Tomocube

HT-X1
Holotomography System

Operation Manual

Version 1

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

Copyright

Trademarks

Revision history

Revision	Date	Description
Version 1	4 January 2023	Initial version

About this guide

This user guide is intended for researchers who are operating and maintaining Tomocube's high-performance holotomography imaging system, the HT-X1.

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.

Symbols

The following table describes the symbols appearing in this manual related to the use of the HT-X1 imaging system.

Symbol	Indications
	Indicates the need to consult the instructions for use.
	Indicates the need to consult the instructions for use for important information, such as warnings or precautions that do not appear on the instrument and/or test kit.
	Indicates components that need protection from light sources.
	Indicates separate waste collection for electrical and electronic equipment.

Safety instructions

⚠ **WARNING** indicates a potentially hazardous situation which, if not avoided, may result in injury.

- Always wear gloves when working with the instrument.
- Do not use the instrument near sources of strong electromagnetic radiation (e.g., unshielded radiofrequency sources) as this may interfere with its operation. To ensure proper operation, the user must not place radiofrequency emitting devices (e.g., cell phones, computers, microwave ovens, other laboratory equipment, etc.) on top of the instrument or very close to it.
- Dispose of waste in accordance with the relevant waste disposal regulations of the laboratory. Improper waste disposal can cause a biohazard. Biological samples such as tissues, body fluids, and blood of humans or other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves.

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

- Read the user manual provided by the manufacturer before operating the instrument. Keep it for future reference.
- Do not operate the instrument in conditions that do not comply with the specified operating conditions of temperature and humidity level. Doing so could damage the instrument and/or produce incorrect test results.
- Use only the certified power supply provided with the instrument. The use of non-certified power sources may cause damage to the instrument.
- Always place the instrument on a flat, stable platform. Confirm that the system on the platform is level before each use.
- In case of power supply failure, replace the power supply with an authorized power supply. If, after replacing the power supply, the instrument still does not turn on, contact Tomocube for technical assistance.
- Assess the electromagnetic environment before operating the instrument.
- When working with the HT-X1 holotomography system, minimize the impact of electrostatic discharge by properly grounding the system and/or operator.
- Avoid touching the inside of the instrument during use.
- Avoid using harsh cleaning products or liquids to clean the instrument as they may damage or stain the surface of the instrument.
- Before using an external memory device such as a USB thumb-drive or an external hard disc drive, check that the device is free of malware.
- Be aware of installing any software other than that pre-installed on the operating computer as doing so may lead to unintended consequences or system malfunction.

Safety and electromagnetic compatibility (EMC) standards

CE Conformity

This mark indicates that the product has been assessed before being placed on the market and has been found to meet the European Union's EMC Directive 2014/30EU and the environmental protection requirements in IEC 62321.

FCC Radio Frequency Interference Statement

This mark indicates that the equipment has been tested and found to comply with the limits for Class A digital devices, pursuant to Title 47 Part 15 Subpart B of the Code of Federal Regulations (CFR) of the US Federal Communications Commission (FCC). These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with this manual, may cause harmful interference to radio communication. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the measures listed below.

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/television technician for help.

This equipment complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions.

- This equipment may not cause harmful interference.
- This equipment must accept any interference received, including interference that may cause undesired operation.

Korean Radio Research Agency Notification

This mark indicates that the equipment is registered and has passed the conformity assessment system according to Article 58-2 of the Radio Waves Act of the Republic of Korea.

WEEE Statement

This mark indicates that the equipment must not be disposed of with normal waste. It is the responsibility of users to dispose of the waste equipment by arranging to return it to a designated collection point for the recycling of waste electrical and electronic equipment (WEEE). Separating and recycling the waste equipment at the time of disposal will help to conserve natural resources and ensure that the equipment is recycled in a manner that protects human health and the environment. For information about how to recycle waste equipment, please contact Tomocube’s service office in your region or your city’s municipal office for household waste collection service.

Table of Contents

CHAPTER 1. Product Information

1.1 Product description

The Tomocube HT-X1 Holotomography System is a cutting-edge digital holotomography (HT) imaging system that enables label-free three-dimensional (3D) visualization of transparent specimens. The HT-X1 generates 3D refractive index (RI) tomograms representing the distribution of the RIs of specimens, which can then be translated into morphological, chemical, and mechanical properties.

This product contains high-performance, low-coherence HT optics based on an inverted imaging system setup with an objective lens and transmitted light. A motorized stage with an autofocus module facilitates the generation of high-fidelity images with minimal intervention by users. The HT-X1 enables the observation of live specimens in standard multi-well imaging plates up to 24-well.

A fluorescence imaging module can also be incorporated to expand the information about the specimens by adding molecular information. Z-stacked 3D fluorescence images after deconvolution can be overlaid with holotomographic information to identify the localization of the target molecules in three dimensions.

An optional onstage incubator guarantees long observation periods of days for monitoring the dynamic nature of live specimens in a favorable culture environment.

The HT-X1 system is controlled by the operating software TomoStudio™ X through an intuitive graphical user interface (GUI) that can be accessed with the included computer mouse and keyboard. The key features of TomoStudio X software include the following.

- Digital live view for specimen observation
- Snapshot acquisition of 3D holotomograms and 3D fluorescence microscopy images
- Time-lapse imaging with multi-modalities
- Massive data navigation and image viewer
- Personalized data management

The HT-X1 system is for research use only. It is not for use in diagnostic procedures.

1.2 Standard included items

The package of the HT-X1 contains the following boxes.

- Main box
- Environmental control unit box (optional)
- Workstation box

The main box contains the following components.

- HT-X1 Holotomography system
- Operation manual (Doc. No. TPM-HTX-01)
- Certificate with test report
- AC power cord
- USB 3.0 A-B cable, 2 m
- Test specimens
- Dish attachment (* if the system is not supplied with the incubation chamber)
- Height block (* if the system is supplied with the incubation chamber)

The workstation box contains the following components.

- Operating workstation
- Keyboard
- Mouse

The optional environmental control unit box contains the following components in four smaller boxes.

- Warranty policy
- Instruction manual
- STXG controller
- 220/110V power cord
- Air tube
- Dish attachment for well plate
- Dish attachment for 35/60mm dish
- Precision screwdriver
- Disposable syringe
- Dish fixing lid for 60mm dish
- Dish fixing lid for 35mm dish

- Lid for well plate
- Temperature sensor and extension wire
- Spare cover glass
- Chamber (* The chamber is pre-installed in the instrument.)
- Lens heater
- Communication cable

Note: A monitor is not included in the workstation box. A 27-inch Full HD (1920 x 1080p) or higher resolution display monitor and an HDMI cable to connect with the operating workstation (PC) should be prepared by the user. Contact your local distributor for more details.

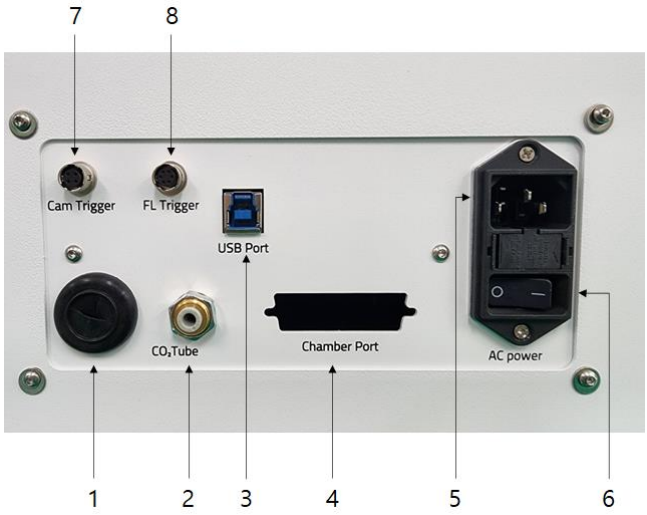
Please confirm that all the items listed above are included in the delivered package. If any of the above components are missing or damaged, contact Tomocube for support at support@tomocube.com.

1.3 Exterior components and mechanical control

Front view

1. Status lamp
2. Motorized door
3. Work light
4. Cableveyor
5. Door button
6. Work light button
7. Water reservoir
8. XY sample positioning stage and vessel holder
9. Condenser optics

Rear view



1. USB 3.0 port to PC (for camera)
2. Air fitting to the chamber
3. USB 3.0 port to PC (for system control)
4. Control port for the chamber
5. Power connector (AC 100–240V / 50–60Hz / 5 A (max.))
6. Main power switch
7. Camera trigger output
8. Fluorescence trigger output

Status lamp color codes

Color	Mode	Indication
Black	Solid	System off
White	Solid	Device initialization stand-by
Teal	Solid	Ready for system operation
Green	Blinking	System in operation
Orange	Blinking	System door in motion (opening or closing)
Red	Solid	Error

Important! If the status lamp turns red at any stage in operation, refer to the Troubleshooting section in Appendix E on Page 89 of this manual and follow the instructions.

1.4 Operating workstation

The operating workstation (PC) has the following specifications*.

- CPU: Intel® Core™ i7 or equivalent
- RAM: 128 Gigabytes
- Storage: Two SSDs (500 GB and 2 TB) and two HDDs (8 TB and 1 TB)
- GPU: NVIDIA GeForce RTX 3090 (VRAM: 24 GB)
- OS: Windows 10 IoT

* The specifications of the operating workstation are subject to change without notice.

1.5 Optional accessories

- Stage-top environmental controller
- Environmental controller for hypoxia
- 2-dish (35 mm) vessel holder
- 4-dish (35 mm) vessel holder
- 6-dish (35 mm) vessel holder
- Vessel holder for microscopic slides

The optional accessories can be purchased separately. Contact your distributor for more information about the items.

CHAPTER 2. Installation

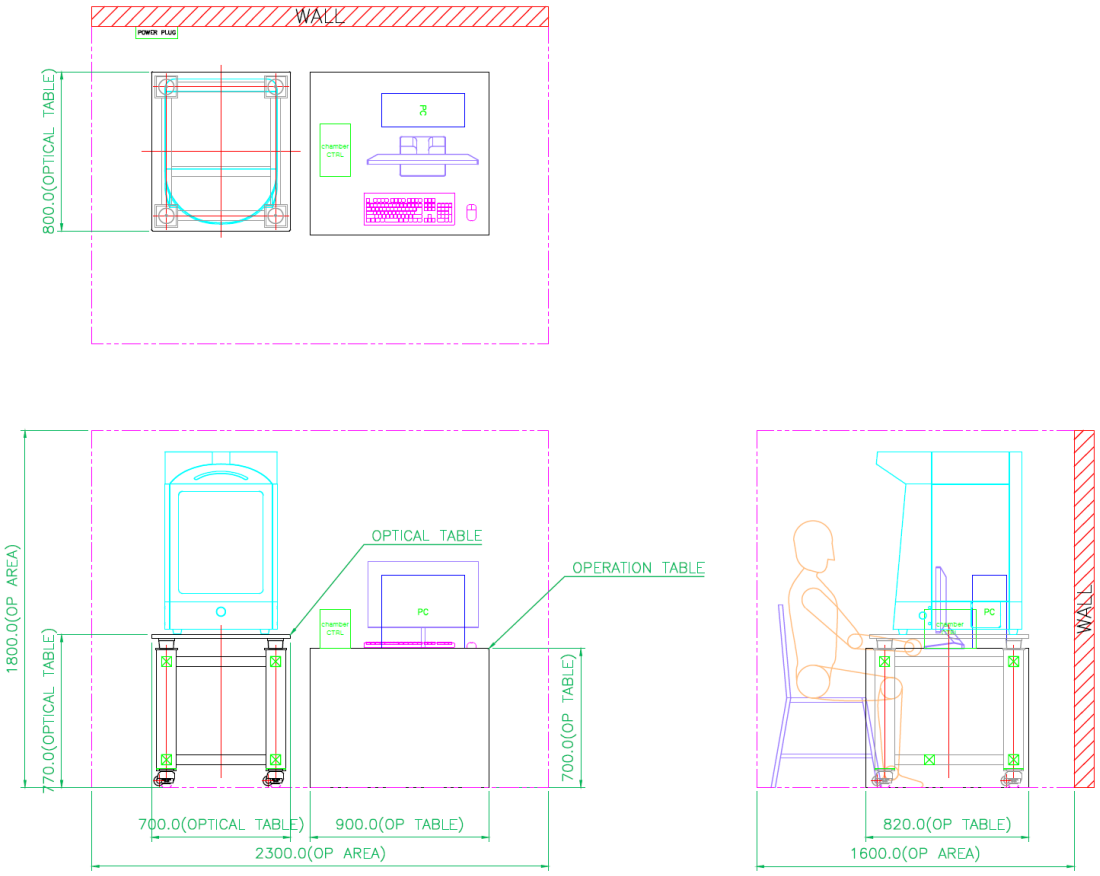
When the product is received, be sure to inspect the package box for any damage. If possible, do not remove the instrument from the box; an installation engineer will unpack and install the instrument.

Important! The HT-X1 system must be installed by a professional technician authorized by Tomocube.

2.1 Operating site requirements

The system must be placed and operated on a stable benchtop of 700 mm x 800 mm (28 in x 32 in) or larger. A pneumatic optical table with vibration isolation is preferable but not required.

It is highly recommended to set up alongside the instrument benchtop an additional worktable of approximately 1200 mm x 800 mm (32 in x 48 in) or wider to place the operating workstation (PC), monitor, and environmental controllers.



The instrument needs at least 5 cm (2 in) of clearance on each side to prevent overheating of the electronic components. Place the instrument on a level surface away from other laboratory equipment that can cause vibration of the instrument during image acquisition.

The AC power supply should be 100–240V, 50/60Hz, single phase, and 2 kW.

⚠**CAUTION** Always place the instrument on a flat, stable platform. Check the positioning on the platform before each use.
⚠**CAUTION** Use only the authorized power supply provided with the instrument. The use of unauthorized power sources may cause damage to the instrument.
⚠**CAUTION** The electromagnetic environment must be assessed before operating the instrument.
⚠**CAUTION** When working with the HT-X1 holotomography system, minimize the impact of discharge events by properly grounding the system and the operator.

2.2 Preparation before installation

1. Inspection after receiving the package

   When the product is received, be sure to inspect the package box for any damage. If possible, leave the instrument in its box. An installation engineer authorized by Tomocube will unpack and install the instrument.

   Verify that the items on the shipping list are identical with what was ordered at the time of purchase.

2. Moving the instrument to the installation site

   Clear the installation site before the installation.

   If possible, move the crated instrument and other shipping containers (boxes) to the installation site.

⚠**CAUTION** Physical injury hazard. Lift or move the instrument using proper lifting techniques. It is strongly recommended to lift and move the crated instrument with the assistance of sufficient persons with the use of appropriate moving equipment that can protect the persons from unintended dropping or falling. Improper lifting of the instrument can cause permanent injury.

Important! Do not unpack the instrument from the package without the assistance of a technical professional authorized by Tomocube.

For more information, refer to the HT-X1 installation manual or inquire to Tomocube at support@tomocube.com.

CHAPTER 3. Sample Preparation

3.1 Overview

The HT-X1 system is intended to capture refractive index (RI) tomograms of a sample placed in a vessel with a bottom thickness of #1.5 (0.17 mm). Imaging vessels with a different bottom thickness can be a source of aberration or noise in the image results.

It is recommended to fill the vessel with the appropriate volume of cell culture medium as listed in the table below. The amount of cell culture medium can influence the light transmission efficiency through the condenser.

Vessel type	Well diameter	Volume of medium	Access area
50-mm imaging dish	50 mm	3 mL	20 mm x 20 mm
35-mm imaging dish	35 mm	2 mL	10 mm x 10 mm
6-well imaging plate	35 mm	2 mL	4 mm x 4 mm
12-well imaging plate	22 mm	1 mL	4 mm x 4 mm
24-well imaging plate	15 mm	0.5 mL	4 mm x 4 mm

Important! Do not use multi-well culture plates made of plastics.

Important! Use the recommended volume of medium corresponding to each vessel type. An excessive amount of medium may decrease the illumination efficiency.

Note: Do not write or make any marks on the center of the vessel lids. Ink or stickers on the center of the lid may block light transmission and interfere with the results.

3.2 Compatible vessels

The products below have been verified as valid for successful holotomography imaging with the HT-X1 system.

Type	Brand	Cat. Number	Bottom
TomoDish (50 mm)	Tomocube	901002-01	#1.5H glass
35-mm imaging dish	Ibidi	82041	#1.5H polymer
6-well imaging plate	Cellvis	P06-1.5H-N	#1.5H glass
12-well imaging plate	Cellvis	P12-1.5H-N	#1.5H glass
24-well imaging plate	Cellvis	P24-1.5H-N	#1.5H glass
24-well imaging plate	Ibidi	82401	#1.5H polymer
24-well imaging plate	Greiner	662892	#1.5H glass

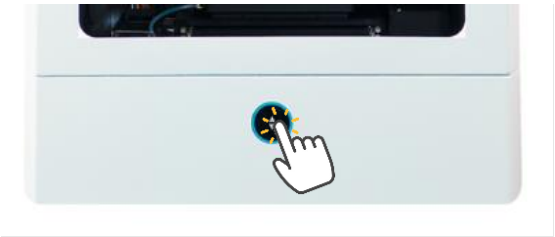
Important! Imaging plates with well diameters smaller than 15 mm, such as 48-well or 96-well plates, are not compatible with the HT-X1 system.

