Tomocube

TomoStudioX
HT-X Operating Software

TomoStudioX 2.1 User Manual

Version 1

For research use only. Not for use in diagnostic procedures.

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Trademarks

Revision history

Revision	Date	Description
Version 1	30 July 2025	Initial version

About this guide

This user guide is intended for researchers and technical users operating TomoStudioX, the software used to control and analyze imaging data from Tomocube's HT-X series holotomography systems.

User attention words

Two types of user attention words appear in this manual. Each attention word signals a particular level of observation or action, as described below.

Note: provides information that may be of interest or help but is not critical to the use of the product.

Important! provides information necessary for proper instrument operation, accurate installation, or safety.

Safety alert words

Two types of safety alert words related to the awareness of relevant hazards appear in this manual. Each safety alert word signals a particular level of observation or action, as described below.

⚠ **CAUTION** indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate harm to users. This alert may also be used to warn against unsafe practices.

⚠ **WARNING** indicates a potentially hazardous situation which, if not avoided, may result in injury. This alert may also be used to indicate the possibility of erroneous data that could result in an incorrect analysis.

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CHAPTER 1. Product Information

1.1 Purpose and scope of the document

This manual is a user guide for TomoStudioX, the operating software for Tomocube's HT-X series systems. It covers the core functionalities of the software—such as system control, 3D holotomography image acquisition, data management, and workflow setup—to support users in both basic and advanced operations.

The guide is intended to ensure efficient, accurate, and reliable use of the software across a wide range of research applications.

1.2 Product description

TomoStudioX is the dedicated software platform for controlling Tomocube's HT-X series systems, enabling advanced 3D holotomography imaging. It provides an integrated environment for microscope control, image acquisition, and data management—allowing users to set up and run experiments with accuracy and efficiency.

The software supports real-time live preview and precise control of the motorized XYZ stage, enabling image acquisition at user-defined positions. A variety of imaging modes are available, including multi-point acquisition and time-lapse imaging, with streamlined setup for complex or repetitive workflows.

After acquisition, TomoStudioX offers powerful tools for navigating and managing large-scale datasets through high-performance viewers and flexible data organization features.

Key features of TomoStudioX include:

- **Stage Control**: Motorized movement along XYZ axes with automated focus support
- **Digital Live View**: Real-time visualization for specimen observation and focus refinement
- **Image Acquisition Modes**: Single, tile scan, time-lapse, and multi-point imaging
- **Multi-Modality Imaging**: Snapshot acquisition of 3D refractive index (RI)-based holotomography, brightfield (color or grayscale), and fluorescence images
- **Correlative Imaging**: Fluorescence deconvolution and 3D overlay with holotomography data for integrated molecular and structural analysis
- **Workflow-Based Interface**: Intuitive, step-by-step operation via a graphical user interface tailored for researchers
- **Large-Scale Data Management**: Efficient browsing and organization of high-content imaging datasets

TomoStudioX is designed to support high-resolution, high-content 3D imaging workflows in research environments.

Important! The HT-X system is for research use only.

1.3 Software components

TomoStudioX

TomoStudioX is the primary control software for holotomography imaging using the HT-X series. It manages the entire imaging workflow, including microscope stage control (XYZ movement, autofocusing), image acquisition (single capture, time-lapse), and experiment configuration. It also provides real-time image preview, as well as tools for navigating, organizing, and managing acquired image data.

HTX Processing Server

The HTX Processing Server is a dedicated module that reconstructs 3D refractive index (RI) tomograms from 2D raw holographic images acquired by the system. It performs high-speed, algorithm-based reconstruction to generate accurate and high-resolution 3D images suitable for downstream analysis.

TA Viewer

TA Viewer is a visualization tool used to explore Tomocube Common Files (TCF) generated by the HTX Processing Server. It allows users to view, capture, and export 2D and 3D images or videos. The software supports basic image operations, volume rendering, and region-of-interest (ROI) analysis for in-depth interpretation of imaging data.

1.4 System requirements

TomoStudioX and all associated software modules are installed and configured by Tomocube on a dedicated workstation provided with each HT-X system. This ensures optimal performance for real-time system control and 3D image reconstruction.

The workstation specifications may differ slightly depending on the HT-X model. Below is a representative example (HT-X1):

- CPU: Intel® Core i7 or equivalent
- RAM: 128 GB
- Storage: Two SSDs (2 TB for OS, 8 TB for data)
- GPU: NVIDIA® GeForce RTX 4090 (24 GB VRAM)
- OS: Windows® 10 IoT

Note: For the latest specifications of your workstation or for other HT-X models, please refer to the system label or contact Tomocube support.

CHAPTER 2. Installation

2.1 Software installation guide

All essential software required for operating the product is pre-installed on the system at the time of installation. If additional installation on a separate PC is needed, or if software updates are required, support must be requested from a service engineer or Tomocube HQ.

2.2 User Account and Permission Settings

2.2.1 User accounts

TomoStudioX supports three types of user accounts:

- **Operator**: For general users. Operators can access and operate the system but cannot change system configurations or manage user accounts.
- **Administrator**: For system administrators. Administrators can create and delete user accounts, modify system parameters, and manage software settings.
- **Service Engineer**: For authorized Tomocube personnel only. This account is used for system maintenance and advanced configuration.

To use TomoStudioX, each user must log in with a valid Operator or Administrator account. The account credentials (username and password) should be issued and managed by the designated System Administrator(s).

Note: Guest Login: A temporary guest account is also available using the credentials:

- *Username: default*
- *Password: (leave blank)*

Administrator accounts can be created either by the Service Engineer during initial installation or by an existing Administrator. TomoStudioX supports multiple Administrator accounts.

User Role	Operate TomoStudio X	Manage User Account	Adjust Preferences	Configure System Settings
Service Engineer	O	O	O	O
Administrator	O	O	O	X
Operator	O	X	X	X

2.2.2 Create/delete user account

After logging in, Administrators can create user accounts in the [Register] menu located on the bottom-right side of the login window.

User accounts can be deleted from the *Preference* menu. (Refer to Chapter 7.5 for details.)

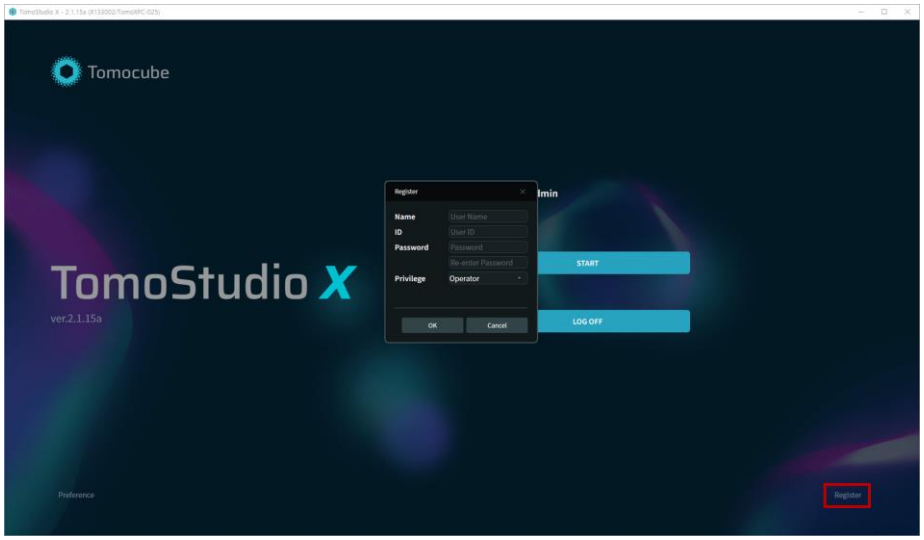


Figure 2.1 Register menu

CHAPTER 3. Device Control and Status

TomoStudioX is not a standalone software—it is designed to control and operate the HT-X series imaging systems.

Therefore, confirming a proper connection between the software and the hardware is essential for using most features.

If the HT-X system is not connected or powered on, the majority of control features will be disabled. However, certain modules of the software—such as Data Navigation (for reviewing previously acquired data) and HTX Processing Server (for processing raw data into holotomography images)—remain available for use.

3.1 Startup and Initialization

1. Before starting an experiment, ensure that the HT-X system is powered on.

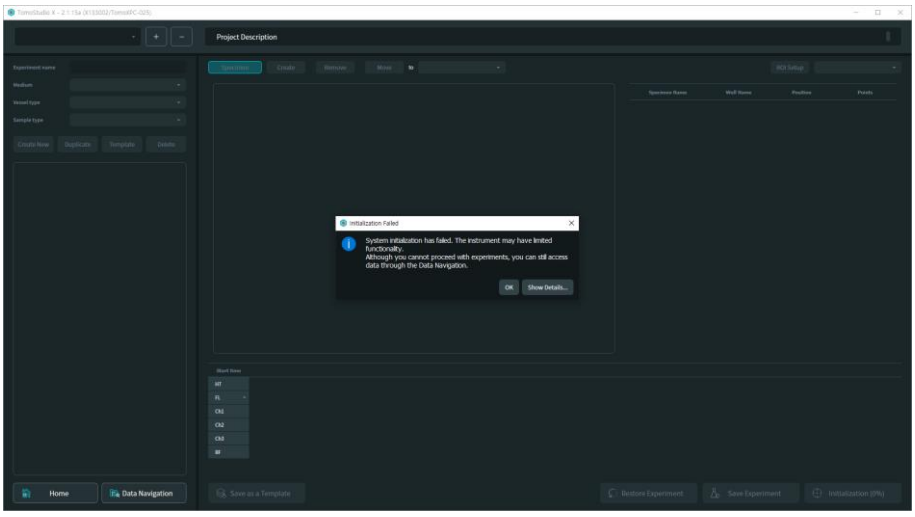
   If the device is off, press the power switch located below the power cable on the lower-left rear panel of the main unit. When powered on, a white status LED will appear on the upper front panel of the device.

2. Launch TomoStudioX, log in, and click START to begin the experiment. This will open the Experiment Manager window, and the system will begin automatic initialization.

   Note: During this process, key components—including the sample stage, camera, and lenses—are initialized and reset to a ready state. Initialization typically takes less than 5 minutes.

3. Once complete, the front indicator light will turn blue, indicating that the system is ready for imaging.

Important! If the device is not powered on, or if a required cable (e.g., camera cable) is not properly connected to a USB 3.0 port on the PC, an error message may appear. Refer to Chapter 8: Troubleshooting for guidance in resolving connection issues.



CHAPTER 4. Initiation and Experimental Setup

4.1 Workflow

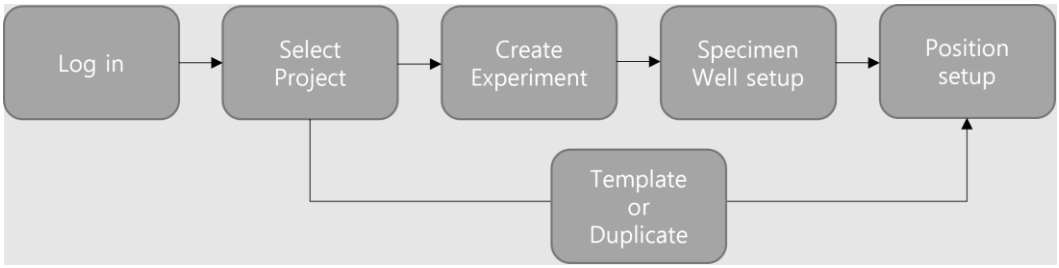


Figure 4.1 Experimental setup workflow

The experimental setup procedure performed before imaging consists of four main steps:

1. Log in to the software using a registered user account.
2. Create or select a project to group related experiments.
3. Create and configure an experiment, including imaging mode and acquisition settings.
4. Set up the specimen and well configuration, including vessel type and sample layout.

These steps can be simplified by importing predefined templates or duplicating previous experiments to streamline recurring workflows.

There are two methods for creating a new experiment in TomoStudioX:

- Simple Method: A basic setup method covering standard configurations and commonly used settings.
- Wizard Method: A step-by-step setup guide that includes advanced options, ideal for optimizing specialized experiments or custom research workflows.

4.2 Starting the software

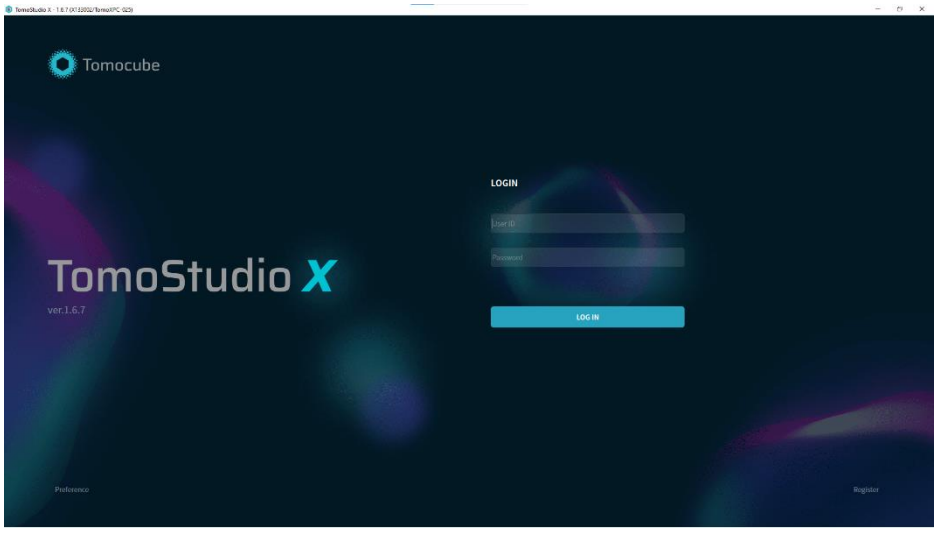
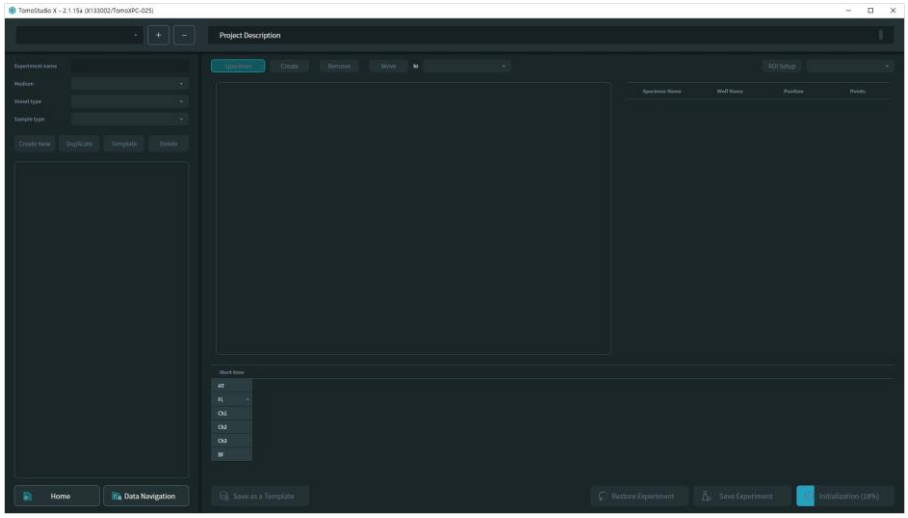


Figure 4.2 Login window

Log in with a personal or designated user account. When the system is powered on and TomoStudioX is launched, the initialization process starts automatically. The progress is displayed as a percentage in the status bar located at the bottom-right corner of the screen. Once the initialization is complete, the status icon changes to "Run Experiment," indicating that the system is ready to start an experiment



4.3 Experiment Manager

After logging in to TomoStudioX with an account and pressing the START button, the Experiment Manager window will appear. In the window, the experimental parameters can be set up.

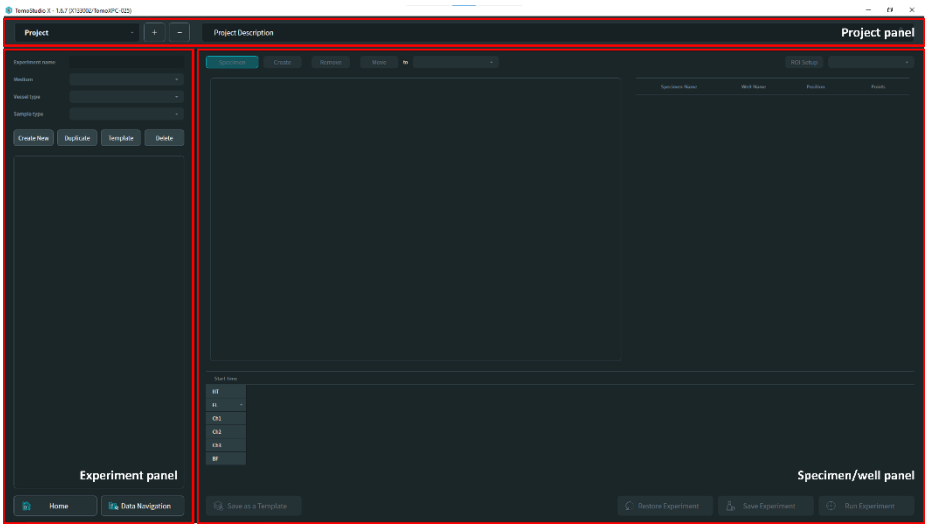


Figure 4.3 Experiment Manager

Project panel

In TomoStudioX, the term *project* refers to a collection of related experiments. Projects have the purpose of organizing experiments by research topic. Projects can be managed using the Project panel along the top of the Experiment Manager window.

The equipment initialization status bar is located on the right side of the project panel. It will display the loading progress until the device and stage are ready for use, after which you can proceed to the next setup.

Figure 4.4 Project panel

Experiment panel

In TomoStudioX, the term *experiment* refers to a set of data acquired for a sample by the HT-X1 system. Different measurements in one experiment share the information of the medium RI, the vessel type, the sample type, and the specimens for imaging (groups of wells to be analyzed). This information is stored in the experimental configuration setting.

Experimental configurations can be set up using the Experiment panel along the left side of the Experiment Manager window.

In the Experiment panel, names for the experiments can be assigned, and the RI of the medium and the vessel type can be selected. The Experiment panel has four buttons—Create New, Copy, Template, and Delete—to manage the experiments in the current project. The list of experiments in the current project can be easily accessed in the middle window of the Experiment panel.

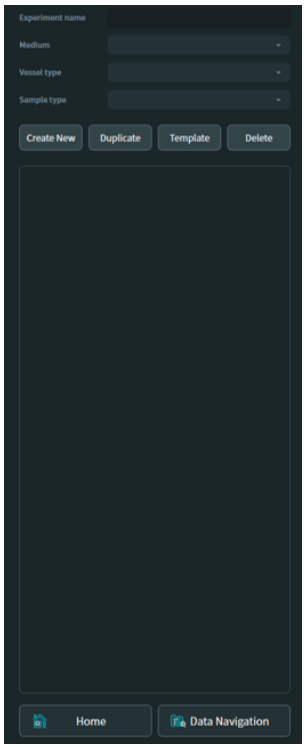


Figure 4.5 Experiment panel

Specimen/Well panel

Several different wells containing specimens can be grouped and named for experiments in the Specimen/Well panel at the right of the Experiment Manager window. The Specimen/Well panel also provides other experimental information such as the number of imaging points, fluorescence settings, and time-lapse conditions.

The Specimen/Well panel has four buttons along the top—Specimen, Create, Remove, and Move. The Specimen button is a toggle button to switch between two modes to manage the wells in the plate. The other buttons are used to manage the wells containing specimens.

Additionally, the panel also has the [ROI Setup] button on the top. It is used to assign regions of interest (ROIs) for imaging when the sample is located in narrow parts of a wide-field imaging vessels. It is recommended to set ROIs for samples in such vessels as tissue slides especially if the sample is not around the center of the vessel.

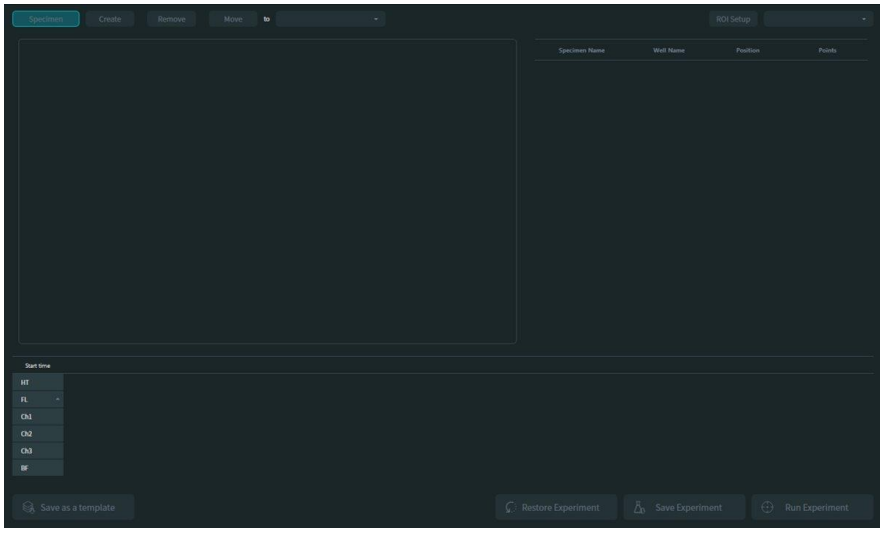
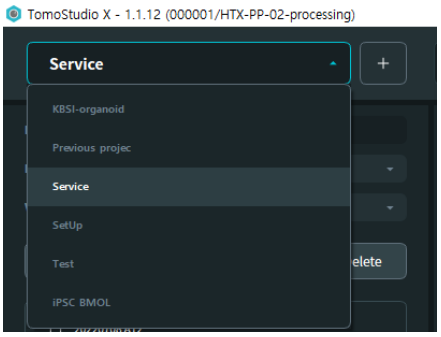


Figure 4.6 Specimen/Well panel

4.4 Project

Create New Project: To create a new project, click the [Add Project] button  in the Project panel.

Select Existing Project: To select an existing project saved to the account, click the drop-down menu in the Project panel and select the project, as shown below.

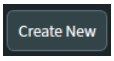


Note: To add notes or brief information on the project, click the Project Description field

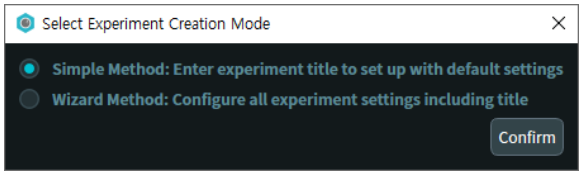
in the Project panel and write a description of the project.

4.5 Simple Method for Experimental Setup

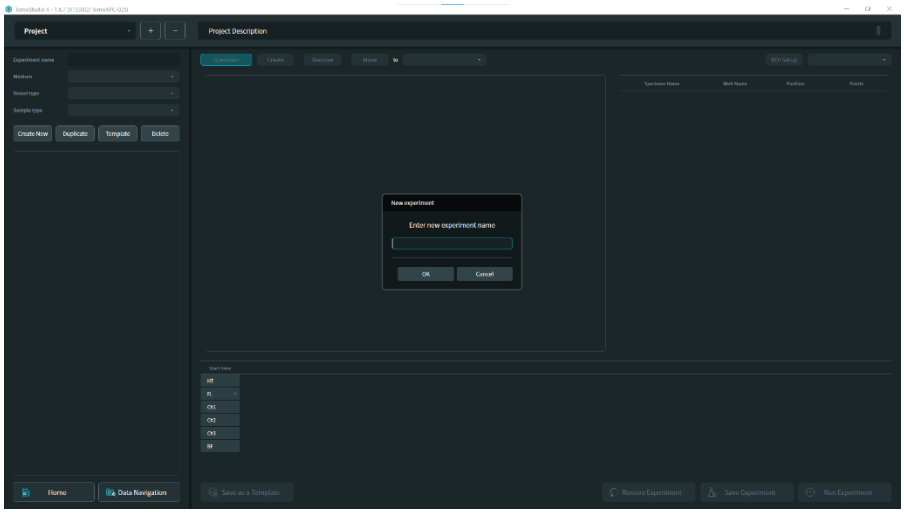
4.5.1 Create a new experiment using Simple Method

1. Click the [Create New] button 

in the Experiment panel.
2. Select Simple Method in the pop-up window and click [Confirm].

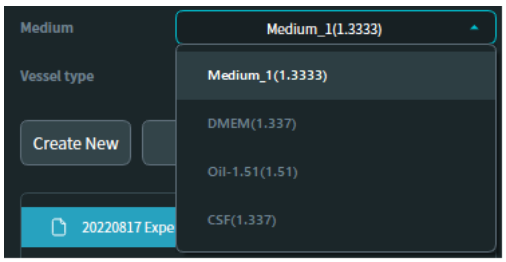


3. Type in a name for the experiment in the pop-up window and click OK.



Note: When naming the experiment, it is not necessary to add the date to the experiment name. The date is automatically recorded in the experiment list.

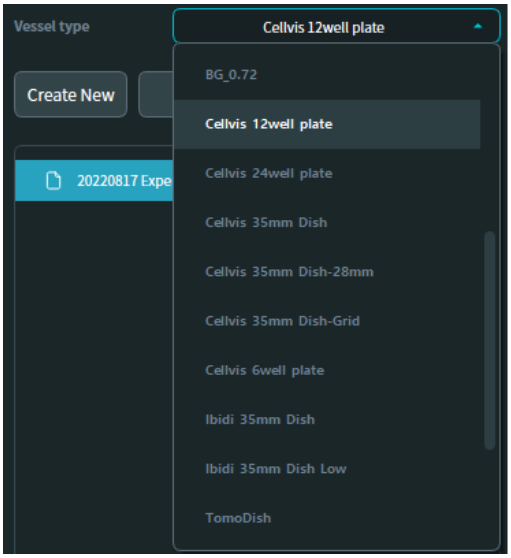
4. Select the appropriate medium RI for the experiment from the Medium drop-down menu in the Experiment panel.



Important! The value for the medium RI is critical in image processing of raw image data acquired by the HT-X1. Choose a proper medium RI value before starting the image acquisition.

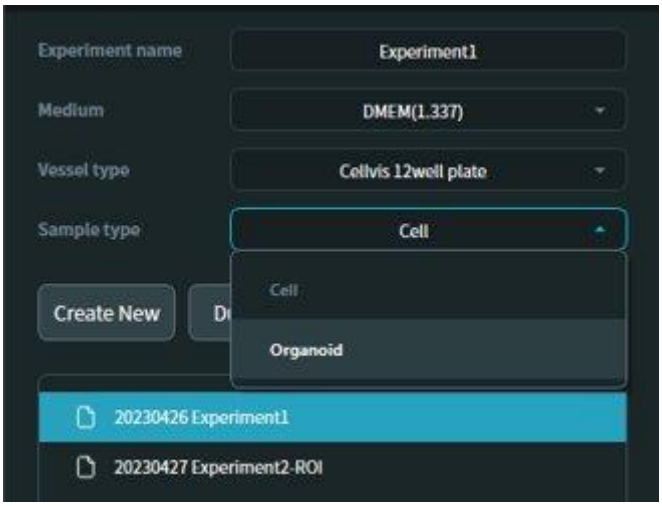
5. Select the appropriate vessel type for the experiment from the Vessel type drop-

down menu in the Experiment panel. A simple vessel map is displayed in the Specimen/Well panel.



Important! If the intended medium RI or vessel type for the experiment is not listed in the related drop-down menu, please contact your Administrator to update the setting. Additional medium RIs and vessel types can be added via the Preference menu accessible with an Administrator account.

6. Select the appropriate sample type for the experiment from the Sample type drop-down menu in the Experiment panel.



Note: Depending on the sample type, TomoStudioX facilitates optimal conditions to acquire holotomographic data for the specified sample type. Please refer to the table below for guidance on selecting the sample type.

Sample type	Light source	Z range	Processing Algorithm
Cell	Blue	60 μm	Standard with regularization
Organoid	Red	140 μm	Advanced without regularization
Bacteria	Blue	30 μm	Standard without regularization
Plant	Green	140 μm	Advanced without regularization

Note: The availability of certain sample types may vary depending on the system configuration. For example, systems that do not support multi-wavelength holotomography can only be used with *cell* and *organoid* sample types.
In such cases, the illumination source for *organoid* samples is automatically switched from red to blue.

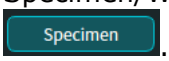
4.5.2 Specimen and Well Setup

In a multi-well plate setup, TomoStudioX provides two options for registering the wells and corresponding specimens for imaging.

These two registration modes are called Specimen Mode and Individual Mode.

- Specimen Mode allows multiple wells to be grouped and treated as a single specimen.
- Individual Mode registers each well as a separate, independent specimen

4.5.2.1 Register a specimen using the Specimen Mode

1. Toggle the mode button, which is the first button located at the top of the Specimen/Well panel in the Experiment Manager window, to the Specimen Mode 

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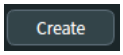
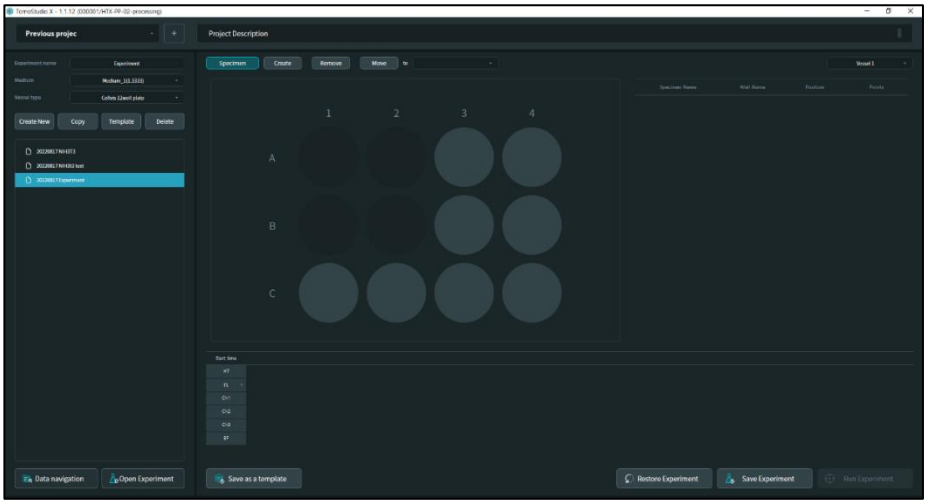
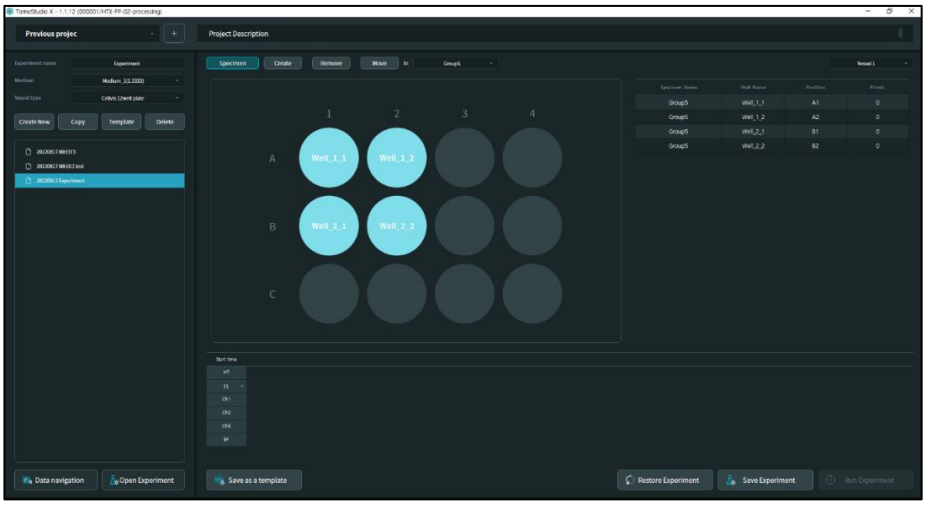
2. To group some or all of the wells on the vessel map as a single specimen, click the desired wells while holding the Ctrl key or select the wells by right-clicking and dragging the mouse cursor over the desired wells on the vessel map.

   Click the [Create] button 

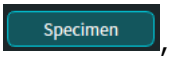
to register the selected wells as a group under a single specimen. To register other wells as a different group of specimens, repeat this process.



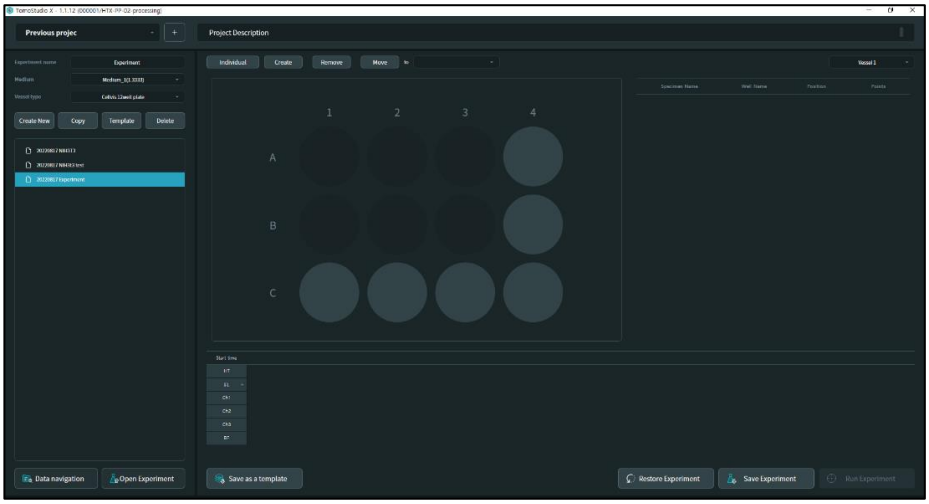
3. When a specimen is created, it automatically appears in the specimen/well table to the right of the vessel map. The names of the specimens and wells can be changed by clicking on each specimen/well name in the specimen/well table.

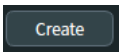


4.5.2.2 Register a specimen using the Individual Mode

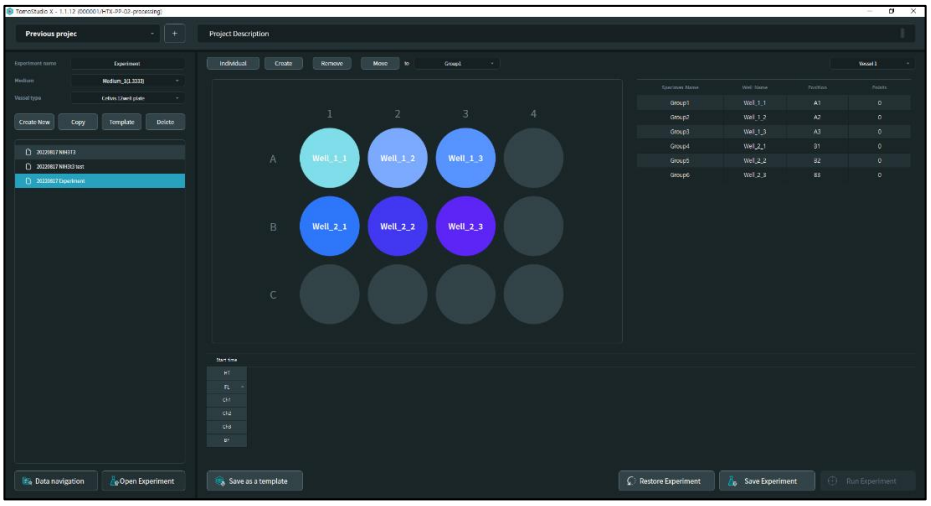
1. Click the mode button 

, which is the first button located at the top of the Specimen/Well panel in the Experiment Manager window, to switch to the Individual Mode from the Specimen Mode.
2. To register individual wells as single specimens, click individual wells while holding the Ctrl key or select the wells by right-clicking and dragging the mouse cursor over the desired wells on the vessel map.



3. Click the [Create] button 

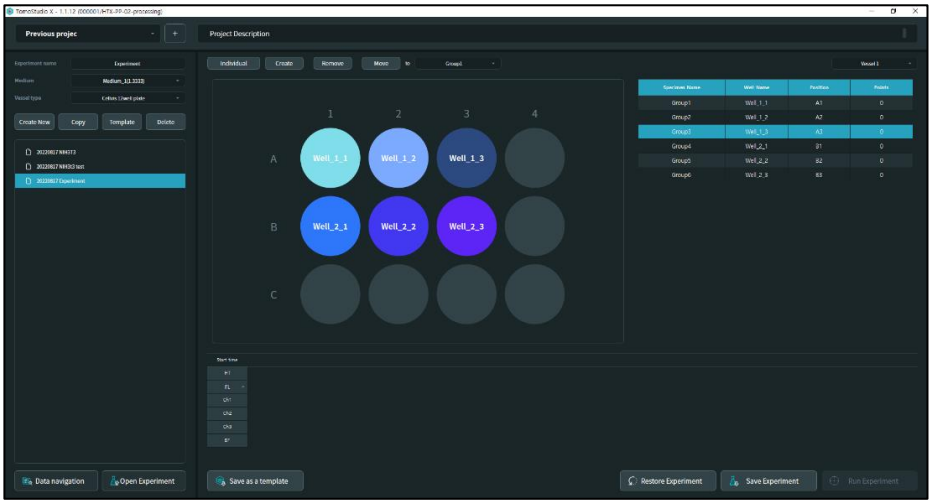
to register the selected wells as individual specimens. Each of the selected wells is assigned as an individual specimen. To register other wells as different individual specimens, repeat this process.

