX5 G/O/NIR Op	ptical Module						- 0	×
$\equiv \bigcirc$ Schedule	⊚ View _	🔑 Manage	Device	- Archi	ve 🙆 Security	(i) Status	Scannin	ng (98%)	
Users	User Name				Permission Level		User Type	Active *	
💄 Add User	Admin1				Admin		Local User	4	
💄 Add Domain User	AdminHarvey				Admin		Local User	4	
😌 Refresh	Admin@taua				Admin		Local Lloar		

2. Click Vessel Workgroups.

The Vessel Workgroups window opens.

Figure D-22: Vessel Workgroups window

IC50000 - sartorius\a.wilson - Incucyte Si	X5 - SX5 G/O/NIR Optical Module					- D >
$\equiv \bigcirc$ Schedule	🔊 View 🤔 Manage	Device	- Arch	ive (6) Security	(i) Status	Next Scan: 5 min
👤 Users	Group Name			User Name	Permission Level	User Type
Vessel Workgroups	CF Test Work Gp			Bryan	Admin	Local User
+ Add Workgroup	Dr. Hunter's Lab			Charishma	Admin	Local User
Refresh				David	Standard User	Local User
C Renesir				Takes	Chandred House	L and L laws

-`@`

The standard Incucyte filter and sort functions are available to assist you in searching for the vessel workgroup that you are deleting. See "Filtering and Sorting Data in an Incucyte Window" on page 21.

To the far right of the entry for the workgroup that you are deleting, click the Ellipsis (. . .) icon.
 A drop-down menu opens with two options: Edit and Delete.

Figure D-23: Vessel Workgroups window with drop-down menu for selected vessel workgroup

IC50000 - sartorius\z.wilson - Incucyte SX	- SX5 G/O/NIR Optical Module			
≡ C Schedule @	View 🔏 Manage 🔲 Device	- Arch	ive Security (i) Status	5
Lusers	Group Name		User Name	Permission
Vessel Workgroups	Bioavailability Study 1		Bryan	Admin
+ Add Workgroup	CF Test Work Gp		Charishma	Admin
Refresh	Dr. Hunter's Lab		David	Standard Us
* ~ ~			Edit	Standard Us
Settings	First Group		Delete	Admin

4. Click Delete.

A Delete Workgroup message opens, asking you if you are sure that you want to delete the selected workgroup.

Figure D-24: Delete Workgroup dialog box



5. Click Yes.

The Delete Workgroup dialog box closes. You return to the Vessel Workgroups window. The deleted vessel workgroup is no longer displayed in the Group Name pane.

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