Note: For any inquiries about the use of other imaging dishes or plates not listed above, please contact Tomocube at support@tomocube.com.

Note: To observe a microscopic slide or a microfluidic device with dimensions of 25 x 75 mm, contact Tomocube for details.

3.3 Mounting a sample onto the stage

1. Push the door button on the front of the instrument to open the door.

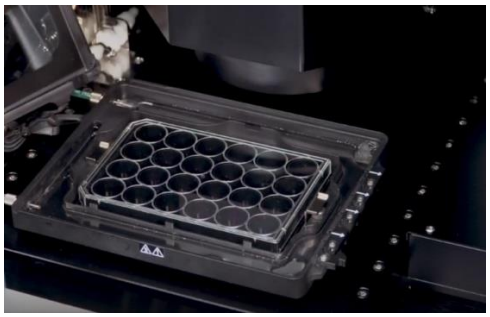


⚠ **CAUTION** Physical injury hazard: Do not touch the door and keep hands and fingers away from the path of the door when the door is opening or closing.

2. Place a proper vessel holder onto the stage.

   Note: To use a dish holder or a slide holder, always place a microplate holder into the chamber in advance.

3. Place the specimen into the vessel holder.



   Important! Make sure that the dish or microplate is firmly attached to the vessel holder without tilting.

4. Close the system door for long-term time-lapse imaging (longer than overnight).

   Note: Images can be successfully captured when the door is open. Closing the door is not required, although it is recommended, to operate the instrument.

CHAPTER 4. Initiation and Experimental Setup

4.1 Workflow

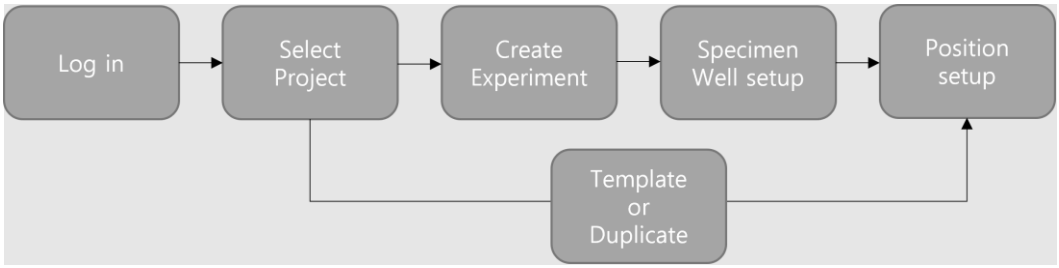


Figure 4.1 Experimental setup workflow

The procedure of the experimental setup to be conducted prior to imaging consists of four steps: 1) Log in to the operating software with a registered account, 2) Create or select a project, 3) Create and set up an experiment, and 4) Set up the specimen and well configuration. These steps can be simplified by importing templates or duplicating previous experiments.

4.2 Starting the software

4.2.1 User accounts

To use TomoStudio™ X, three types of accounts are available: Operator, Administrator, and Service Engineer.

The Operator account is for general users of the system. The Administrator account is for the System Administrator(s) with the ability to create and delete users and configure the system parameters. The Service Engineer account is reserved for system maintenance.

General users need an Operator account (username and password) to log in to the operating software, TomoStudio X. Please contact the System Administrator(s) for log-in information.

An Administrator account can be created by the Service Engineer during the initial system installation or by other System Administrators. TomoStudio X software allows for multiple Administrator accounts.

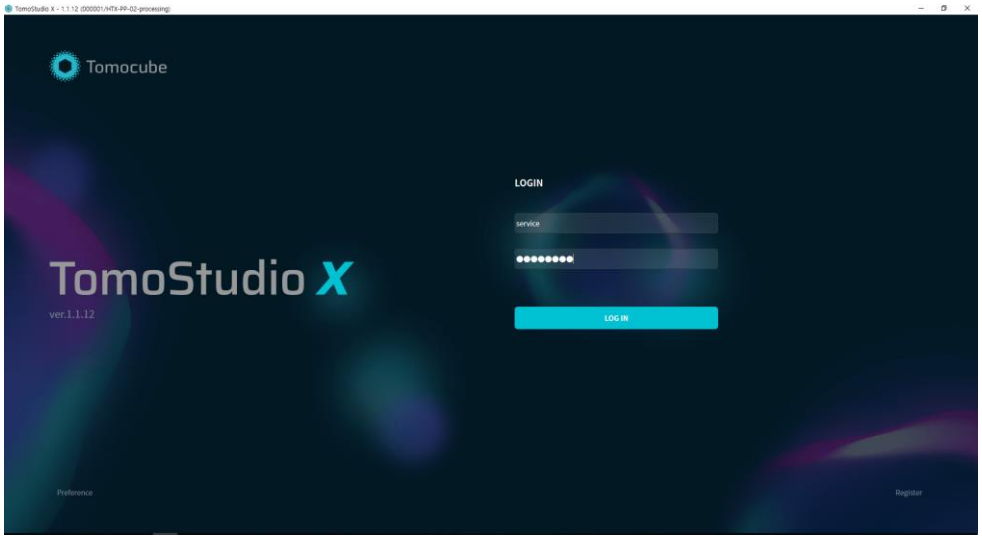


Figure 4.2 Login window

4.2.2 Create/delete user accounts

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

4.3 Experiment Manager

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

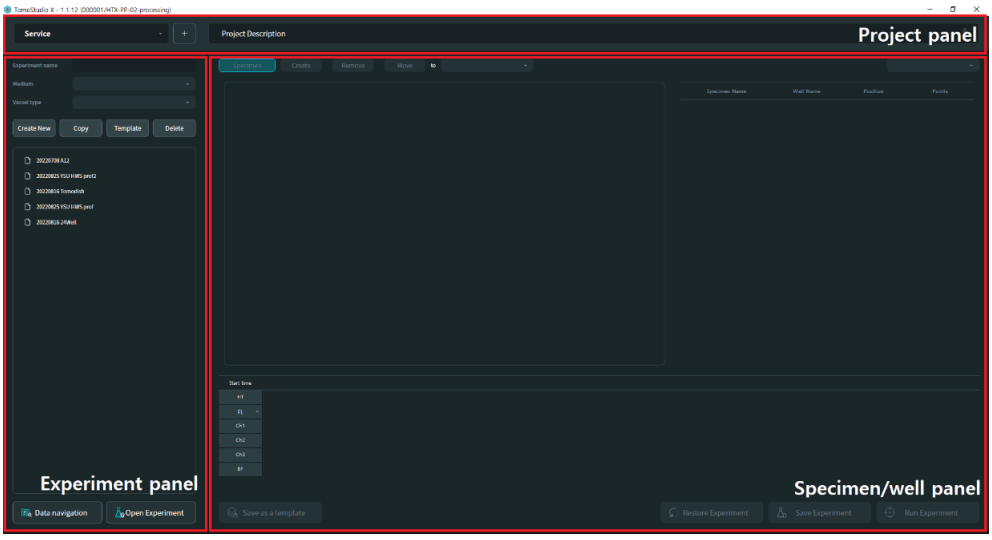


Figure 4.3 Experiment Manager

Project panel

In TomoStudio X, 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.

Figure 4.4 Project panel

Experiment panel

In TomoStudio X, 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 refractive index (RI), the vessel 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.

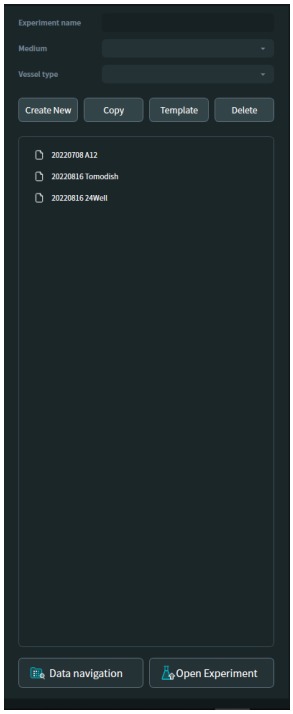


Figure 4.5 Experiment panel

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.

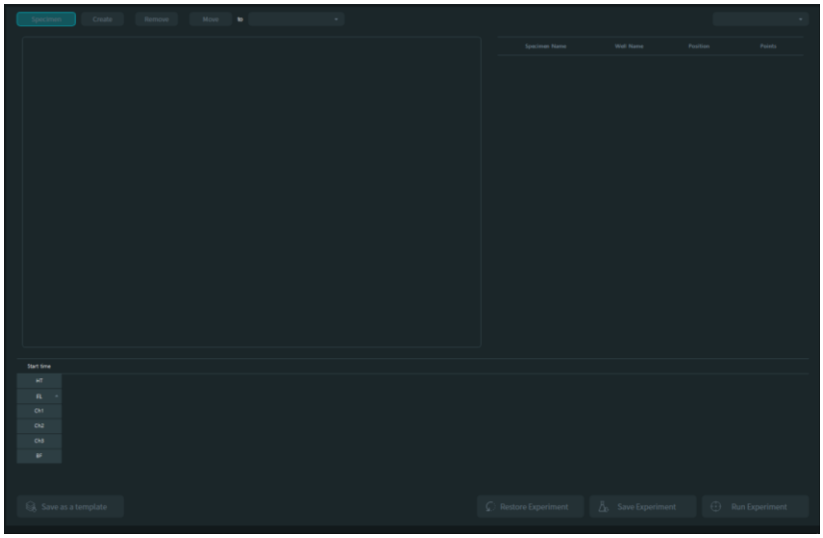
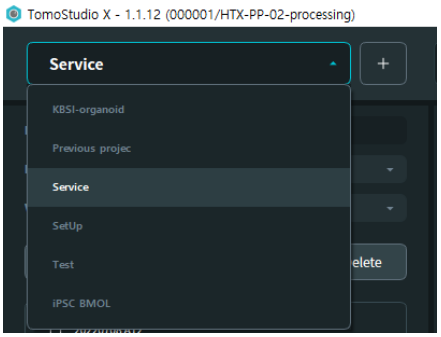


Figure 4.6 Specimen/Well panel

4.4 Project

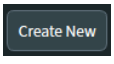
Create New Project: To create a new project, click the [Add Project] button  in the Project panel.

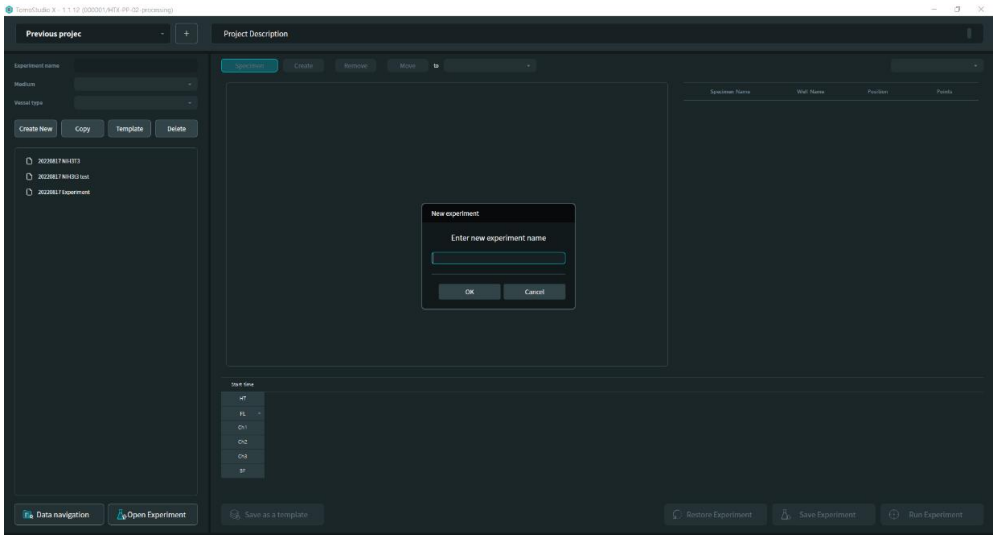
Select Existing Project: To select an existing project saved to the account, click the drop-down menu in the Project panel and select the project, as shown below.



Note: To add notes or brief information on the project, click the Project Description field in the Project panel and write in a description of the project.

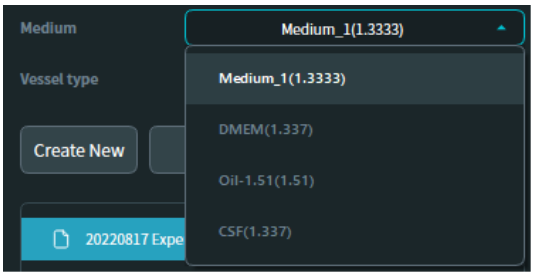
4.5 Experiment

1. To create a new experiment, click the [Create New] button  in the Experiment panel.
2. Type in a name for the experiment in the pop-up window and click OK.



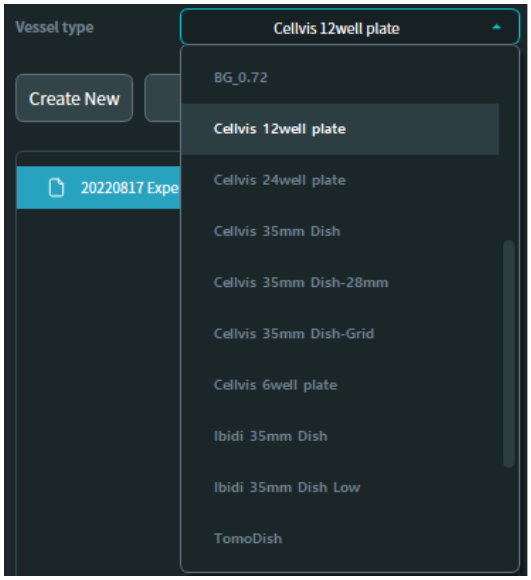
Note: When naming the experiment, it is not necessary to add the date to the experiment name. The date is automatically recorded in the experiment list.

3. 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.

4. 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.

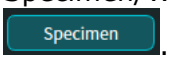


Important! If the intended medium RI or vessel type for the experiment is not listed in the related drop-down menu, please contact your System Administrator to update the setting. Additional medium RIs and vessel types can be added via the Preference menu accessible with an Administrator account.

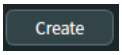
4.6 Specimen and well set up

Before obtaining images of the samples in a multi-well plate, TomoStudio X provides two options to register the wells of the vessel and the specimens. The two modes available for registering specimens are the Specimen Mode and the Individual Mode. To group and register multiple wells as a single specimen, use the Specimen Mode. To register each individual well as a different specimen, use the Individual Mode.

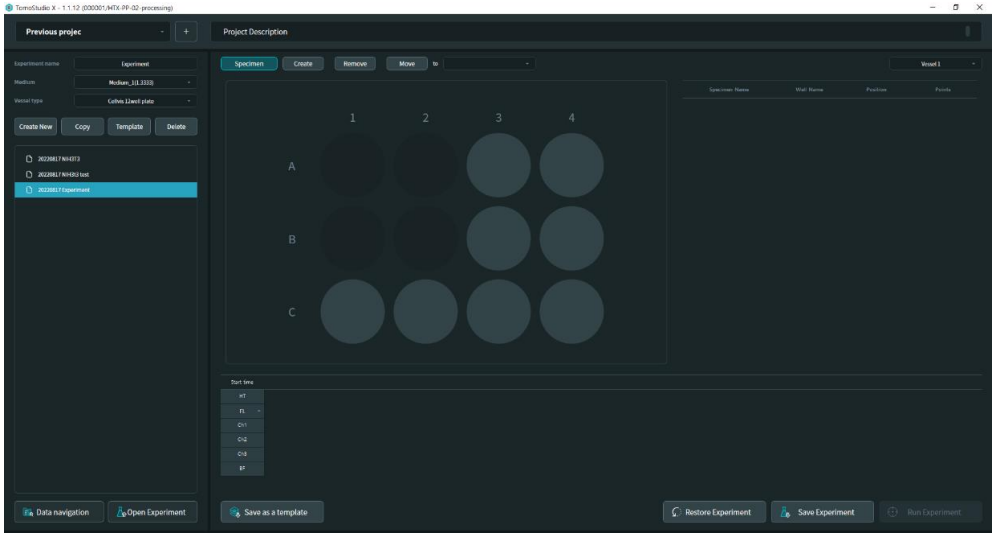
4.6.1 Register a specimen using the Specimen Mode

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 

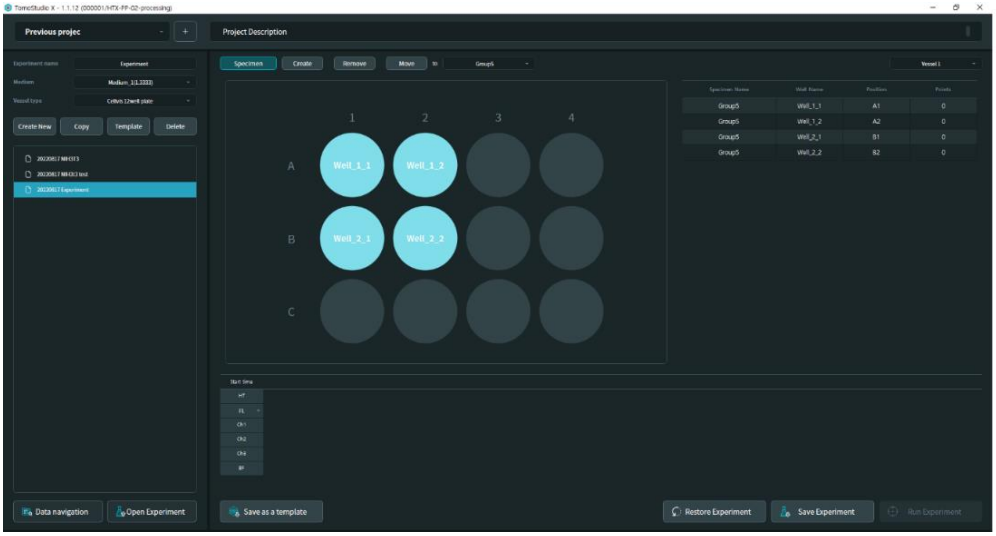
.