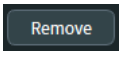


4.5.2.3 Unregister wells as specimen

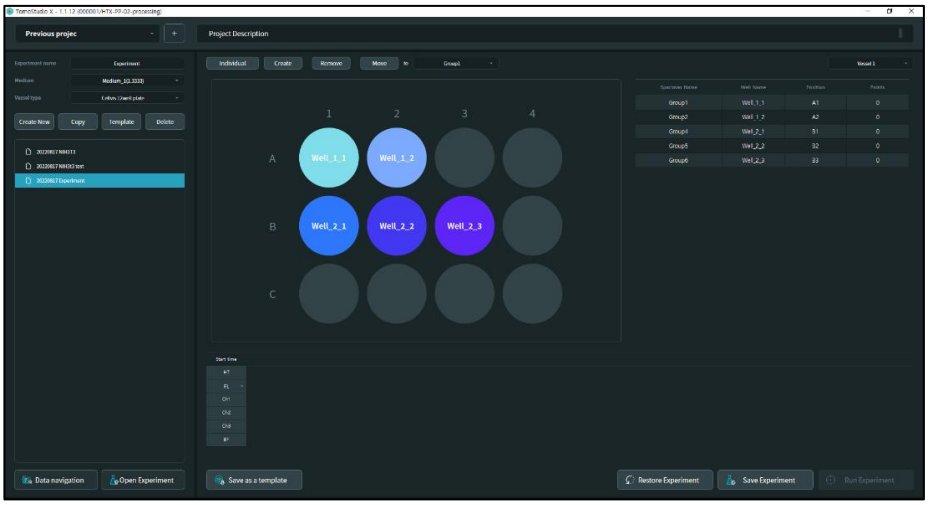
Wells can be unregistered as specimens in case some of the registered wells are not registered properly.

1. Click the well or wells to unregister from the listed specimens in the specimen/well table while holding the Ctrl key or select the wells by right-clicking and dragging the mouse cursor over the desired wells on the vessel map.



2. Click the [Remove] button 

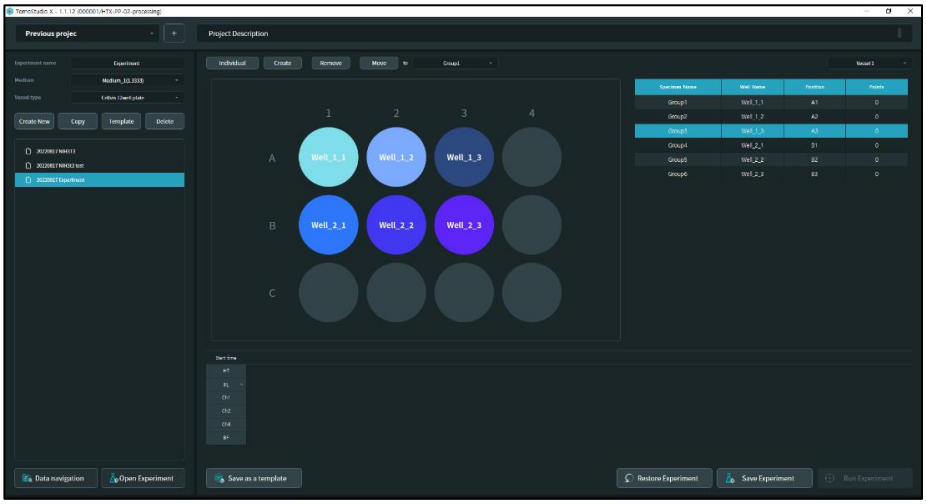
to unregister the selected wells. The change is automatically reflected in the vessel map and the specimen/well table.



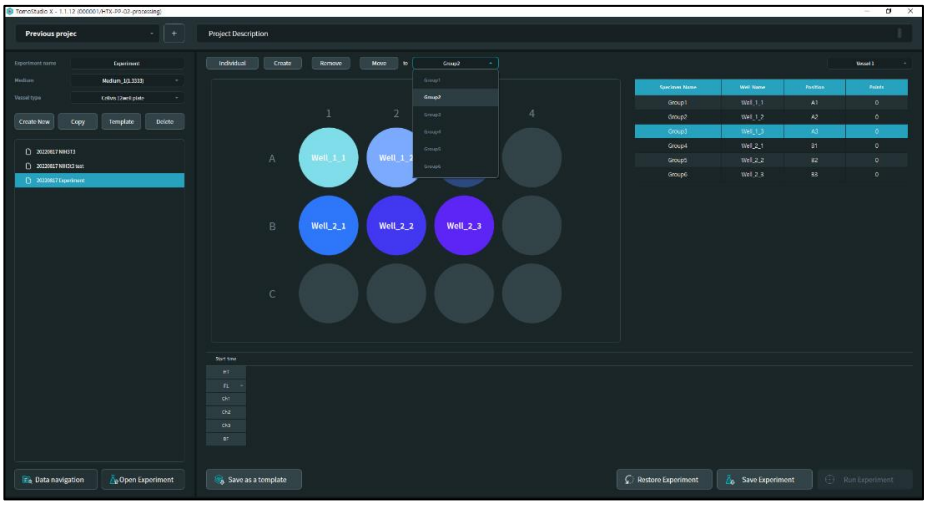
4.5.2.4 Reassign wells to a different specimen

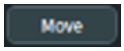
Wells that have been registered as a specimen can be reassigned to a different specimen.

1. Click the well or wells to reassign from the listed specimens in the specimen/well table while holding the Ctrl key or select the wells by right-clicking and dragging the mouse cursor over the desired wells on the vessel map.

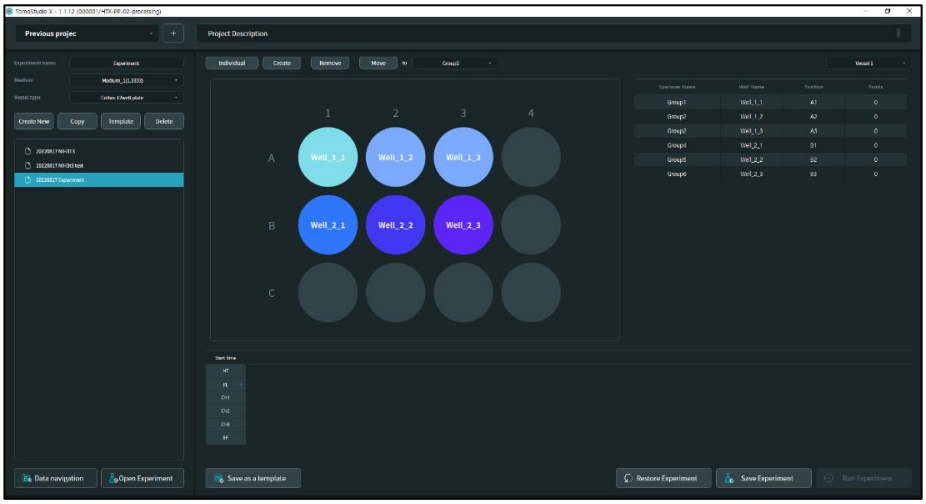


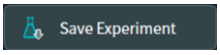
2. Click the drop-down menu for the specimen list and choose the specimen to which the selected specimen(s) will be reassigned.



3. Click the [Move] button 

to reassign the selected well(s) to the selected specimen. The change is automatically reflected in the vessel map and the well/specimen table.



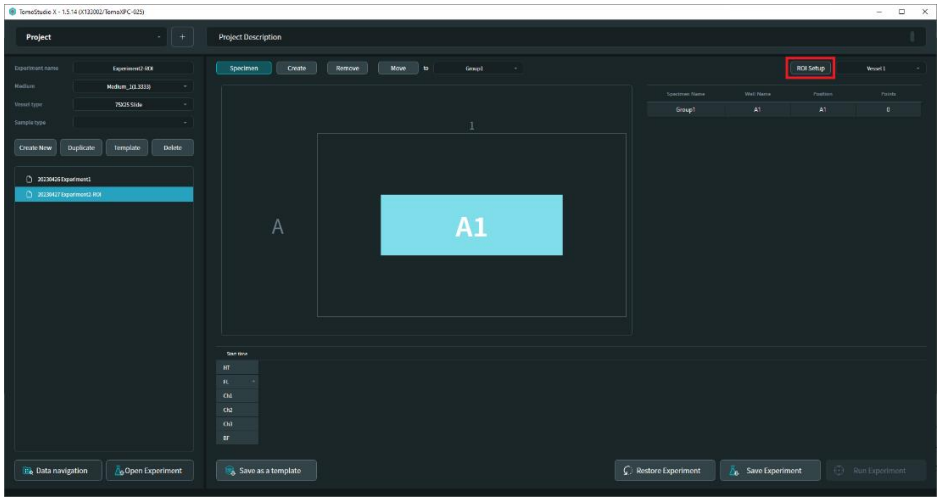
4. Click the [Save Experiment] button 

to assign the wells.

4.5.2.5 ROI setup for slide and wide-field vessel imaging

When imaging samples such as slides or microfluidic devices, setting a ROI is often necessary as not all the sample area needs to be accessed for the imaging. In this circumstance, ROIs can be assigned and displayed on a well map during the experiment.

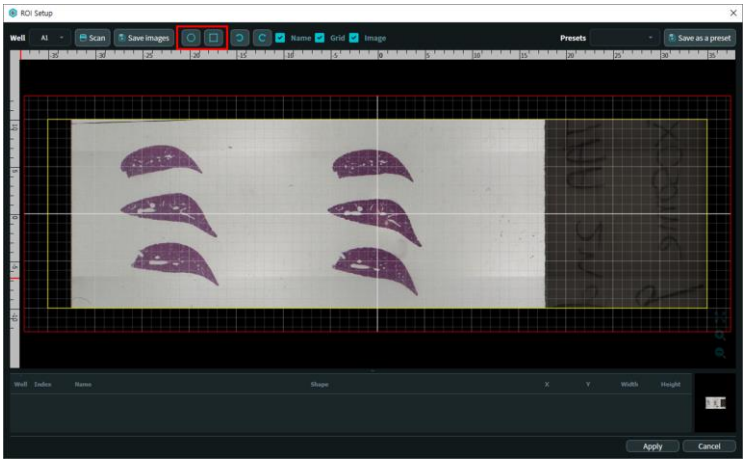
1. After adding the specimen, click the [ROI setup] button on the top-right side of the Specimen/Well panel to assign ROIs.



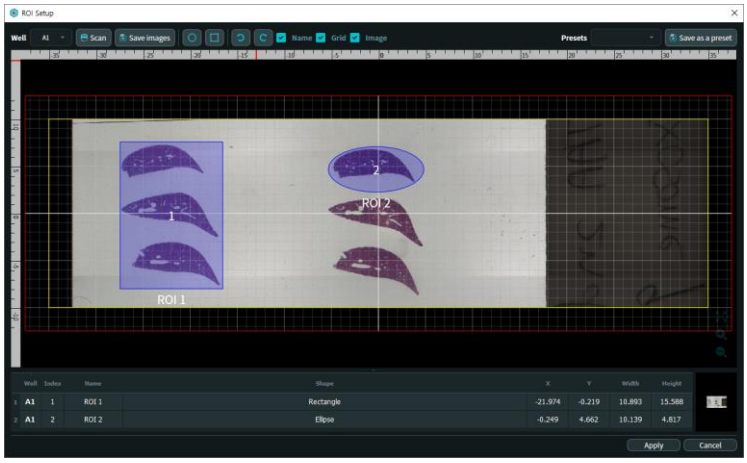
2. The ROI setup panel will appear on the pop-up window. Press the [Scan] button to capture a wide preview image. Progress can be monitored on the status bar.

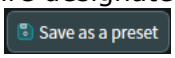
   Note: The [Scan] function is only available on systems equipped with the wide preview capability. It will be disabled on configurations that do not support this feature.

3. Once the scan is complete, you can view the wide preview image. To add a ROI, click either the circle or the rectangle ROI button on the ROI setup panel to add a ROI of the corresponding shape.

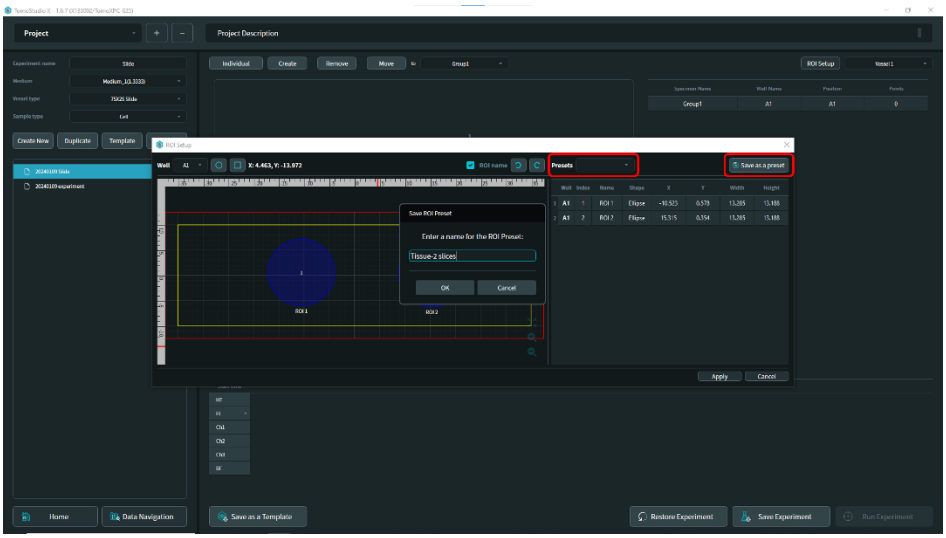


4. Then, designate the location to place the ROI by clicking the position on the well map. Adjust the width and height (or the radius) of the placed ROI by clicking and moving its edges or circumference. The horizontal and vertical spans of the selected ROI can be identified by blue lines on the rulers of the well map.

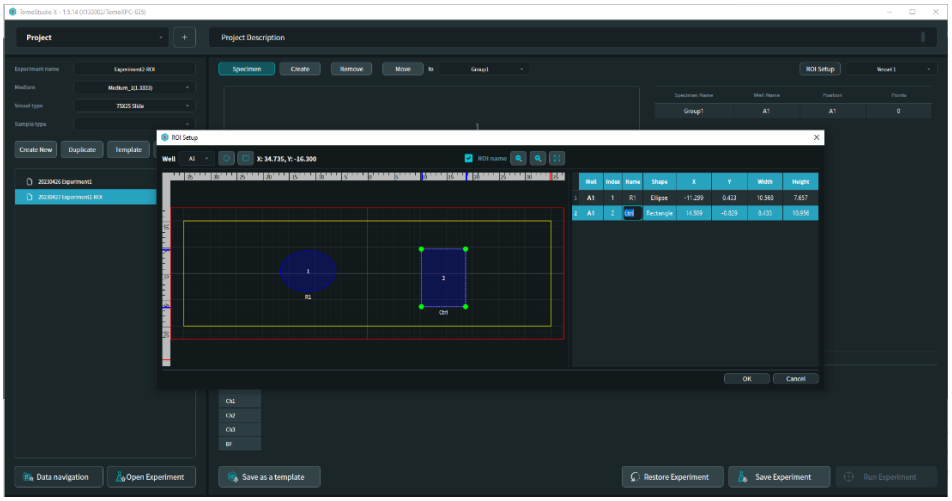


5. The entire designated ROI can be saved in preset format. Click the [Save as preset] 

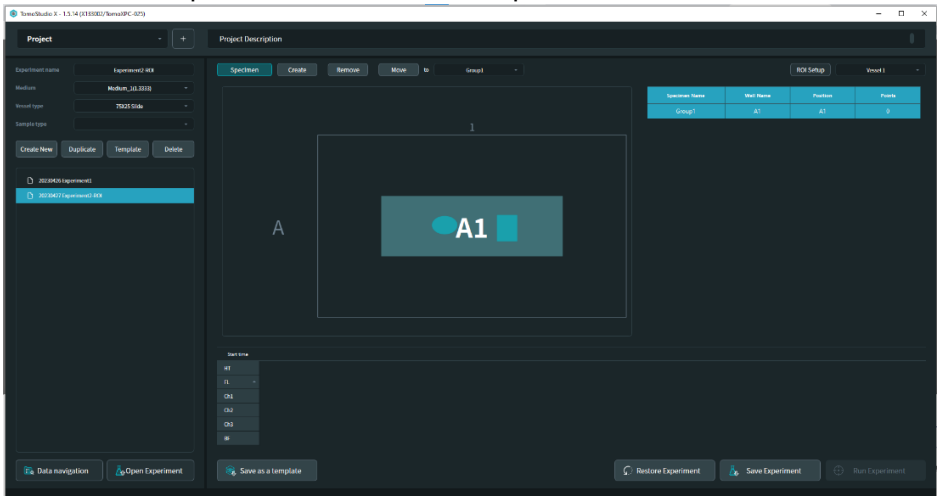
button located in the upper right side, and you can save the ROI setup as a preset. The saved preset can be loaded through the dropdown menu in the upper right side.



6. If the name of a registered ROI needs to be changed, double-click the cell of the name column of the ROI to be renamed on the ROI table. When the cell is ready for user input, key in a new name for the ROI.

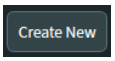


7. Click [Apply] button to apply the ROI setup. The registered ROIs can be seen in the vessel map as well as the well map.

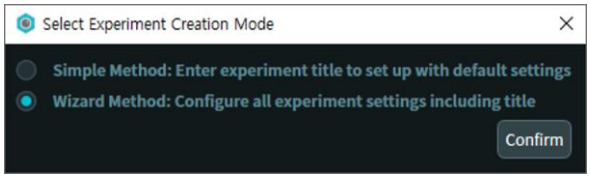


4.6 Wizard Method for Experimental Setup

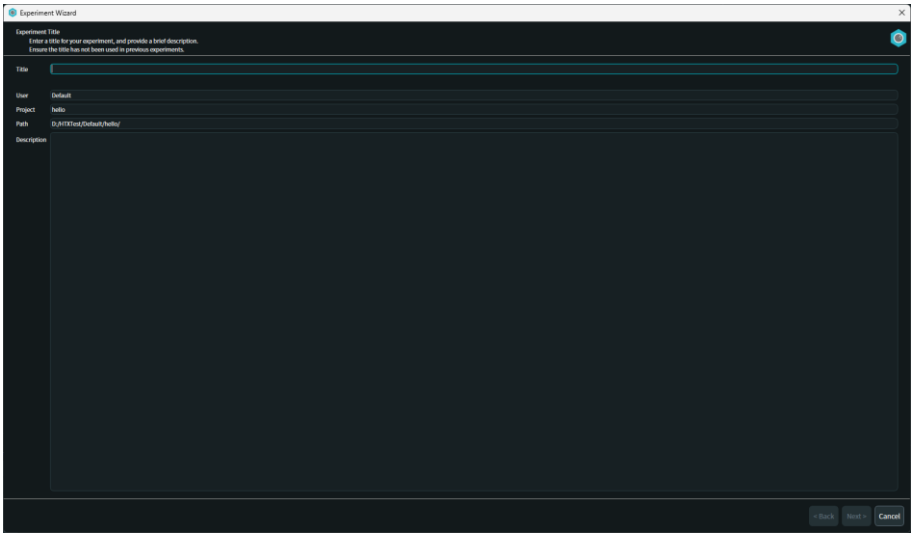
4.6.1 Create a new experiment using Wizard Method

1. Click the [Create New] button  in the Experiment panel.

2. The Select Experiment Creation Mode will appear on the pop-up window. Select Wizard Method and click [Confirm].



3. The Experiment Wizard panel will appear on the pop-up window. Enter the experiment name in the Title field and optionally add a brief description of the experiment in the Description field. Then click [Next].



4. In the sample type setup step, you can choose between two options: Standard Sample Type and Custom Sample Type.

    - Standard Sample Type

      This option allows you to select from pre-defined sample types. For more information on standard types and their recommended conditions, please refer to Chapter 4.5.1.

    - Custom Sample Type

      This option allows you to define a new sample type by configuring the following parameters:

        - Light Source:

          Choose from Blue, Green, or Red.

            - Blue provides the highest resolution and is suitable for general live-cell imaging.
            - Red offers enhanced contrast for thick or complex samples such as organoids.
            - Green is appropriate for photosynthetic samples such as plant cells.

        - Processing Algorithm:

          Select from Cell, Bacteria, Organoid, or Plant. Each algorithm is optimized for the respective sample morphology and imaging requirements. See Chapter 4.5.1 for details.

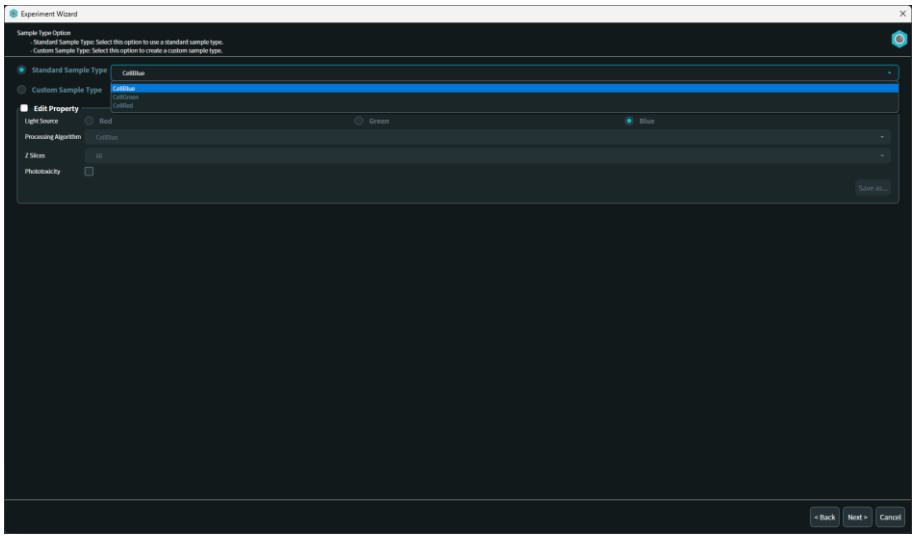
        - Z Slices:

          Choose the number of Z slices based on sample thickness.

            - For thin samples such as single cells (~10 μm), 60 slices are recommended.
            - For thicker samples such as organoids, 140 slices are typically used.

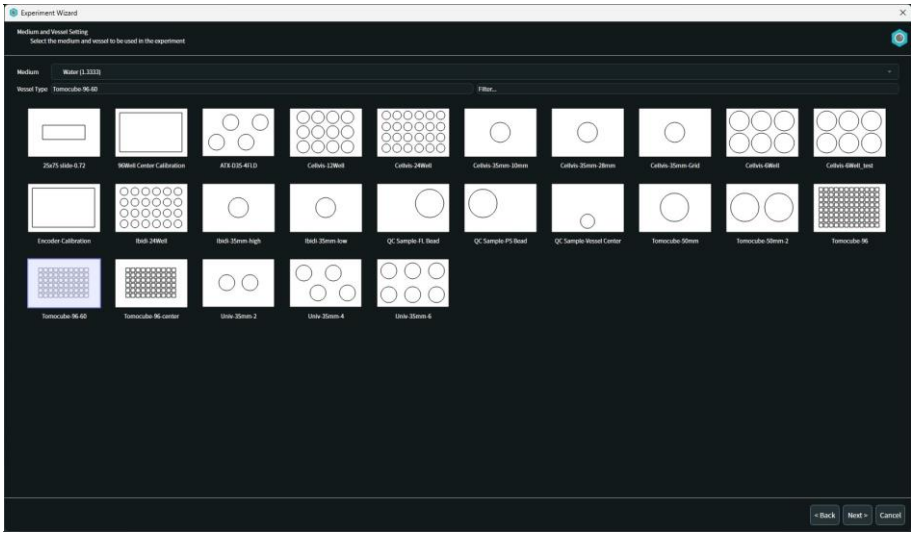
- Fluorescence Deconvolution:

  You may enable or disable deconvolution for fluorescence channels. For systems equipped with a confocal module, disabling deconvolution is generally recommended.

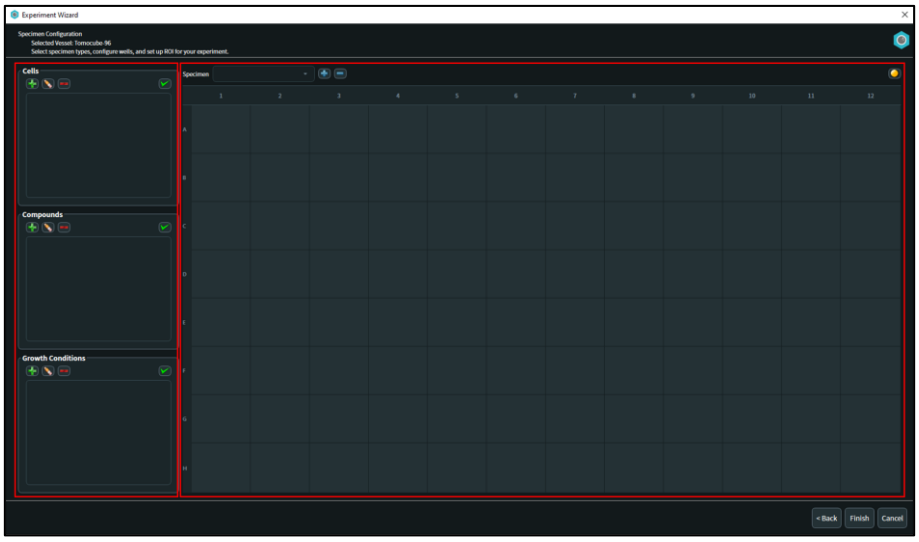


5. After completing the settings, click [Next].
6. Select the appropriate medium RI for the experiment from the Medium drop-down menu in the Medium and Vessel Setting panel.
7. Select the appropriate vessel type for the experiment. Refer to the vessel map image below to choose the correct vessel type for your experiment. You can search and sort for a specific vessel by entering its name in the Filter field.

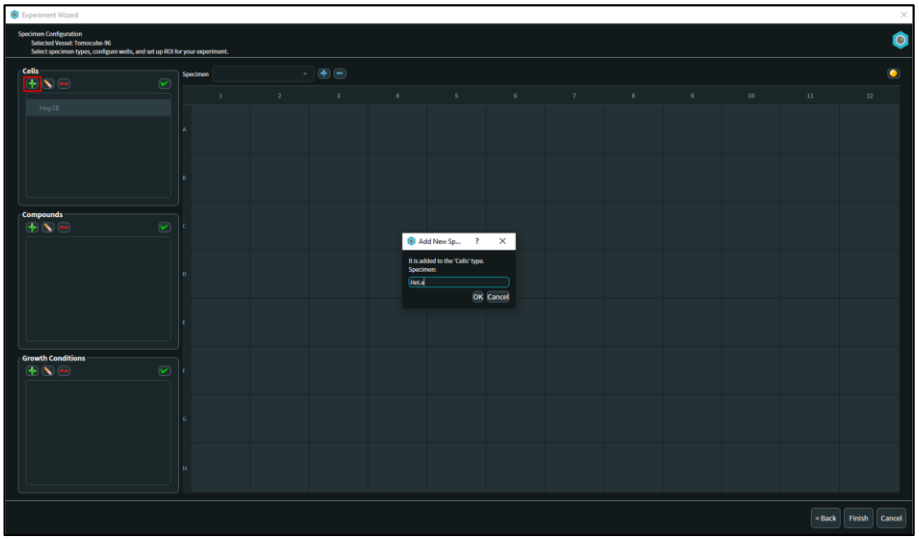
   Important! If the intended medium RI or vessel type for the experiment is not listed in the related menu, please contact your System Administrator to update the setting. Additional medium RIs and vessel types can be added via the Preference menu accessible with an Administrator account.



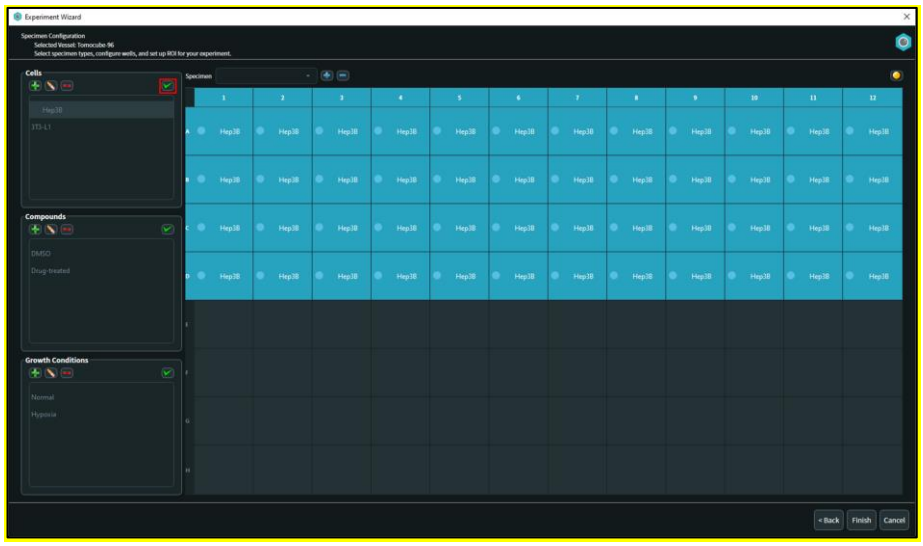
8. Specimen Configuration window will appear. The left panel is used to input experimental conditions and the right panel displays each wells of selected vessel.



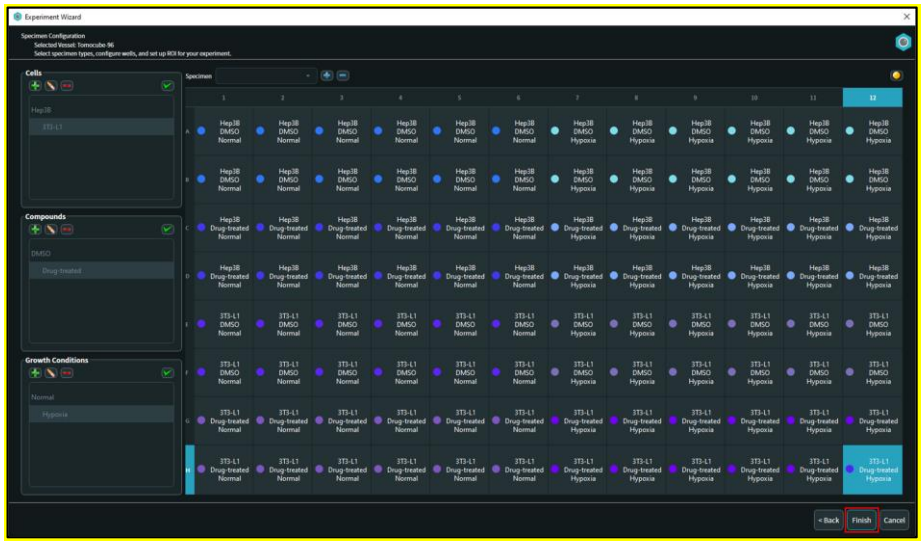
9. To configure well setups, enter the desired conditions like Cells, Compounds, and Growth Conditions into the left panel. Press the [+] button  to enter the sample name. Use the pencil button  to modify, and the [-] button  to delete.



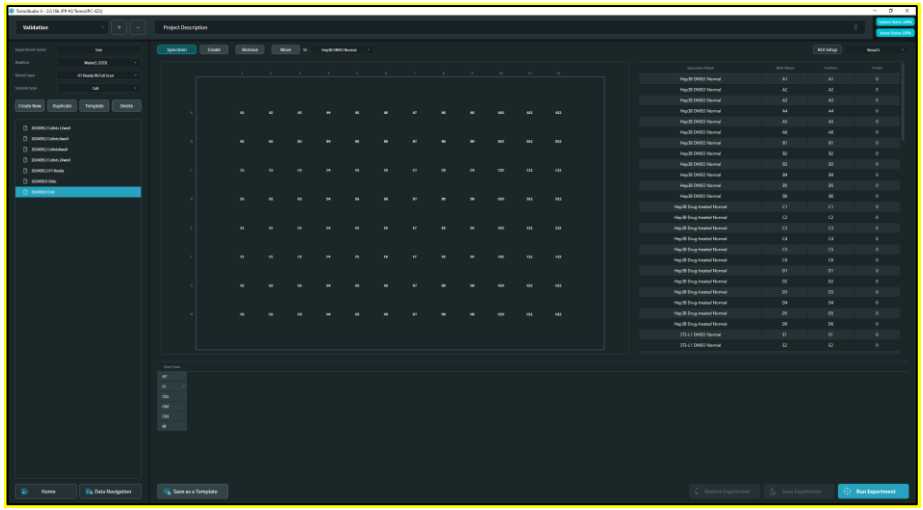
10. To assign conditions to a specific well, click and drag to select the well on the right panel. Then, assign the corresponding conditions by pressing the [✓] checkbox  on the left panel.



11. The sample name will be assigned to the specified well. After setting other conditions, press the [Finish] button  to complete the setup.

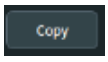


12. In the Experiment Manager window, you can confirm that the settings have been completed in the Specimen/Well panel.

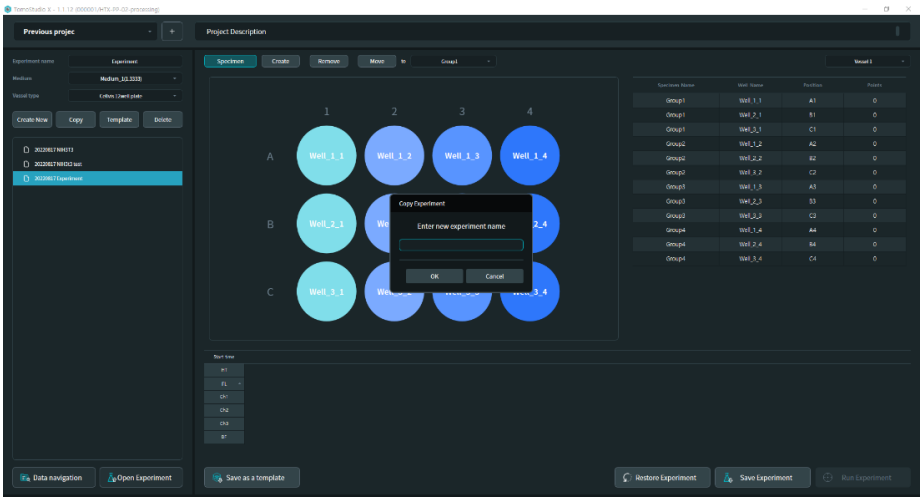


4.7 Create a new experiment by duplicating a previous experiment

A new experiment can be created with the same conditions as a previous experiment.

1. In the Experiment panel, click the previous experiment to be duplicated in the experiment list.
2. Click the [Copy] button 

in the Experiment panel.



3. Type in a name for the new experiment in the pop-up window and click OK.

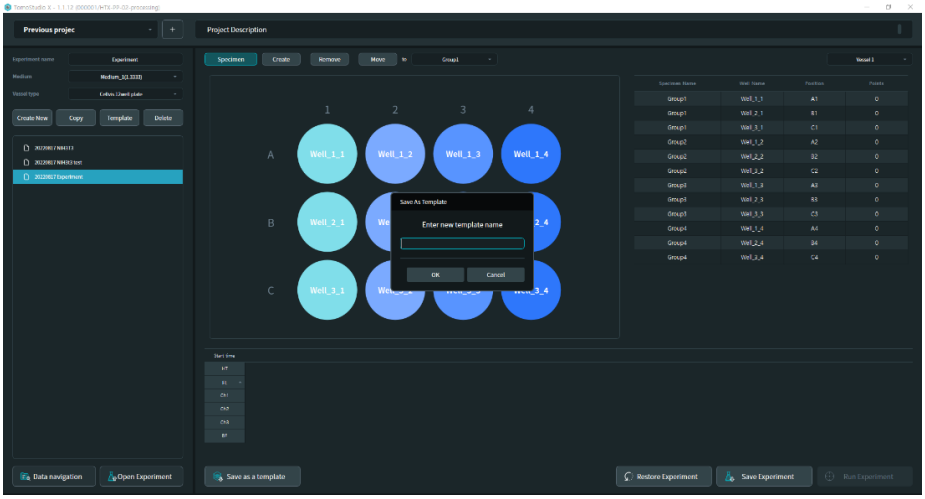
4.8 Create a new experiment by importing a template

If users repeat experiments with the same conditions, the experimental conditions can be saved as a template and a new experiment can be created by importing the template. The template includes the following information: medium RI, vessel type, specimen/well registration, fluorescence parameters, and time-lapse parameters.

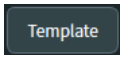
4.8.1 Exporting experimental conditions as a template

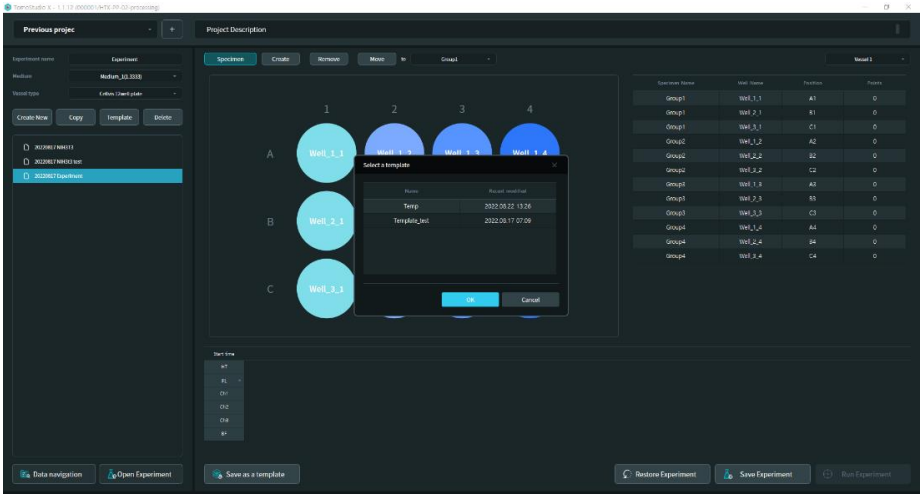
1. In the Experiment panel, click the previous experiment to be exported as a template.
2. Click the [Save as a template] button 

at the bottom of the Experiment Manager window.
3. Type in a name for the template in the pop-up window and click OK.



4.8.2 Importing an experiment template to create a new experiment

1. Click the [Template] button  in the Experiment panel to see a list of stored templates in a pop-up window.
2. Choose the template with the desired experimental conditions to import in the pop-up window and click OK to proceed.



3. Type in a name for the new experiment in the pop-up window and click OK.

CHAPTER 5. Image Acquisition

5.1 Workflow

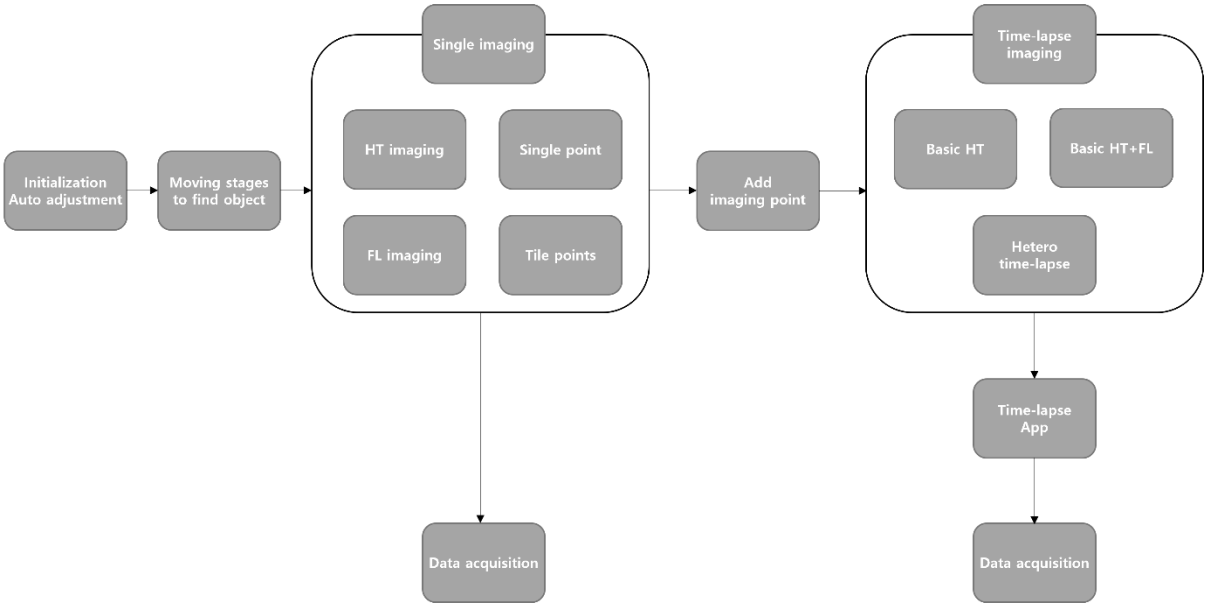


Figure 5.1 Workflow for image acquisition

Image acquisition consists of the following four steps.

1. System initialization and focus adjustment
2. Adjustment of the stage to locate the regions of interest for image acquisition
3. Imaging parameter setup: Single or Time-Lapse Imaging
4. Acquisition

In the third step, or the imaging parameter setup step, the parameters for Fluorescence Imaging, Tile Imaging, and Time-Lapse Imaging can be controlled.

5.2 User interface

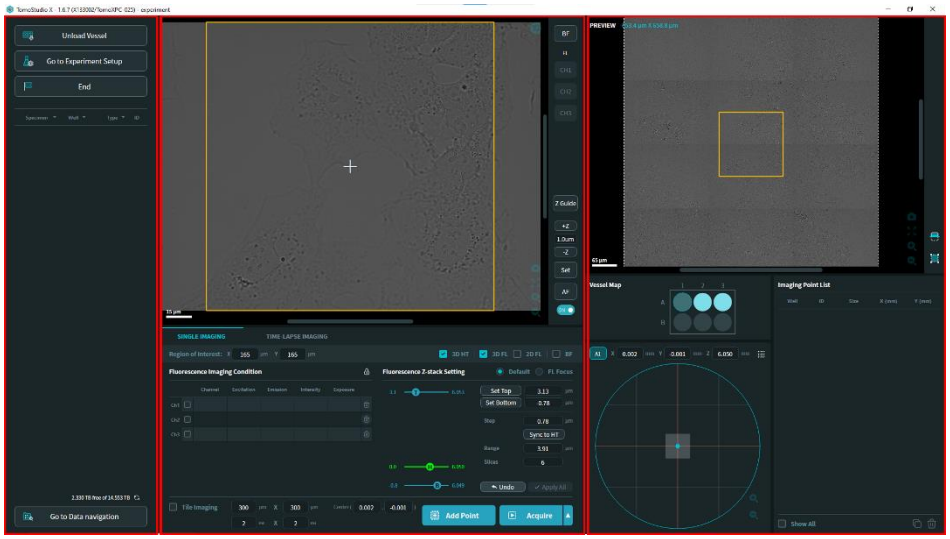


Figure 5.2 Image Acquisition window

Clicking the [Run Experiment] button in the Experiment Manager window will open the Image Acqusition window. In the Image Acqusition window, the experimental parameters for data acquisition can be set up. The window is ready for user inputs following an initialization procedure, if required. See Chapter 5.3.

Tool Area (left)

The Tool Area on the left of the image acquisition interface is intended for managing the experiment status and monitoring the acquired data from the current experiment. From the Tool Area, it is possible to access other windows such as the Experiment Manager window and the Data Navigation screen.

Acquisition Area (center)

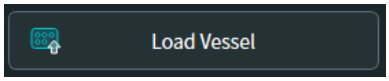
The main Acquisition Area in the center of the image acquisition interface is divided into two panels: Live View and Acquisition. The Live View panel displays a live image from the camera. In the Acquisition panel, the image acquisition parameters can be set up and images can be acquired.

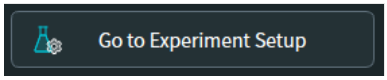
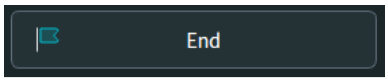
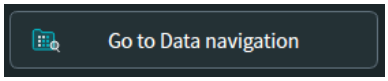
Utility Area (right)

The Utility Area on the right of the image acquisition interface allows users to move the positioning stage to navigate the sample. This area is divided into three panels: Preview, Vessel/Well Map, and Imaging Point list.

5.2.1 Tool Area

Experiment management

Parameter	Function	Description
	Vessel load/unload	Moves the sample stage to place the vessel into the loading or unloading

		position. The button toggles between Load Vessel and Unload Vessel depending on the position of the sample stage.
	Go to the Experiment Manager window	Goes back to the Experiment Manager window.
	End experiment	Finishes the current experiment.
	Go to Data Navigation	Switches to the Data Navigation screen.

Data list

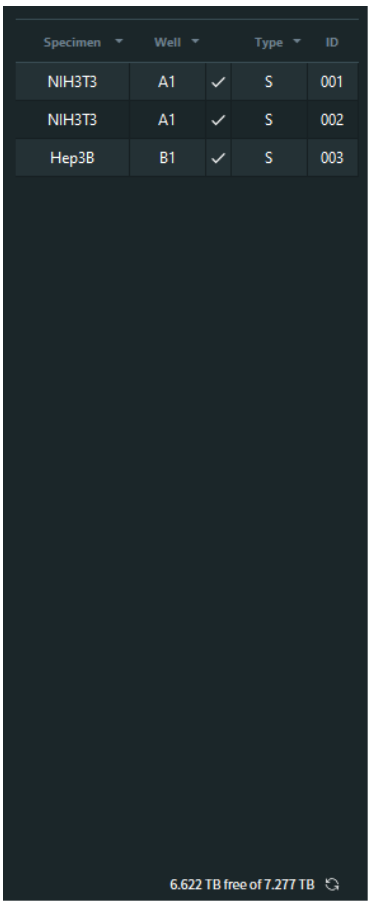


Figure 5.3 Data List panel

After each image acquisition is completed, the acquired data from the experiment is automatically listed in the Data List panel. In the Data List panel, the acquired data files are shown in a table with brief information including the specimen's name, well name, nature of the data regarding time and dimension, and the data ID. At the bottom part of the panel, it shows an available space to store acquired data.

5.2.2 Acquisition Area

Live View panel

The Live View panel shows a live image of the current location that the system is imaging.

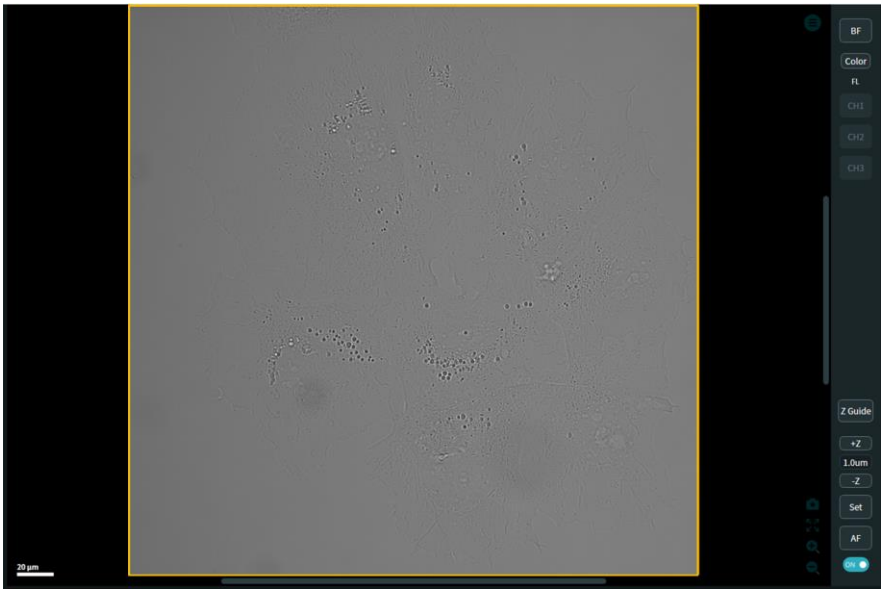
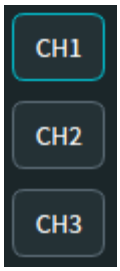
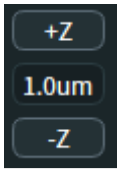
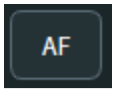
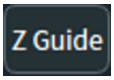


Figure 5.4 Live View panel

Icon	Function	Description
	Brightfield mode	Shows a brightfield image in the Live View panel. The brightfield image can be viewed in grayscale or color.
	Fluorescence mode	Shows an image from the selected fluorescence channel in the Live View panel. Each fluorescence channel button is activated when the corresponding fluorescence channel is activated in the Fluorescence Imaging Condition panel.
	Z control buttons	Adjust the Z position by the specified amount displayed in the middle text box.
	Set Z position	Sets the current Z position as the intended focus position.
	Autofocusing	Finds the best focus position automatically by the system.
	AF on/off button	Turns the autofocus function on or off while navigating.

	Z guide	Load the Z guide panel: This allows to find the bottom of the vessel and indicate the current location and the maximum height that can be reached in relation to the vessel's bottom.
	Zoom in/Zoom out	Zooms in or out in the Live View panel.
	Reset view	Resets the size of the Live View to the default size.
	Capture	Saves the Live View as a PNG file.

Acquisition panel

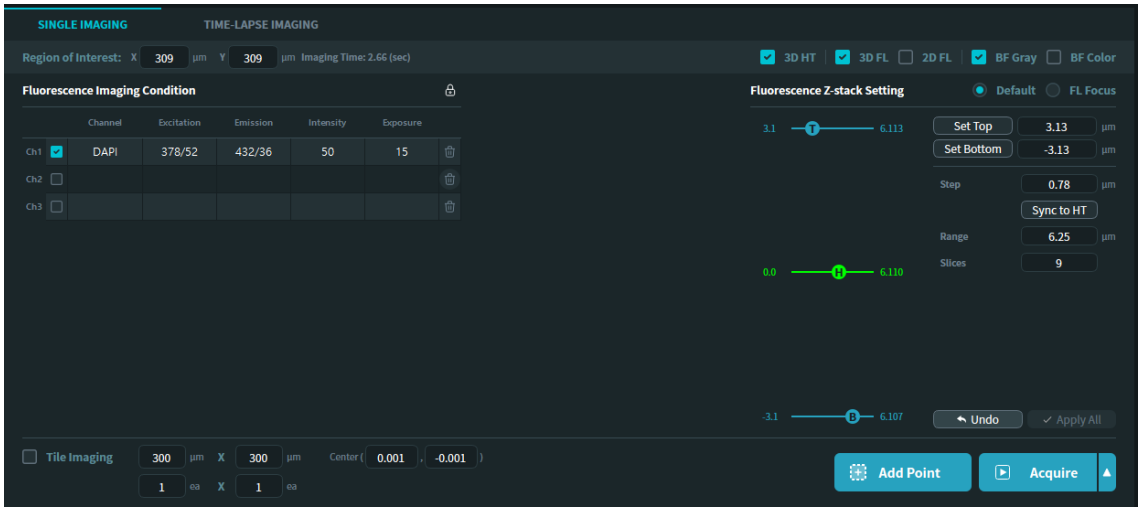
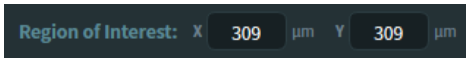
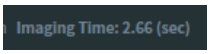
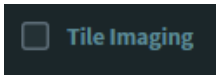
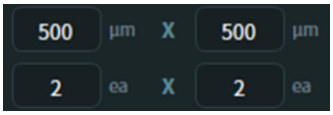
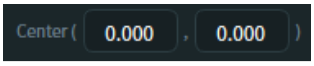
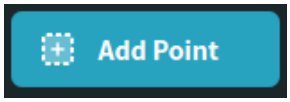


Figure 5.5 Acquisition panel (Single Imaging)

In the Acquisition panel, the parameters for holotomography and fluorescence image acquisition can be set up, and imaging can be performed. The two imaging acquisition modes are Single Imaging and Time-Lapse Imaging, which can be selected by clicking the corresponding tab on the top of the Acquisition panel. Single Imaging is for image acquisition at the current location of the Live View and adjusting the imaging conditions as desired. Time-Lapse Imaging is for programmed imaging with utilities for time-lapse sequences.

Single Imaging tab

Parameter	Function	Description
	ROI setup	Sets the size of the ROI.
	Acquisition Time	It displays the time required for imaging.
	Imaging mode setup	Activates the desired imaging mode(s).

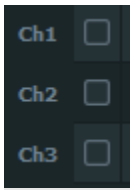
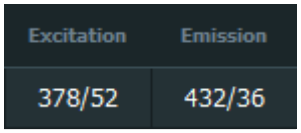
	Tile Imaging checkbox	Toggles the Tile Imaging mode on and off.
	Tile size	Sets the width and height of the Tile Imaging area.
	Tile location	Displays the center position of the Tile Imaging area.
	Add point	Registers the current position in the Imaging Point List.
	Acquire	Acquires an image at the current position in the activated imaging mode(s).

Fluorescence setup panel

Figure 5.6 Fluorescence setup panel (imaging conditions and Z-stack settings)

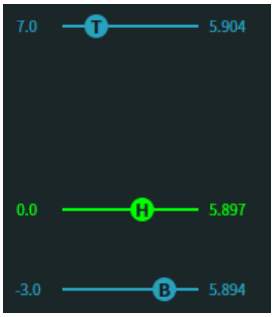
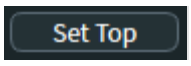
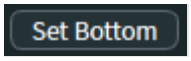
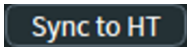
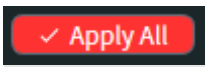
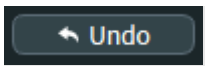
The Fluorescence setup panel is divided into two sub-panels: Fluorescence Imaging Conditions for configuring each fluorescence channel, and Fluorescence Z-stack Settings for 3D fluorescence imaging acquisition.

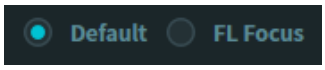
Fluorescence Imaging Conditions

Parameter	Function	Description
	Channel selection	Activates the desired fluorescence channel(s).
	Excitation and emission filter settings	List the excitation and emission filter wavelength information for each channel.
	Intensity	Displays the intensity value for each channel.

	Exposure	Displays the exposure time for fluorescence detection for each channel (unit: msec).
	Delete	Deletes the activated channel from the Fluorescence Imaging Conditions panel.
	Sync to Live View panel	Locks or unlocks the current fluorescence excitation intensity and exposure time settings to synchronize them to those in the Live View panel.

Fluorescence Z-stack Settings

Parameter	Function	Description
	Z-stack histogram	Illustrates the current fluorescence Z-stack settings for three Z positions: Top (T), Bottom (B), and focus position (H) for holotomography imaging.
	Set top	Sets the current Z position to the highest position in the Z scan range for the fluorescence imaging.
	Set bottom	Sets the current Z position to the lowest position in the Z scan range fo fluorescence imaging.
	Step	Sets the step size in the Z direction for the fluorescence imaging.
	Sync to HT	Sets the fluorescence imaging step size to be same as the HT imaging step size.
	Range	Sets the calculated span in the Z direction for the Z-stack imaging settings. The range depends on the Top/Bottom parameters set by the user.
	Apply	Applies the current Z-stack setup as the acquisition condition.
	Undo	Restores the previous Z-stack settings.

	Z-stack mode selection	Selects the method to determine the Z range of the Z-stack for fluorescence imaging.

Two Z-stack modes are available in TomoStudioX: Default mode and FL Focus mode.

Time-Lapse Imaging tab

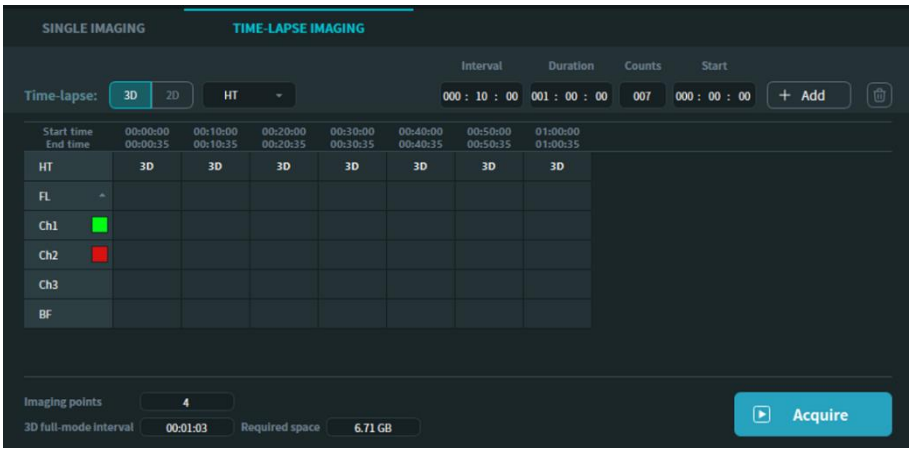
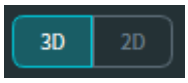
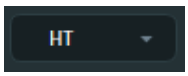
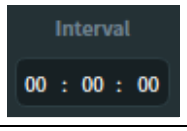
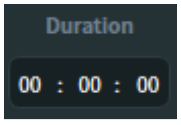
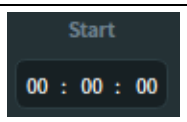
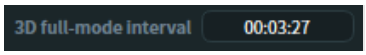
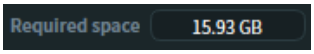
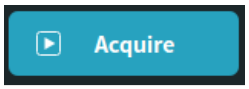


Figure 5.7 Acquisition panel (Time-Lapse Imaging)

In the Time-Lapse Imaging tab, images can be acquired from multiple points simultaneously at more than one time frame. Various combinations of time-lapse sequences can be configured via the configuration interface. In this tab, the Time-Lapse Schedule Table displays the timeframes for each imaging mode. For more information on how to configure the schedule for Time-Lapse Imaging, please refer to section 6.1.

Parameter	Function	Description
	Dimension	Sets the data dimension of the imaging mode for acquisition in the time-lapse sequence.
	Mode	Sets the imaging mode type for acquisition in the time-lapse sequence.
	Interval	Sets the measurement interval of each imaging mode in the time-lapse sequence.
	Duration	Sets the acquisition duration of each imaging mode in the time-lapse sequence.
	Start time	Sets the start time of each imaging mode in the time-lapse sequence.
	Imaging points	Displays the number of imaging time points in the Imaging Point List.
	Minimum interval	Displays the estimated time required

		to complete the longest cycle in the sequence.
	Required space	Displays the amount of the storage space for the time-lapse acquisition
	Acquire time-lapse images	Performs the scheduled measurements in the Time-Lapse Schedule Table.

5.2.3 Utility Area

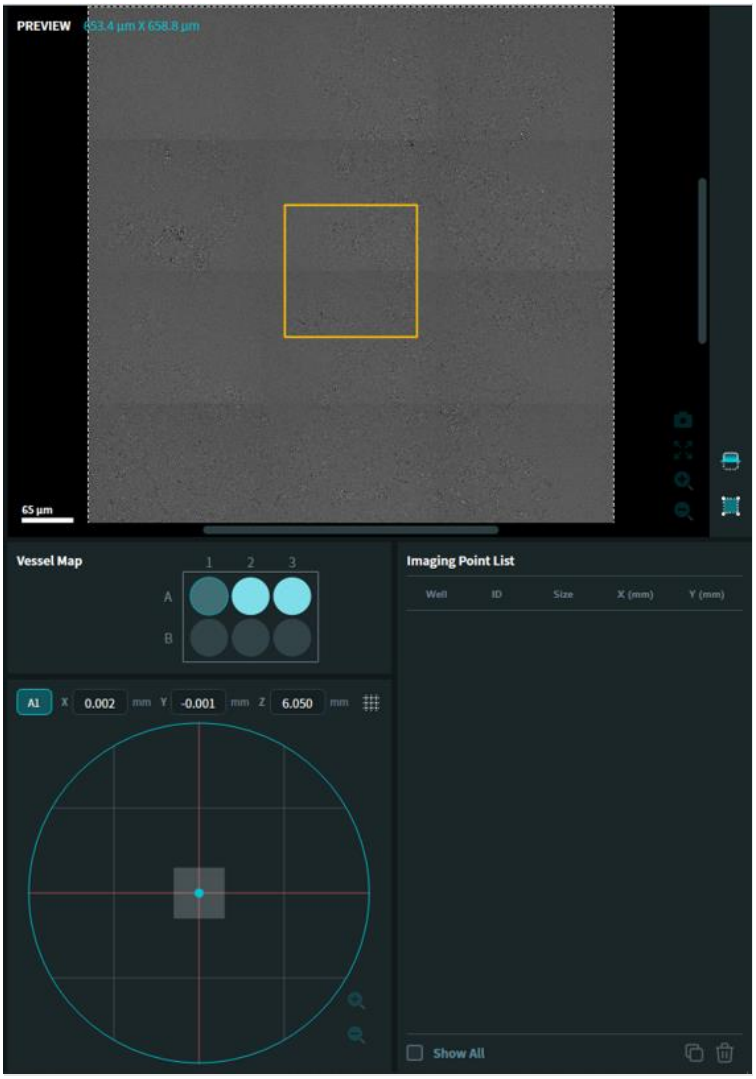


Figure 5.8 Utility Area layout

Preview panel

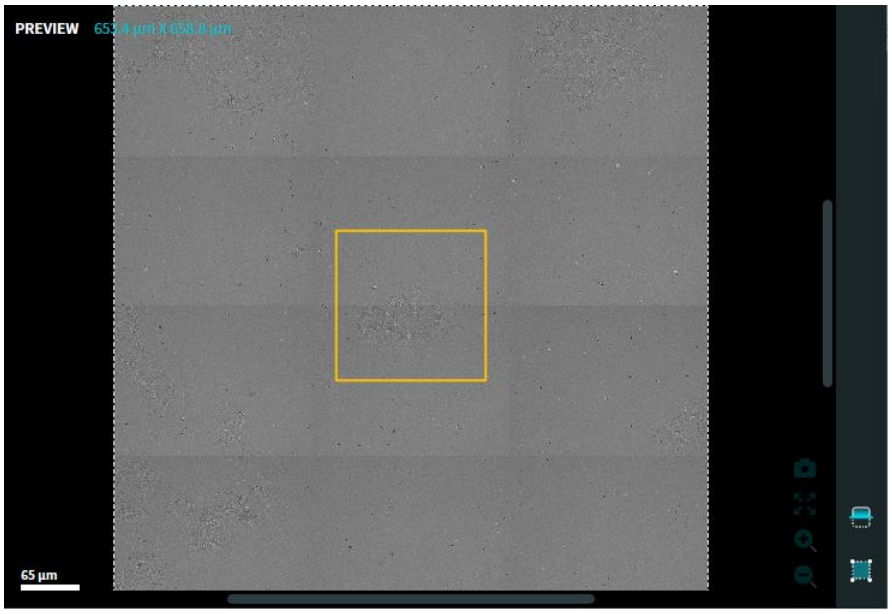
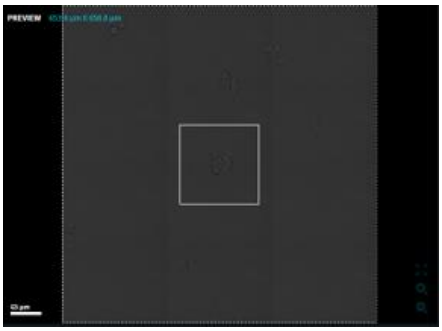


Figure 5.9 Preview panel

In the Utility Area in the image acquisition interface, the Preview panel displays an extended view of the current field of view (FOV) by capturing multiple FOVs around the current one.

Parameter	Function	Description
	Scan preview	Captures a pre-defined area in brightfield imaging mode around the current FOV.
	Change preview size	Adjust the area for the preview scan other than the default size.
	Preview result	Displays the acquired image for the brightfield preview.
	Zoom in/Zoom out	Adjust the level of zoom on the Preview image.
	Reset view	Resets the size of the Preview to the default size.
	Capture	Saves the Preview as a PNG file.

Vessel/Well Map panel

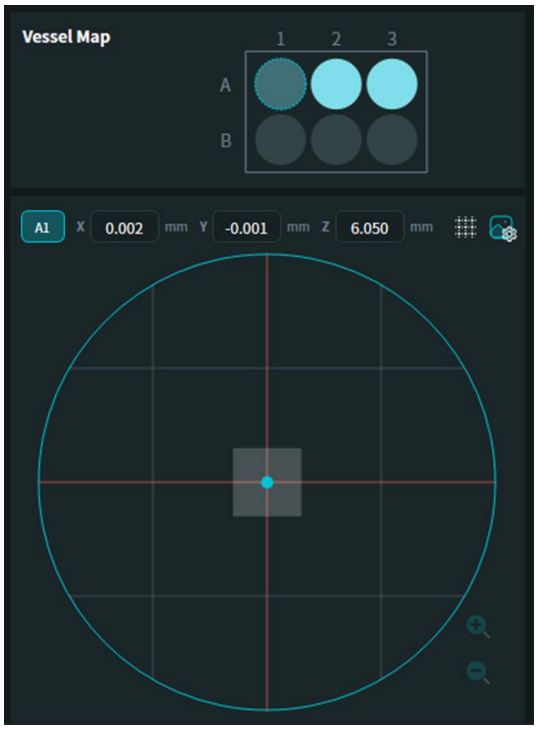
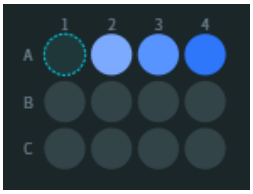
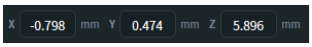
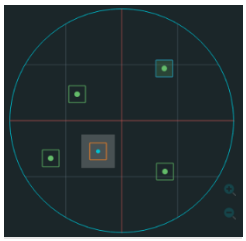


Figure 5.10 Vessel/Well Map panel

The Vessel/Well Map panel in the Utility Area shows the position of the current FOV in the well and in the vessel. In this panel, the position inside the well as well as across the wells can be controlled.

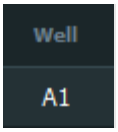
Parameter	Function	Description
	Vessel map	The dotted circle indicates the well where the current FOV is indicated. The FOV can be switched to another well by double-clicking the desired well.
	Current XYZ position	Shows the current X, Y, and Z positions in the current well. The XY coordinates of the well center are each defined as 0 mm.
	Well map	Displays the positions of the imaged area of the well in the current FOV. The current FOV location is indicated by a blue dot.
	Show/Hide grid	Shows or hides a grid on the Well Map.
	Show/Hide image	The scanned wide preview image can be shown or hidden in the

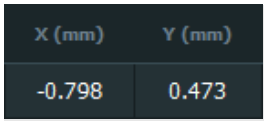
		Well Map.
	Zoom in/Zoom out	Adjust the level of zoom on the displayed Well Map.

Imaging Point List panel

Figure 5.11 Imaging Point List panel

The Imaging Point List panel displays the points registered for image acquisition with brief information including the well position, the size of the image to be acquired, and the XY coordinates in the well. Each point has its own unique ID to easily distinguish different points.

Parameter	Function	Description
	Well position	Displays the well position of each imaging point.
	Point ID	Displays the ID assigned to each imaging point.
	Size	Displays the width and height of the acquired images at each imaging point in micrometers.

	Center point	Displays the XY coordinates of the center of the acquired images at each imaging point.
	Advanced adding	Function to add matrix points or copy the selected imaging points to other wells.

5.3 System calibration

By clicking the [Run Experiment] button in the Experiment Manager window and then pressing [Load Vessel], the stage moves the vessel holder to the imaging position. The system then automatically performs a system calibration procedure to optimize imaging conditions for the current sample. This procedure consists of the following three steps:

1. Objective Lens Autofocus – The system adjusts the focus of the objective lens to locate the sample.
2. Condenser Lens Autofocus – The condenser lens is automatically aligned to match the focus plane of the sample.
3. HT Illumination Intensity Adjustment – The illumination is optimized to ensure appropriate contrast for holotomography.

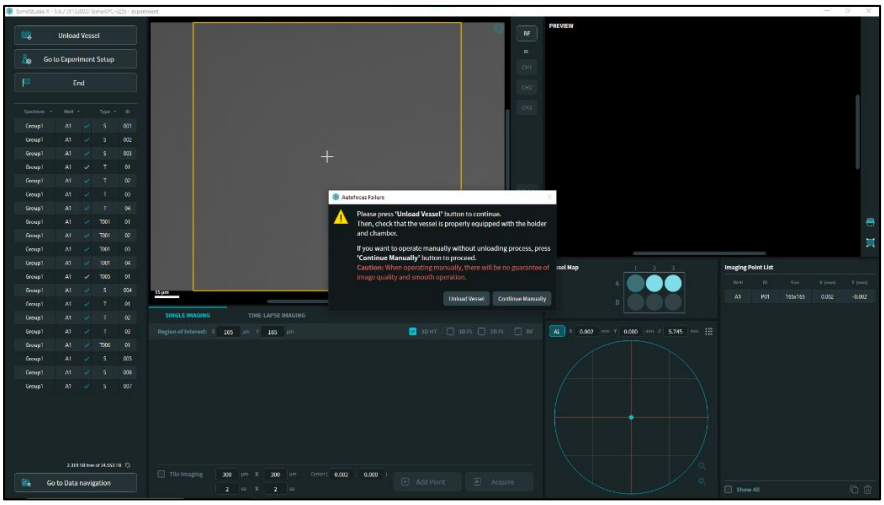
Important! The system calibration may fail if the imaging dish/vessel is not compatible with the system or is not correctly placed in the chamber. In this case, unload the vessel holder and confirm that the vessel is a compatible type, and then repeat the placement step. If the autofocus failure persists despite the vessel being correctly mounted, please see section 5.3.1.

5.3.1 Manual system calibration

The autofocus failure may arise even when the vessel is correctly mounted. This usually happens if the vessel type used is not compatible with the specifications registered in the system or if the sample type is not conventional. In such cases, you can manually perform the system initialization procedure to continue the experiment.

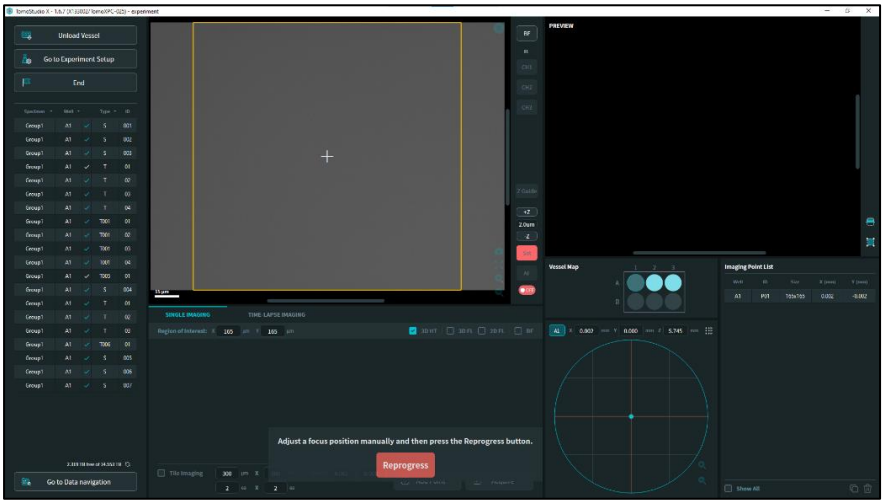
Important! In a manual initialization, unlike the normal automatic initialization, the quality of the HT data may not be optimal. Therefore, it must be conducted under the guidance of Tomocube personnel. Additionally, operations after a manual calibration may have limitations on certain features of TomoStudioX, including the autofocus.

1. If the autofocus fails during the initialization process, click the [Continue Manually] button in the popup window.

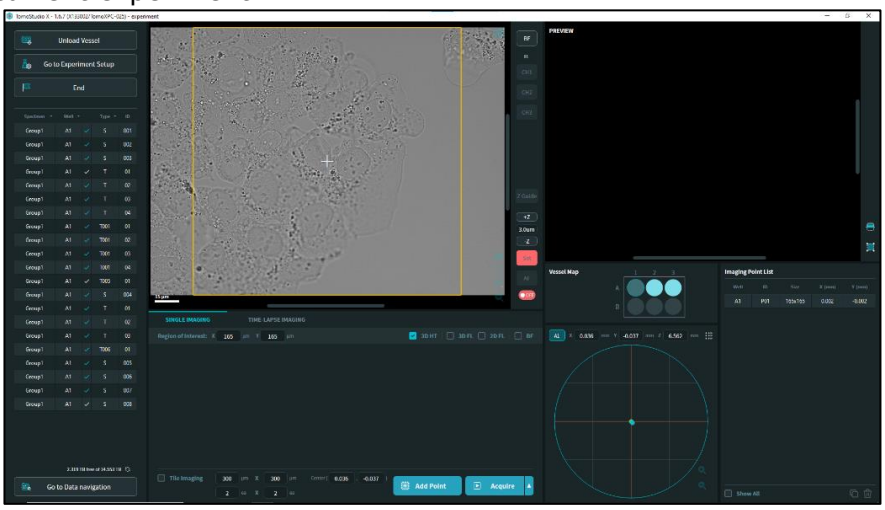


2. Click the Z adjustment buttons on the right side of the live view panel to adjust the focal position of the sample.

3. Once you have found the Z position where the sample appears sharp on the live view panel, press the [Calibrate] button at the bottom of the screen to start the calibration process.



4. After the calibration process is complete, it is possible to acquire images in the current experiment.



5.4 Moving the sample stage: Locating an object

There are four ways to move the measurement position within a vessel.

1. Double-click the Live View panel.
2. Double-click the Well Map.
3. Double-click the Preview panel.

   The Preview panel displays an extended area beyond the current FOV. Click the [Preview Scan] button to display a preview image. By default, the Preview panel shows an area of roughly 600 µm x 600 µm around the current FOV.
4. Enter the desired XY coordinate in the Well Map.

5.5 Single imaging

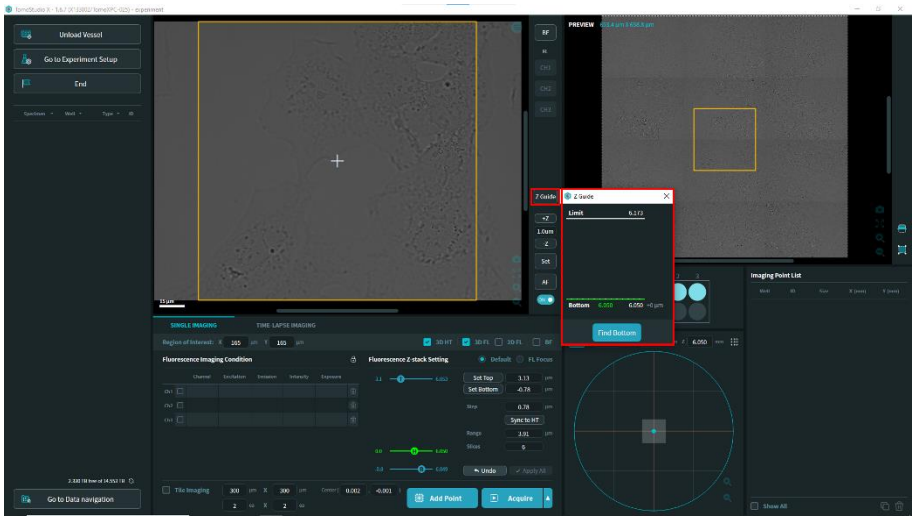
5.5.1 Holotomography

After the initialization and pre-adjustment procedures are complete, holotomography images can be acquired. Single HT images of the current FOV can be acquired in the Single Imaging tab.