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 

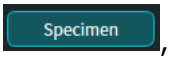
to register the selected wells as a group under a single specimen. To register other wells as different groups of specimens, repeat this process.



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.

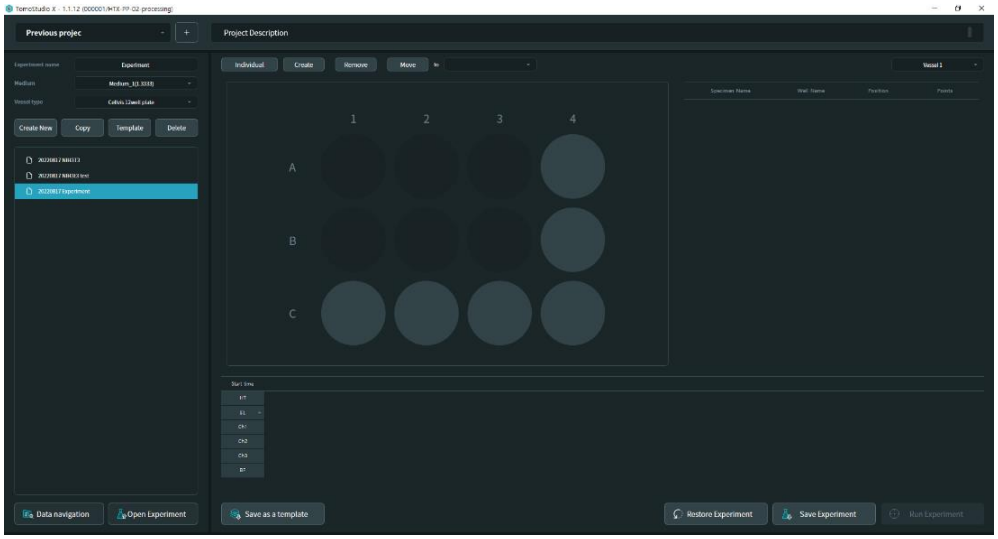


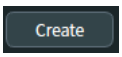
4.6.2 Register a specimen using the Individual Mode

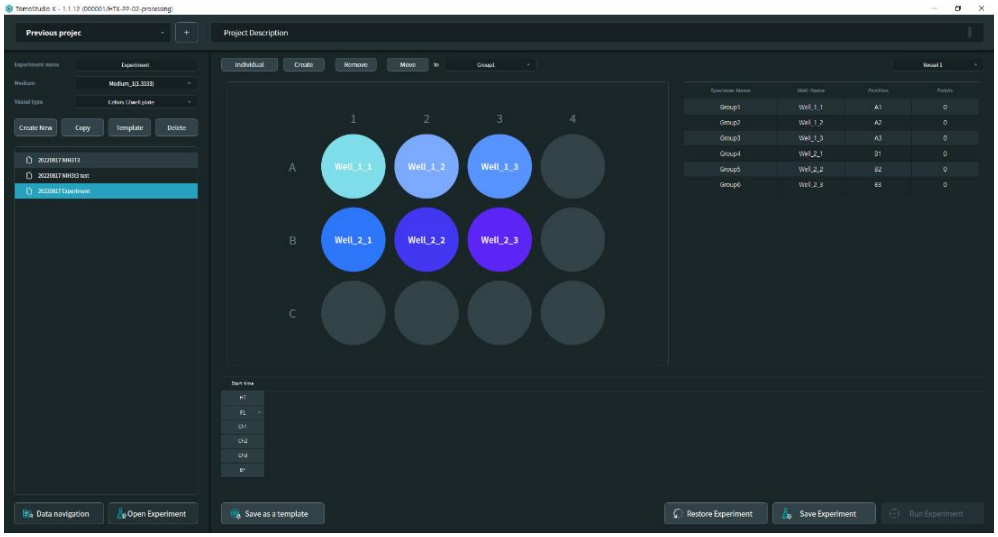
1. Click the mode button 

, 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.



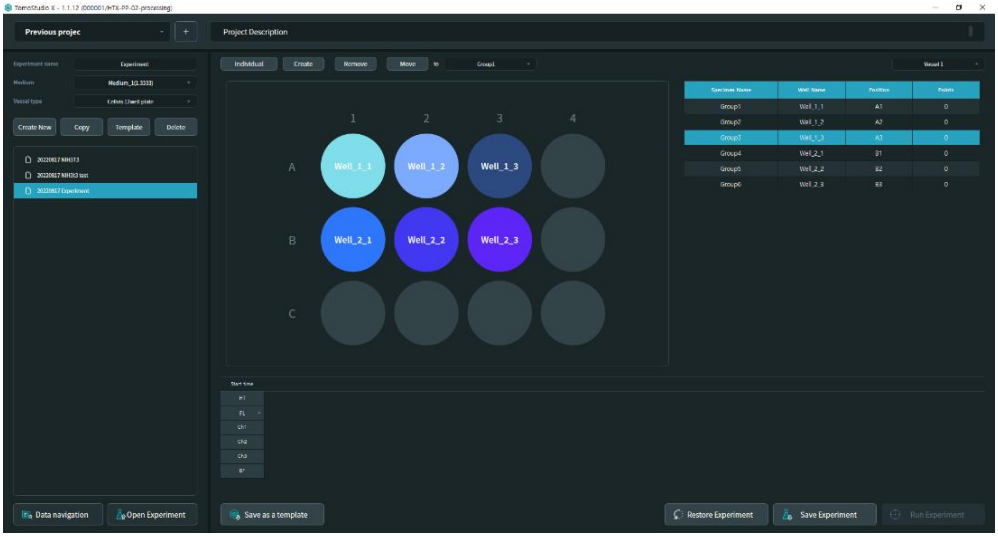
3. Click the [Create] button  to register the selected wells as individual specimens. Each of the selected wells is assigned as an individual specimen. To register other wells as different individual specimens, repeat this process.

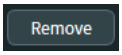


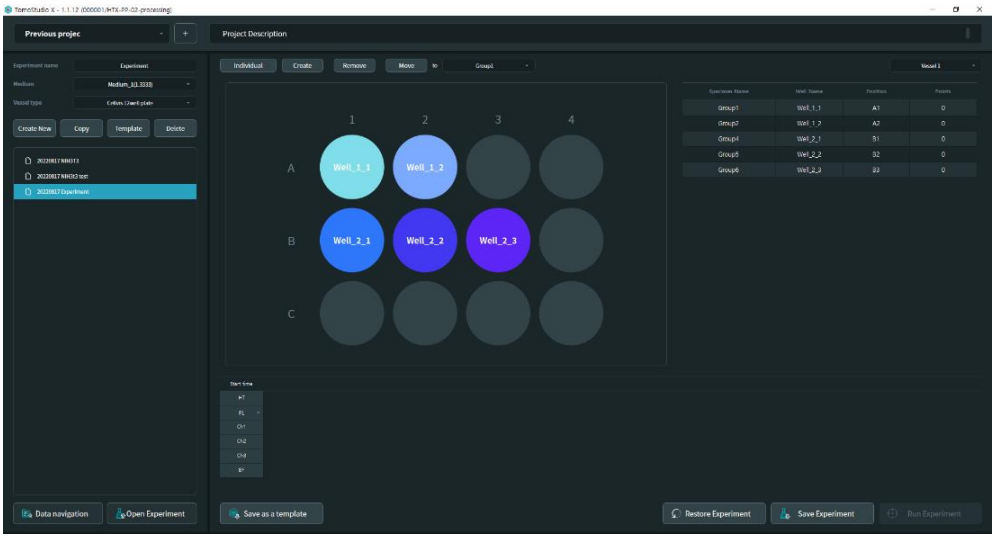
4.6.3 Unregister wells as specimens

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.



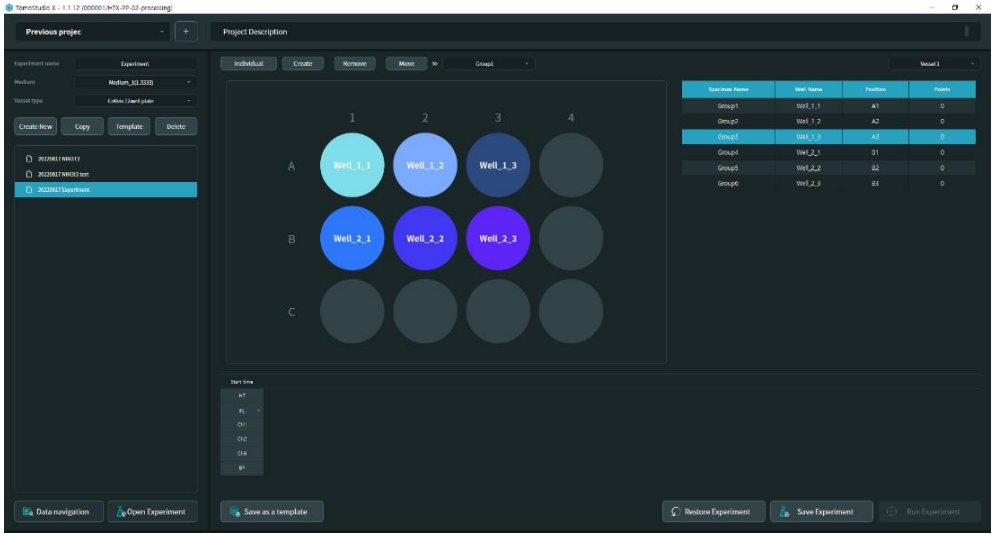
2. Click the [Remove] button  to unregister the selected wells. The change is automatically reflected in the vessel map and the specimen/well table.



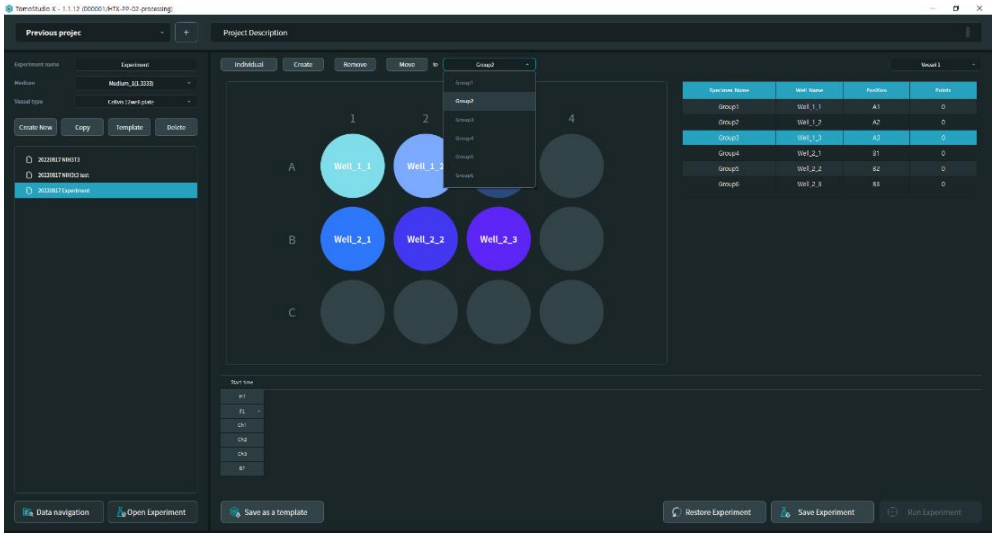
4.6.4 Reassign wells to a different specimen

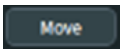
Wells that have been registered as a specimen can be reassigned to a different specimen.

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.

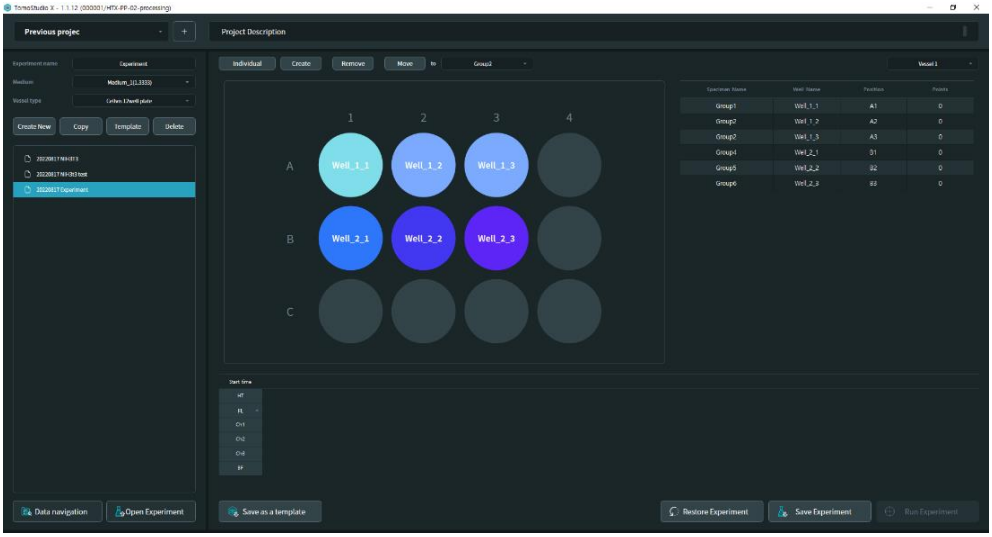


2. Click the drop-down menu for the specimen list and choose the specimen to which the selected specimen(s) will be reassigned.



3. Click the [Move] button 

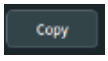
to reassign the selected well(s) to the selected specimen. The change is automatically reflected in the vessel map and the well/specimen table.

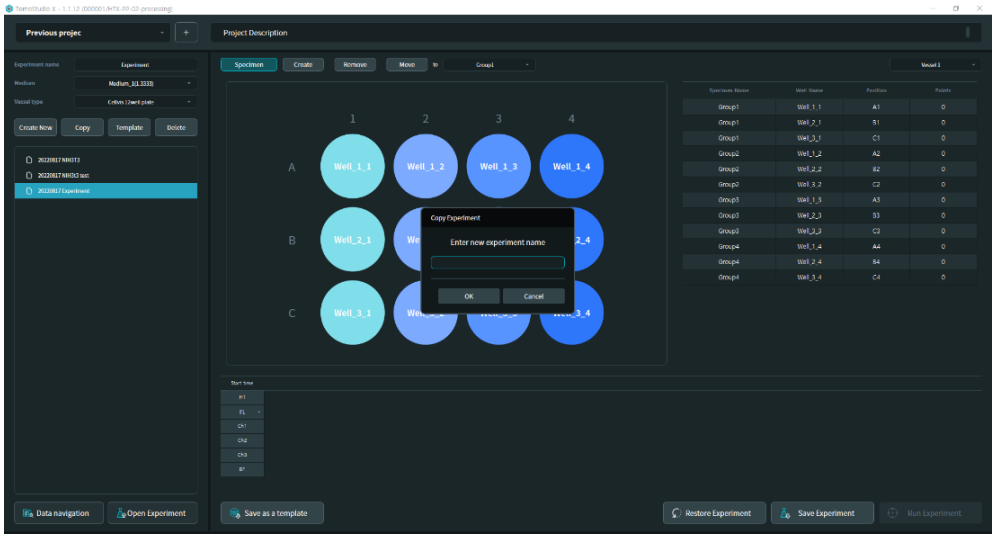


4.7 Create a new experiment by duplicating a previous experiment

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.

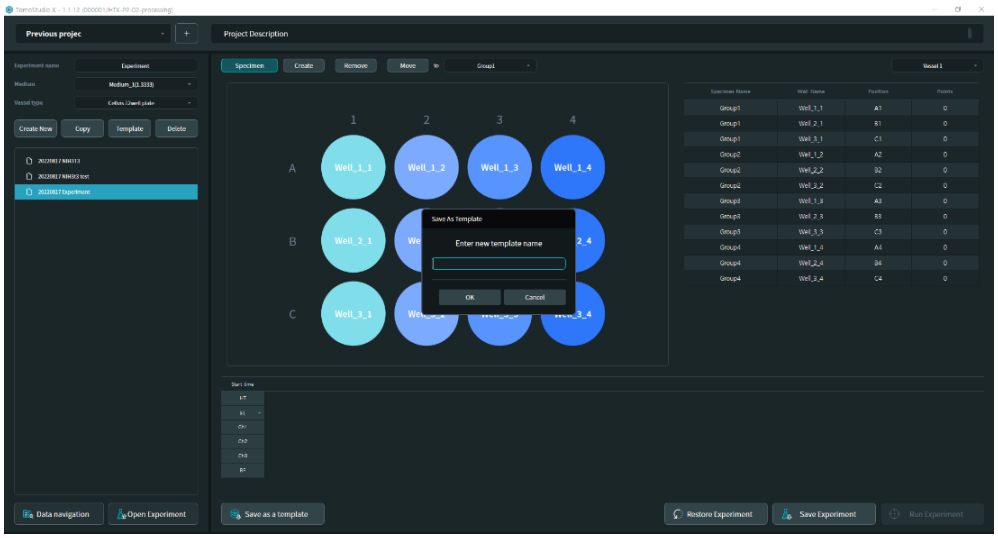
4.8 Create a new experiment by importing a template

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.

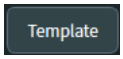
4.8.1 Exporting experimental conditions as a template

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

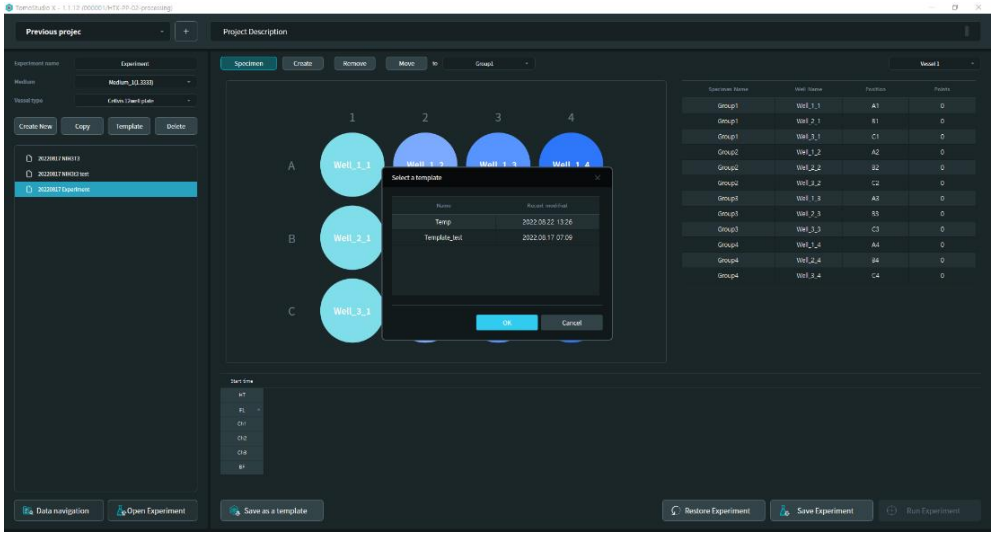
at the bottom of the Experiment Manager window.
3. Type in a name for the template in the pop-up window and click OK.



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

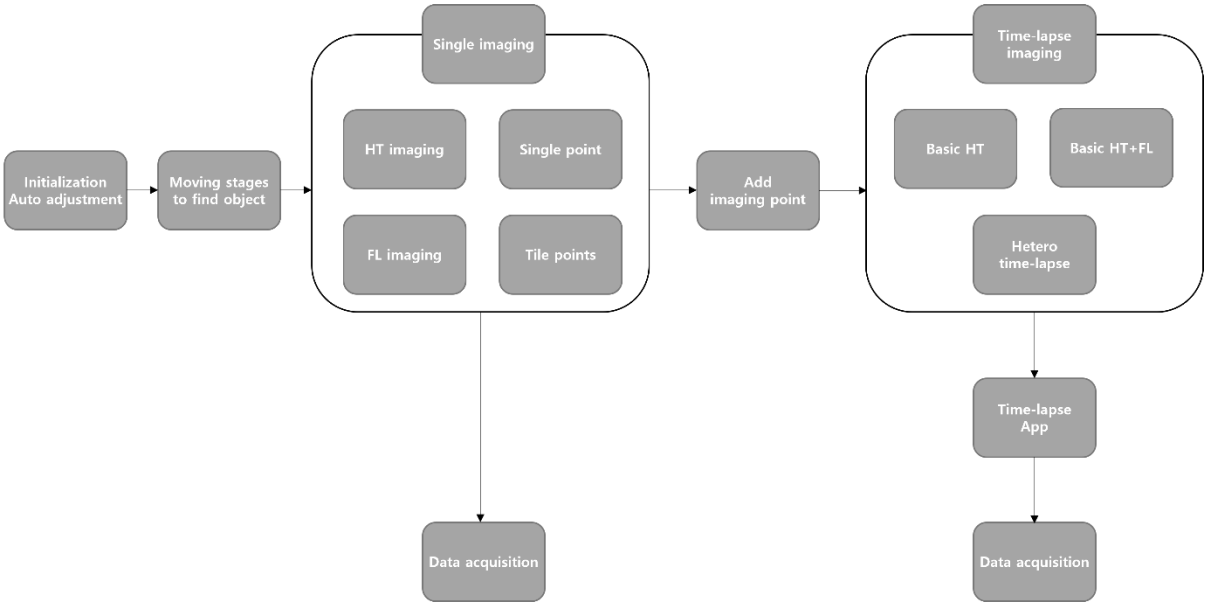


Figure 5.1 Workflow for image acquisition

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

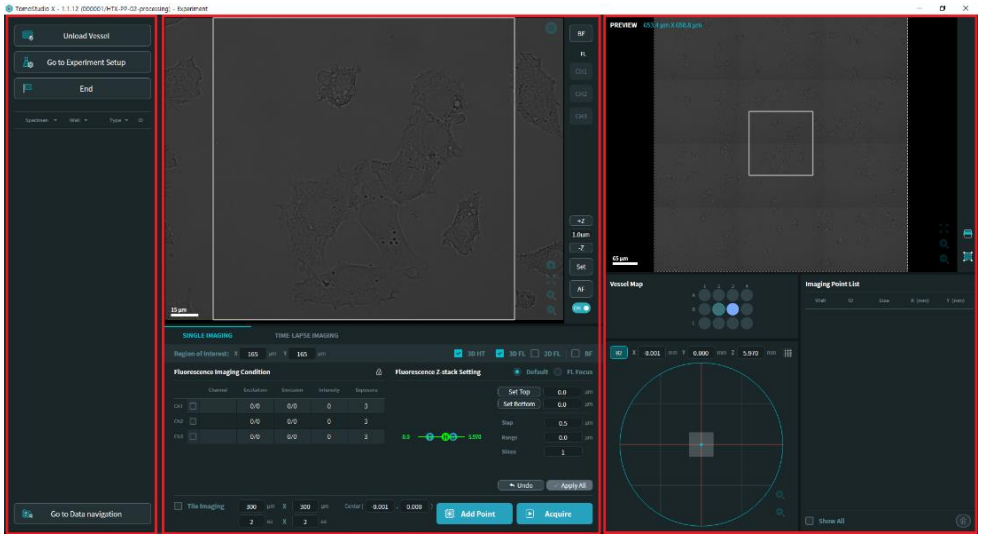


Figure 5.2 Image Acquisition window

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)

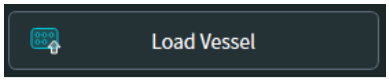
The main Acquisition Area in the center of the image acquisition interface is divided into two panels: Live View and Acquisition. The Live View panel displays a live image from the camera. In the Acquisition panel, the image acquisition parameters can be set up and images can be acquired.

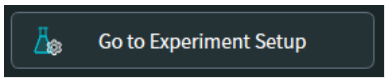
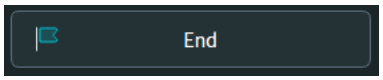
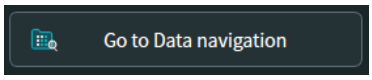
Utility Area (right)

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

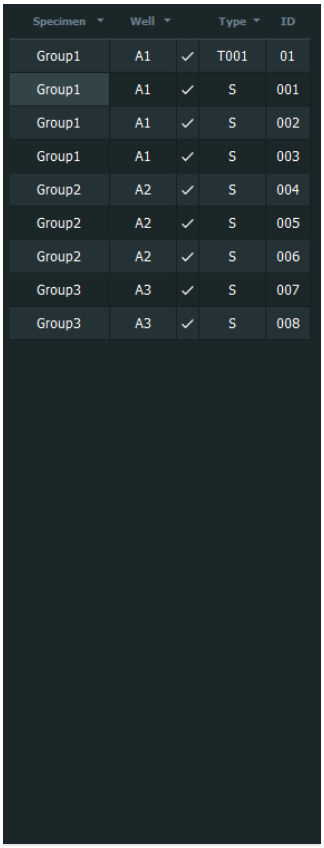


Figure 5.3 Data List panel

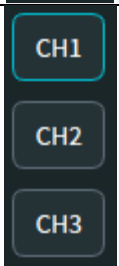
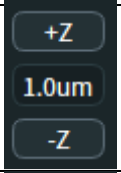
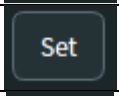
After each image acquisition is completed, the acquired data from the experiment is automatically listed in the Data List panel. In the Data List panel, the acquired data files are shown in a table with brief information including the specimen name, well name, nature of the data regarding time and dimension, and the data ID.

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.
	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 FL Acquisition 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.
	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

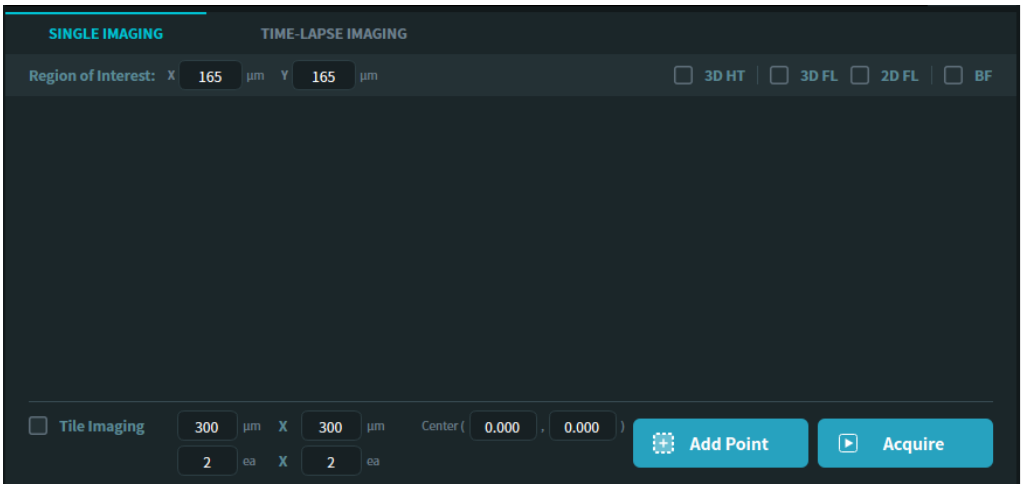
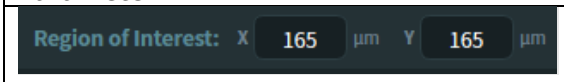
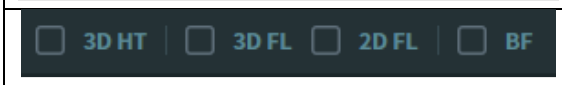
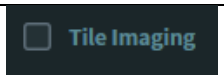
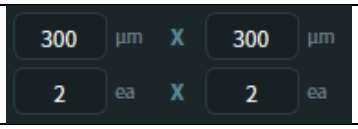
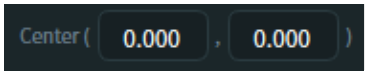
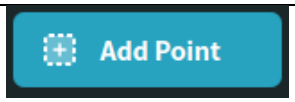
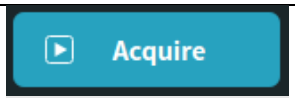


Figure 5.5 Acquisition panel (Single Imaging)

In the Acquisition panel, the parameters for holotomography (HT) 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		Description
	Region of interest (ROI) setup	Sets the size of the ROI.
	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

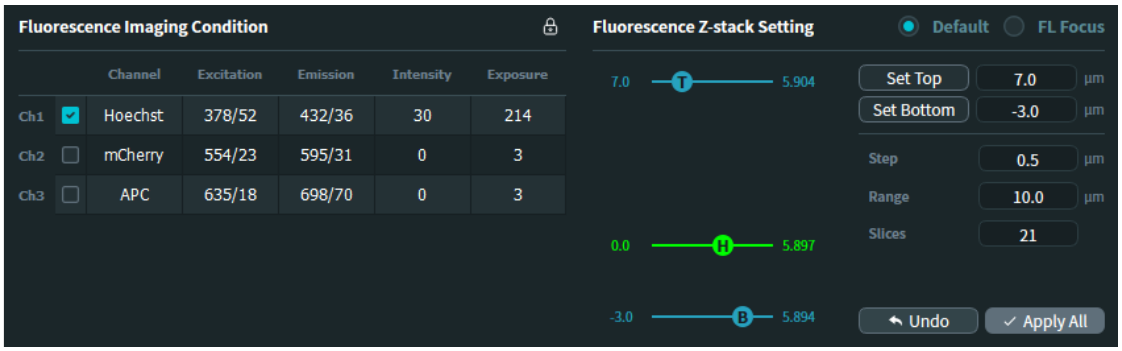
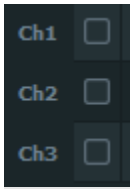
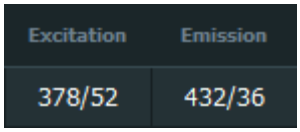


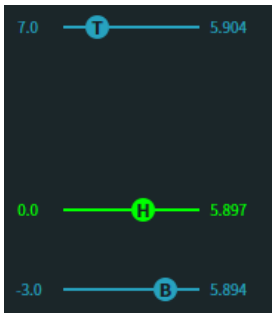
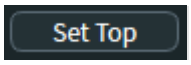
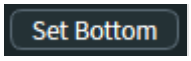
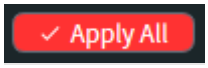
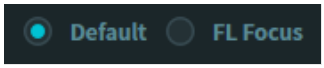
Figure 5.6 Fluorescence setup panel (imaging conditions and Z-stack settings)

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		Description
	Channel selection	Activates the desired fluorescence channel(s).
	Excitation and emission filter settings	Lists 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).
	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		Description
	Z-stack histogram	Illustrates the current fluorescence Z-stack settings for three Z positions: Top (T), Bottom (B), and focus position (H) for HT measurement.
	Set top	Sets the current Z position to the highest position in the Z scan range for the fluorescence measurement.
	Set bottom	Sets the current Z position to the lowest position in the Z scan range for the fluorescence measurement.
	Step	Sets the step size in the Z direction for the fluorescence measurement.
	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 measurement.

Two Z-stack modes are available in TomoStudio™ X: Default mode and FL Focus mode

Time-Lapse Imaging tab

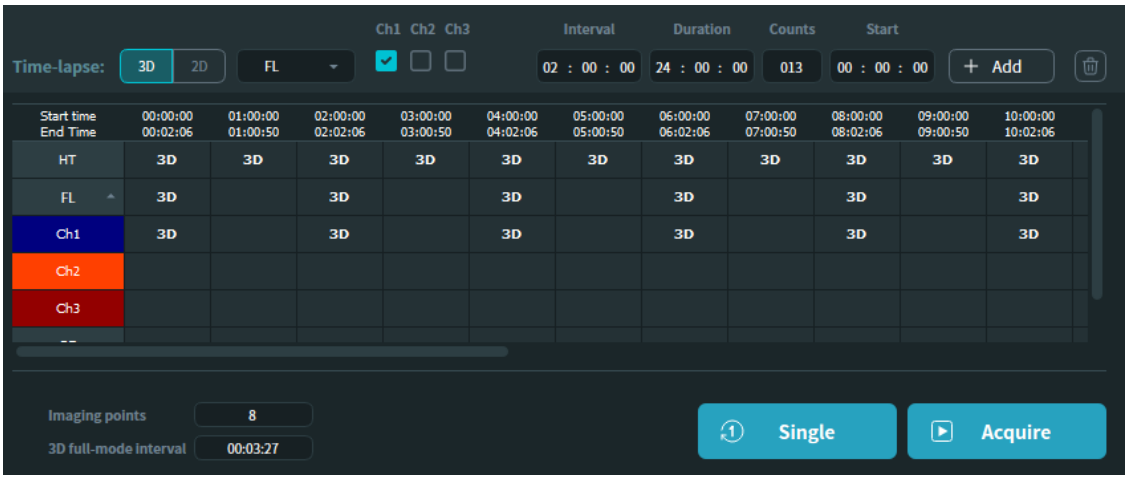
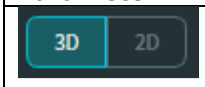
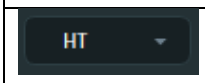
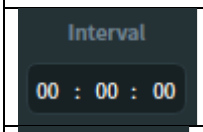
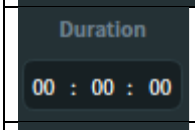
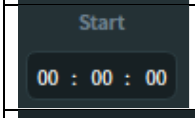
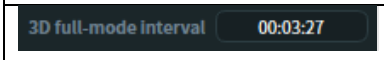
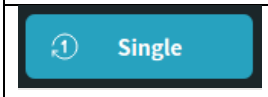
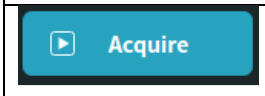


Figure 5.7 Acquisition panel (Time-Lapse Imaging)

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 time frames for each imaging mode. For more information on how to configure the schedule for Time-Lapse Imaging, please refer to Page 54.

Parameter		Description
	Dimension	Sets the data dimension of the imaging mode for measurement in the time-lapse sequence.
	Mode	Sets the imaging mode type for measurement in the time-lapse sequence. The three mode options are HT, FL, and BF.
	Interval	Sets the measurement interval of each imaging mode in the time-lapse sequence.
	Duration	Sets the measurement 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.
	Acquire once	Captures a single image for each imaging mode at the registered measurement points.
	Acquire time-lapse images	Performs the scheduled measurements in the Time-Lapse Schedule Table.