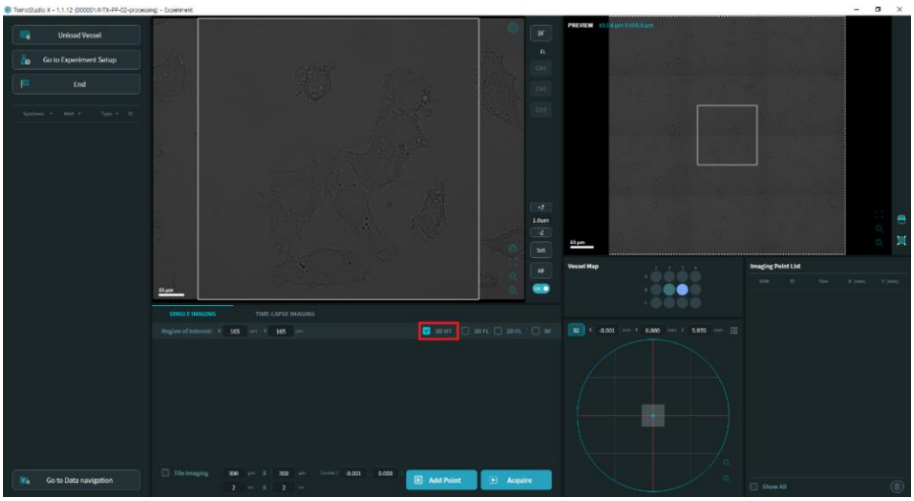
1. Move to the desired position using the Well Map or Preview panel.

   Note: For more information about positioning within a well, please see section 5.4.
2. Check the focus of the brightfield image on the Live View panel if the contrast is acceptable. If required, adjust the Z position by using the [+Z] or [-Z] buttons on the right side of the Live View panel to have an optimal contrast of the image. Or click the [AF] button to find the best focus automatically. If the focus is acceptable, click the [Set] button to make the Z position the baseline of the focus for the following image acquisition.

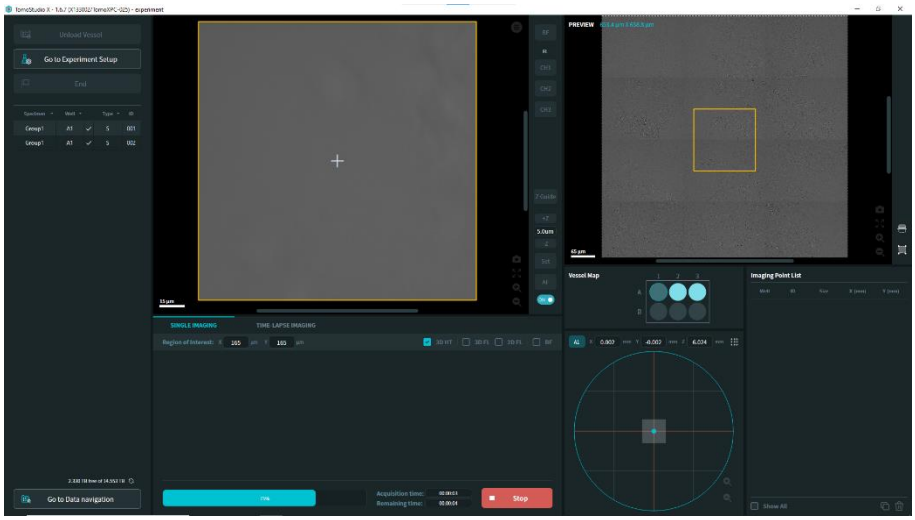
   Note: For samples with a relatively high position along the Z-axis, it is advisable to use the [Z Guide]. Clicking the [Z Guide] will indicate how much above the bottom of the dish or plate the current position is located. It also indicates the maxi height visible from the current position. If you have moved the XY position, you can update the dish's bottom position by pressing the [Find bottom] button.



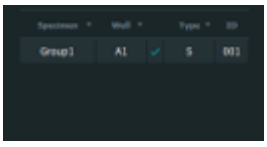
3. Check the [3D HT] checkbox to activate the holotomography imaging mode.



4. Click the [Acquire] button to perform holotomography imaging.
5. During the image acquisition, the current imaging progress, elapsed time, and the remaining time until completion are displayed at the bottom of the imaging tab. If you want to cancel the acquisition, click the [stop] button.



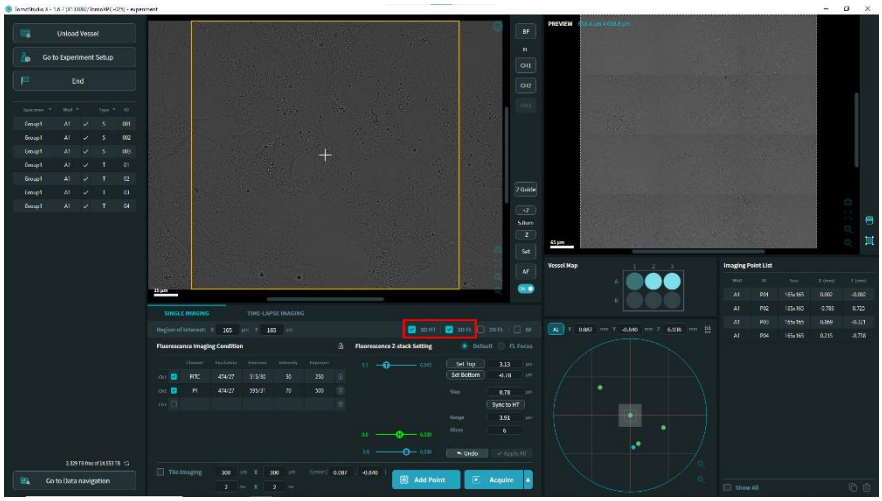
6. The acquired image is automatically registered in the data list table. Note that the color of the check mark on the data list changes from grey to blue when the data processing of the image has been completed.



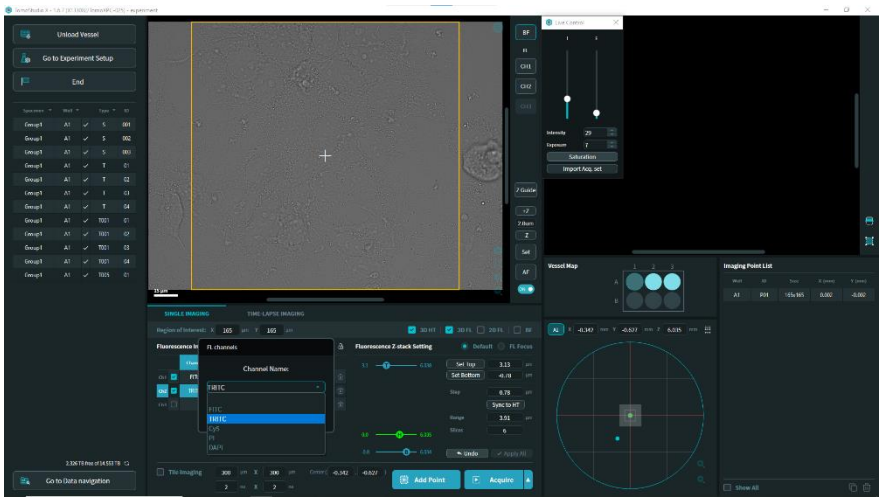
5.5.2 Holotomography with fluorescence

If the sample generates fluorescence signals, fluorescence images can be acquired along with holotomography images through TomoStudioX.

1. Check both the [3D HT] and [3D FL] checkboxes to enable both imaging modes for acquisition.



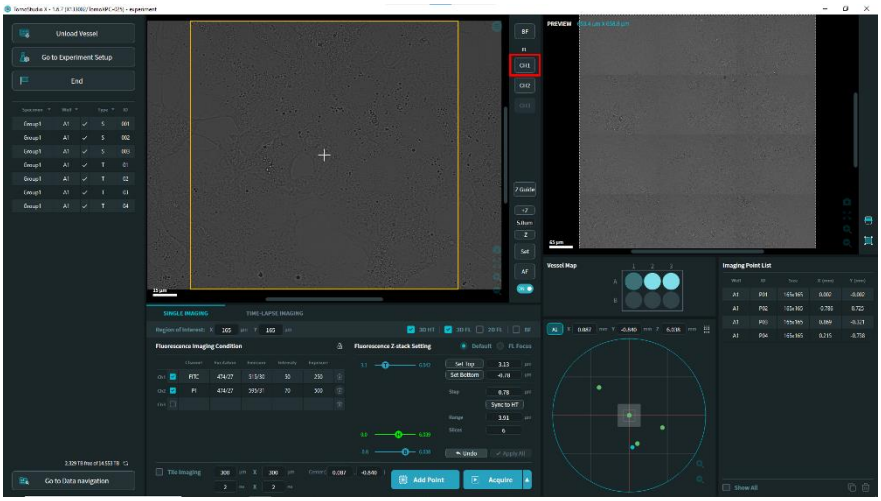
2. Check one of the channel checkboxes on the Fluorescence Imaging Conditions table to add fluorescence channels. On the pop-up menu, select the appropriate channel name for acquisition.



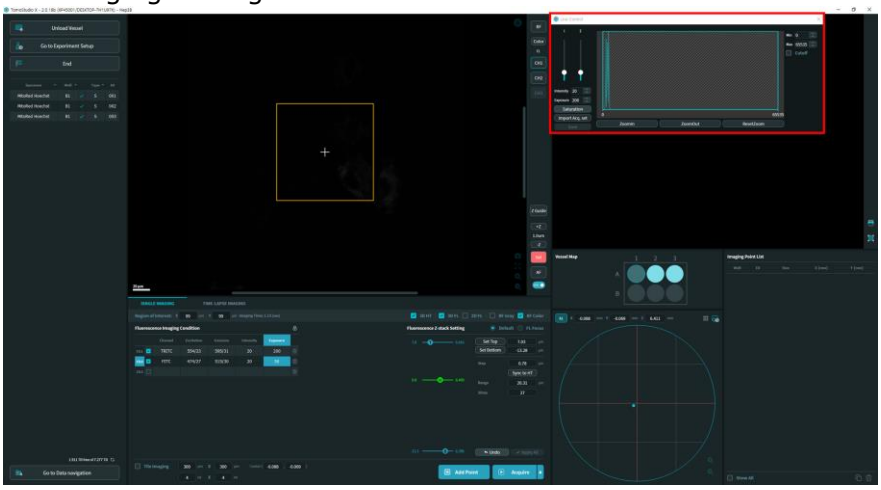
Important! TomoStudioX provides four fluorescence channels to image: DAPI, FITC, TRITC, and Cy5. To add a new channel with a different combination of excitation and emission filters, please contact Tomocube at support@tomocube.com.

3. When Channel is added, the channel selector for each Channel in the Live View mode is available for image acquisition.

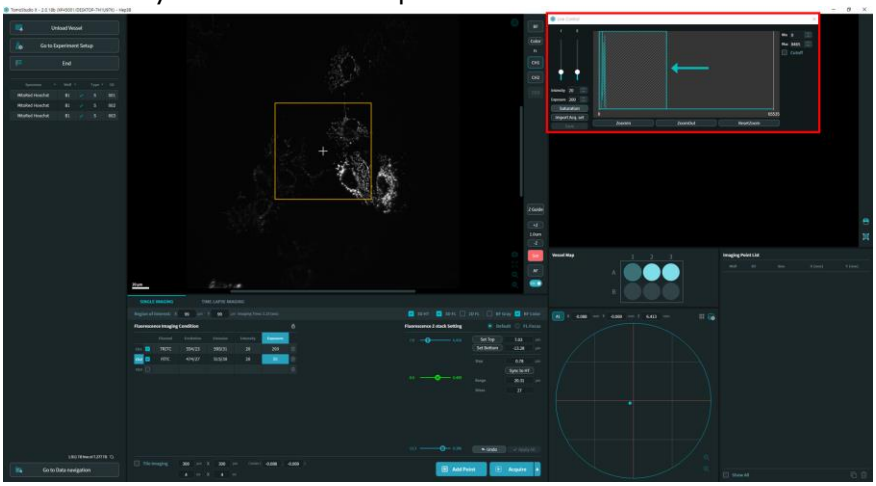
Note: In the Fluorescence Imaging Condition panel on the right, you can select either Widefield or Confocal mode. The Confocal mode is only available on systems equipped with an external fluorescence module configured with SDC (Spinning Disk Confocal).

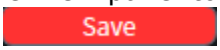


4. Click the selector for Channel 1 on the right side of the Live View panel to adjust the imaging settings for the fluorescence channel.

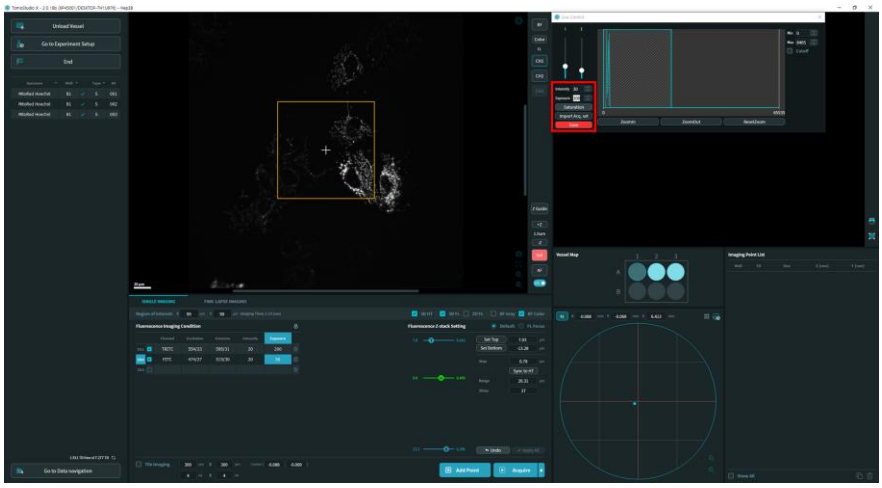


5. In the histogram window, you can adjust the display range by setting the Min and Max to the area with signal. By dragging to resize the box, you can detect signals more clearly in the Live view panel.

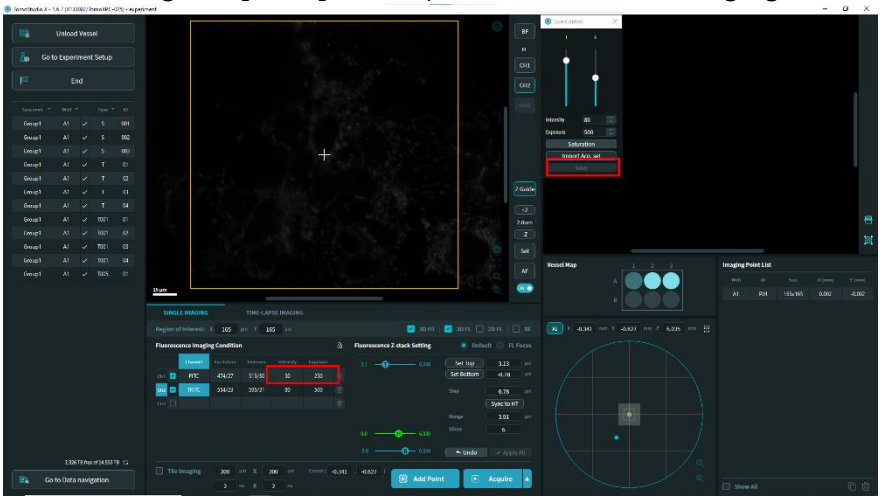


6. Enter the intensity and the exposure time in the Live View panel to optimize the fluorescence signal. Then click the [Save] button  to save and

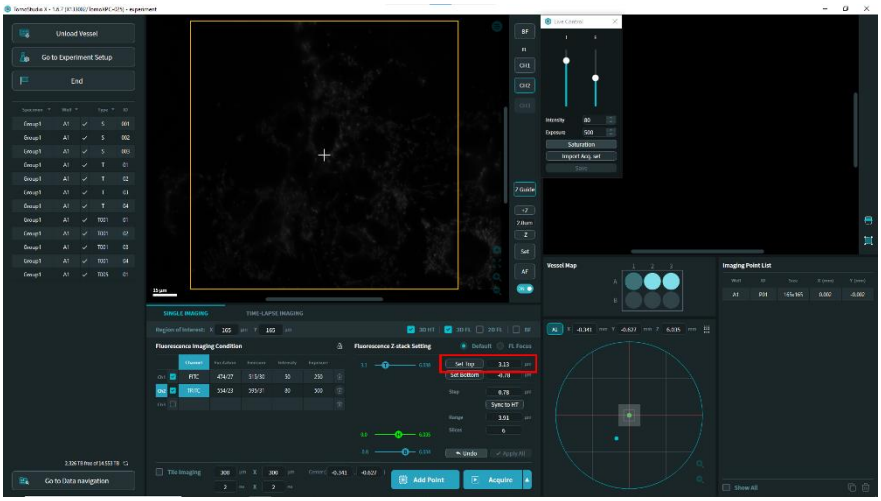
apply the parameters as the fluorescence imaging condition.



7. After clicking the [Save] button, the fluorescence imaging condition is updated.



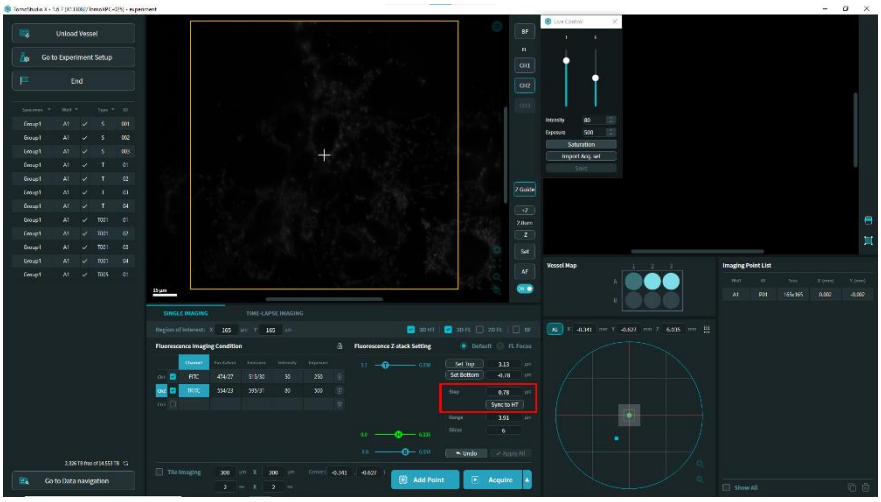
8. To acquire fluorescence images in 3D, set the top and bottom positions of the fluorescence acquisition in the Z direction. To set the uppermost position for fluorescence acquisition, move the focus of the current FOV to an appropriate Z position by using the Z control buttons. Then click the [Set Top] button to apply the current position as the top position. The Z-stack information is automatically updated.



9. To set the bottommost position for fluorescence acquisition, follow the same procedure as in the previous step and click the [Set Bottom] button.

10. Set the step size of the fluorescence imaging in the Z direction for the 3D fluorescence images.

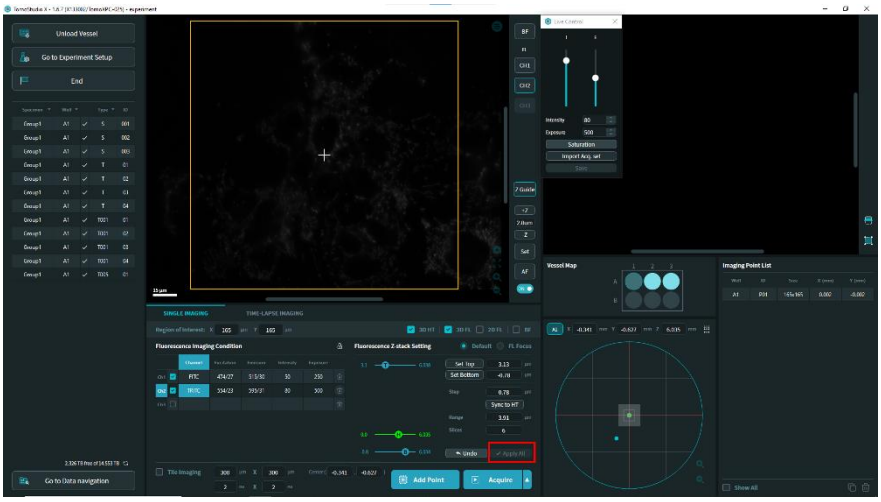
    Note: If you want to set the step size to be the same as the current holotomography imaging conditions, click the [Sync to HT] button to automatically configure the step size.



11. After entering the step size, the number of fluorescence slices is automatically calculated.

    Note: A large number of slices can lengthen the imaging time and may result in photobleaching and phototoxicity of the sample.

12. Click the [Apply] button to save and apply the fluorescence Z-stack parameters.

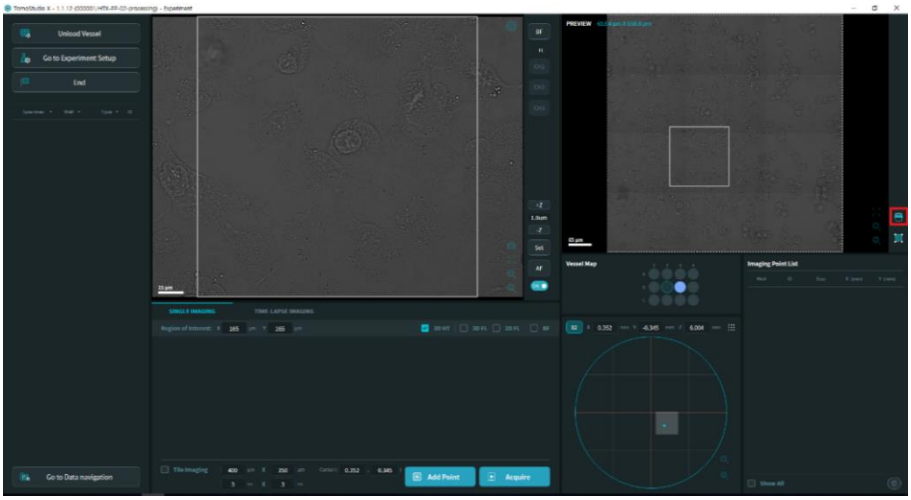


13. Click the [Acquire] button to perform holotomography and fluorescence imaging. Acquired images are updated automatically on the data list table.

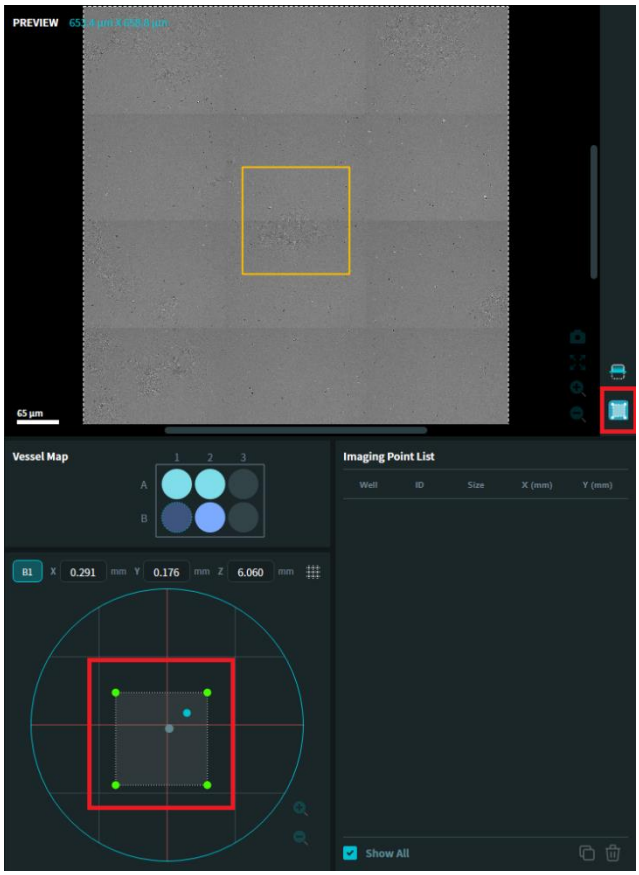
5.5.3 Tile acquisition

To acquire an image of a wider area than the maximum single ROI (165 µm × 165 µm), the Tile Imaging option can be selected in the Single Imaging tab.

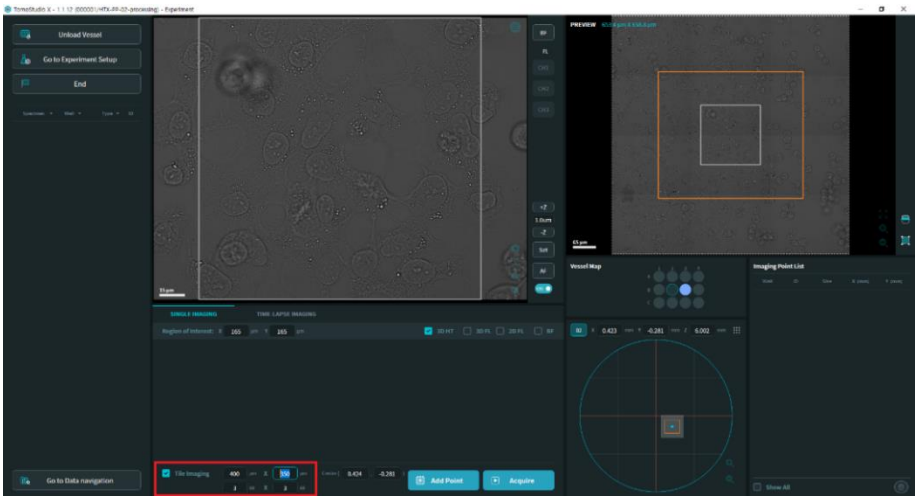
1. Move the current location to the desired position and click the [Scan] button in the Preview panel to display a preview image.



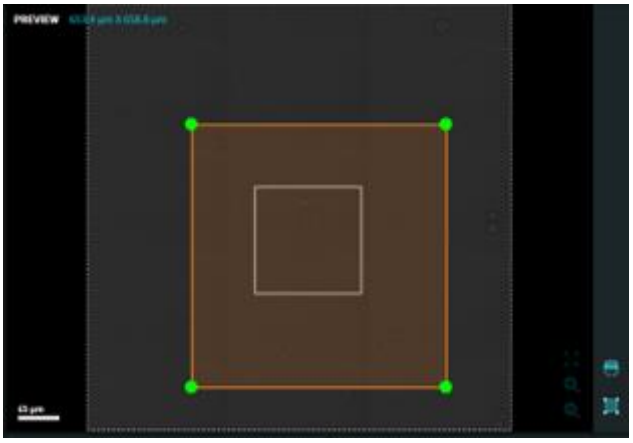
2. If a wider scan area is desired for the preview image than the default, click the [Change Preview Size] button at the bottom-right side of the Preview panel to adjust the preview scan size. When clicked, you can change the scan area by moving the edges of the preview size box on the well map. After adjusting the scan area, click the [Scan] button to have a preview image on the chosen area.



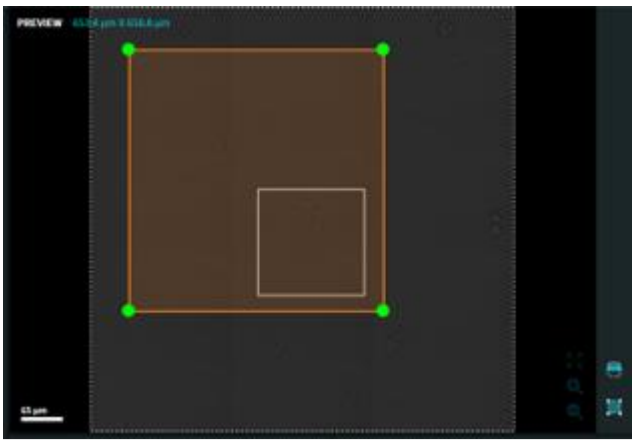
3. Click the [Tile Imaging] checkbox to activate the Tile Imaging mode. The tile imaging area will appear as an orange box in the Preview and the Well Map.



4. Set the imaging size for tile imaging by clicking one of the four green dots on the corners of the tile imaging area box in the Preview panel and dragging it to the desired size.
   Note: The tile size can also be set by entering width and height values in the Tile Imaging panel.



5. Adjust the location of the tile imaging area by clicking the tile imaging area box in the Preview panel and dragging it to the desired location.



6. Confirm that the imaging modes and settings are properly set and then click the [Acquire] button to start the imaging.

5.6 Multi-point imaging

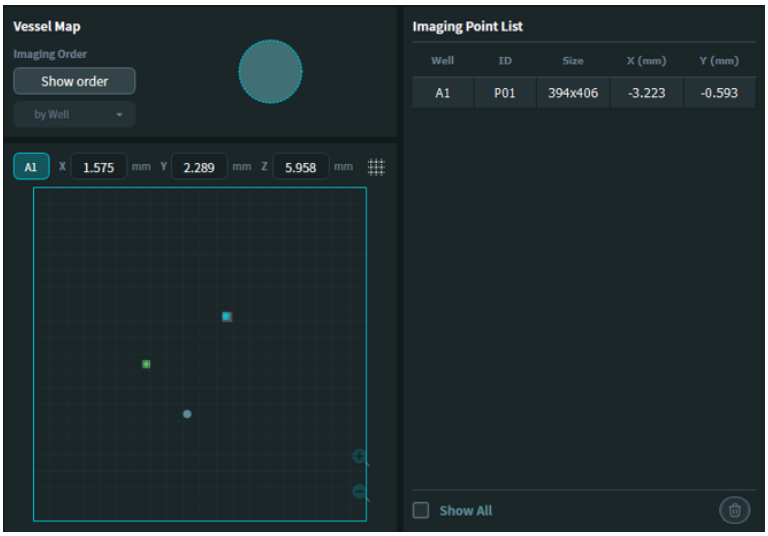
You can register multiple imaging points in the [imaging point list] and acquire data all at once in a batch.

5.6.1 Add imaging points – Basic

To perform multi-point imaging, register the desired locations to add them as imaging points. The imaging points can be registered or added in the Single Imaging tab.

1. Move the current position of the Live View to the desired location to register it as an imaging point and click the [Add Point] button. The current location is now added as an imaging point in the Imaging Point List. The registered imaging points can be seen in the Imaging Point List panel.

   Note: Registered imaging points appear as green dots on the Well Map. The Live View can be changed to show each imaging point by double-clicking the imaging points displayed in the Well Map or listed in the Imaging Point List panel.



2. To register a tile as an imaging point, click the Tile Imaging checkbox to activate the Tile Imaging mode. Adjust the imaging area for tile imaging and click the [Add Point] button.

   Note: Registered imaging points appear as green dots on the Well Map. The Live View can be changed to show each imaging point by double-clicking the imaging points displayed in the Well Map or listed in the Imaging Point List panel. After registering all desired imaging points on the current well, move to the next well by double-clicking the desired well in the Vessel Map.

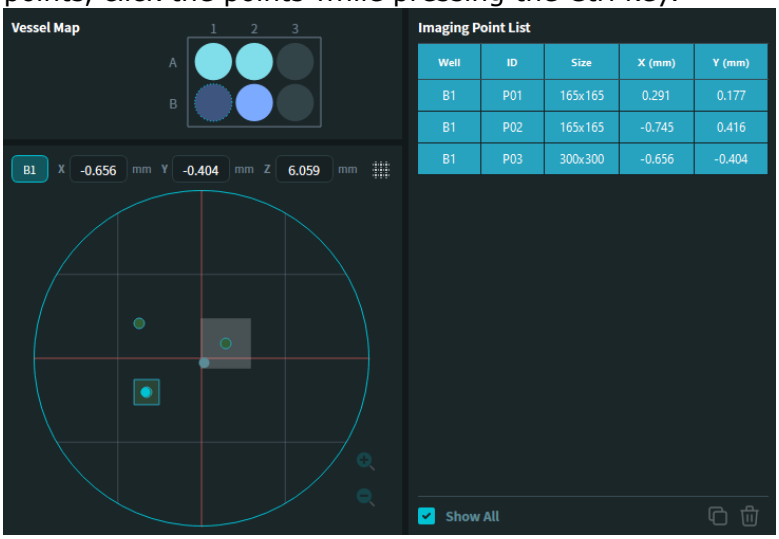
3. To register imaging points in the next selected well, repeat Step 1.

   Note: To see all the registered imaging points in the wells, click the [Show All] checkbox. All the registered imaging points of the current experiment will appear in the Imaging Point List.

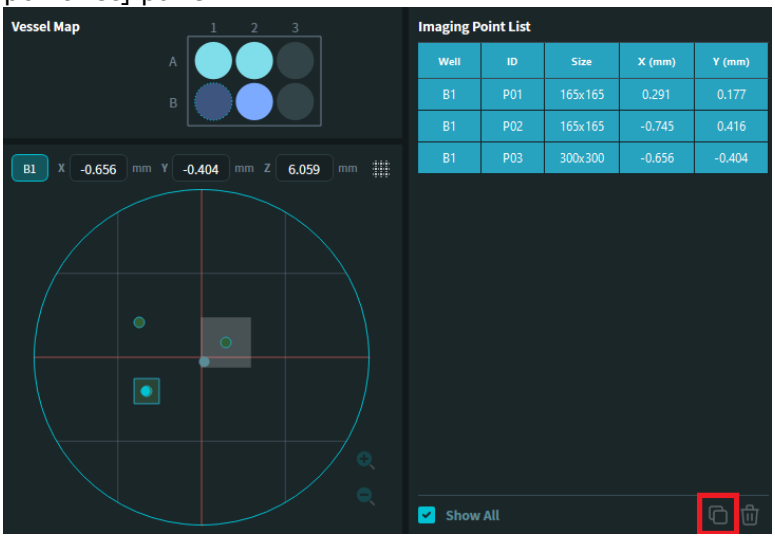
5.6.2 Add imaging points – Duplicating imaging points to other wells

When the vessel is a multi-well plate, you can add imaging points in other wells by duplicating the imaging points registered in the current well.

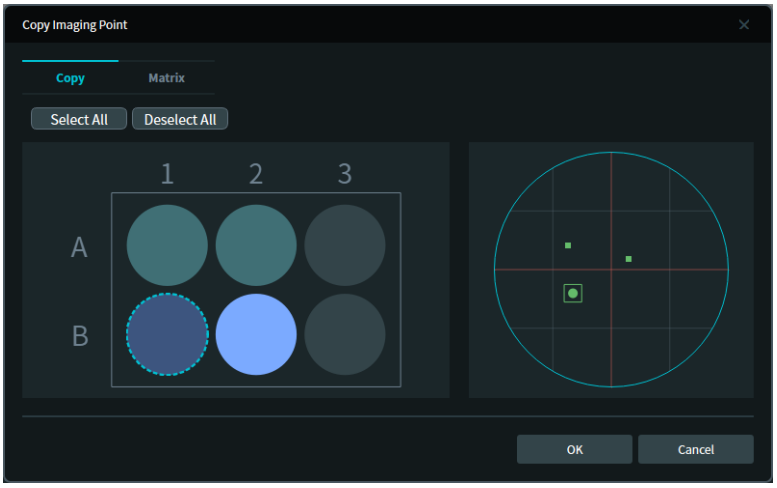
1. From the Imaging Point List, select the points to be copied. To select multiple points, click the points while pressing the Ctrl key.



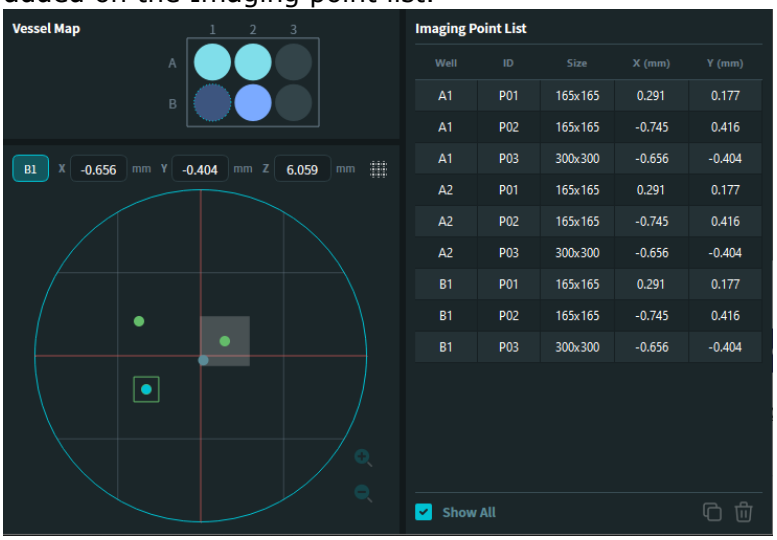
2. Click the [Advanced adding] button at the bottom-right side of the [Imaging point list] panel.



3. Click the wells to copy to on the vessel map of the pop-up window. If multiple wells are to be selected, press the Ctrl key while clicking the wells.



4. Click the [OK] button to apply. The points duplicated to the other well(s) will be added on the Imaging point list.

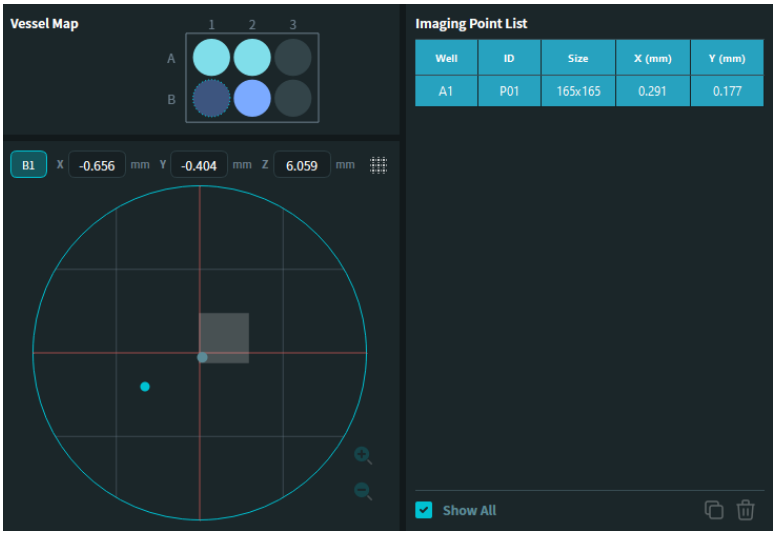


Well	ID	Size	X (mm)	Y (mm)
A1	P01	165x165	0.291	0.177
A1	P02	165x165	-0.745	0.416
A1	P03	300x300	-0.656	-0.404
A2	P01	165x165	0.291	0.177
A2	P02	165x165	-0.745	0.416
A2	P03	300x300	-0.656	-0.404
B1	P01	165x165	0.291	0.177
B1	P02	165x165	-0.745	0.416
B1	P03	300x300	-0.656	-0.404

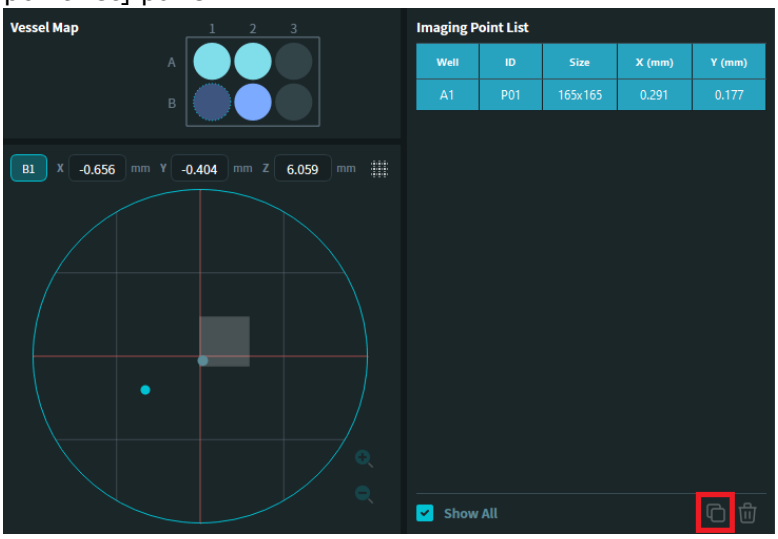
5.6.3 Add imaging points – Matrix points

It is possible to add multiple imaging points arranged by regular spacing, which is called the matrix points.