5.2.3 Utility Area

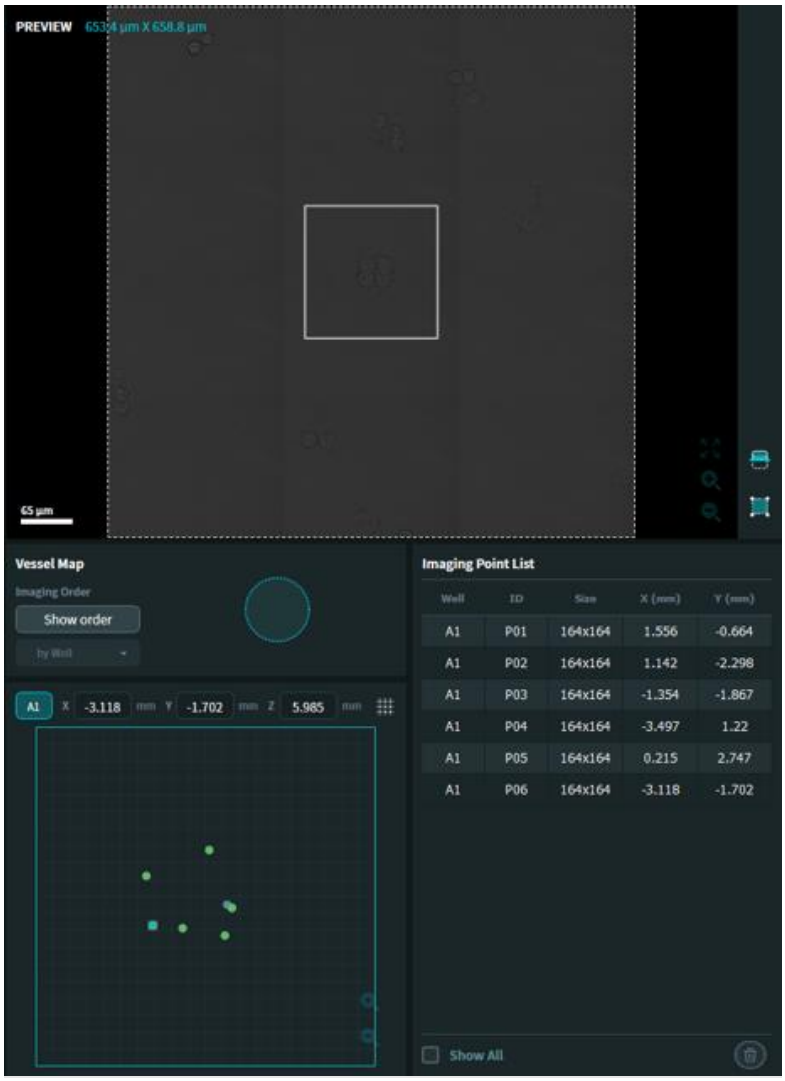


Figure 5.8 Utility Area layout

Preview panel

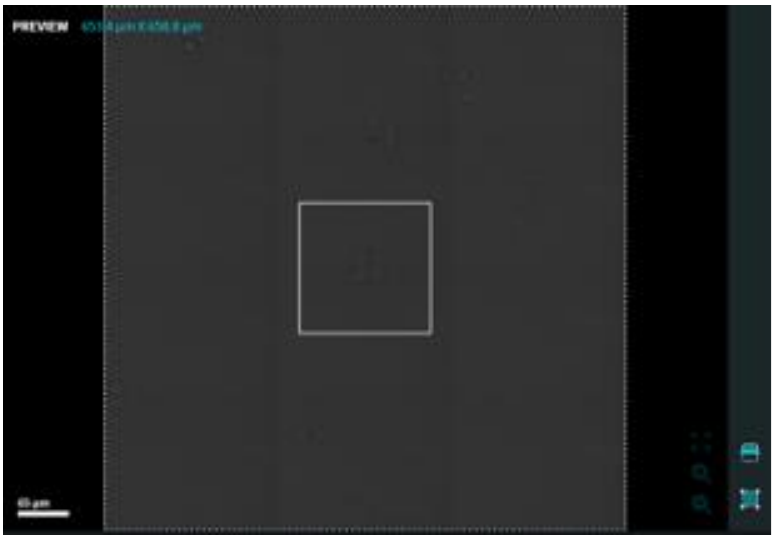
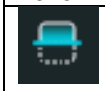
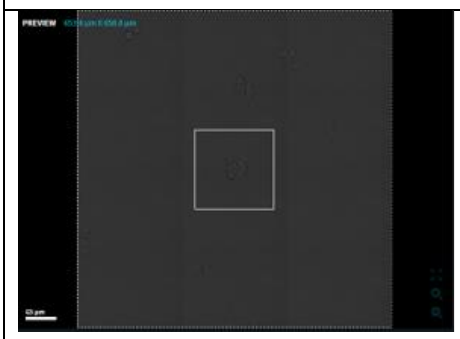


Figure 5.9 Preview panel

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		Description
	BF preview	Captures a pre-defined area in a 600 μm × 600 μm square in the BF imaging mode around the current FOV.
	Change preview size	Adjusts the area for the BF preview other than the default size.
	Preview result	Displays the acquired image for the BF preview.

Vessel/Well Map panel

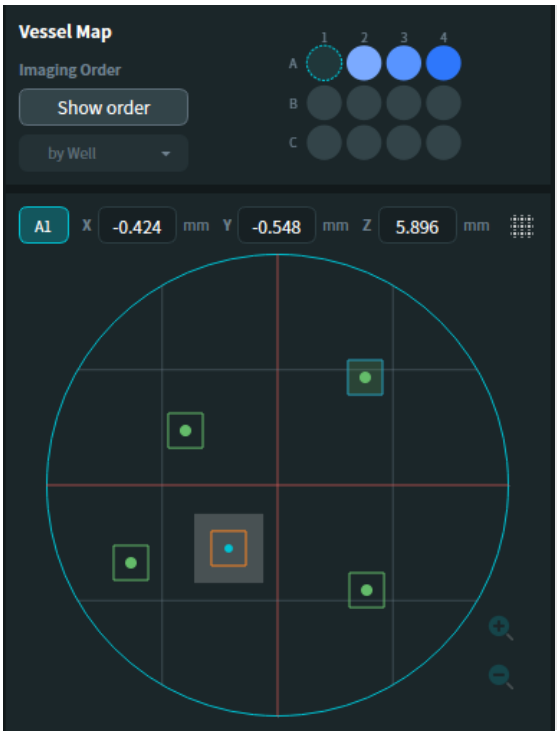
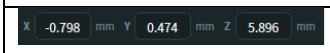
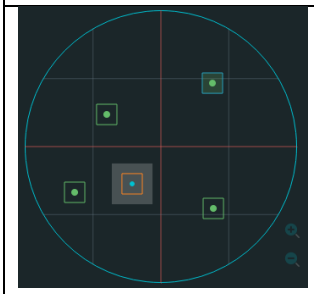


Figure 5.10 Vessel/Well Map panel

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

Imaging Point List panel

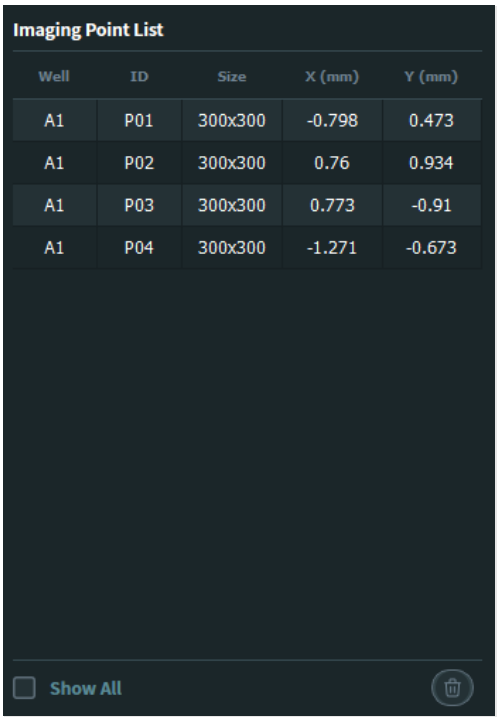
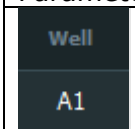
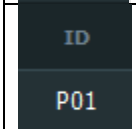
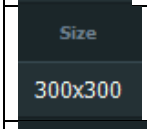
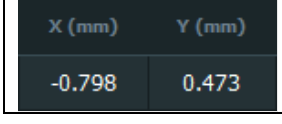


Figure 5.11 Imaging Point List panel

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		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.

5.3 System initialization

By clicking the [Run Experiment] button in the Experiment Manager window, the HT-X1 system goes through an initialization procedure with the current sample to optimize the best configuration for image acquisition. All steps of the initialization procedure listed below are performed automatically.

1. Upon the [Run Experiment] command, the HT-X1 system performs the initialization procedure if it has not already gone through the procedure after it has been turned on. This step takes about 5–10 minutes.
   The initialization procedure is skipped if the system has already been initialized after it has been turned on.
2. The vessel holder moves to the unloading position automatically.
3. Place the imaging dish/vessel on the vessel holder that sits inside the incubation chamber. Ensure that the holder fits well into the chamber without tilting, such that no corner of the vessel is raised from the holder.
4. Click the [Load Vessel] button.
5. After the stage moves the vessel holder to the imaging position, the instrument automatically adjusts the focus of the objective lens. After the objective lens autofocusing process, the system automatically adjusts the focus of the condenser lens and the illumination intensity for HT. This autofocusing step takes about 5 minutes.

Important! The autofocus 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 Step 3.

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

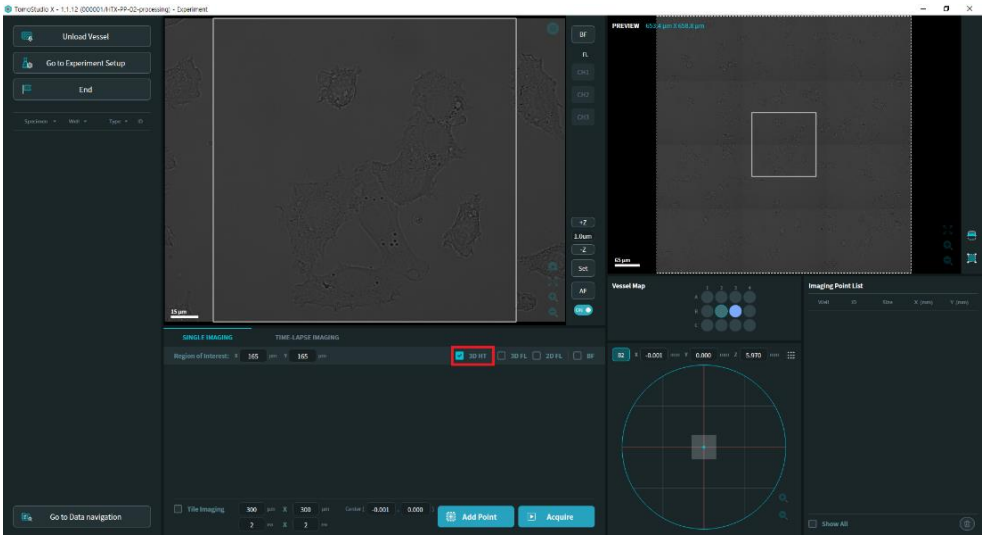
After the initialization and pre-adjustment procedures are complete, HT images can be acquired. Single HT images of the current FOV can be acquired in the Single Imaging tab.

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

   Note: For more information about positioning within a well, see [Moving the sample stage] section on Page 47.

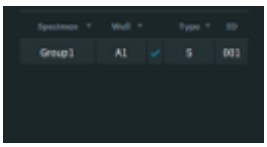
2. Check the focus of the brightfield image on the Live View panel if the contrast is acceptable. If required, adjust the Z position by using the [+Z] or [-Z] buttons on the right side of the Live View panel to have an optimal contrast of the image. Or click the [AF] button to find the best focus automatically. If the focus is acceptable, click the [Set] button to make the Z position the baseline of the focus for the following image acquisition.

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



4. Click the [Acquire] button to perform HT imaging.

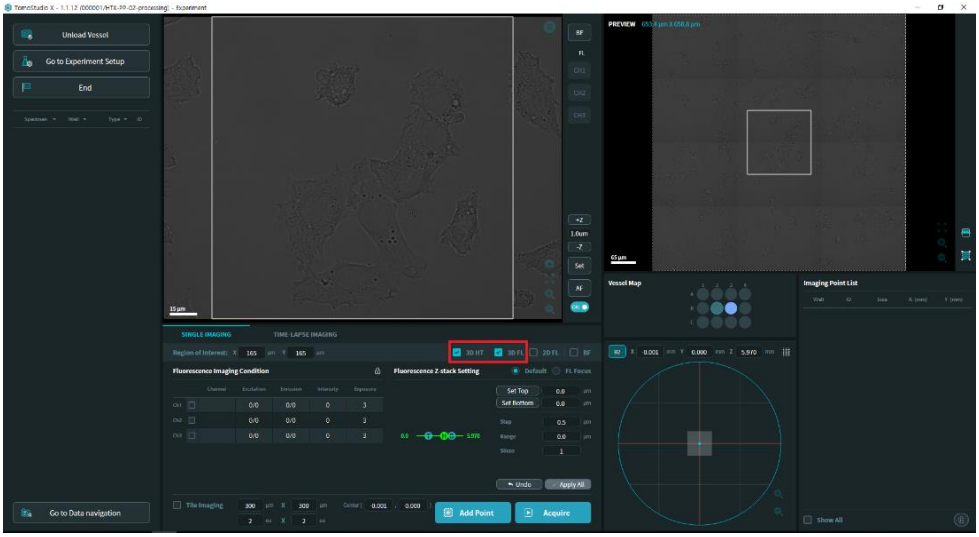
5. The acquired image is automatically registered in the data list table. Note that the color of the check mark on the data list changes from grey to blue when the data processing of the image has been completed.



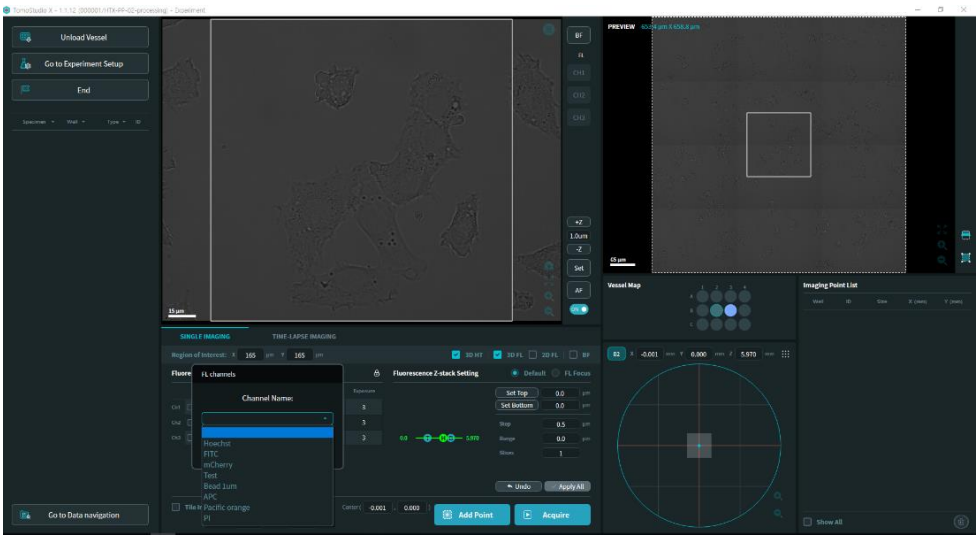
5.5.2 Holotomography with fluorescence

If the sample generates fluorescence (FL) signals, fluorescence images can be acquired along with HT images through TomoStudio X.

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

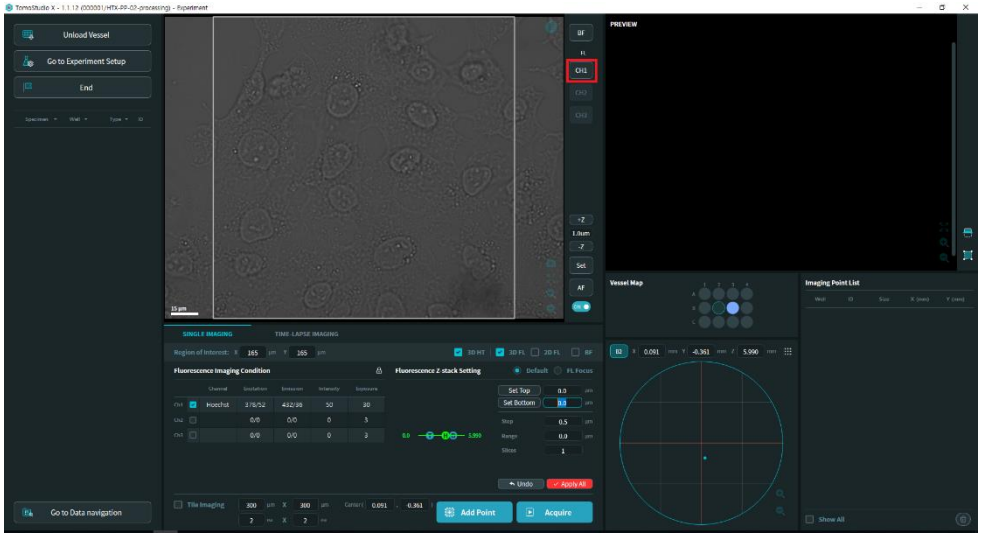


2. Check one of the channel checkboxes on the Fluorescence Imaging Conditions table to add FL channels. On the pop-up menu, select the appropriate channel name for acquisition.

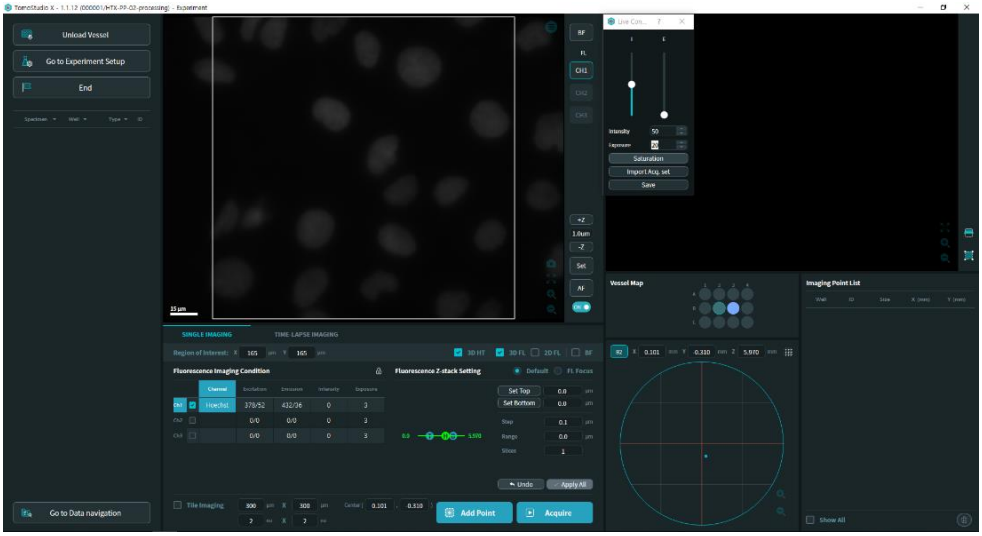


Important! TomoStudio X provides four fluorescence channels to image: DAPI, FITC, TRITC, and Cy5. To add a new channel with a different combination of excitation and emission filters, please contact Tomocube at support@tomocube.com.

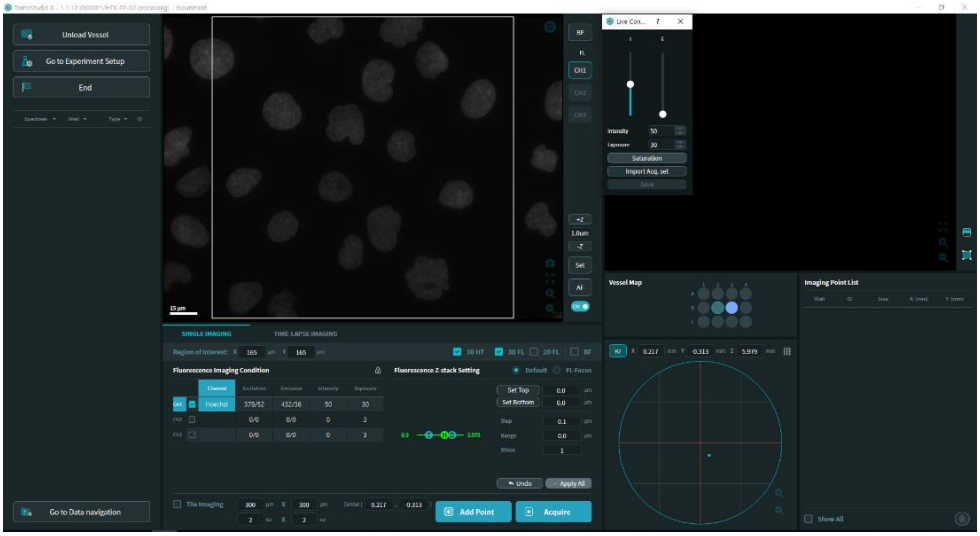
3. When Channel 1 is added, the channel selector for Channel 1 in the Live View mode is available for image acquisition.



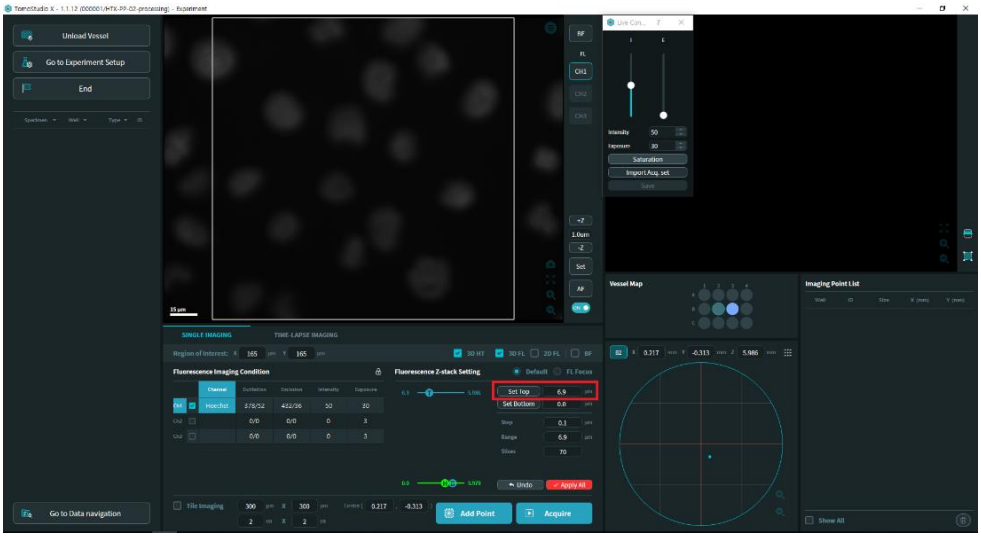
4. Click the selector for Channel 1 on the right side of the Live View panel to adjust the imaging settings for the FL channel. Enter the intensity and the exposure time in the Live View panel to optimize the FL signal. Then click the [Save] button to save and apply the parameters as the FL imaging condition.



5. After clicking the [Save] button, the FL imaging condition is updated.

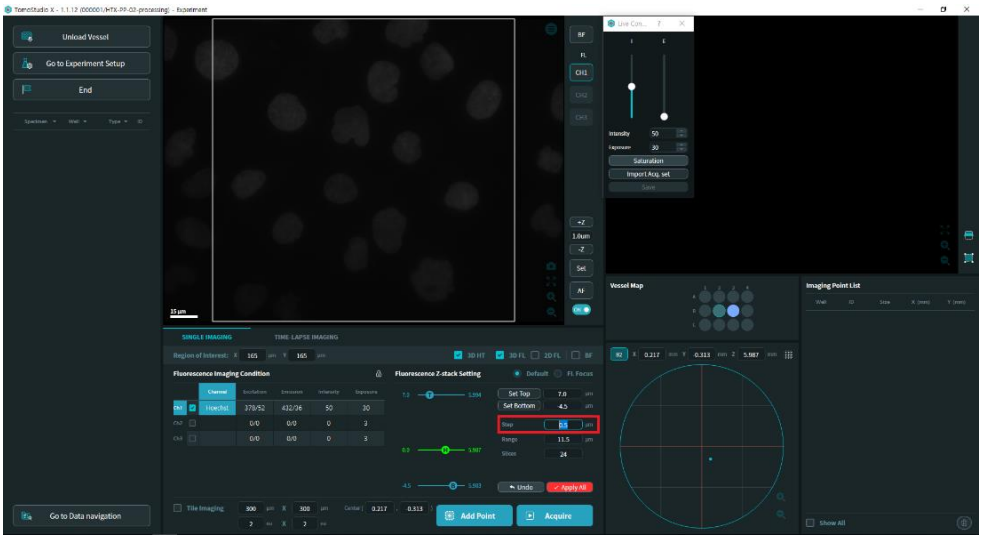


6. To acquire FL images in 3D, set the top and bottom positions of the FL acquisition in the Z direction. To set the uppermost position for FL acquisition, move the focus of the current FOV to an appropriate Z position by using the Z control buttons. Then click the [Set Top] button to apply the current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



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

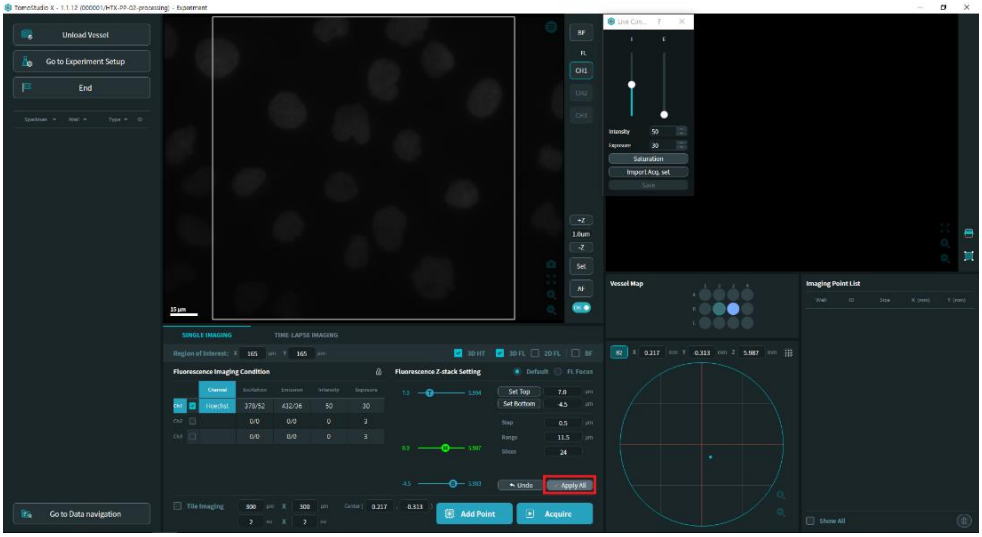
8. Set the step size of the FL imaging in the Z direction for the 3D fluorescence images. A step size of 0.5 μm is recommended for imaging mammalian culture cells.



9. After entering the step size, the number of FL 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.

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

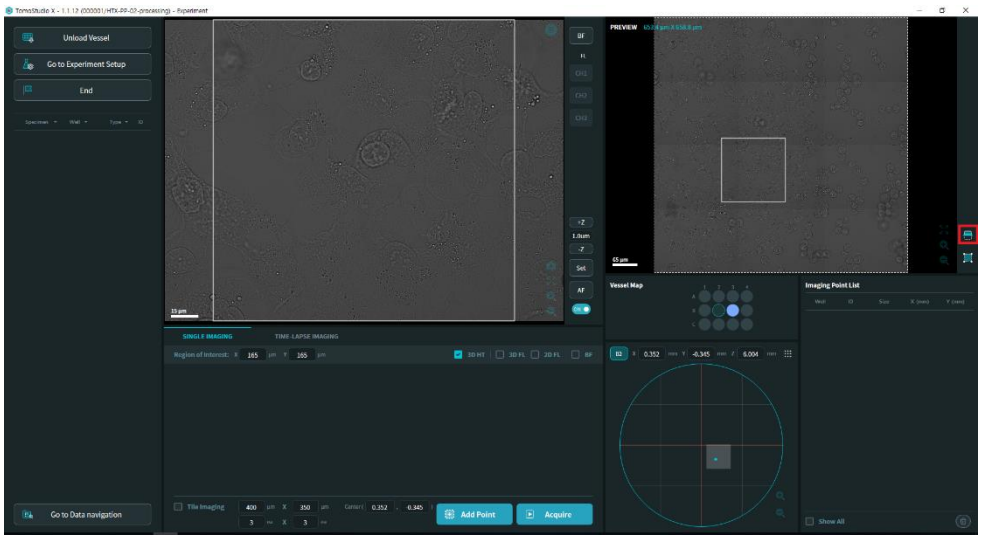


11. Click the [Acquire] button to perform HT and FL imaging. Acquired images are updated automatically on the data list table.

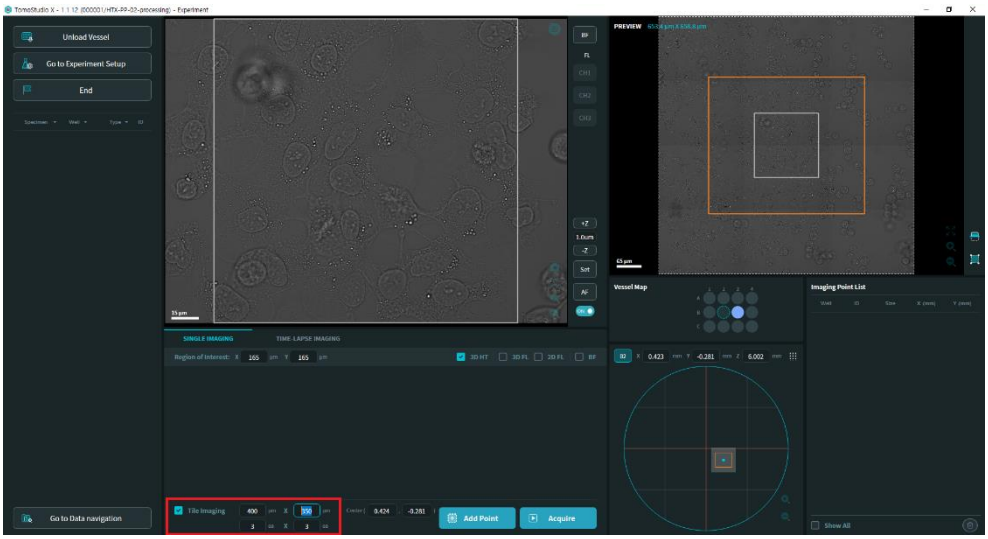
5.5.3 Tile acquisition

To acquire an image of a wider area than the maximum FOV (around 200 μm × 160 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.

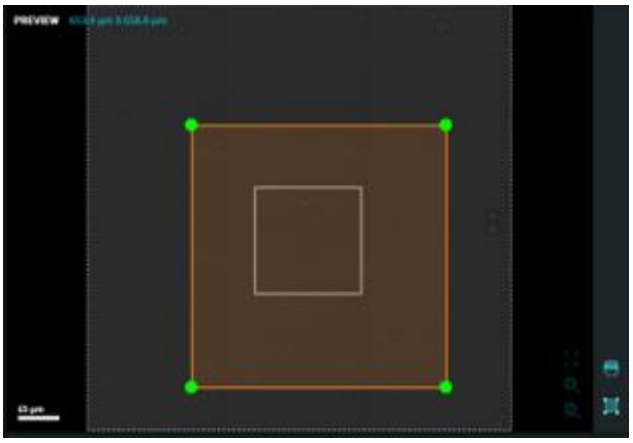


2. Click the [Tile Imaging] checkbox to activate the Tile Imaging mode. The tile imaging area will appear as an orange box in the Preview panel and on the Well Map.

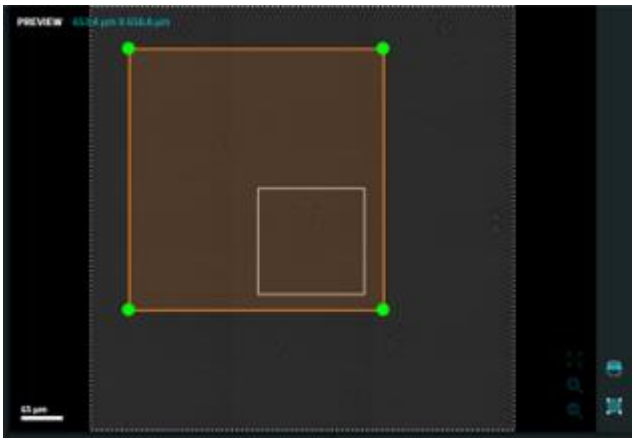


3. Set the imaging size for tile imaging by clicking one of the four green dots on the corners of the tile imaging area box in the Preview panel and dragging it to the desired size.

   Note: The tile size can also be set by entering width and height values in the Tile Imaging panel.



4. 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.



5. Confirm that the imaging modes and settings are properly set and then click the

[Acquire] button to start the imaging.