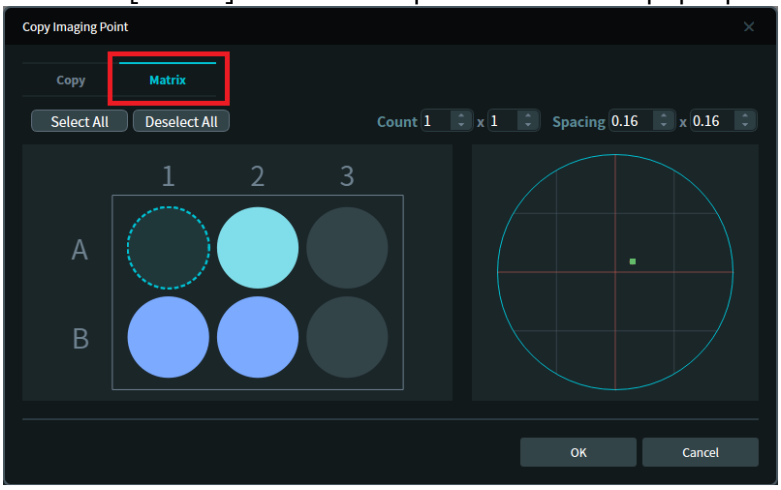
1. To generate matrix points, choose an imaging point in the Imaging Point List that will be the center of the matrix points. If there is no imaging point registered in the Imaging Point List, register at least one imaging point in the list beforehand.



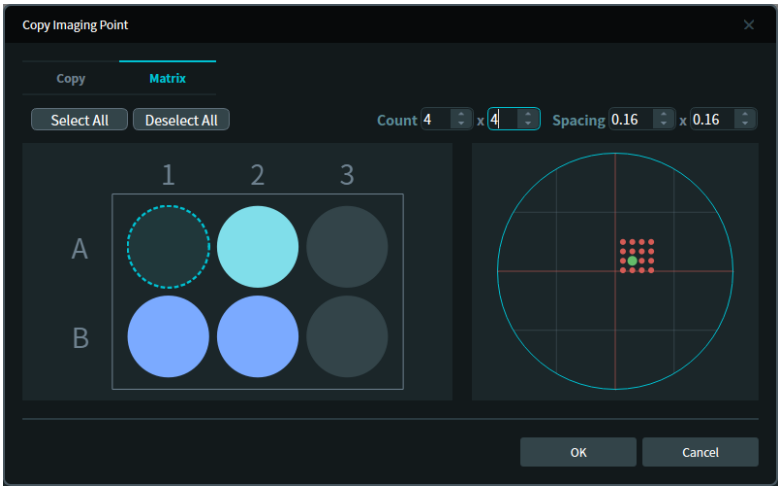
2. Click the [Advanced adding] button at the bottom-right side of the [Imaging point list] panel.



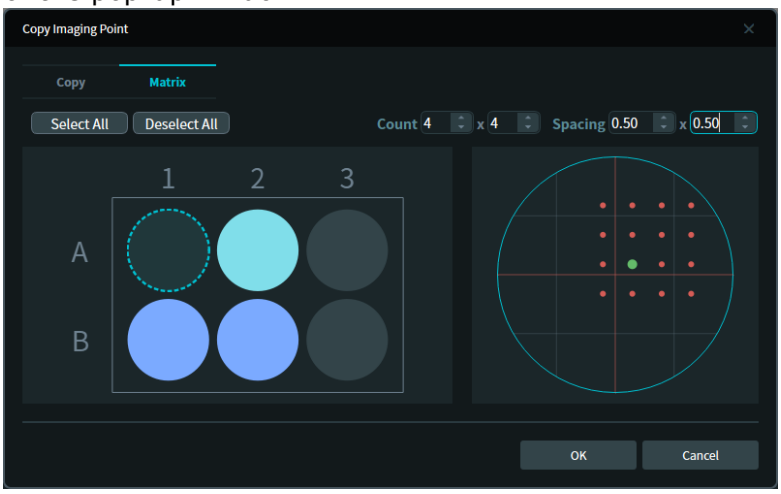
3. Click the [Matrix] tab at the top-left side of the pop-up window.



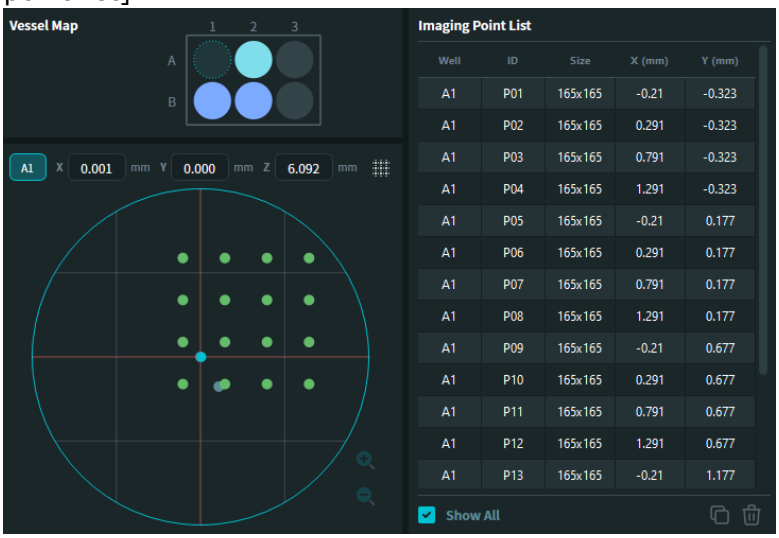
4. Set the number of rows and columns for the matrix points.



5. Set the spacings in the X and Y directions of the matrix points. The preview of the arrangement of the matrix points appears on the well map at the right side of the pop-up window.



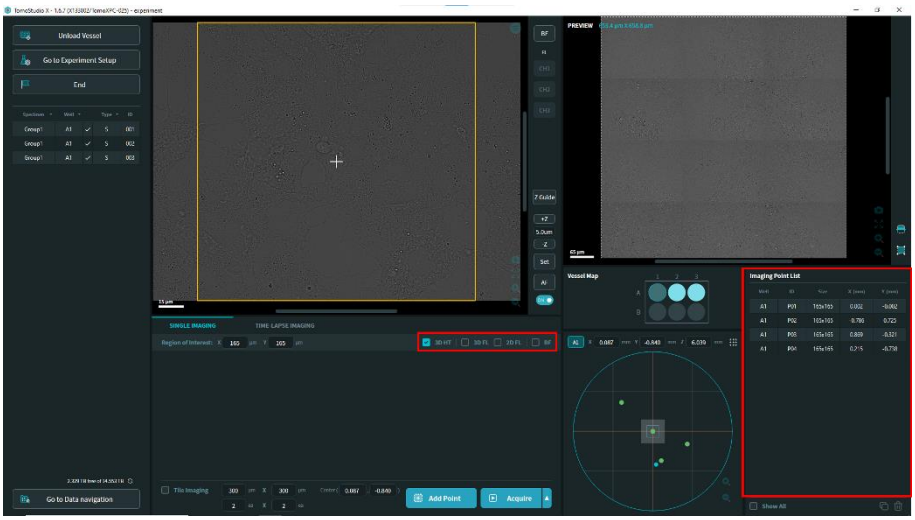
6. Click the [OK] button to apply. The matrix points will be added on the [Imaging point list].



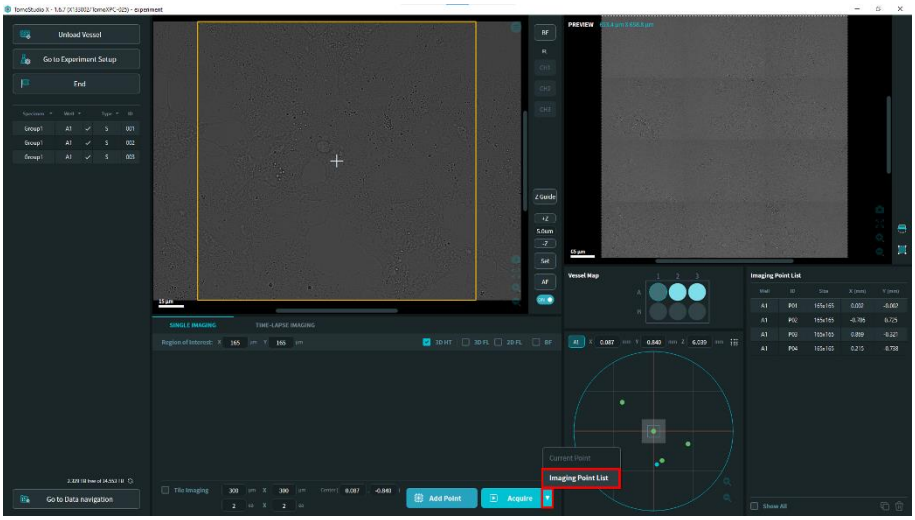
5.6.4 Multi-point acquisition

Once you have completed registering the imaging points on [Imaging point list], you can acquire each of the imaging points once on [Single imaging] tab.

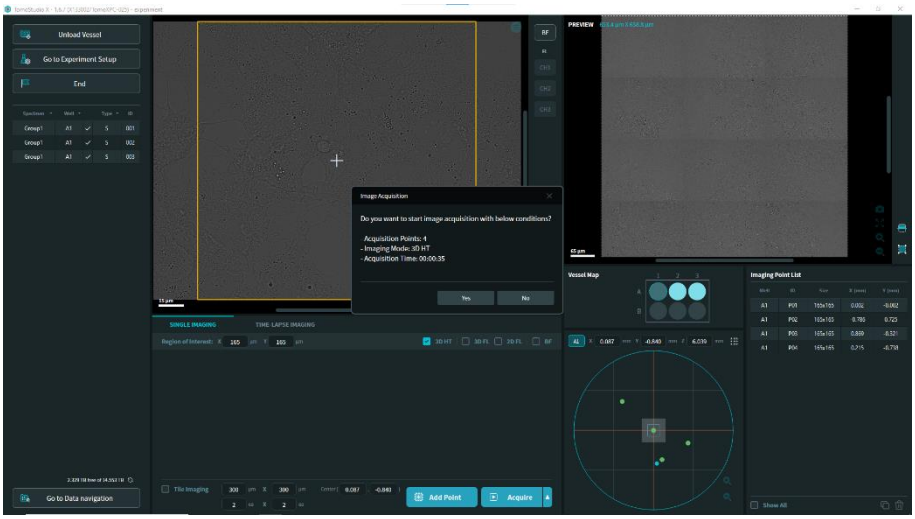
1. After adding the imaging points, confirm that the imaging modes and settings are properly set.



2. Click the additional arrow of [Acquire] button to show advanced menu. Then, select the [Imaging point list] on the menu.



3. Clicking on [imaging point list] will bring up a popup displaying the number of registered imaging points and the estimated time required for the acquisition. After confirming the indicated imaging conditions, click the [OK] button to start multi-point acquisition.



4. During the image acquisition, the current imaging progresses, elapsed time, and the remaining time until completion are displayed at the bottom of the imaging tab. If you want to cancel the acquisition, click the [stop] button.

5.7 Time-lapse imaging

You can not only acquire the registered imaging points once but also image how they change over time on [Time-lapse imaging] tab.

5.7.1 Add imaging points for time-lapse imaging

For time-lapse imaging, just like multi-point imaging, you must register the imaging points you want to capture in the [imaging point list]. Please see the section 5.6.1 to 5.6.3 for more details.

5.7.2 Time sequence setup — Basic

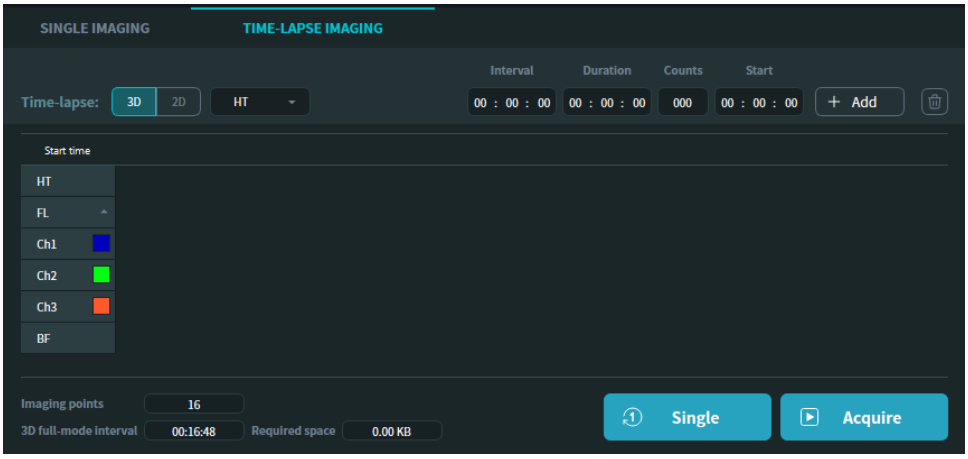
After registering all desired imaging points in all wells, the time-lapse sequence can be scheduled by setting the appropriate intervals and imaging modes. Basic guidelines for setting various time-lapse imaging types are provided below.

Standard Holotomography time-lapse

Among the various types of time-lapse sequences supported by the HT-X1 system, the simplest and most frequently used is the standard holotomography time-lapse: a time-lapse sequence in holotomography mode with a uniform interval.

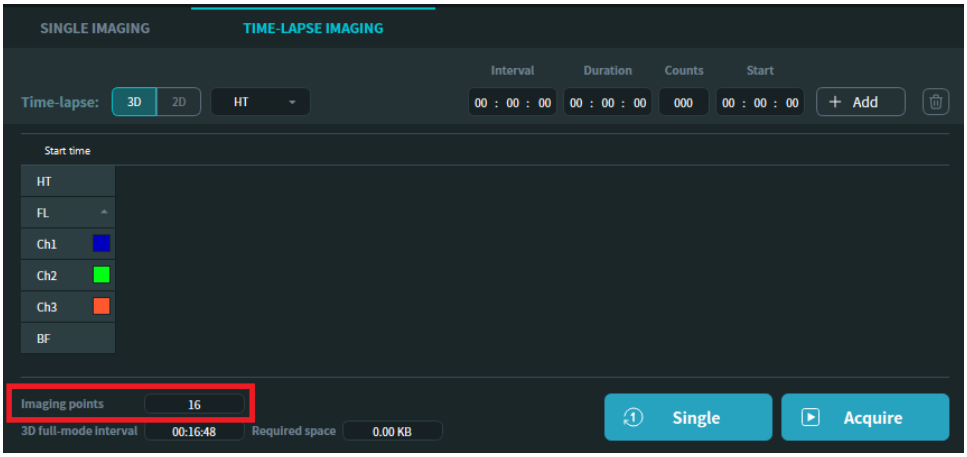
Prerequisite ▶ To enable a time-lapse sequence, at least one imaging point needs to be registered in the Imaging Point List.

1. Click the Time-Lapse Imaging tab in the Acquisition panel.

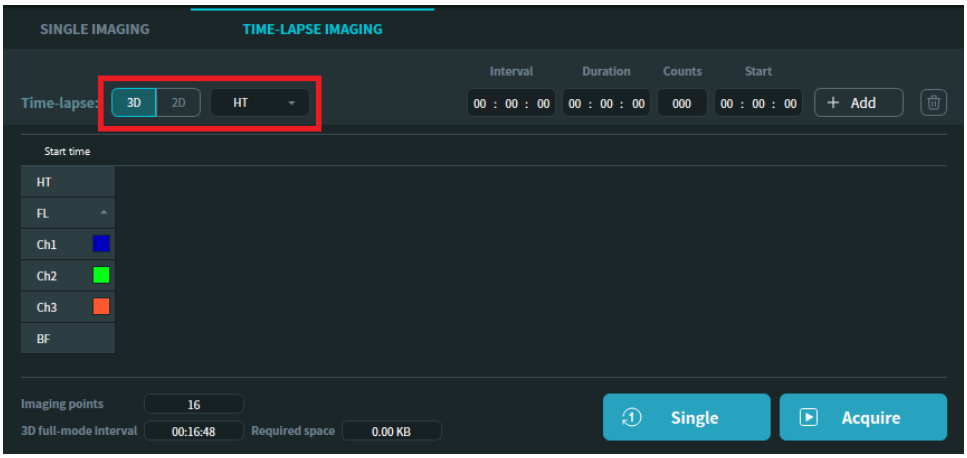


2. Confirm that the imaging points are registered properly.

   Note: The number of registered imaging points is shown on the bottom-left side of the Time-Lapse Imaging tab.



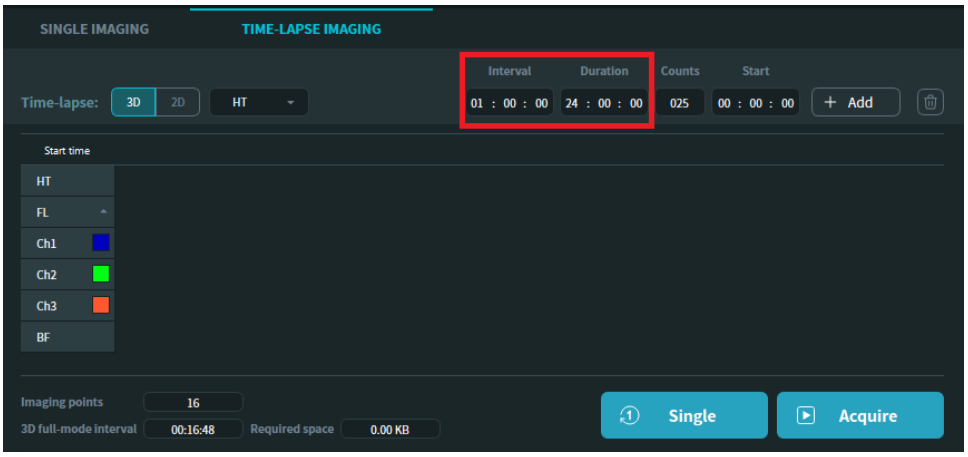
3. To add a time frame for the holotomography time-lapse sequence, set the sequence mode to 3D and HT.



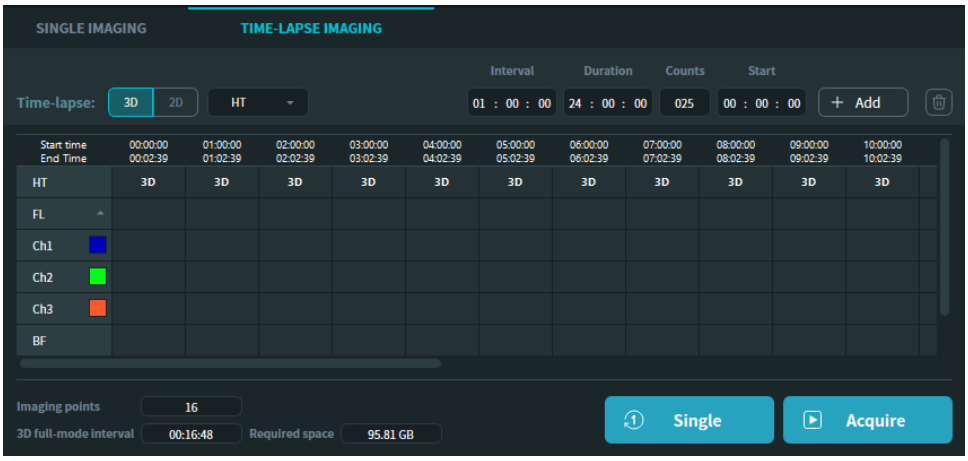
4. Enter the imaging interval and duration for each time frame in the corresponding

input fields, hereafter referred to as the Sequence time input field.

Note: The counts of the sequence are calculated automatically.



5. Click the [Add] button to apply the sequence setup into the Time-Lapse Schedule Table.



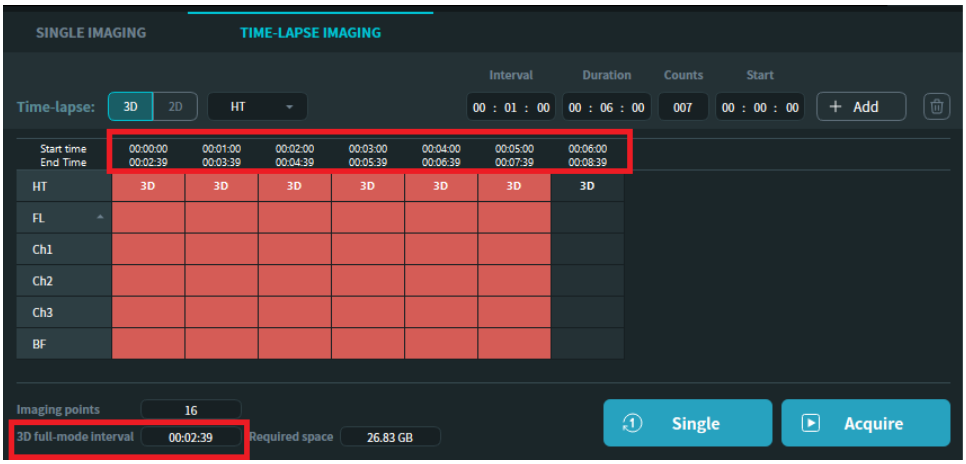
6. Confirm that no error has occurred in all time frames. Time frames with an error are highlighted in red.

Important! Errors in the Time-Lapse Schedule Table appear when the interval set by the user is insufficient to complete one cycle of the longest time frame in the time-lapse sequence. Check the [Minimum interval] (3D full-mode interval) shown on the bottom-left side of the Time-Lapse Imaging tab and set the interval longer than the displayed minimum interval.

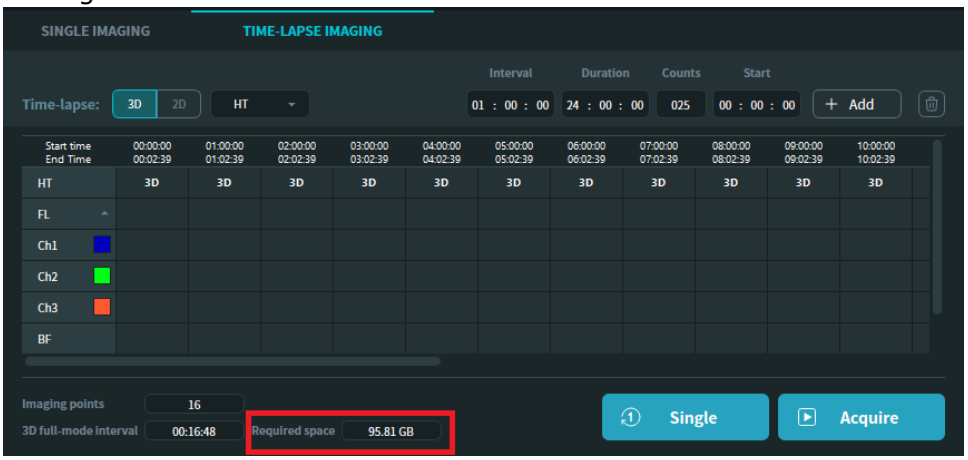
Important! If the number of imaging points registered in the Imaging Point List exceeds 300, it may cause an unstable progress for the experiment due to the computer memory when the time-lapse interval is set to the 3D full-mode interval. In such circumstances, follow the guideline below to mitigate the instability.

1. Set the timelapse interval about twice the value of the 3D-full mode interval.
2. Reduce the number of imaging points.
3. Avoid HT reconstruction data processing or copying data to other storage

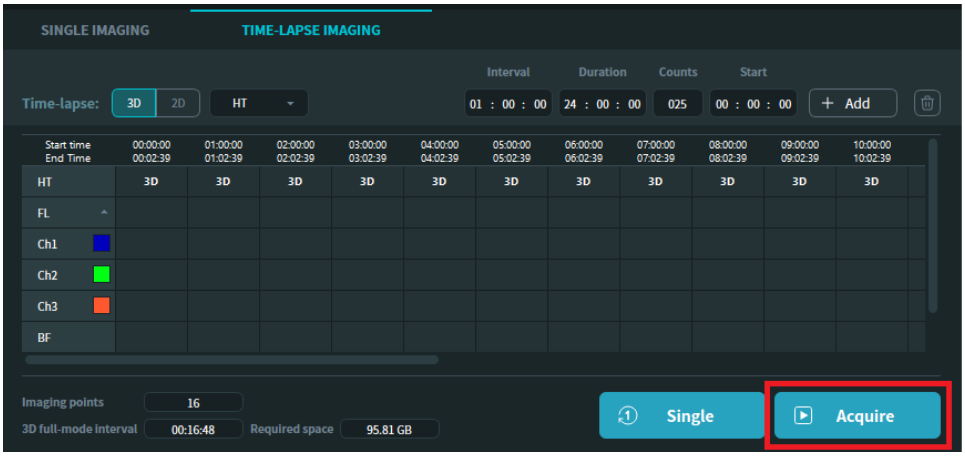
devices during the time-lapse imaging.



7. Check the Required space at the bottom-left of the Time-lapse Imaging panel. If the amount of the required space exceeds the available storage space, please free up the storage space to meet the required space or modify the time-lapse setting to reduce it.



8. Click the [Acquire] button to start the time-lapse imaging.

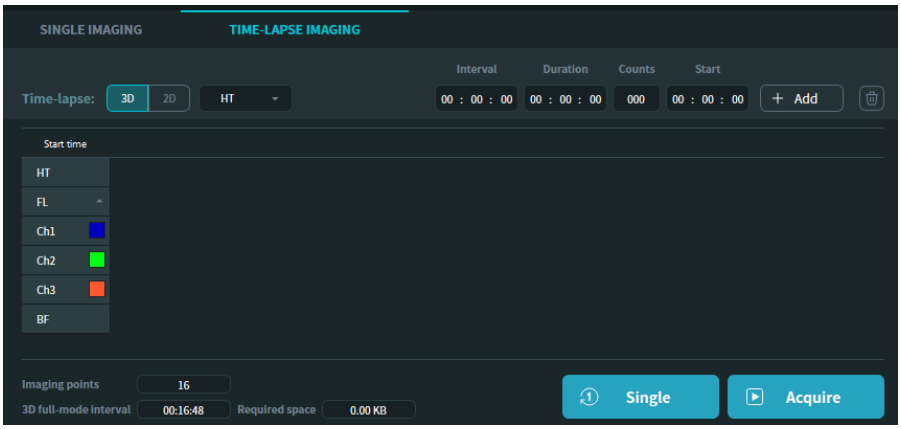


Standard Holotomography time-lapse with Fluorescence or Brightfield

Prerequisite

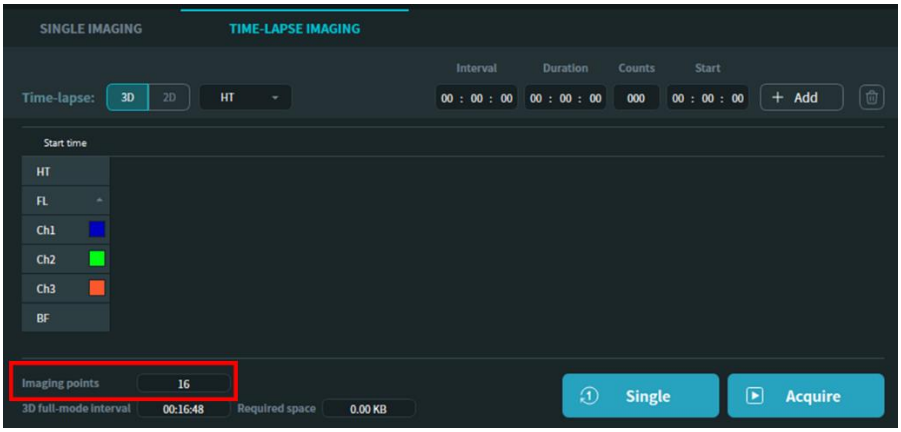
- ▶ At least one point is registered in the Imaging Point List.
- ▶ The Fluorescence mode is activated in the Single Imaging tab.

1. Click the Time-Lapse Imaging tab in the Acquisition panel.

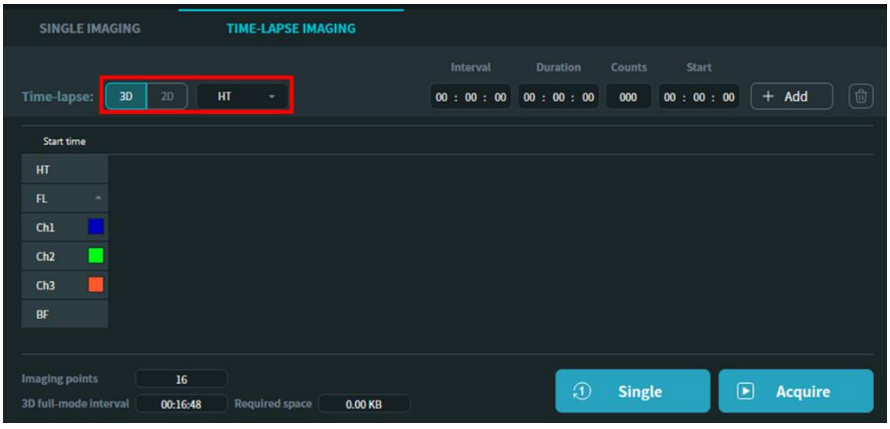


2. Confirm that the imaging points are registered properly.

   Note: The number of registered imaging points is shown on the bottom-left side of the Time-Lapse Imaging tab.

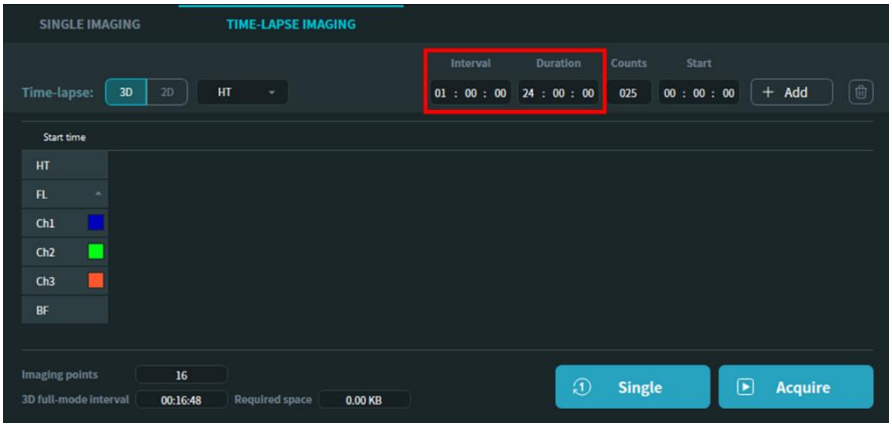


3. To add a timeframe for the holotomography time-lapse sequence, set the sequence mode to 3D and HT.

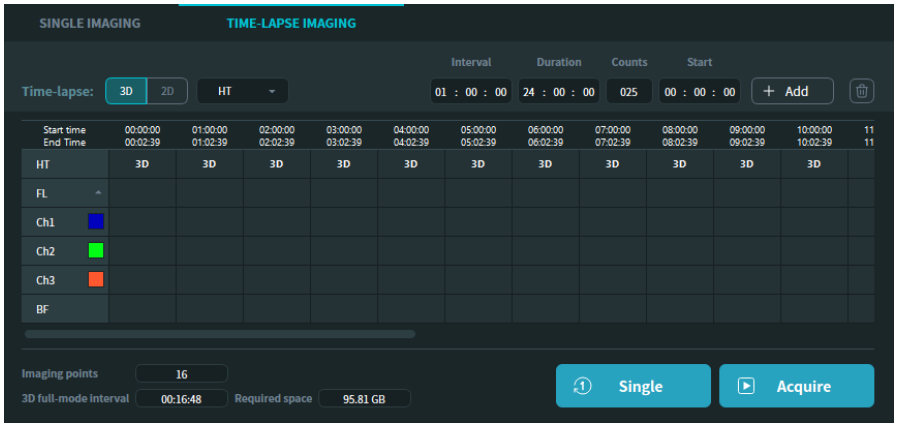


4. Enter the imaging interval and duration for each time frame in the Sequence time input field.

   Note: The counts of the sequence are calculated automatically.

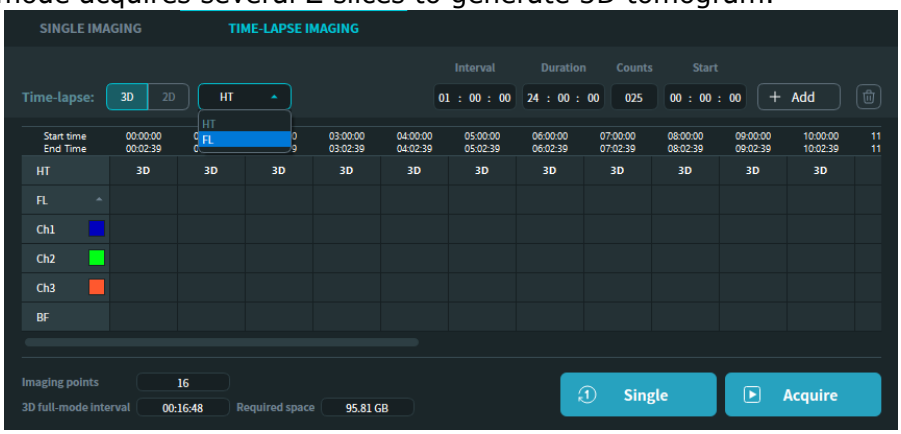


5. Click the [Add] button to apply the sequence setup into the Time-Lapse Schedule Table.

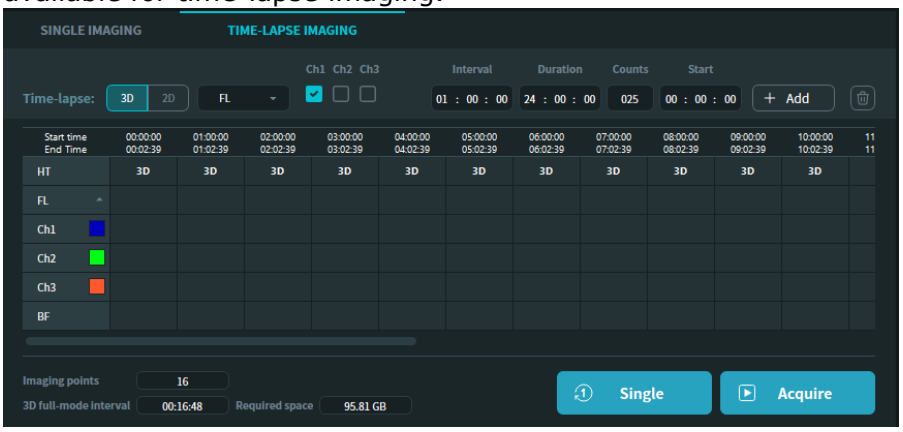


6. To include fluorescence imaging in the time-lapse sequence, set the mode to FL.

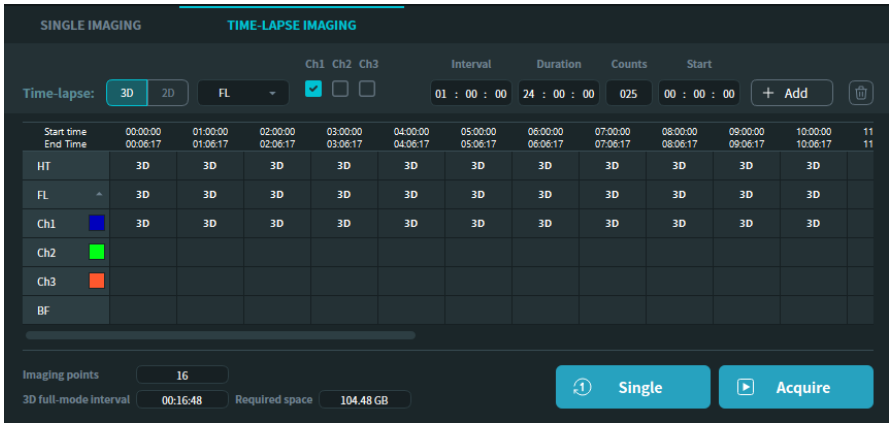
   Note: The Fluorescence mode has two options in the time-lapse sequence: 2D and 3D. The 2D fluorescence mode may result in less phototoxicity and require less time for image acquisition because it acquires only a single Z slice. The 3D mode acquires several Z slices to generate 3D tomogram.



7. Click the desired fluorescence channel checkbox for time-lapse imaging.
   Note: Only the channels that are activated in the Single Imaging tab are available for time-lapse imaging.