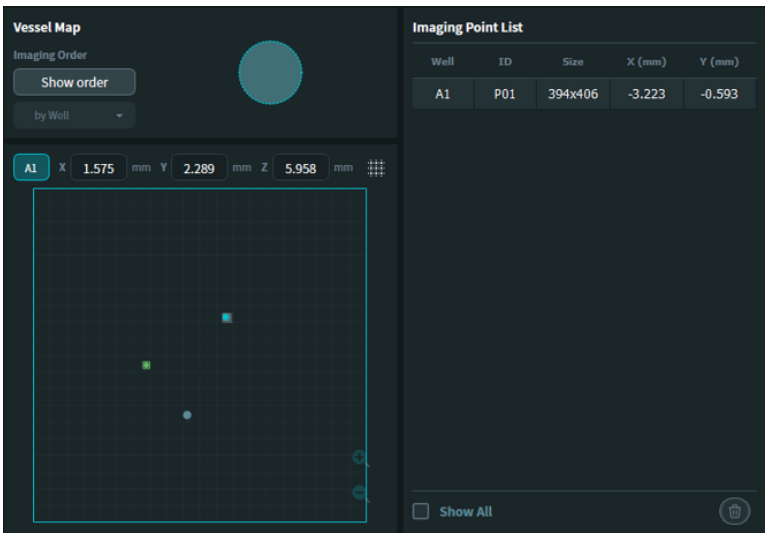
5.6 Time-lapse imaging

5.6.1 Add imaging points

To perform time-lapse sequence 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 Time sequence setup — Basic

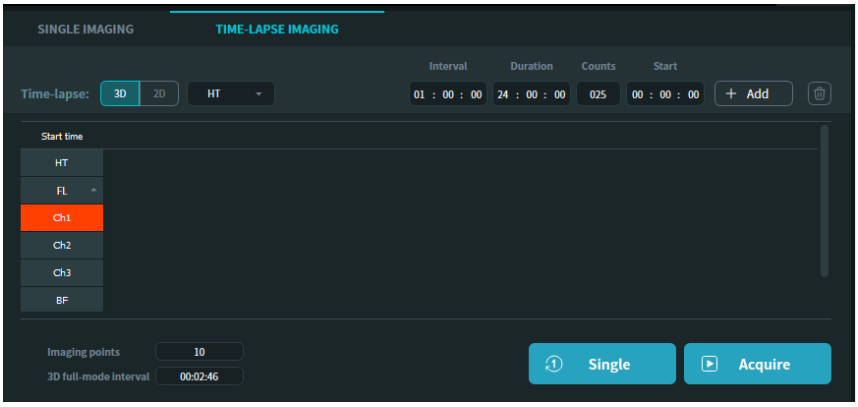
After registering all desired imaging points in all wells, the time-lapse sequence can be scheduled by setting the appropriate intervals and imaging modes. Basic guidelines for setting various time-lapse imaging types are provided below.

Standard HT 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 HT time-lapse: a time-lapse sequence in HT mode with a uniform interval.

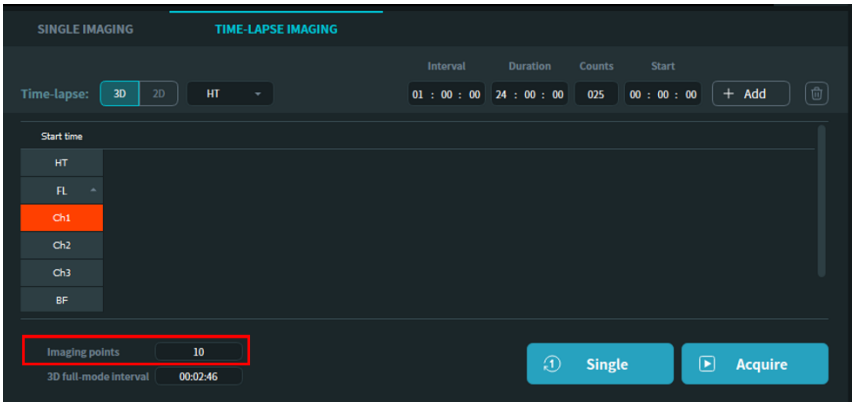
Prerequisite ▶ To enable a time-lapse sequence, at least one imaging point needs to be registered in the Imaging Point List.

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

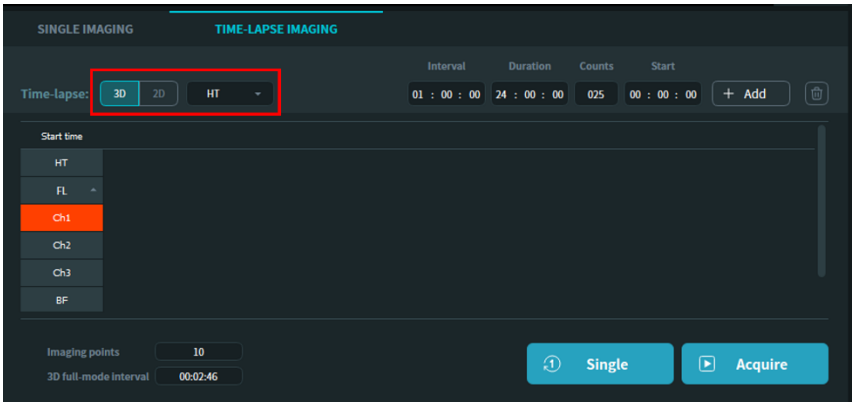


2. Confirm that the imaging points are registered properly.

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

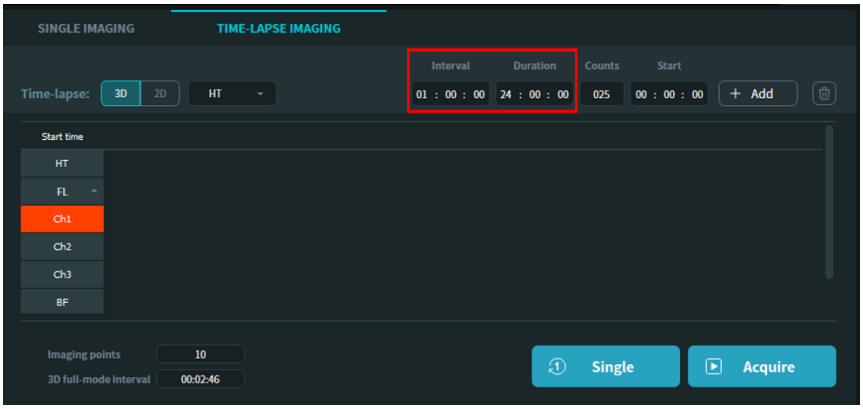


3. To add a time frame for the HT time-lapse sequence, set the sequence mode to 3D and HT.

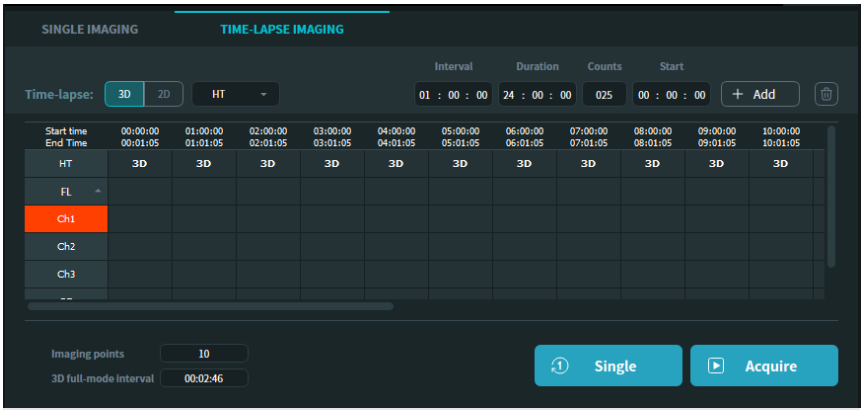


4. Enter the imaging interval and duration for each time frame in the corresponding input fields, hereafter referred to as the Sequence time input field.

   Note: The counts of the sequence are calculated automatically.



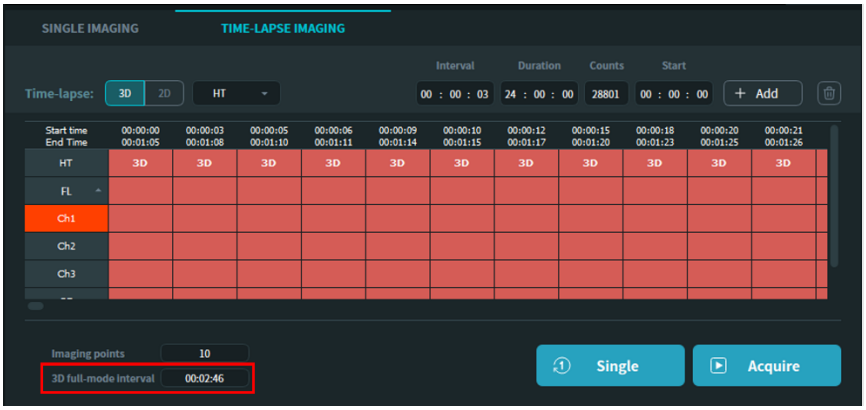
5. Click the [Add] button to apply the sequence setup into the Time-Lapse Schedule Table.



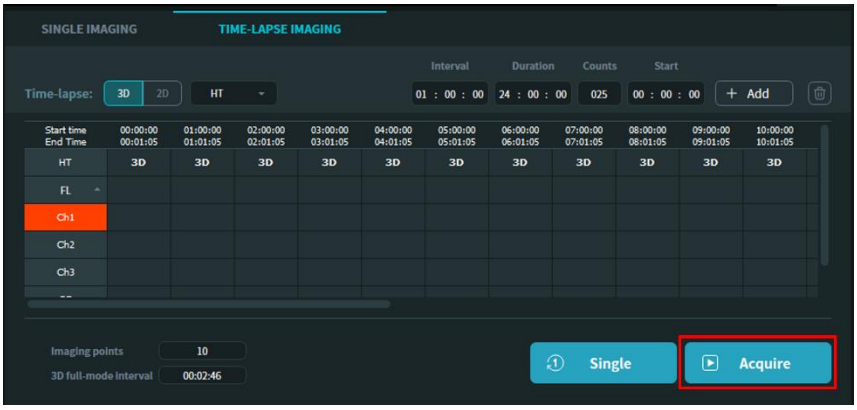
6. Confirm that no error has occurred in all time frames. Time frames with an error are highlighted in red.

   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.



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

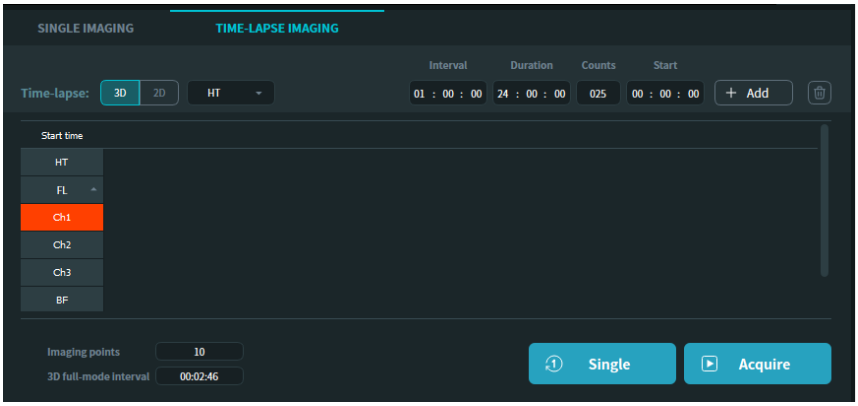


Standard HT time-lapse with FL or BF

Prerequisite

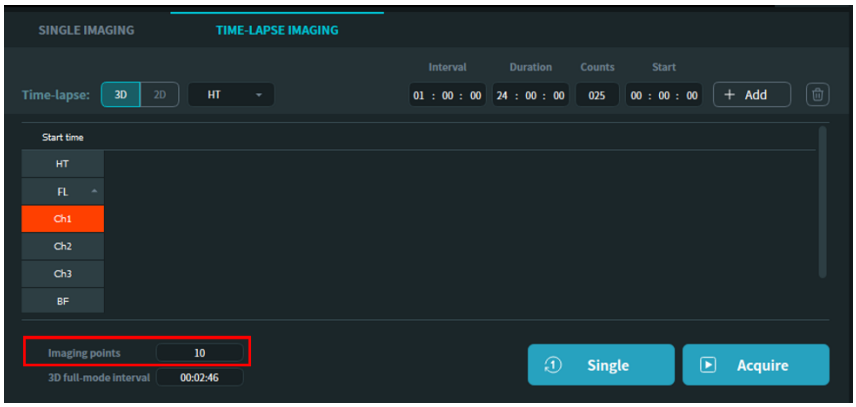
- At least one point is registered in the Imaging Point List.
- The FL or BF mode is activated in the Single Imaging tab.

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

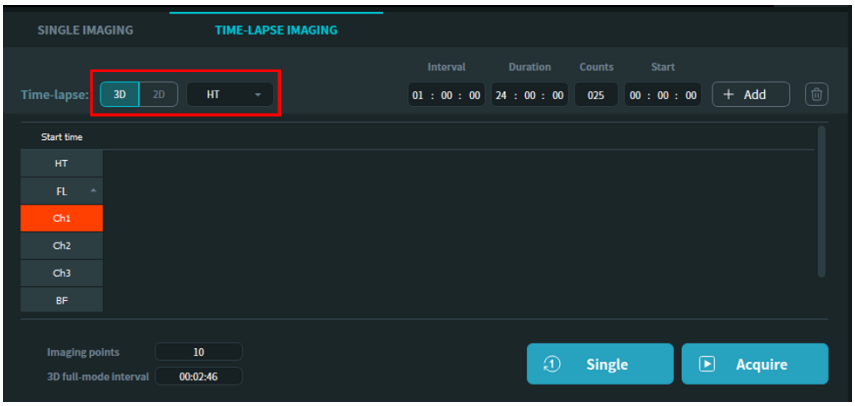


2. Confirm that the imaging points are registered properly.

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

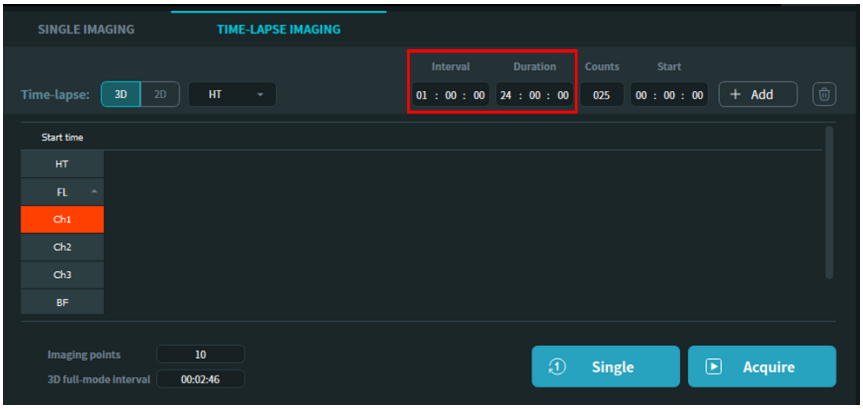


3. To add a time frame for the HT time-lapse sequence, set the sequence mode to 3D and HT.

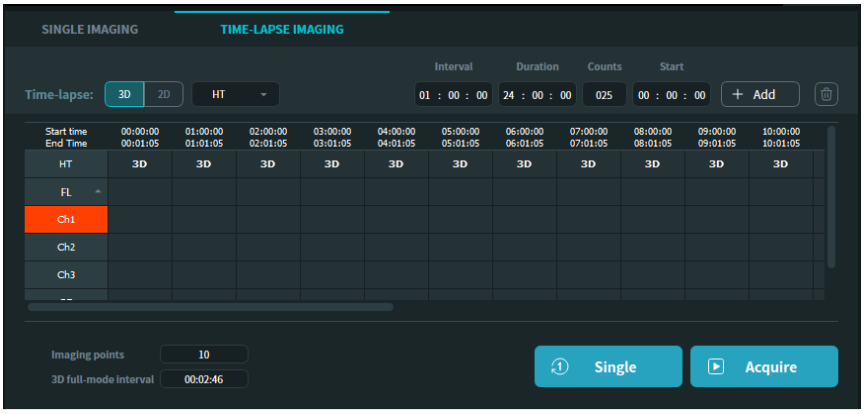


4. Enter the imaging interval and duration for each time frame in the Sequence time input field.

   Note: The counts of the sequence are calculated automatically.

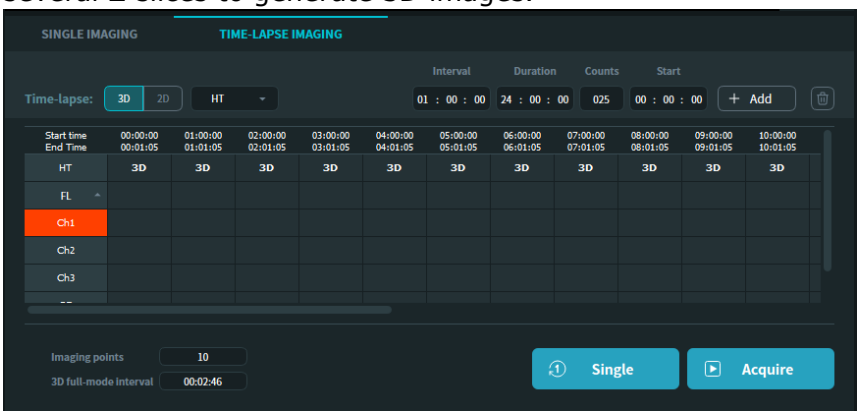


5. Click the [Add] button to apply the sequence setup into the Time-Lapse Schedule Table.



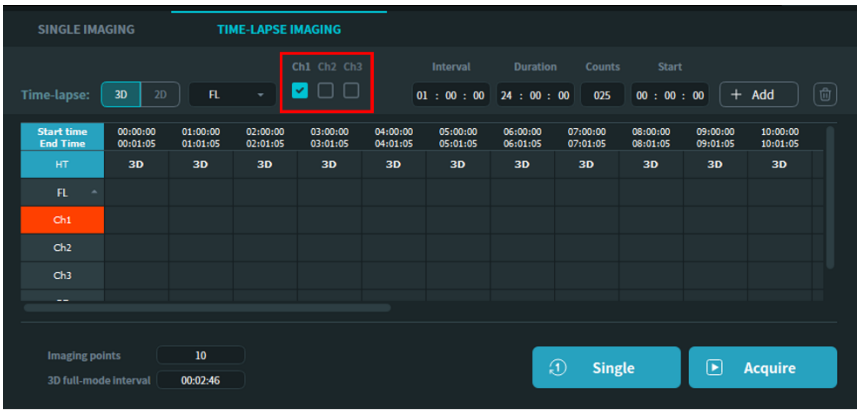
6. To include fluorescence imaging in the time-lapse sequence, set the mode to FL.