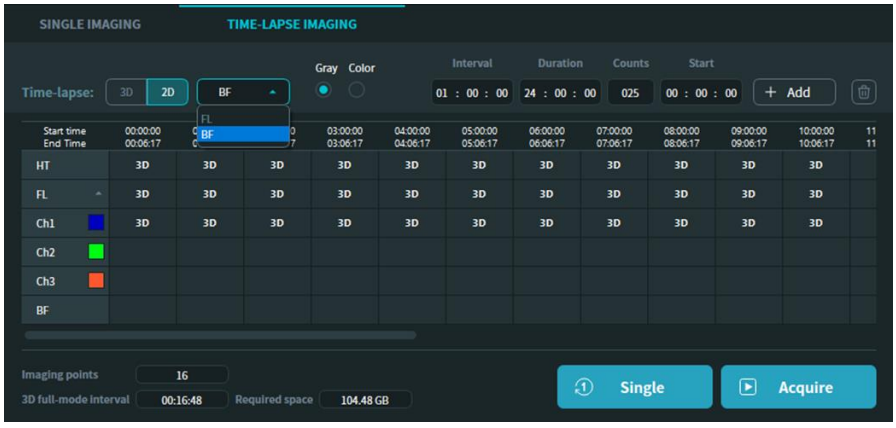


8. Click the [Add] button to apply the sequence setup into the Time-Lapse Schedule Table.

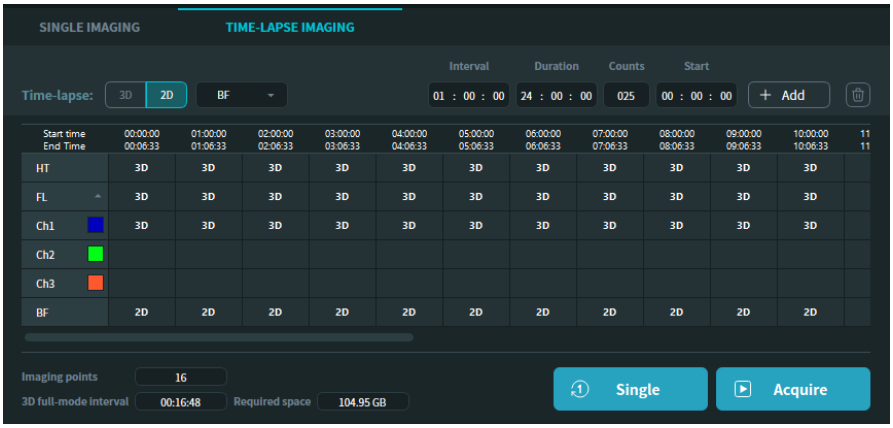


9. To add brightfield channel data into the time-lapse sequence, set the mode to BF.

   Note: The brightfield mode can be set to grayscale or color with HT-X1™ Plus and is available only in 2D mode.



10. Click the [Add] button to apply the sequence schedule of the brightfield images into the Time-Lapse Schedule Table.



11. Confirm that no error has occurred in all time frames. Time frames with an error are highlighted in red.

    Important! Errors in the Time-Lapse Schedule Table appear when the interval set by the user is insufficient to complete one cycle of the longest time frame in the time-lapse sequence. Check the 3D full-mode interval shown on the bottom-left side of the Time-Lapse Imaging tab and set the interval longer than the displayed minimum interval.

12. Click the [Acquire] button to start the time-lapse imaging.

5.7.3 Sequence setup — Advanced

Advanced Holotomography time-lapse with hetero imaging of Fluorescence or Brightfield

In addition to basic time-lapse imaging, TomoStudioX provides more advanced **hetero time-lapse imaging** that consists of multiple imaging modes with a different interval for each mode. Although the setup for hetero time-lapse imaging is more complicated than for basic time-lapse imaging, a wide range of experimental settings are available with hetero time-lapse imaging, such as lowering fluorescence imaging or setting irregular imaging intervals. Guidelines for setting three frequently used hetero time-lapse imaging modes are provided below.

HT time-lapse imaging with Fluorescence at the start and the end of the sequence

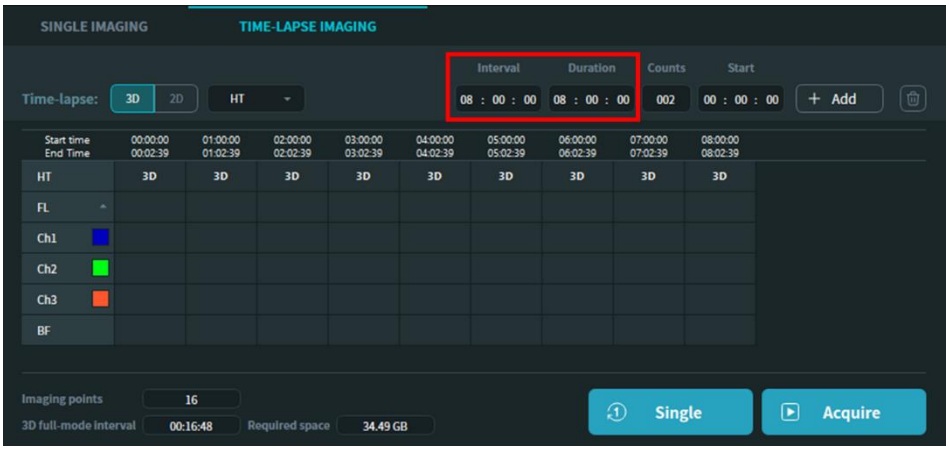
Prerequisite
- ▶ At least one point is registered in the Imaging Point List.
- ▶ The Fluorescence mode is activated in the Single Imaging tab.

1. Click the Time-Lapse Imaging tab in the Acquisition panel and confirm that the imaging points are registered properly.
2. Add an holotomography time-lapse schedule with an appropriate interval and duration in the Sequence time input field.
3. Click the [Add] button to apply the sequence schedule into the Time-Lapse Schedule Table.
4. To include fluorescence imaging in the time-lapse sequence schedule, set the mode

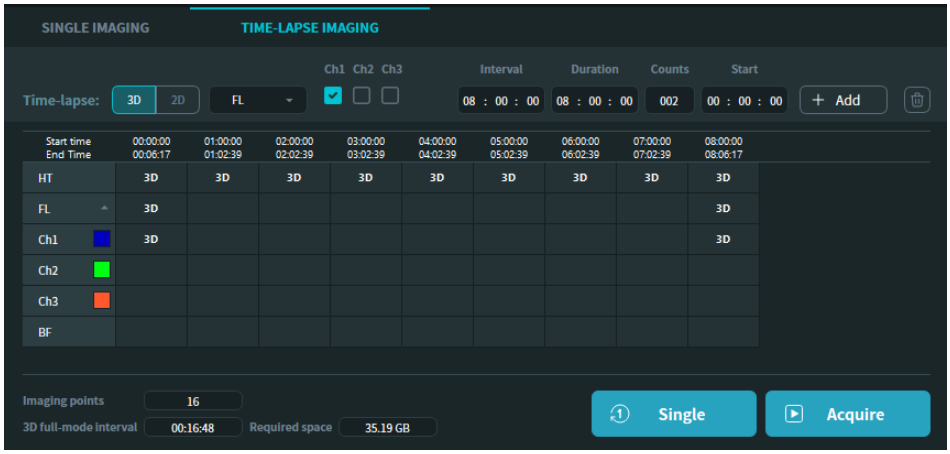
to FL and click the desired fluorescence channel checkbox for time-lapse imaging.

5. Enter the same values for the interval and duration in the Sequence time input field as those set in Step 2.

   Note: For fluorescence imaging only at the start and end point of the time frame, set the fluorescence interval to the same value as the duration.



6. Click the [Add] button to apply the sequence setup into the Time-Lapse Schedule Table.



   Important! When the fluorescence channels are added into the Time-Lapse Schedule Table, an error may occur if the interval set for HT is not long enough. Two methods to resolve this issue are as follows: shorten the imaging time by deregistering some of the imaging points or remove some of the time frames that are overlapped.

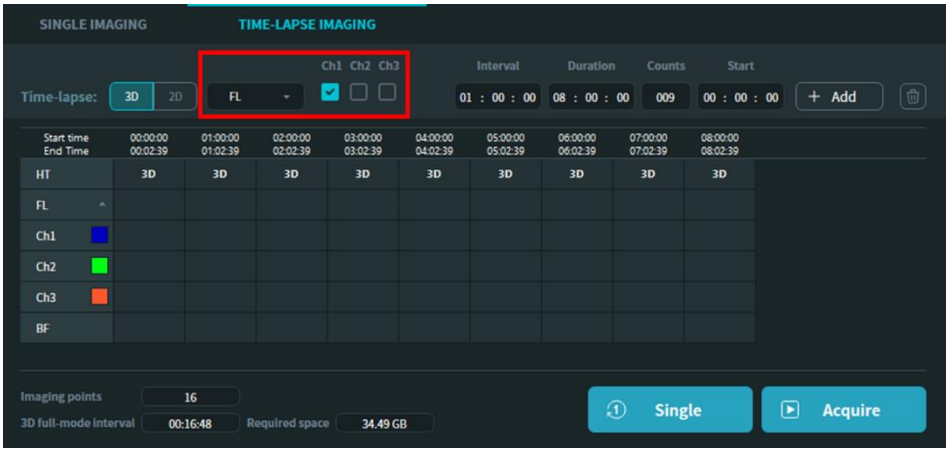
7. Click the [Acquire] button to start the time-lapse imaging.

Repetitive imaging of Holotomography with single Fluorescence

Prerequisite

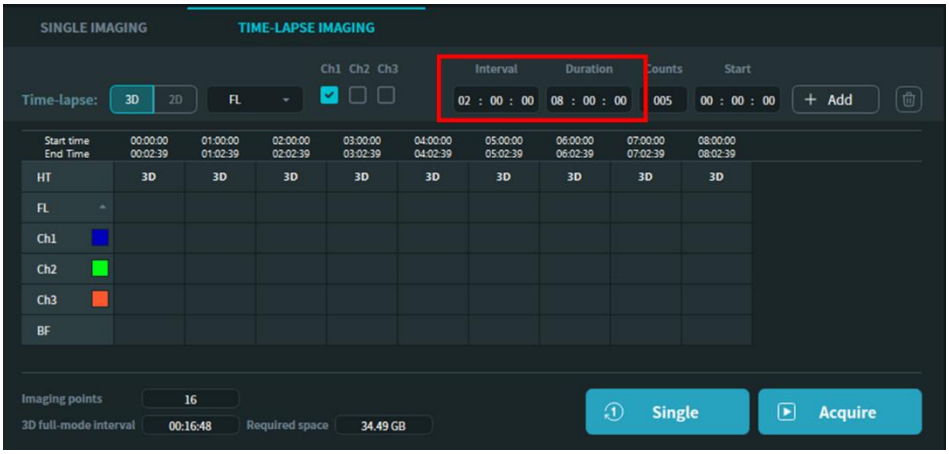
- ▶ At least one point is registered in the Imaging Point List.
- ▶ The Fluorescence mode is activated in the Single Imaging tab.

1. Click the Time-Lapse Imaging tab in the Acquisition panel and confirm that the imaging points are registered properly.
2. Add a holotomography time-lapse sequence with an appropriate interval and duration in the Sequence time input field.
3. Click the [Add] button to apply the sequence schedule into the Time-Lapse Schedule Table.
4. To include fluorescence imaging in the time-lapse sequence schedule, set the mode to FL and click the desired fluorescence channel checkbox for time-lapse imaging.

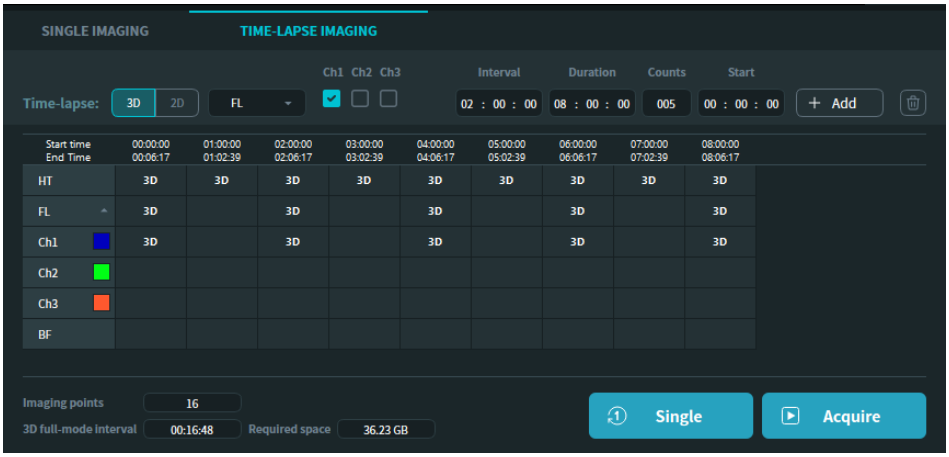


5. Enter the imaging interval for the FL channel as a multiple of the value set for the holotomography interval in the Sequence time input field.

   Note: For example, to obtain one FL image for every three holotomography images, set the fluorescence interval to three times the HT interval.



6. Click the [Add] button to apply the sequence schedule into the Time-Lapse Schedule Table.



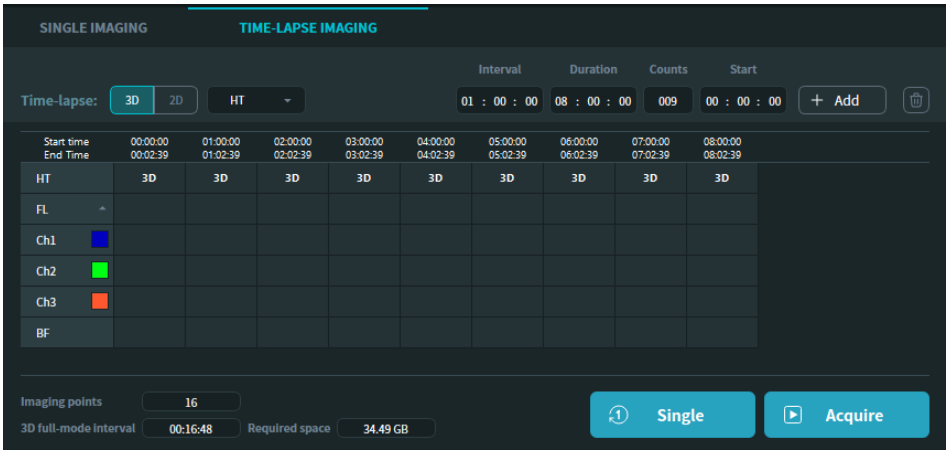
Important! When the fluorescence channels are added into the Time-Lapse Schedule Table, an error may occur if the interval set for holotomography is not long enough. Two methods to resolve this issue are as follows: shorten the imaging time by deregistering some of the imaging points or remove some of the time frames that are overlapped.

7. Click the [Acquire] button to start the time-lapse imaging.

Two different intervals for Holotomography imaging with different start time points

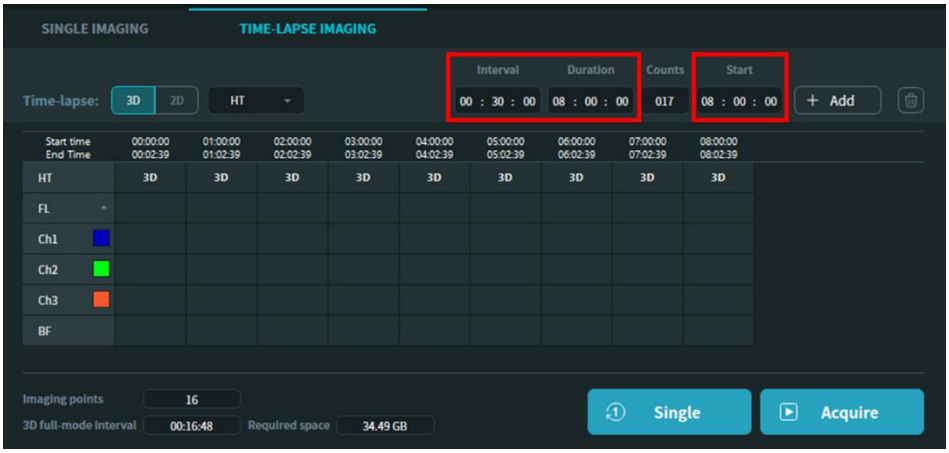
Prerequisite ▶ At least one point is registered in the Imaging Point List.

1. Click the Time-Lapse Imaging tab in the Acquisition panel and confirm that the imaging points are registered properly.
2. Add an Holotomography time-lapse sequence with an appropriate interval and duration in the Sequence time input field.
3. Click the [Add] button to apply the sequence schedule into the Time-Lapse Schedule Table.

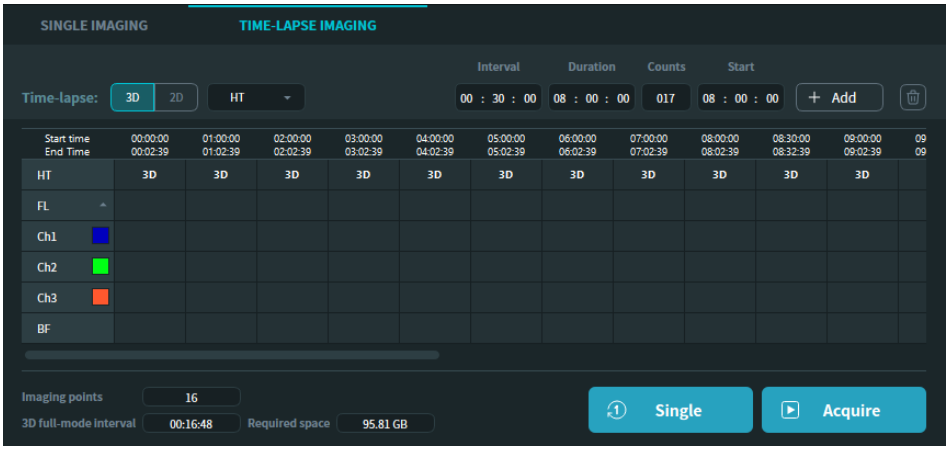


4. Set a second holotomography time-lapse sequence with a different interval and duration in the Sequence time input field.

5. Enter the desired Start time of the second holotomography time-lapse sequence in the corresponding input field.



6. Click the [Add] button to apply the sequence schedule into the Time-lapse Schedule Table.



7. Click the [Acquire] button to start the time-lapse imaging.

5.8 Time-lapse progress window

After starting a time-lapse acquisition, TomoStudioX displays the Time-Lapse Progress window to show the progression of the time-lapse imaging in real time. Most of the components in the Time-Lapse Progress window are similar to those in the Image Acquisition window in Figure 5.2 with minor differences in several user interface elements.

5.8.1 User interface description

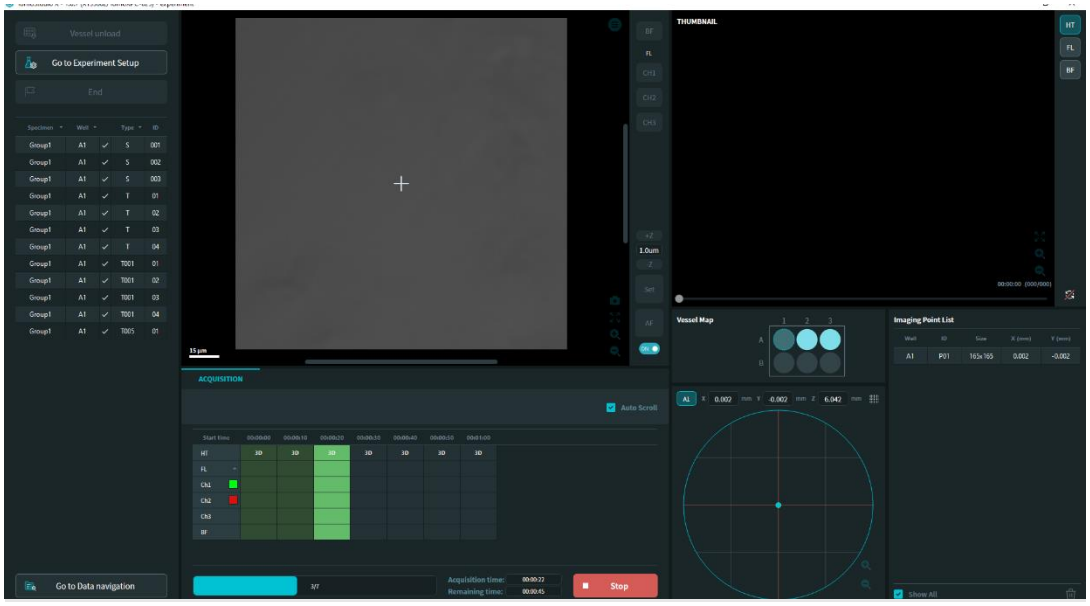
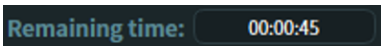
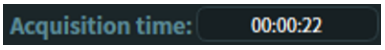
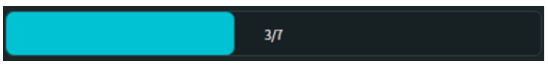
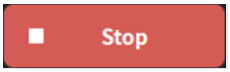
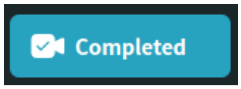


Figure 5.12 Time-Lapse Progress window

During the time-lapse imaging, the Time-Lapse Schedule Table displays the status of the time-lapse schedule with various colors. The current imaging timeframe is indicated in light green, and the time frames already executed are shown in dark green. Several user interface components for the time-lapse schedule setting are disabled.

Parameter	Function	Description
	Remaining time	Shows the remaining time to finish the time-lapse sequence.
	Acquisition time	Shows the elapsed time after starting the acquisition.
	Progress bar	Shows the current progress of time-lapse imaging: number of acquired timeframes and the total timeframes.
	Stop	Immediately stops and exits the time-lapse sequence.

	Completed	Exits the Time-Lapse Progress window after finishing the time-lapse sequence.

Thumbnail viewer

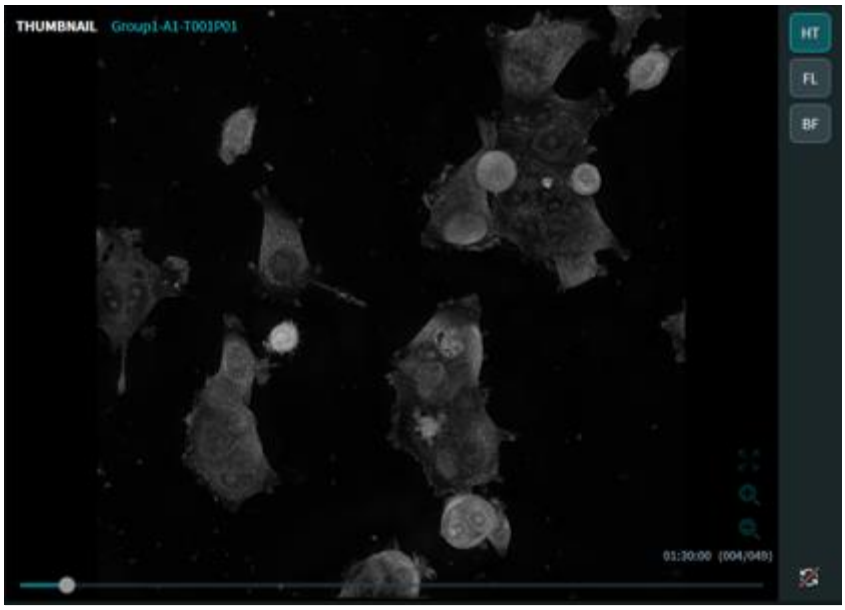
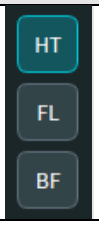


Figure 5.13 Thumbnail viewer

In the Time-Lapse Progress window, the Thumbnail viewer replaces the Preview panel and displays MIP (maximum intensity projection) images of the time frames already acquired. The Thumbnail viewer can show a thumbnail of each imaging point separately, which can be selected in the Imaging Point List panel.

Important! During time lapse imaging, a thumbnail for each time frame will appear in the Thumbnail viewer after the corresponding data is processed. If the viewer does not show a thumbnail, check that the image acquired at the current time frame is processed properly by the HTX processing server. For the processing server, refer to section 6.1 for Image processing.

Parameter	Function	Description
	View HT/FL/BF mode	Shows and toggles between thumbnails of the selected imaging mode.
	Sync ON	The Thumbnail viewer displays in sync with the latest image processed. While the sync mode is enabled, it is not possible to display images of other time frames or other imaging points in the same time frame.
	Sync OFF	When the sync mode is disabled, the Thumbnail viewer can display other images from different time frames or different imaging points.

CHAPTER 6. Image Review

6.1 Image processing

TomoStudioX utilizes a proprietary file format, TCF (Tomocube Common File), for recording the image data. A TCF file stores not only images but also annotation datasets that include multiple types of images such as RI tomograms, MIP, brightfield, and fluorescence images. The RI tomogram represents the 3D RI distribution of a sample, and the 2D MIP shows the highest RI value for each lateral position in the XY plane.

The process to generate a TCF file from the raw image data acquired by the HT-X1 system, called *Image Processing*, is an essential step to visualize HT, FL, and BF. Image Processing is performed by the HTX processing server, which runs as a separate module.

6.1.1 User interface description

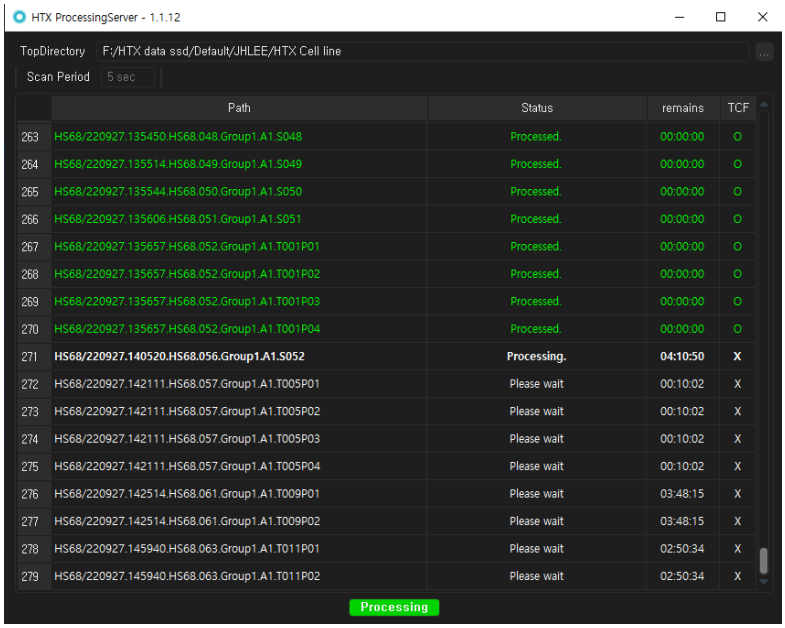
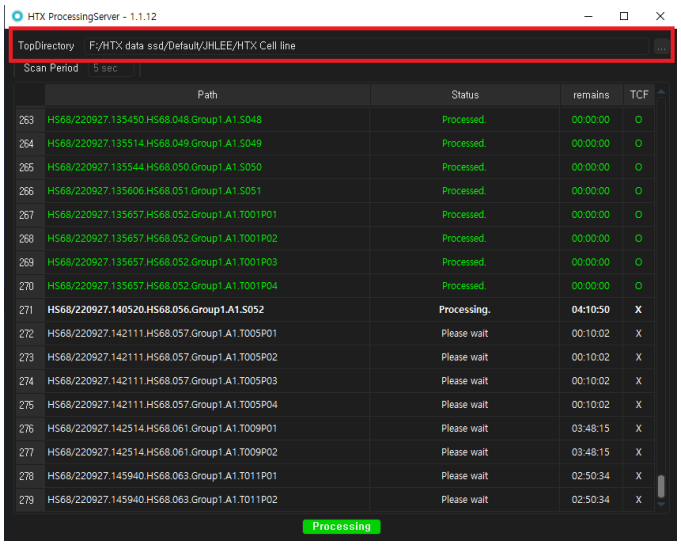


Figure 6.1 Processing server

Parameter	Function	Description
TopDirectory	Top directory path	Displays the path of the top directory to be processed.
Path	Data path	Displays the path of the files to be processed.
Status	Status	Displays the status of image processing.
remains	Remaining time	Displays the required time to complete the processing for each data.
TCF	TCF status	Shows the presence of a processed TCF for each data path.
Processing	Run processing	Starts the image processing at the top directory path specified by the user.

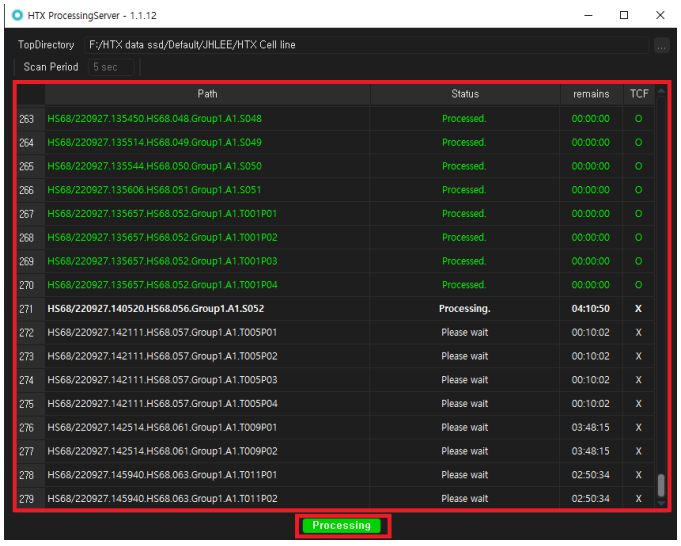
6.1.2 Running image processing

1. Double-click the [HTX processing server.exe] button to start the program.
2. Set the top directory path of the experimental datasets to be processed.



Note: The top directory for processing can be set by drag-and-dropping the desired experiment folder in the HTX processing server window.

3. Check the list of data paths updated in the HTX processing server window. Once the data paths are updated properly, click the [Processing] button to run the image processing.



4. Each data processing status is categorized into five states. Refer to the table below for descriptions of each state.

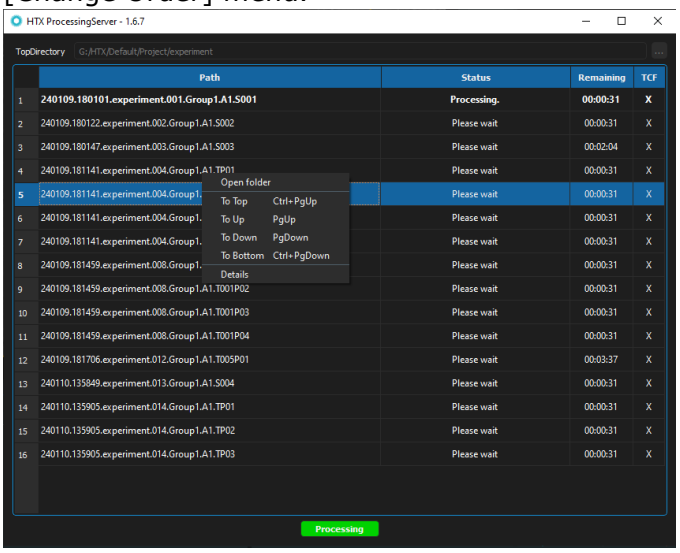
UI	Name	Description
Processing.	Processing	Displayed on the data currently undergoing processing.

Processed.	Processed	Displayed on the data that has completed processing.
Please wait	Please wait	Displayed on the data that has not yet undergone processing.
Data count does not match	Wait for acquisition	Displayed when the processing server is waiting for the next timeframe data acquisition to begin.
PSF Generating	PSF generating	Displayed when generating the Point Spread Function (PSF) for data processing. This occurs typically during the initial processing of uncommon media RI or when the software is updated. It may take under 20 minutes.
Processing failed	Processing failed	Processing failure due to an error.

6.1.3 To change the processing order

To change the processing order and prioritize the processing of specific data for quicker result verification.

1. Right-click on the data you want to change the order for and open the [Change Order] menu.



2. Select the button on the [Change Order] menu to change the processing order. The function of each button is described on the table below.

UI	Name	Description
To Top	To top	Change the priority of the selected data to the top-most position.
To Up	To up	Move the processing priority one step forward.

To Down	To down	Move the processing priority one step backward.
To Bottom	To bottom	Change the priority of the selected data to the last position.

6.2 Data navigation

After the acquired images from the experiment are processed, the data can be reviewed in the Data Navigation screen. The Data Navigation screen can be accessed by clicking the [Go to Data navigation] button located on the bottom-left side of the Experiment Manager window or the Imaging Acquisition window. In the Data Navigation screen, users can view the data obtained from the current user account and browse each image. Three navigation modes are provided for reviewing the experiments: Summary mode, Gallery mode, and List mode. These modes can be selected by clicking the corresponding tabs located under the Project Description panel.

6.2.1 Mode description

Summary mode

In the Summary mode, brief information is displayed about the experimental settings, such as the name of the experiment, the RI of the medium, the vessel type, and the settings for each group/well. The Summary mode also shows the number of imaging points and the last settings used for time-lapse imaging.

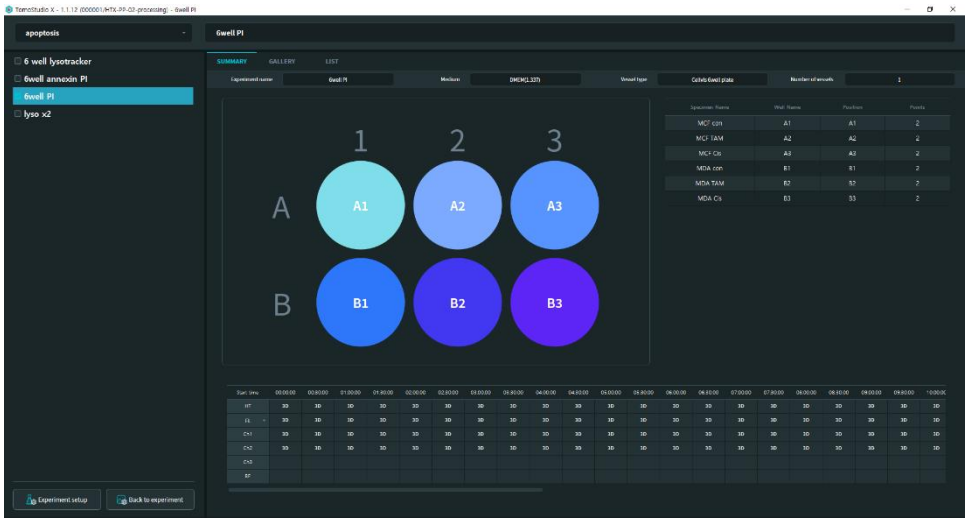


Figure 6.2 Data navigation (Summary mode)

Gallery mode

The Gallery mode displays all the data in the current experiment data path as 2D MIP images from the 3D RI tomograms.

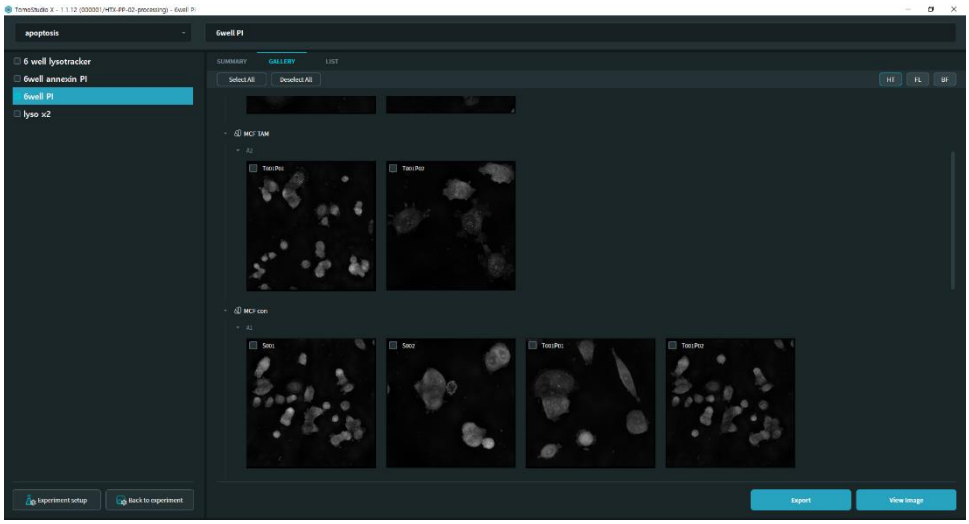


Figure 6.3 Data navigation (Gallery mode, HT view)

The displayed image type can be switched by selecting the desired imaging mode located on the top-right side of the window.

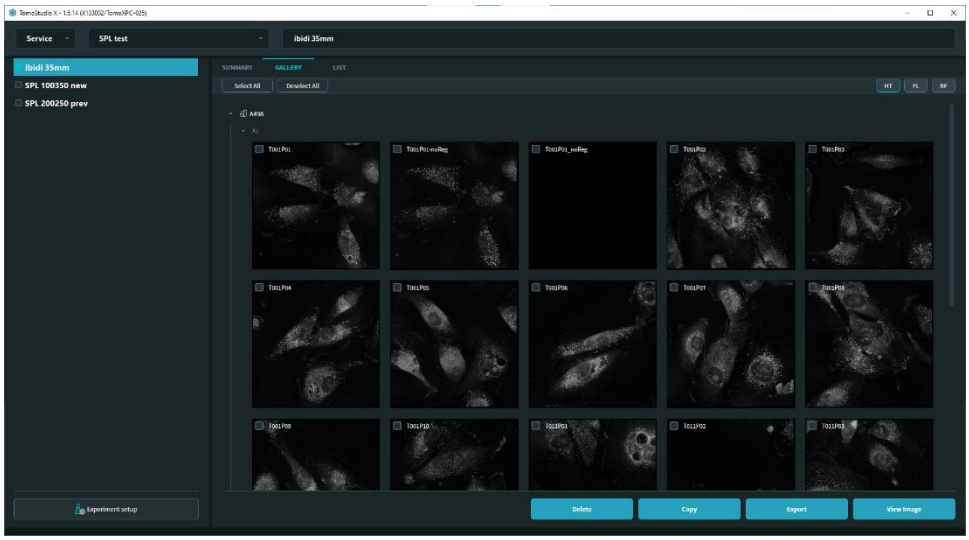


Figure 6.4 Data navigation (Gallery mode, FL view)

Note: If a data file listed in the Gallery mode does not display an image of the selected mode, the data file is indicated as ‘no FL’ or ‘no BF’.

Information of a single data file can be checked by double-clicking the MIP image thumbnail. A pop-up window appears that contains the following file information: image type, imaging mode(s), fluorescence conditions, and location of the imaging points.

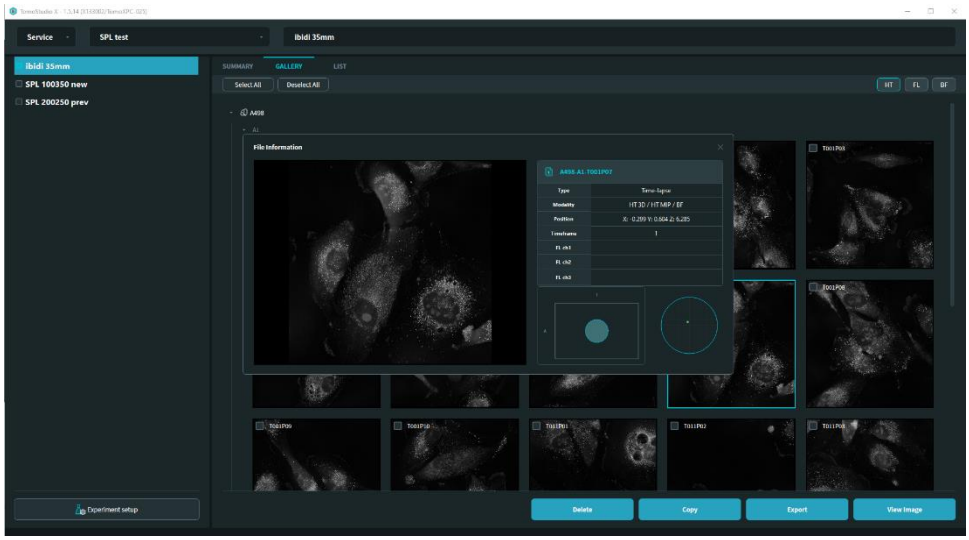


Figure 6.5 Data navigation (Gallery mode, file information window)

List mode

The List mode shows the acquired data files as a list in table format with brief information, such as the specimen/well name, the nature of the data regarding the time-lapse and the dimension, and the point ID. This mode is useful to review an experiment containing large number of data files in a single view. By double-clicking a data file in the list, all relevant file information can be reviewed, as in the Gallery mode.

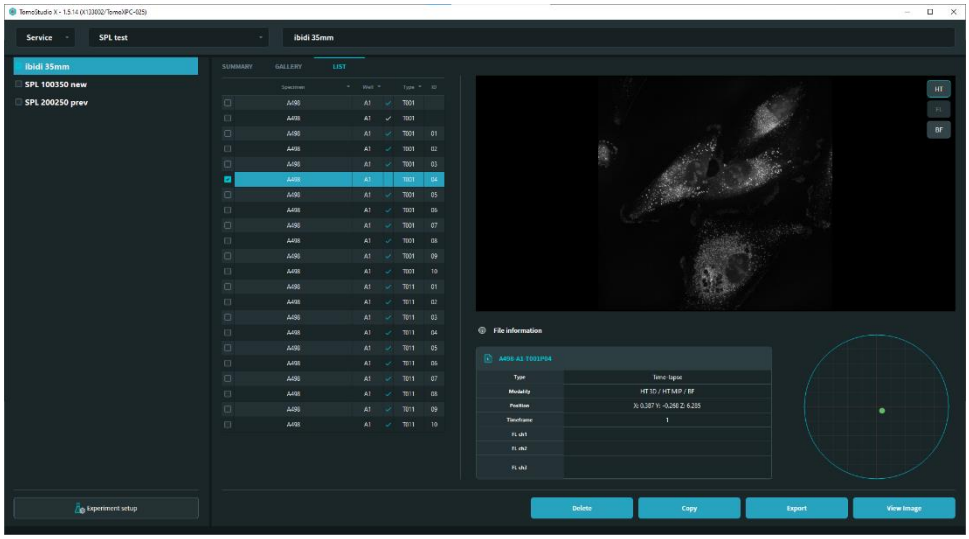
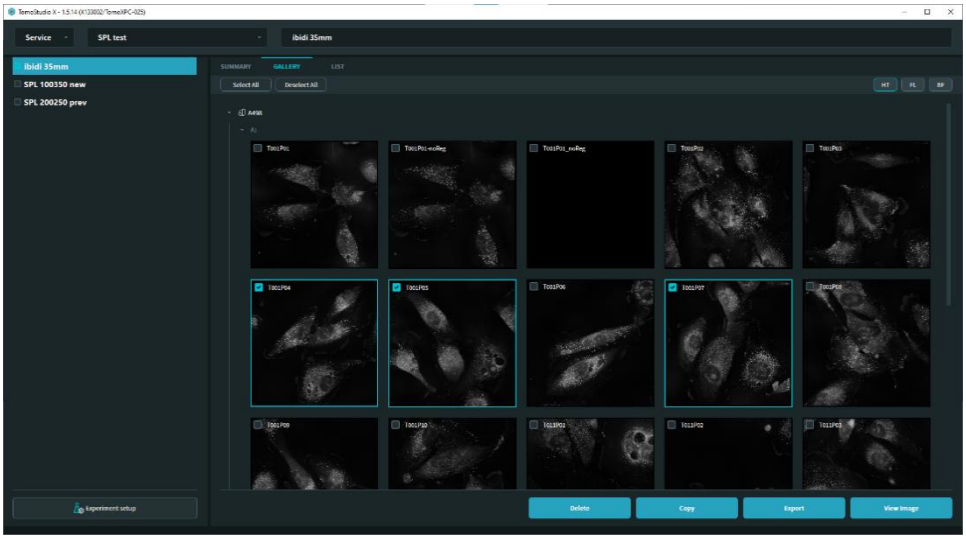


Figure 6.6 Data navigation (List mode)

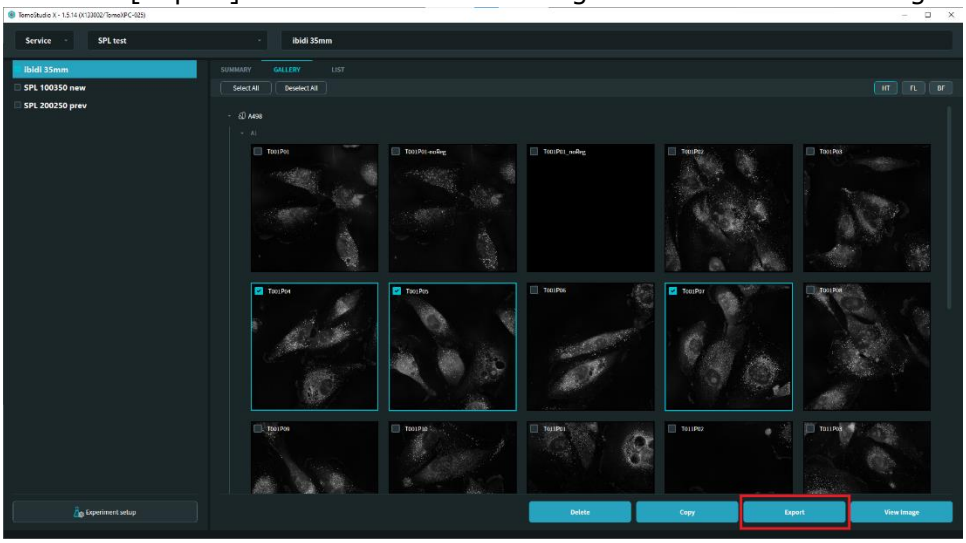
6.2.2 Data export as RAW file

To analyze the data files acquired from the HT-X1 system with a third-party program such as ImageJ or MATLAB, the data can be exported in RAW file format from the Data Navigation screen in TomoStudioX.

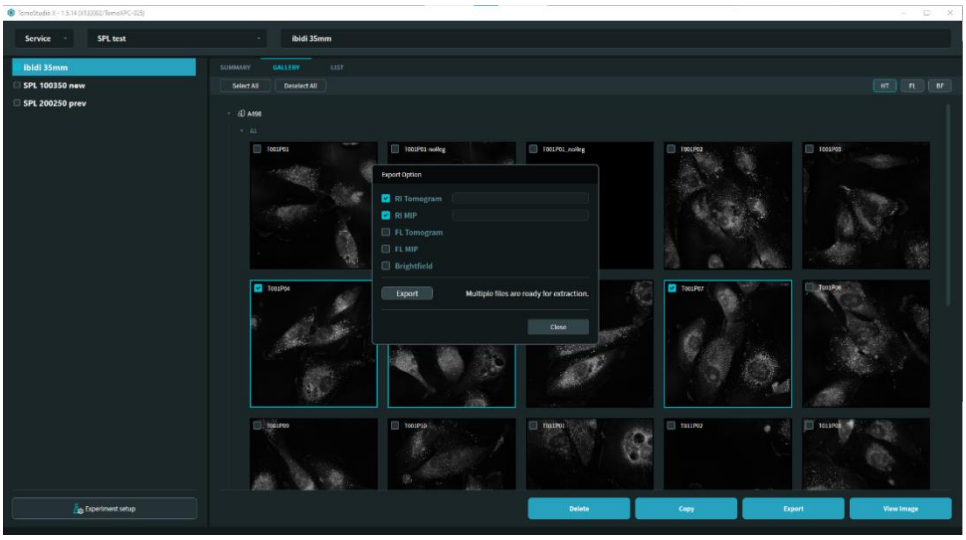
1. In Gallery mode or List mode, click the data file to be exported.



2. Click the [Export] button on the bottom-right side of the Data Navigation screen.



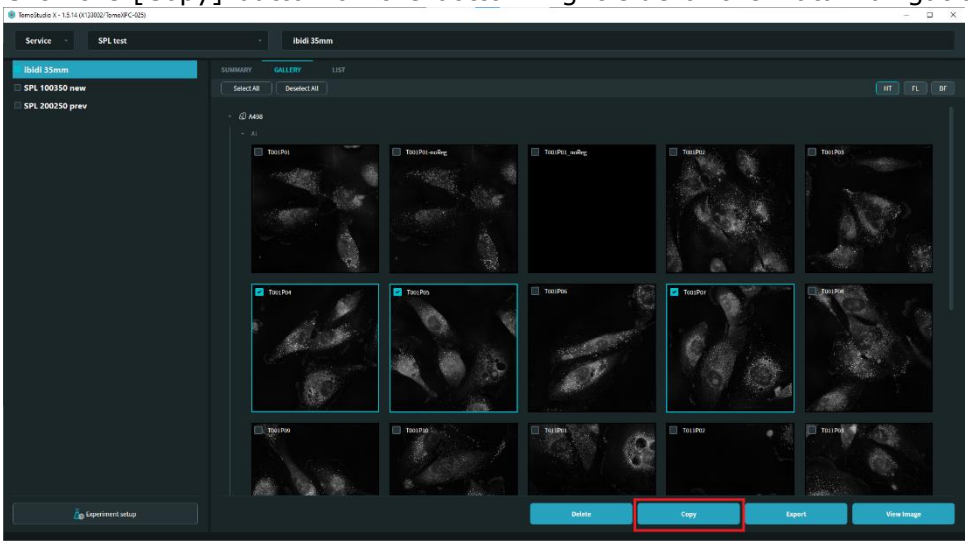
3. In the pop-up window, check the imaging modes to export from the file. Then click the [Export] button.



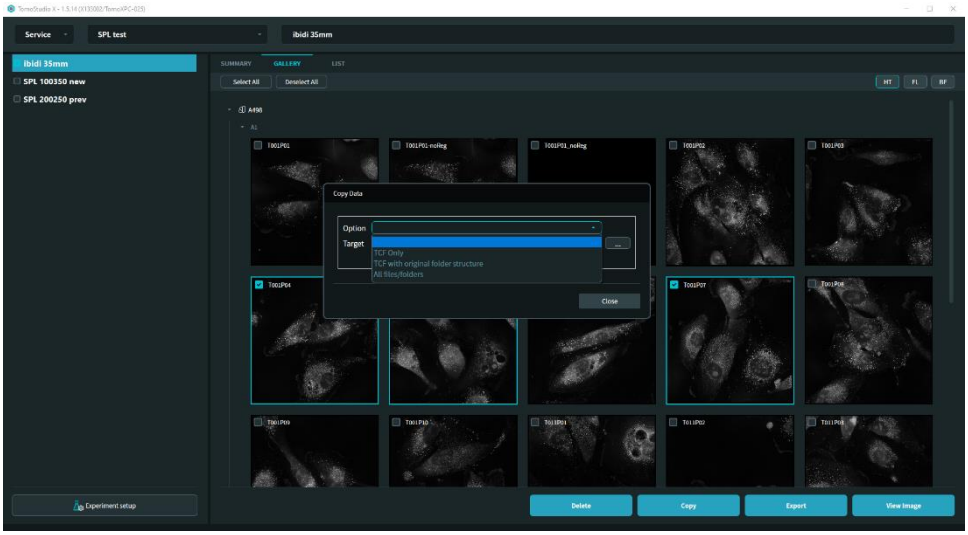
6.2.3 Managing data in Data Navigation: Copy

Acquired data stored can be copied to other folders or external devices by using the Data Navigation function.

1. In the Gallery or List mode, select data files to be copied.
2. Click the [Copy] button on the bottom-right side of the Data Navigation screen.



3. In the pop-up window, select an appropriate option from the drop-down menu. There are three options available, each with a different scope of action:

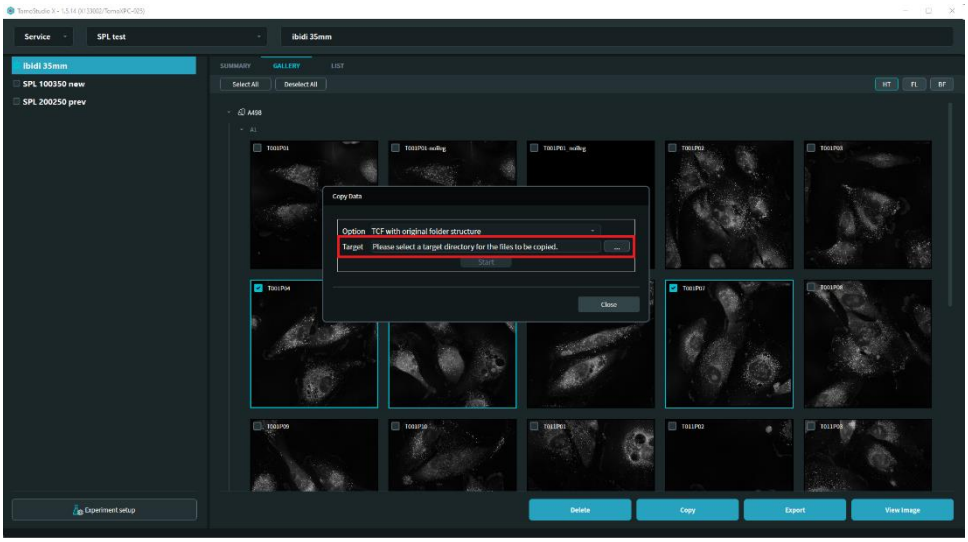


   A. TCF only: It copies only the selected TCF files to the same target location, excluding their configuration files and the folder structure.

   B. TCF with original folders structure: It copies the selected TCF to the target location files while keeping their associated experiment and project information as well as the folders.

   C. All files/folders: It duplicates the whole data and folders of the selected TCF files to the target location, including their raw files.

4. Designate the target location to copy.

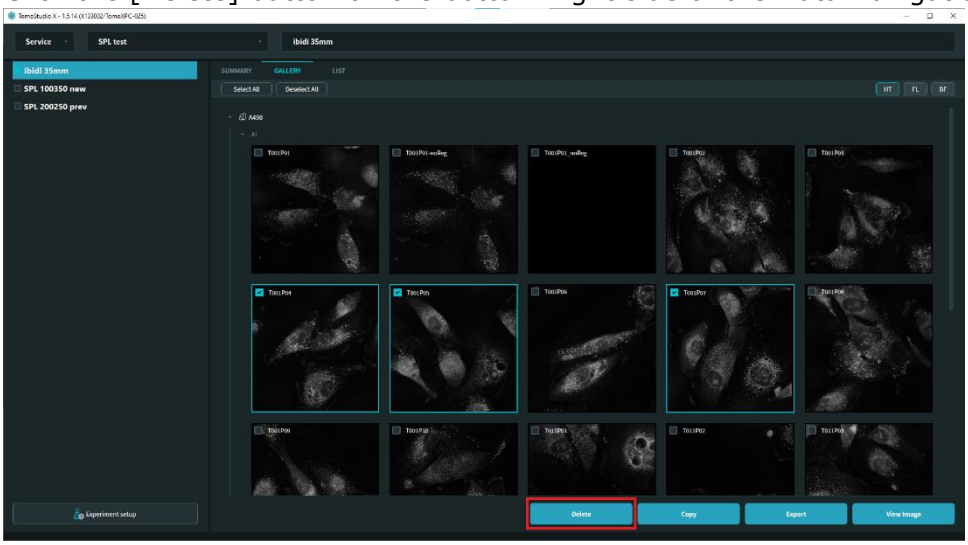


5. Click the [Start] button to initiate the procedure.

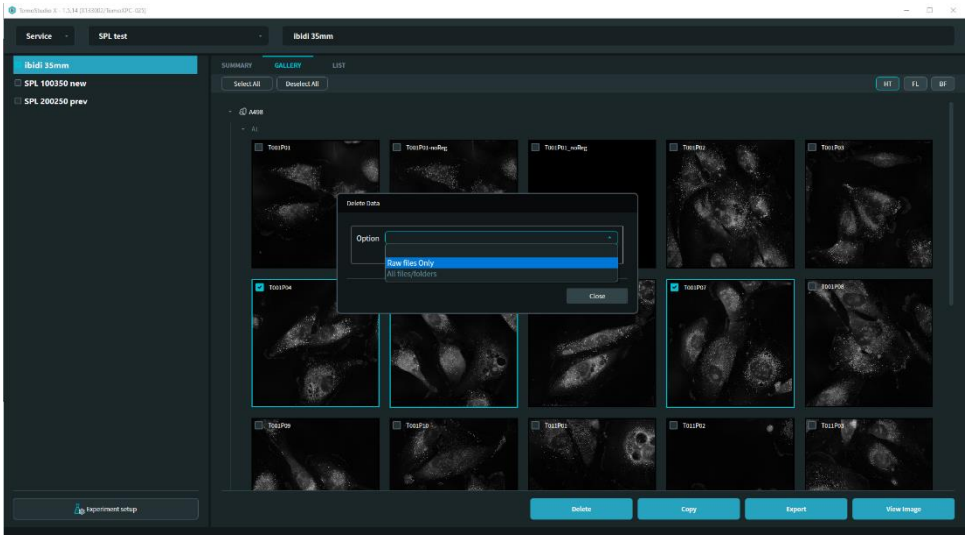
6.2.4 Managing data in the Data Navigation: Delete

Unnecessary data can be removed from the Experiment using the Data Navigation function.

1. In the Gallery or List mode, select data files to delete.
2. Click the [Delete] button on the bottom-right side of the Data Navigation screen.



3. In the pop-up window, select an appropriate option from the dropdown menu. There are two options available, each with a different scope of action:



A. Raw files only: It removes the raw files of the selected TCF files only, while keeping all the other files.

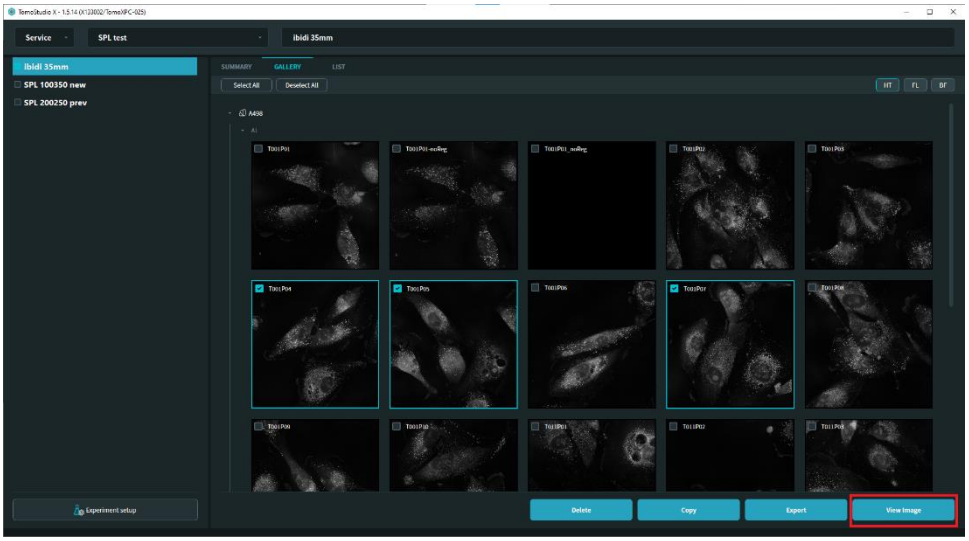
B. All files/folders: It removes all the data and folders of the selected TCF files, including the raw files, thumbnails, and the other related folders as well as the TCF themselves.

4. Click the [Start] button to initiate the deletion with the chosen option.

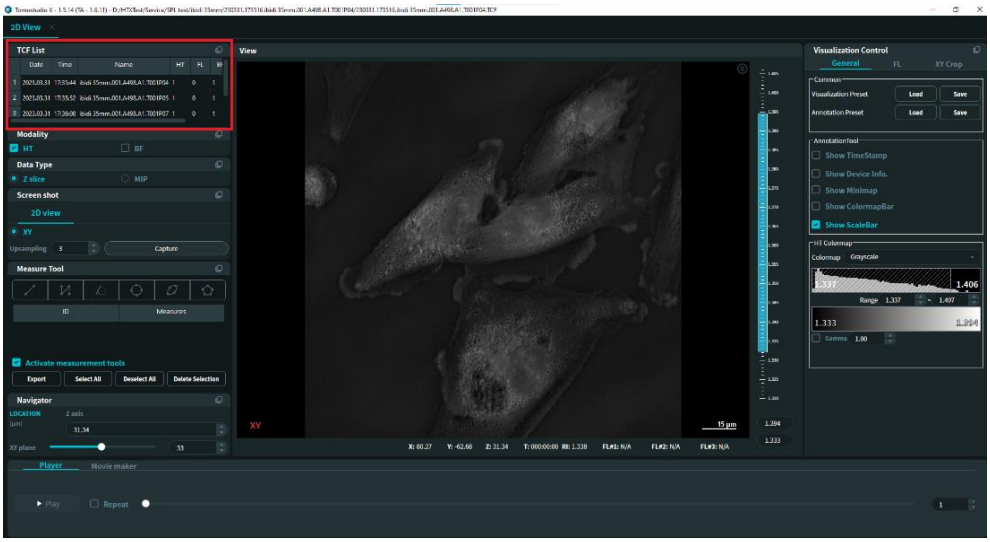
6.2.5 To view TCF files in TA viewer

To examine the TCF files in detail, you can use the “View image” function of the Data Navigation to open TCF files directly in TA viewer. TA viewer visualizes 3D slices, MIPs, and performs simple post-processing or the 3D visualization of the TCF file.

1. In the Gallery or List mode, select the data files to view in TA viewer.
2. Click the [View image] button on the bottom-right side of the Data Navigation screen to start TA viewer.



3. TA viewer appears and the selected TCF files will be listed on the work list of the 2D View screen of TA viewer.

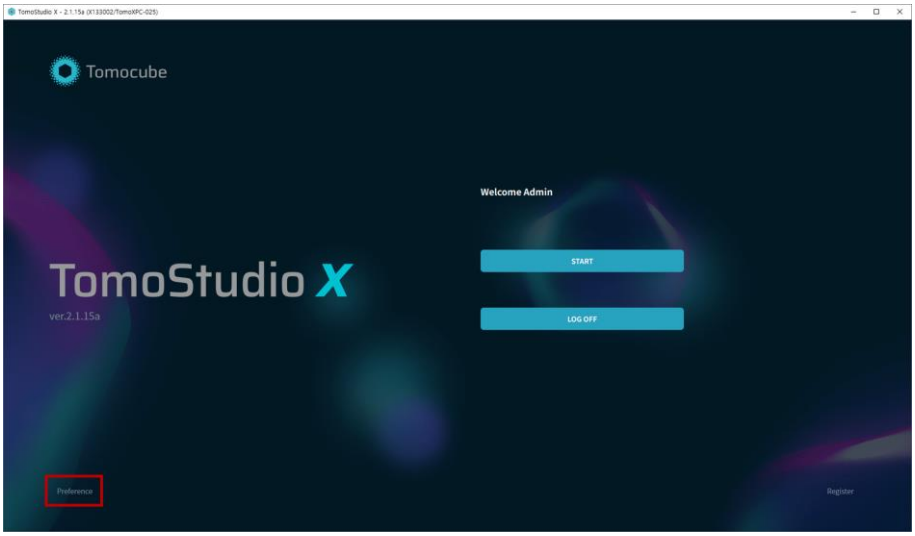

2D View
TCF List
Date
Time
Name
HT
FL
BF
Modality
HT
BF
Data Type
Z slice
MIP
Screen shot
2D view
XY
Upsampling
Capture
Measure Tool
ID
Measures
Activate measurement tools
Export
Select All
Deselect All
Delete Selection
Navigator
LOCATION
Z axis
XY plane
Player
Movie maker
Play
Repeat
View
XY
15 μm
Visualization Control
General
FL
XY Crop
Common
Visualization Preset
Load
Save
Annotation Preset
Load
Save
Annotation Tool
Show TimeStamp
Show Device Info.
Show Minimap
Show ColormapBar
Show ScaleBar
HT Colormap
Colormap
Grayscale
1.406
Range
1.337
1.497
1.333
1.294
Gamma
1.00

CHAPTER 7. Preference

Changing Experimental Settings in the Preferences Menu

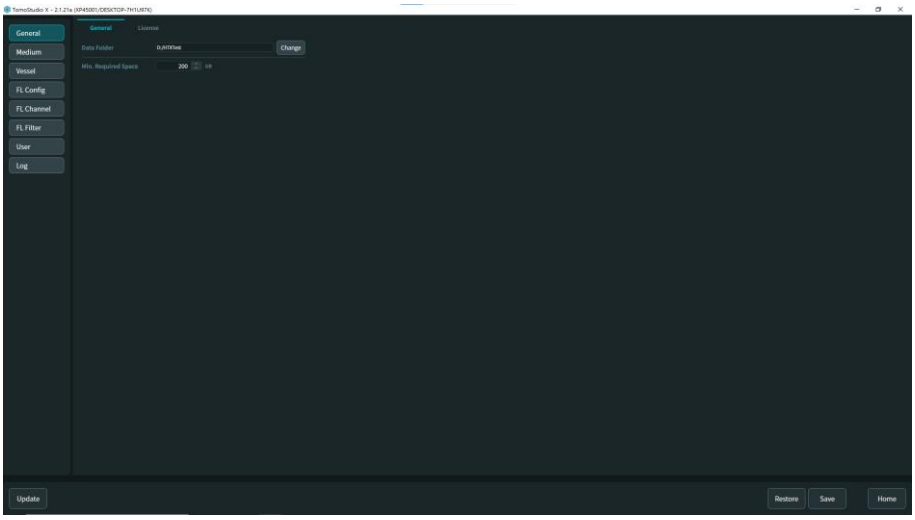
Modifications to key experimental settings—such as data storage path, fluorescence channel configuration, medium RI values, and vessel information—can be made through the Preferences menu. These settings are only editable by users with Administrator. If you would like to change any of these settings, please contact your system administrator.

To access the Preferences menu, log in with an Administrator account and click Preferences in the bottom-left corner of the main window.



7.1 General

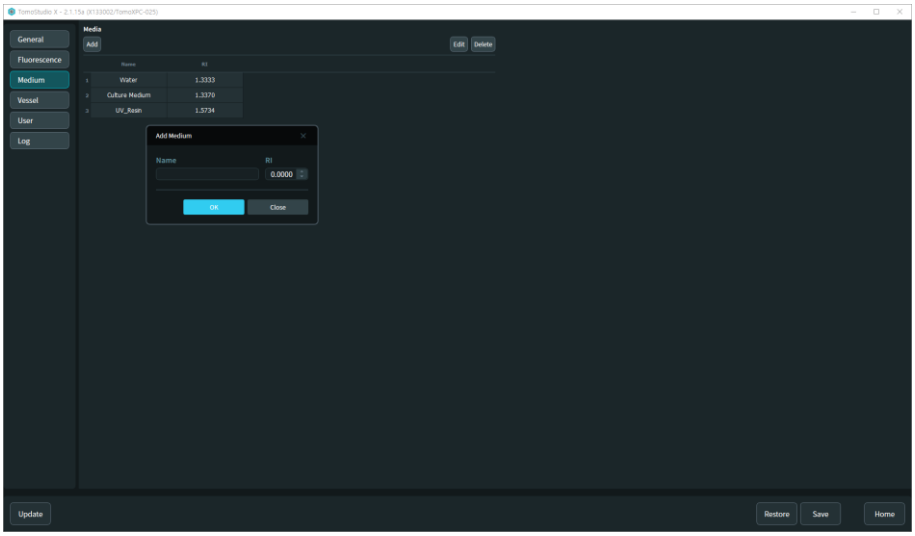
In the General tab, you can change the default data storage path. The initial default save location is set to the D: drive. After making changes, make sure to click [Save] at the bottom right corner Save before returning to Home Home.



Important! Do not set a portable USB drive as the data save location. Slow transfer speeds can cause system malfunctions or failed image acquisition.

7.2 Medium

You may update or add new medium RI values as required for your samples. As always, click [Save] after any modification, and return to the Home.



7.3 Fluorescence

TomoStudioX allows users to configure fluorescence imaging settings through three main menus under Preferences:

FL Channel, FL Config, and FL Filter.

- FL Config:

  Manage the specifications of the four components that constitute the fluorescence imaging path — light source, excitation filter, emission filter, and dichroic mirror. This menu allows users to register or update these elements as needed for their system.

- FL Channel:

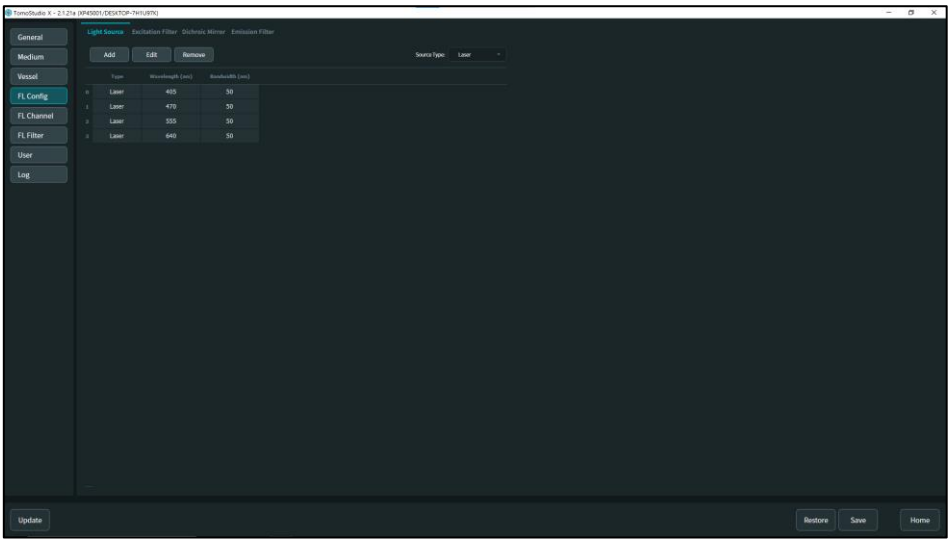
  Create and manage fluorescence channels by combining the four components defined in FL Config, along with a designated channel index. These channels are then used in fluorescence imaging experiments.

- FL Filter:

  Provides a management interface for each filter type by filter wheel position and supports physical replacement operations if applicable.

Each submenu is explained in detail in the following subsections.

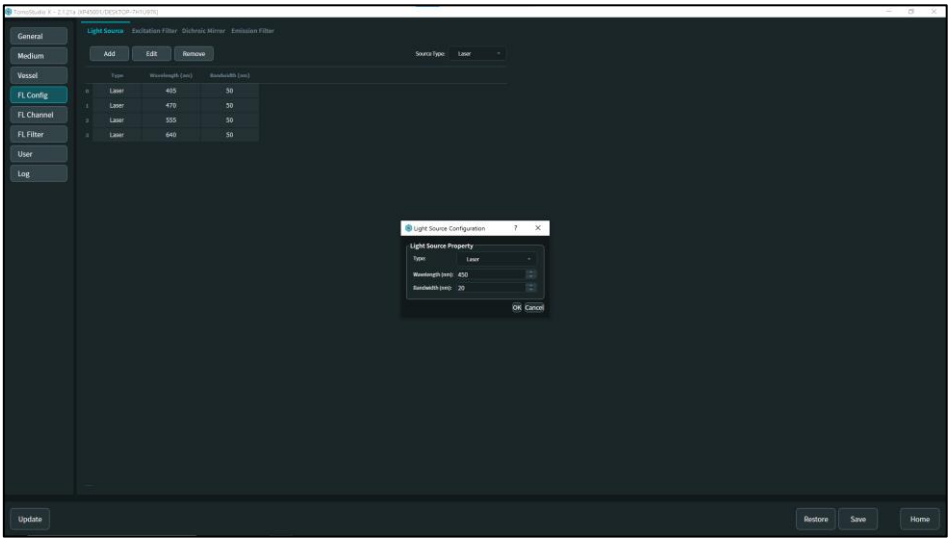
7.3.1 FL Config



The FL Config menu allows users to input and manage the specification details for the four core components of the fluorescence optical path: Light Source, Excitation Filter, Emission Filter, and Dichroic Mirror.

Each component can be accessed via the sub-menu located in the upper-left corner of the FL Config window.

Light Source

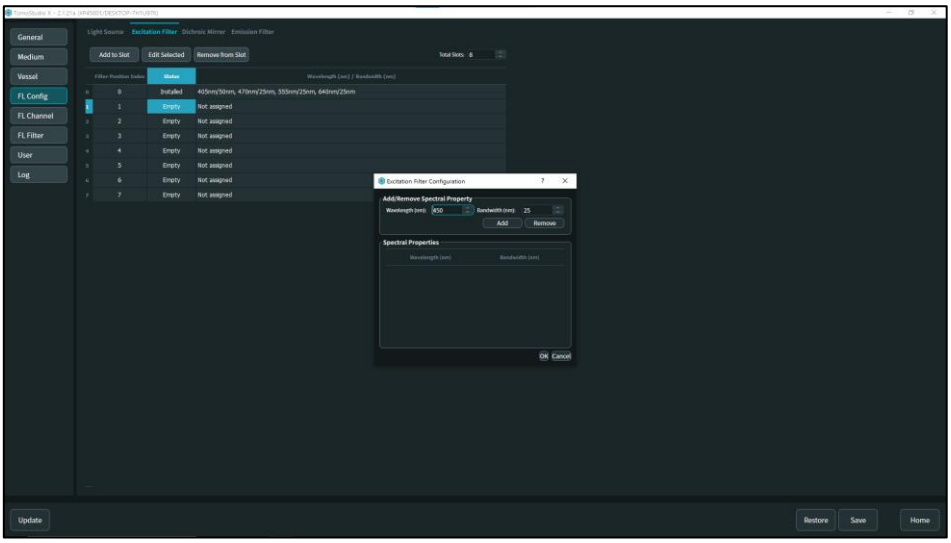


For each light source, users can specify the light type (LED or Laser), center wavelength, and bandwidth.

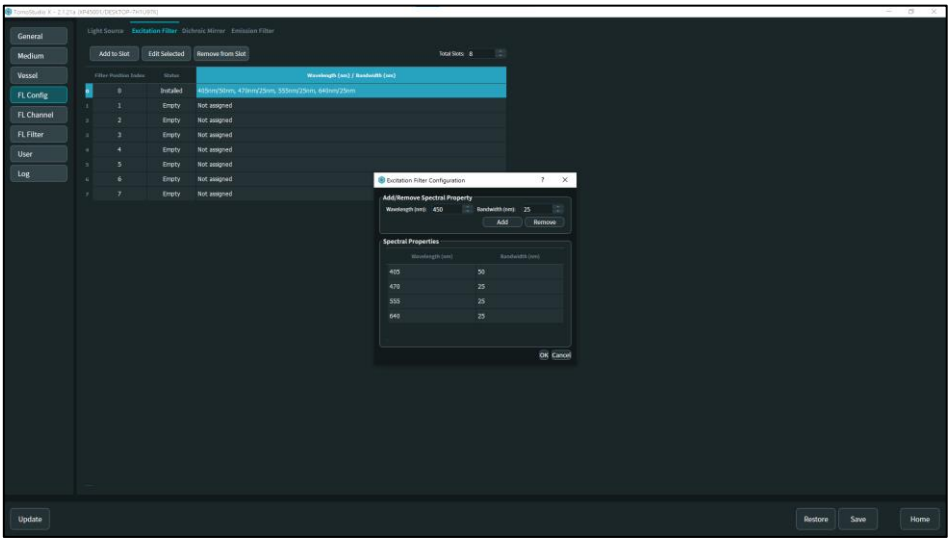
Note: In most systems, LED is used as the default light source. However, in systems equipped with an external spinning disk confocal (SDC) module, laser sources are used. Incorrectly setting the light source type may cause system malfunction.

Note: The number of light sources available is determined by the hardware configuration of the system.

Excitation Filter / Emission Filter / Dichroic Mirror



For each filter component, users can register wavelength and bandwidth values corresponding to the filter installed at each physical position in the filter wheel.



When using multi-band filters, multiple wavelength ranges can be entered for a single filter position.

7.3.2 FL Channel

The FL Channel menu allows users to create, configure, and manage individual fluorescence channels used during acquisition and processing. Each channel is defined by a specific combination of optical components and settings, as detailed below:

Channel Components

1. Channel Name

   User-defined name for the fluorescence channel (e.g., DAPI, GFP, etc.).

2. Light Source / Excitation Filter / Emission Filter / Dichroic Mirror

Optical components selected from the FL Config list. Each channel must be configured using pre-registered component specifications.

3. Color

   Designated display color for the channel in processed images and visualizations.

4. Channel Index

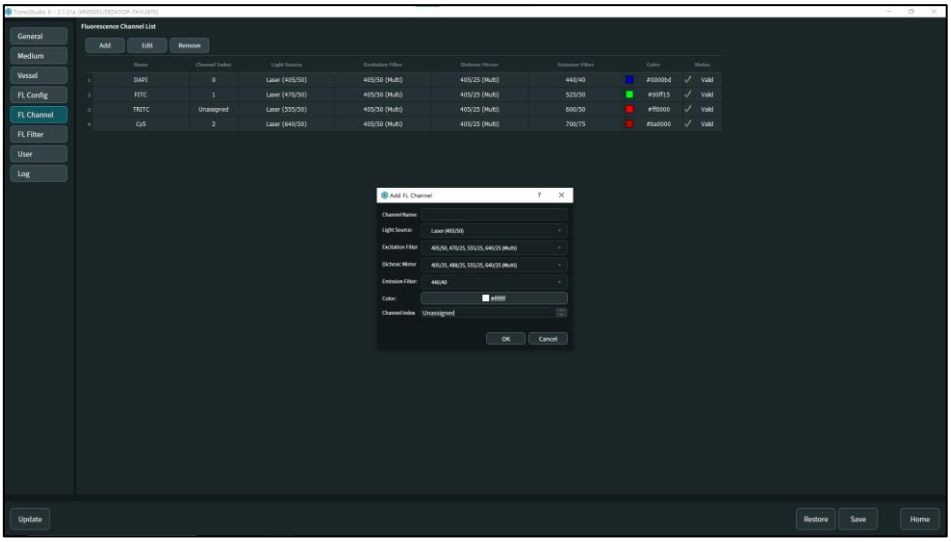
   Index number that maps the fluorescence channel to the corresponding light source.

   - Each light source must be assigned a unique channel index.
   - Multiple channels using the same light source must share the same index.

Note: The order of channel indices must follow ascending wavelength order. The channel with the lowest-wavelength light source must be assigned index 0, and subsequent indices should follow the increasing wavelength sequence.

Note: Channel index limits may vary depending on the system configuration. For example, in systems equipped with an external SDC fluorescence module, a maximum of three channels (index 0 to 2) may be supported.

Note: Different light sources must not share the same channel index.



Changing Light Source Wavelength with Channel Index (for SDC Systems)

In systems equipped with an external SDC module, the number of available light sources may exceed the number of supported channel indices. In such cases, switching between light sources requires physically changing the cable connection along with selecting the appropriate FL channel configuration in the software.

To change the FL channel with a different light source (wavelength), follow the steps below:

1. Connect the fluorescence cables to the TTL input ports on the breakout box.

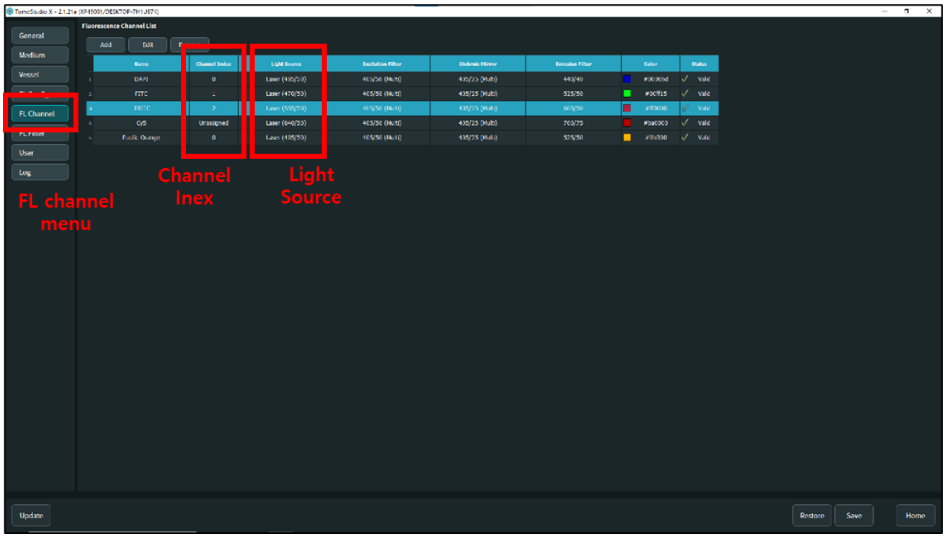
   Connect the cables in ascending order of wavelength (from shortest to longest):

   - Cable 0 → lowest wavelength
   - Cable 1 → middle wavelength

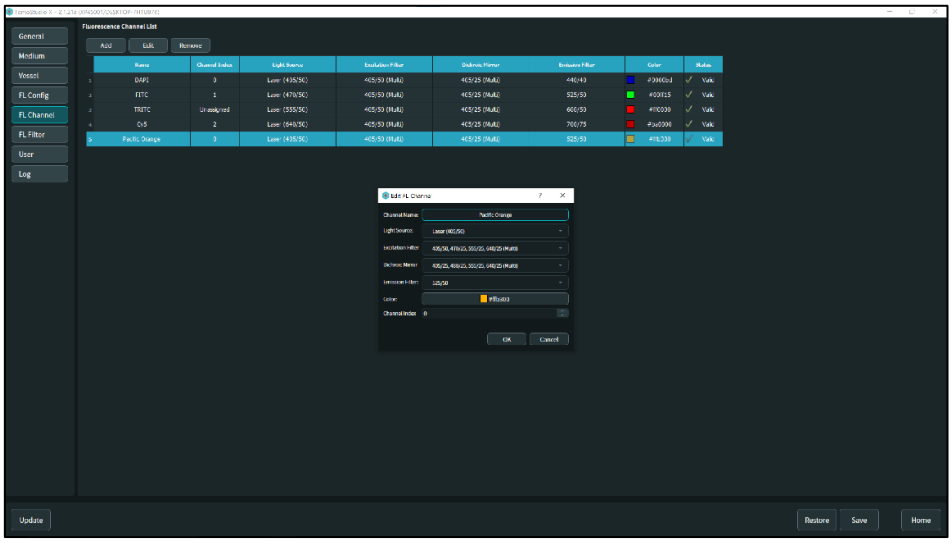
- Cable 2 → highest wavelength

Important! The cables must be connected in order of increasing wavelength. Incorrect wiring may cause the fluorescence channels to function improperly.

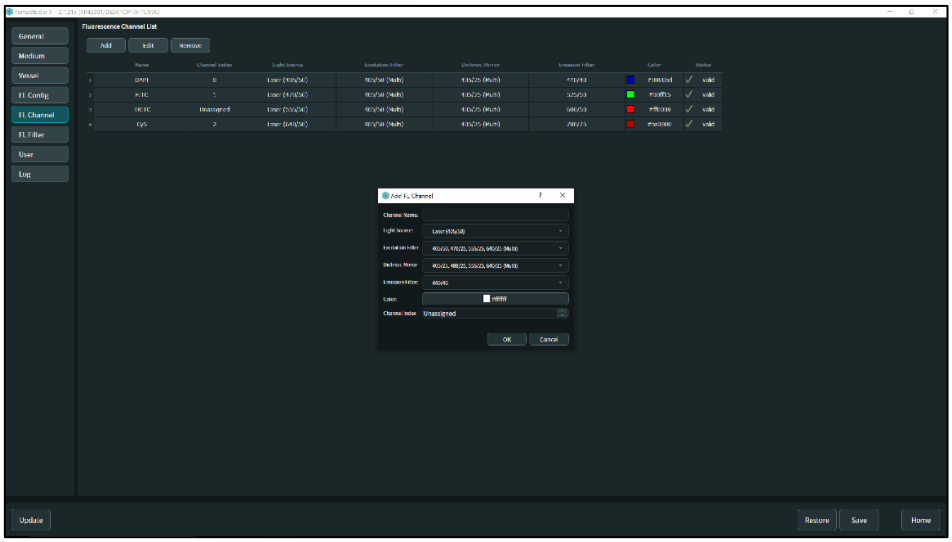
2. In the FL Channel page, assign the correct cable index to each fluorescence channel.
   Proceed in order of increasing wavelength. For each channel, select it from the list and click the Edit button at the top-left corner.



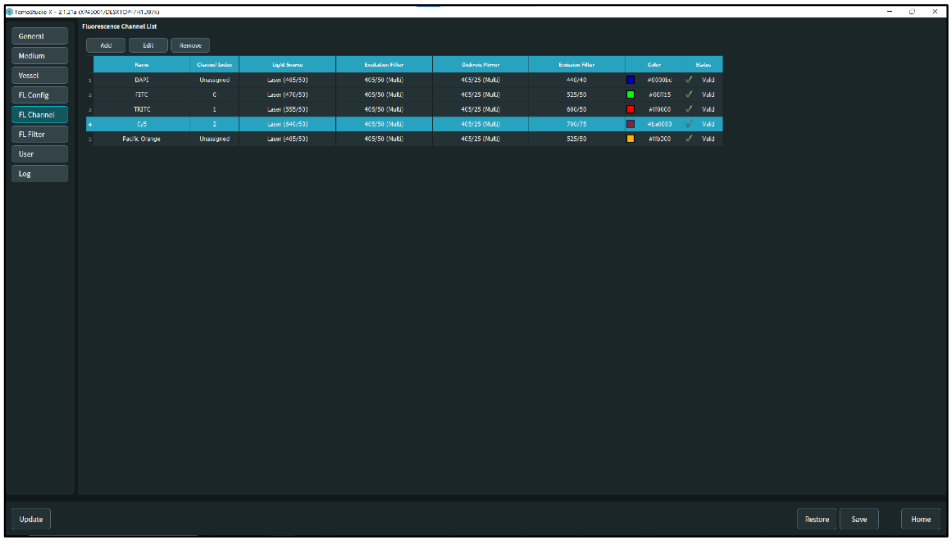
3. In the channel edit popup, select the appropriate light source (laser wavelength), then use the up/down arrows in the *Channel Index* field to set the corresponding cable index, based on the port connection.



4. For any fluorescence channel that is not in use, set its Channel Index to “Unassigned.”
   This can be done by pressing the down arrow until “Unassigned” appears.



5. Once all channels are configured, the Channel Index assignments should follow this rule: Among the four available wavelengths, three should be assigned as 0, 1, and 2, and one should remain Unassigned.



6. Click the Save button, then exit and restart TomoStudioX to apply the changes. After restarting, the selected three fluorescence channels will operate normally with the new configuration.

7.3.3 FL Filter

The FL Filter menu provides both the specification management and physical replacement interface for each filter component used in fluorescence imaging. Users can manage the installed filters and initiate replacement procedures where supported.

Available Functions:

- View and modify the specifications (wavelength, bandwidth, etc.) of filters assigned to each position.

- Perform physical filter replacement (if supported by the system configuration).

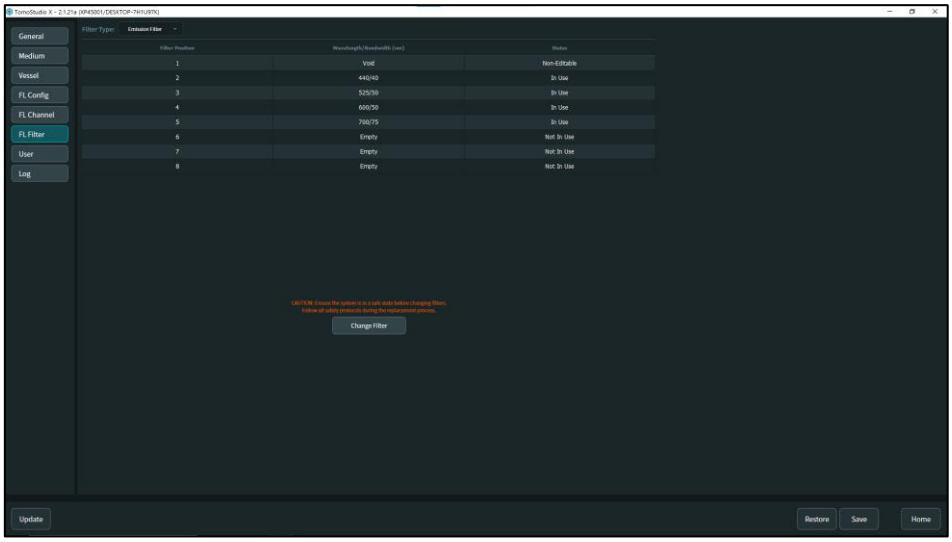
Important! For emission filters, Position 1 must always be left empty (void). Installing a filter in this position may cause system malfunction or optical interference.

Important! The filter replacement function is only supported on systems equipped with an SDC module. For other system configurations except SDC, physical filter replacement should be performed manually, without using this software function.

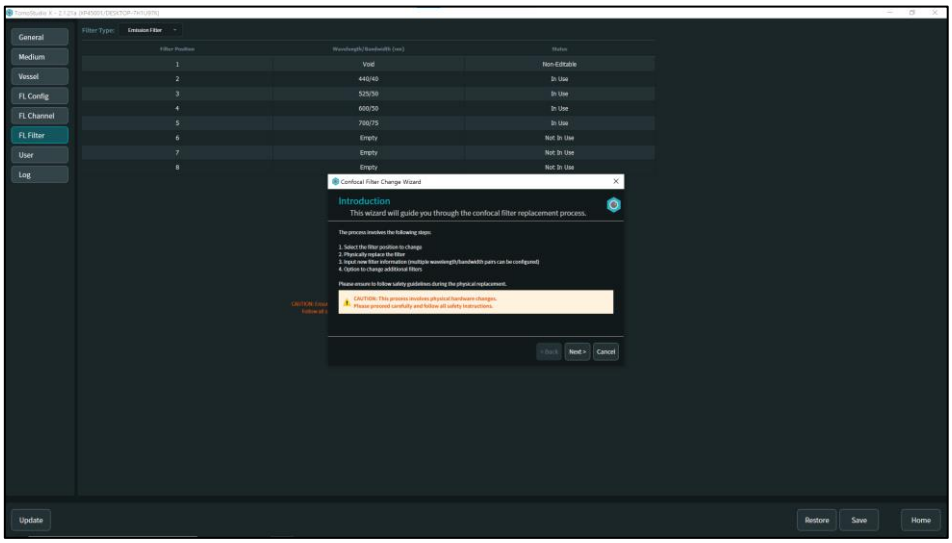
Filter Replacement Guide (For SDC Configuration)

On systems equipped with a SDC module, users can perform filter replacement using the software-guided procedure. Please follow the steps below:

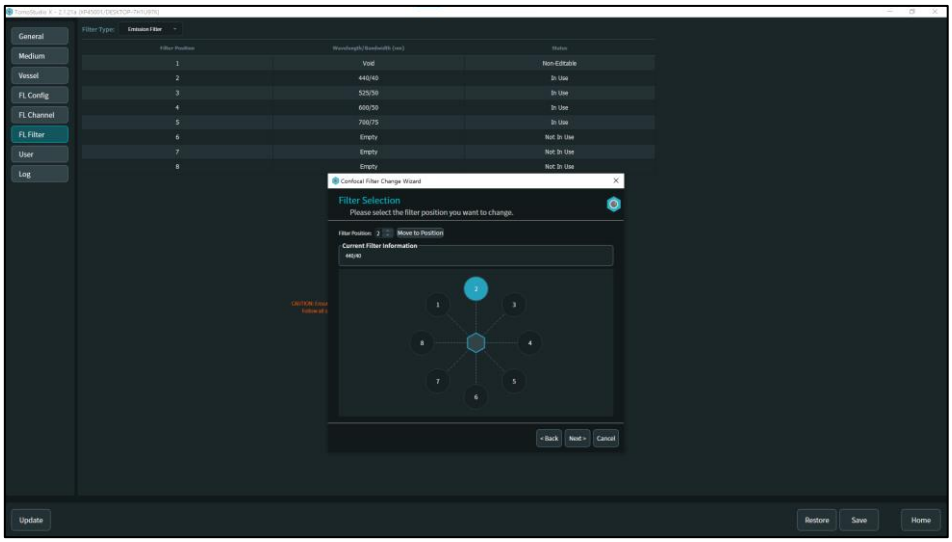
1. Click the [Change Filter] button located at the center of the screen.



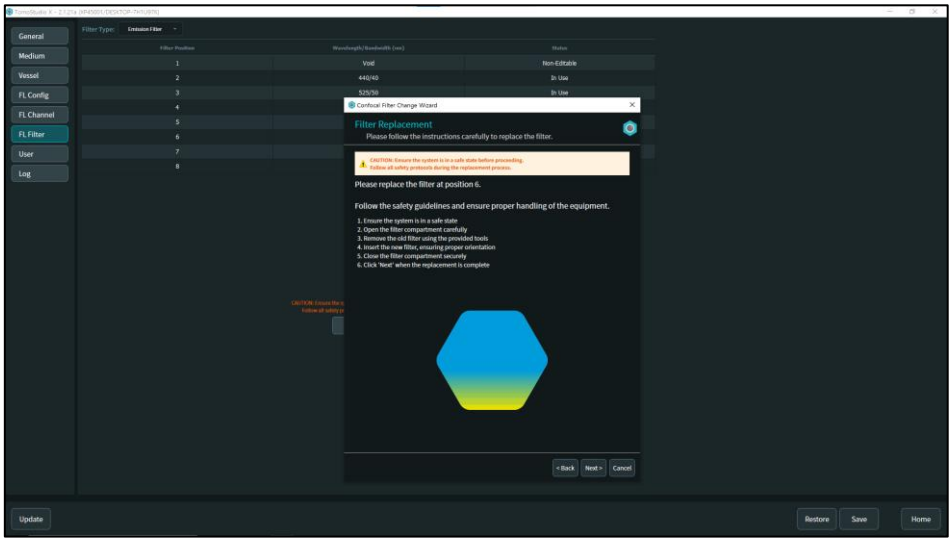
2. A Confocal Filter Change Wizard window will appear. Click [Next] to proceed.



3. Select the target filter position to be changed and click [Move to Position].

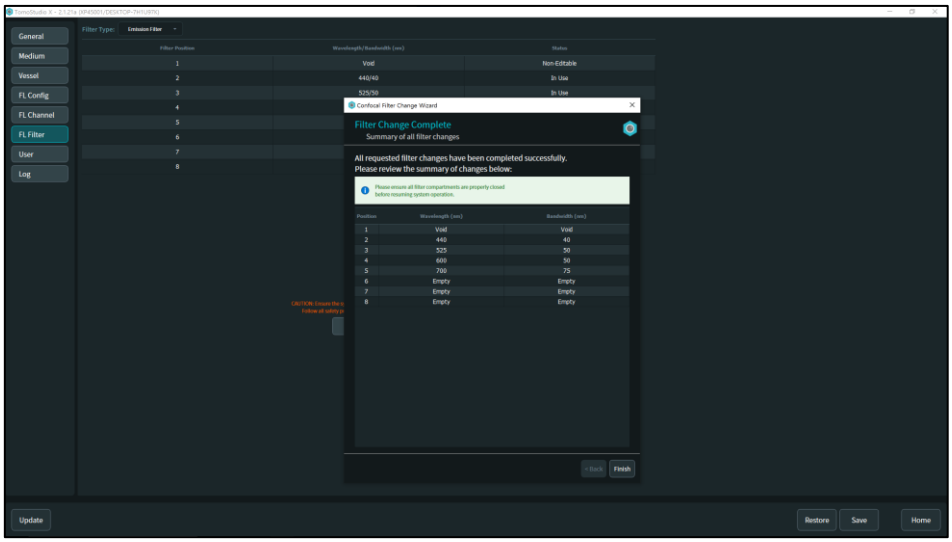


4. The system will rotate the filter turret to the selected position, making it physically accessible. Then, follow the instructions shown in the pop-up window to replace the filter manually.

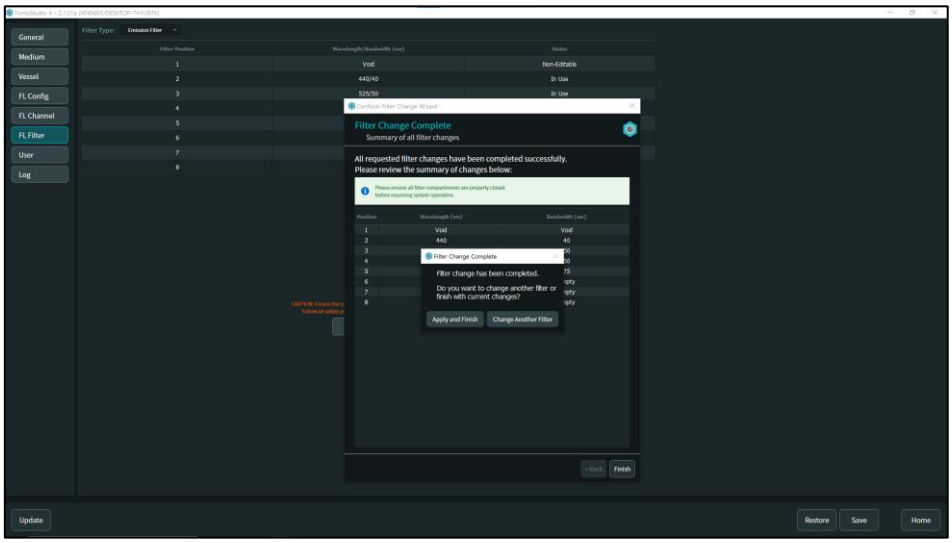


Note: For detailed mechanical instructions, refer to the Hardware Manual – Filter Replacement Section.

5. After the filter is replaced, input the corresponding wavelength and bandwidth values for the new filter.



6. Review the entered specifications and click [Finish]. To replace another filter, click [Change Another Filter] and repeat the process.

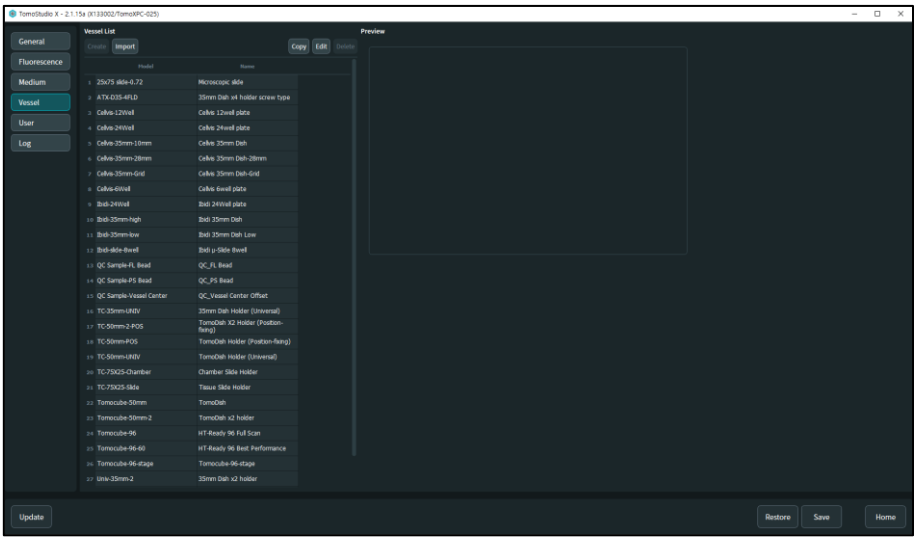


Important! Always confirm that the physical filter has been properly installed before completing the procedure.

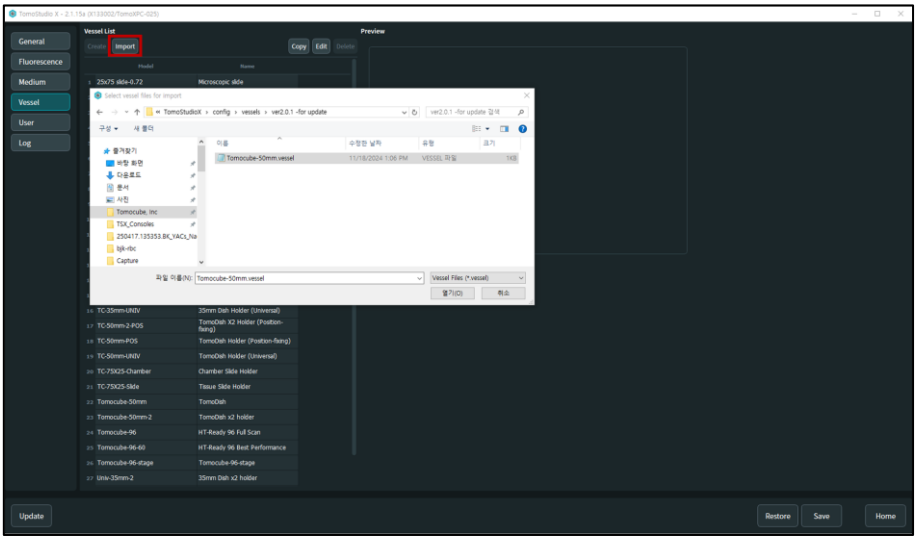
7.4 Vessel

In the Vessel tab, you can view the complete list of all vessels supported by HTX. If your experiment requires a new or custom-made vessel not listed, you can import the corresponding vessel file in this menu.

Note: To use a new vessel, please contact Tomocube HQ. The file will be reviewed and provided if deemed compatible.

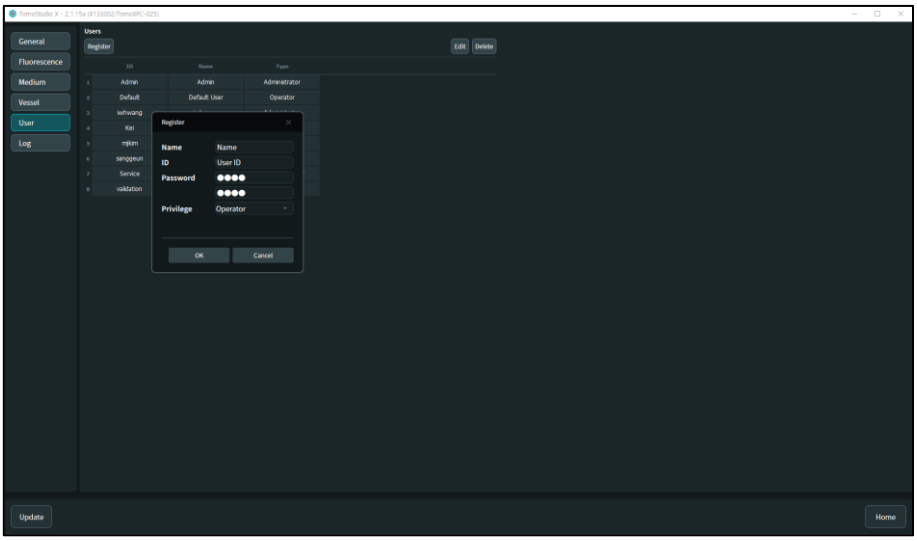


Administrators can add a new vessel by clicking [Import] and selecting the appropriate file. After importing, click [Save] and return to Home.



7.5 User

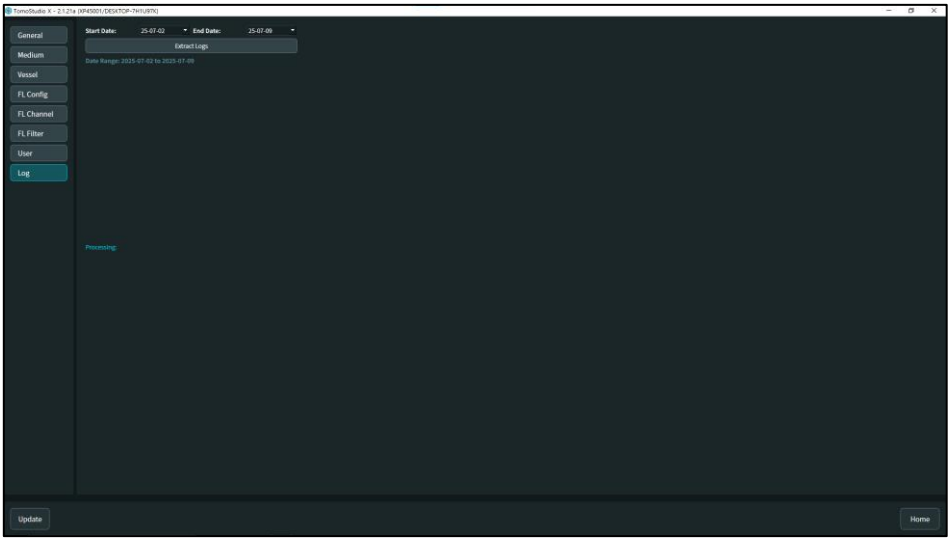
The Preferences menu also displays a list of all registered users and their privileges. Administrators can create new user accounts using [Register] and can [Edit] or [Delete] existing users as needed.



7.6 Log

The Log menu allows users to export usage logs automatically generated by TomoStudioX. Users can specify a desired time range for log collection and save the selected data as a file. This function is particularly useful when sharing logs with technical support engineers during troubleshooting or when reporting unexpected issues encountered while using the software.

Note: Including logs when requesting support can help engineers diagnose problems more accurately and efficiently.



CHAPTER 8. Troubleshooting

8.1 Troubleshooting guide

These troubleshooting guides are designed to help you quickly identify the root cause of unexpected issues and take appropriate corrective actions. By minimizing downtime and ensuring stable system operation, they support efficient maintenance and continuous use.

The table below summarizes common issues and provides troubleshooting guidance for each case.

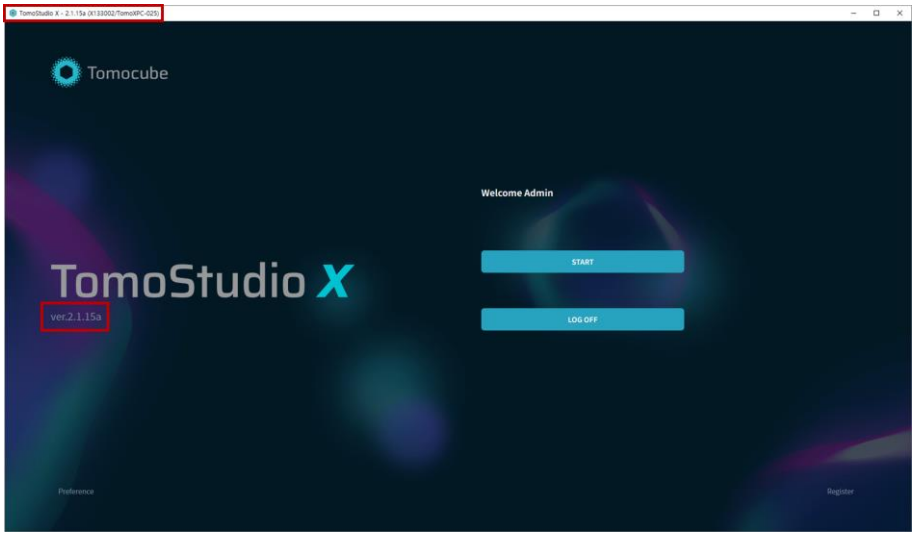
Issue	Possible cause	Solution
The Status LED is illuminated in red.	Camera communication error	Make sure the system connection cable is plugged into a USB 3.0 port on the PC.
	Motion control communication error	Check that there are no foreign objects or materials inside the system. Then, turn the power off and back on.
Calibration failed after sample loading.	The sample is not properly seated and is slightly lifted.	After unloading the stage, check whether the sample is properly seated. If necessary, gently press down to ensure full contact with the chamber bottom.
	Using a vessel with a bottom thickness other than 170 µm (1.5H coverglass).	Using a vessel with a 170 µm (No. 1.5H) optical bottom.
	Incorrect vessel file selected.	Ensure the selected vessel file matches the brand and type of the actual vessel.
Autofocus failure occurs after sample loading.	Trying to focus on a sample located too far above the bottom surface.	Disable autofocus and use the Z Guide feature in TomoStudioX to manually check the maximum focusable height
	Lens contamination or condensation (water droplets on lens)	Clean the lens only under the guidance of Tomocube support.
	Imaging of glass-sandwich structures (e.g., tissue slides)	For tissue slides or glass-sandwich samples, disable autofocus and use manual focus adjustment.
‘Initialize Failed’ warning signs occur in TomoStudioX	System is powered off or the error state was not properly cleared	Power off and then turn the system back on.
	Communication error due to incorrect port connection.	Make sure the system connection cable is plugged into a USB 3.0 port on the PC.
Live view appears dark in the field of view (FOV)	Condensation formed on the dish lid.	Check if the chamber power is on. If it is, ensure the chamber cable is firmly connected.

Processing fails immediately after initiation	Forced cancellation of PSF generation or unexpected software termination	Contact a Tomocube service engineer for further assistance. Recovery may require manual cleanup of temporary files or software reinstallation.
Fluorescence appears as a grid-like pattern in the processed result	Deconvolution was applied to a sample with insufficient fluorescence signal	Increase fluorescence intensity or exposure time during imaging. Alternatively, create a new Sample Type using the "No Decon" option and re-acquire the data.
Processing fails during time-lapse or large dataset reconstruction	Insufficient storage space on the data drive	Ensure enough free space is available on the storage drive and retry processing. High-capacity datasets may require several hundred GB of available space.
Processing fails 30 seconds to 1 minute after initiation	Insufficient free space on the system (C:) drive	Free up space on the C: drive and try again. The processing server uses the system drive for temporary file handling and memory caching.
Time-lapse acquisition stops unexpectedly and a pop-up appears	Autofocus failure or communication error with the device	Check that the vessel is properly mounted and fully seated in the holder. Also verify that the initial focus position is not set too high above the bottom of the vessel, which may prevent successful autofocus.

CHAPTER 9. Support and Contact

9.1 How to check the version of TomoStudioX

When requesting support from Tomocube due to equipment issues or other technical problems, it is often necessary to provide the software version. You can check the version number either below the logo on the main screen or in the title bar of the window.



9.2 Supporting documents

For more information, refer to the following documents.

- HT-X1™ Site Requirement Checklist
- Quick Guide – Organoid Sample Preparation for HT-X1™
- Quick Guide – How to Operate the HT-X1™ Holotomography
- Quick Guide – How to Observe Tissue Slide and Flat Microfluidic Chip with HT-X1™
- Quick Guide – How to Use HT-Ready 96 Well Plate
- Quick Guide – HT-X1™ Compatible Imaging Vessels

9.3 Crash reporting via BugSplat

When TomoStudioX experiences an unexpected crash, a BugSplat error reporting window will automatically appear. Users are encouraged to submit the error report using the [Send Error Report] button to help us identify and resolve the issue as quickly as possible.

To ensure effective support, please include the following information in the report:

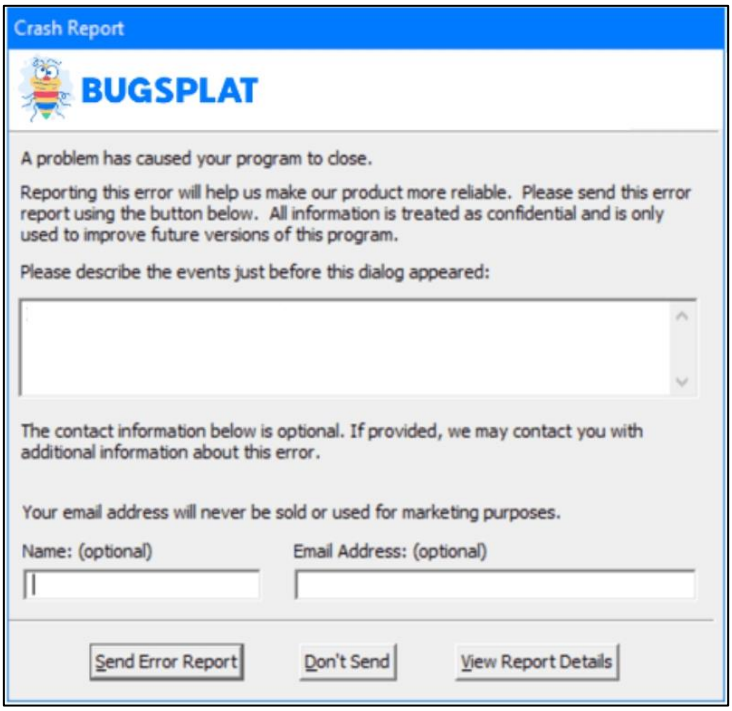
- Affiliated Institution

- Time of Crash Occurrence
- What You Were Doing Just Before the Crash (e.g., starting an experiment, changing vessel settings)
- Your Name or Email Address

  (This will be used as a contact point for follow-up support)

Submitting this information allows our support team to promptly analyze the crash and provide a tailored solution.

Note: All reports are reviewed by our technical team, and follow-up contact will be made when necessary.



9.4 Customer support

If you require assistance, please contact our technical support team and provide the following information to help us respond efficiently:

1. Description of your inquiry or issue
   a. Specify whether the inquiry is related to instrument operation or software usage.
   b. Provide details of any malfunction, error messages, or unexpected behavior.
   c. Describe any specific requests or desired outcomes.
2. Serial number: A 7-character alphanumeric code found on the product label or certificate.

3. Your contact information

Support Channels:

- Technical support team: support@tomocube.com
- Local distributor: https://www.tomocube.com/contacts/distributors/
- Web: www.tomocube.com

For contact information, visit:
https://tomocube.com/about_contact

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