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

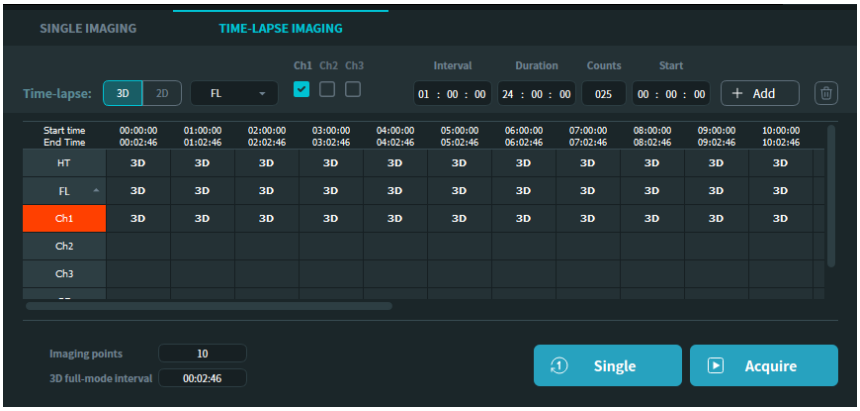


7. Click the desired FL channel checkbox for time-lapse imaging.

   Note: Only the channels that are activated in the Single Imaging tab are available for time-lapse imaging.

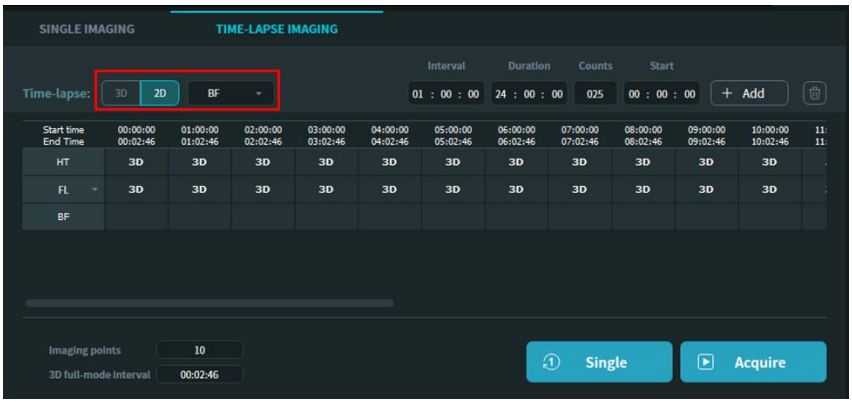


8. Click the [Add] button to apply the sequence setup into the Time-Lapse Schedule Table.

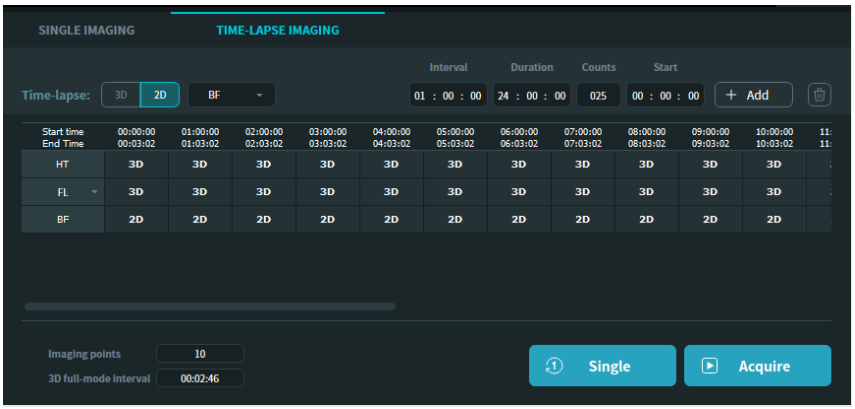


9. To add BF channel data into the time-lapse sequence, set the mode to BF.

   Note: The BF mode is only available in the 2D mode.



10. Click the [Add] button to apply the sequence schedule of the BF 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 [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.

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

5.6.3 Sequence setup — Advanced

Advanced HT time-lapse with hetero-imaging of FL or BF

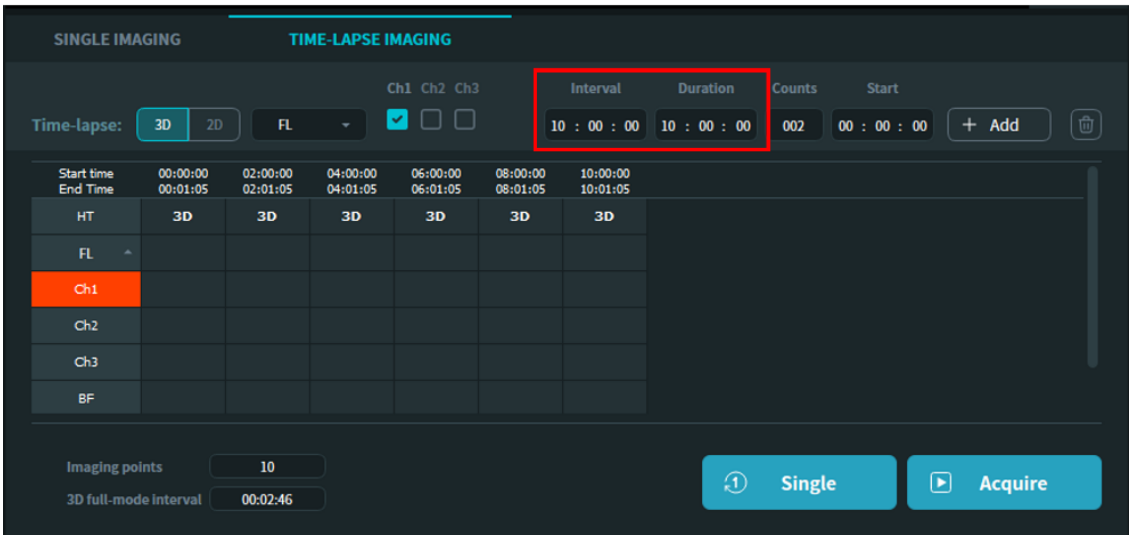
In addition to basic time-lapse imaging, TomoStudio X provides more advanced **hetero time-lapse imaging** that consists of multiple imaging modes with a different interval for each mode. Although the setup for hetero time-lapse imaging is more complicated than for basic time-lapse imaging, a wide range 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 FL at the start and the end of the sequence

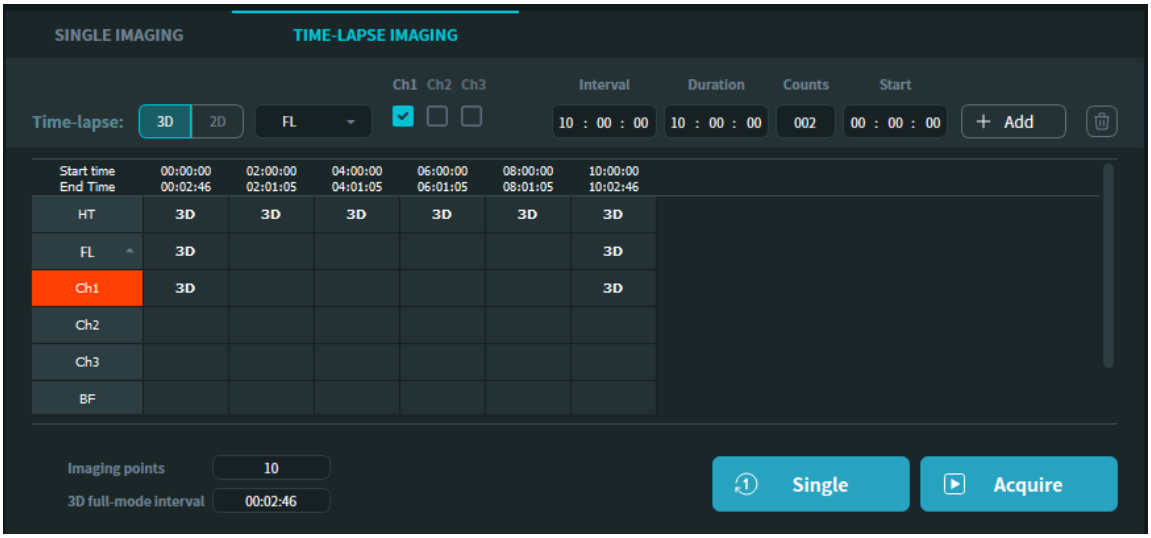
Prerequisite
- At least one point is registered in the Imaging Point List.
- The FL or BF mode is activated in the Single Imaging tab.

1. Click the Time-Lapse Imaging tab in the Acquisition panel and confirm that the imaging points are registered properly.
2. Add an HT 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 FL 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 FL 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 FL 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.

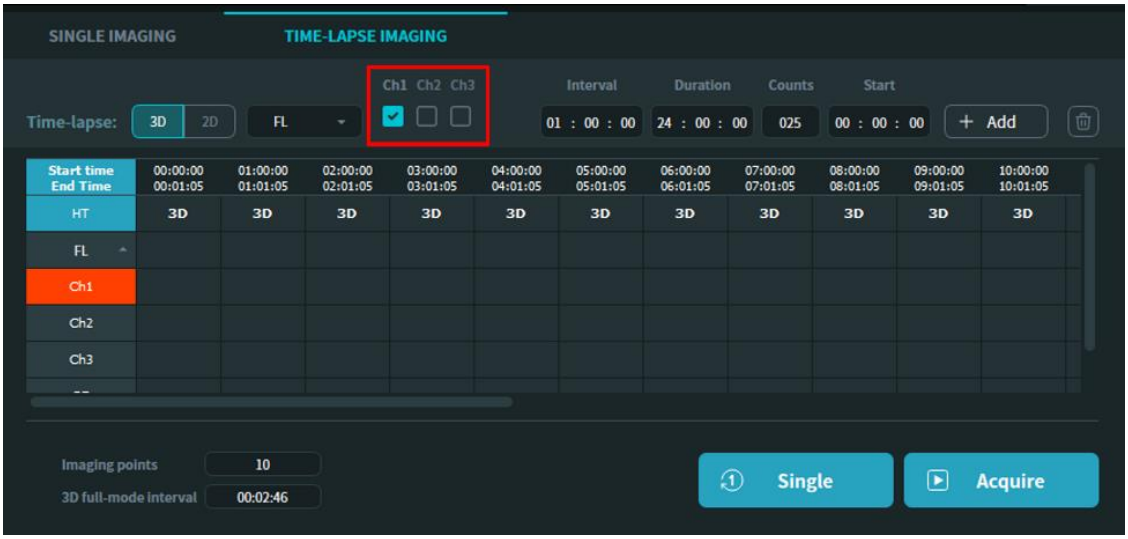
Repetitive imaging of HT with single FL

Prerequisite

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

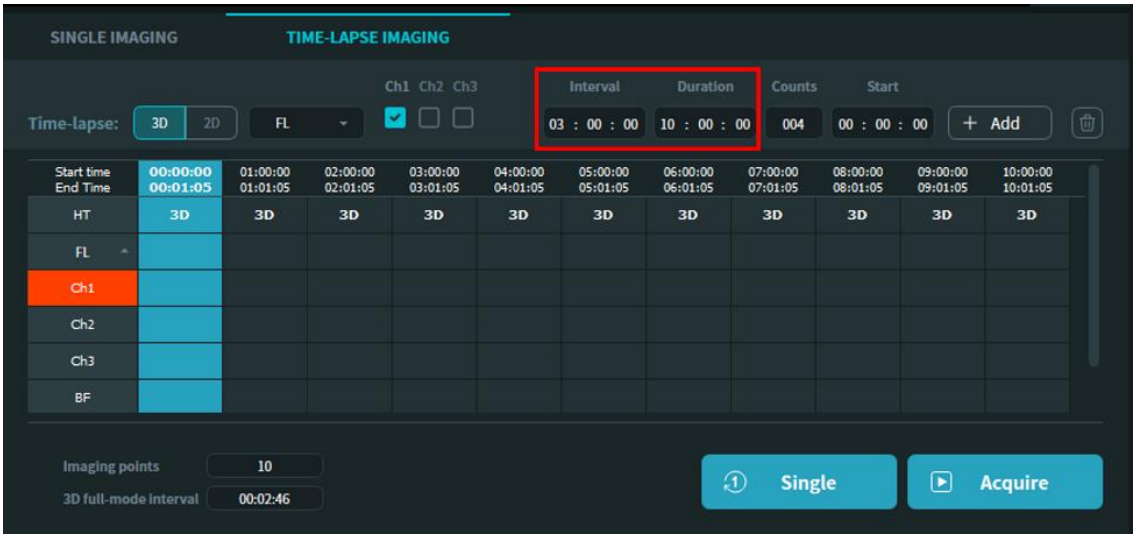
1. Click the Time-Lapse Imaging tab in the Acquisition panel and confirm that the imaging points are registered properly.

2. Add an HT 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 FL channel checkbox for time-lapse imaging.

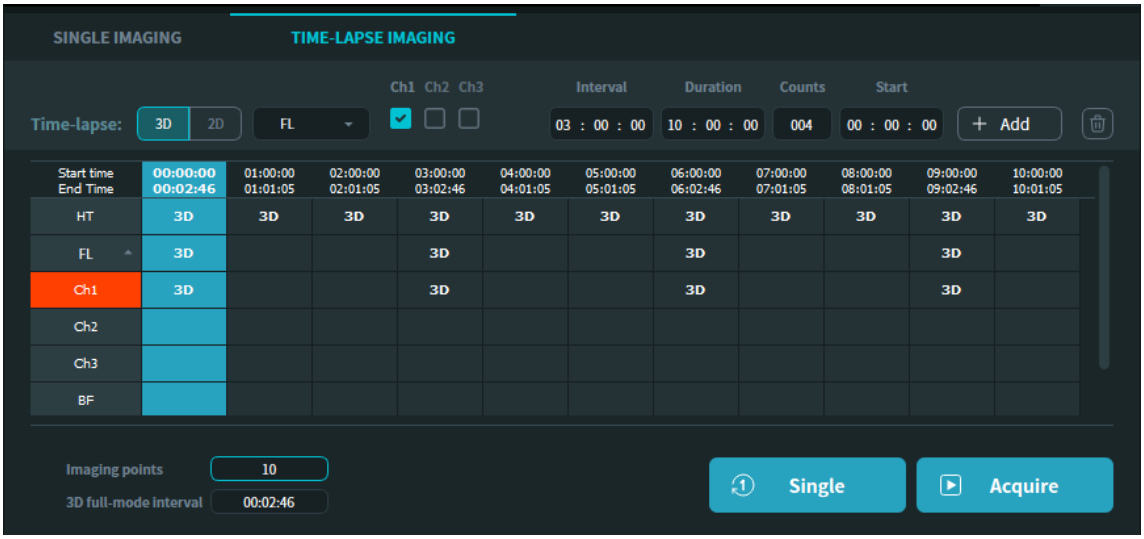


5. Enter the imaging interval for the FL channel as a multiple of the value set for the HT interval in the Sequence time input field.

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



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



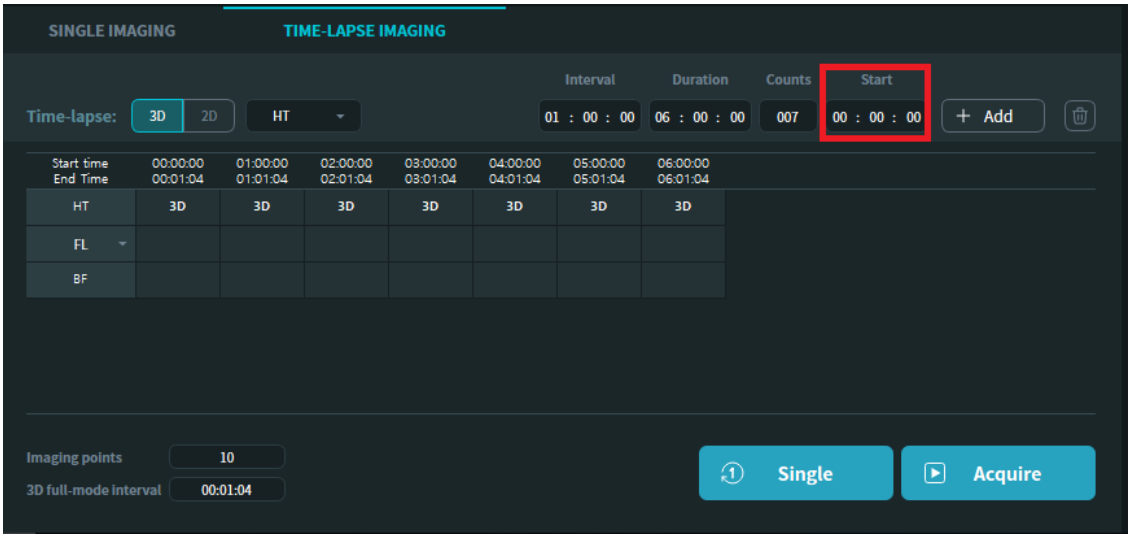
Important! When the FL channels are added into the Time-Lapse Schedule Table, an error may occur if the interval set for HT is not long enough. Two methods to resolve this issue are as follows: shorten the imaging time by deregistering some of the imaging points, or remove some of the time frames that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

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

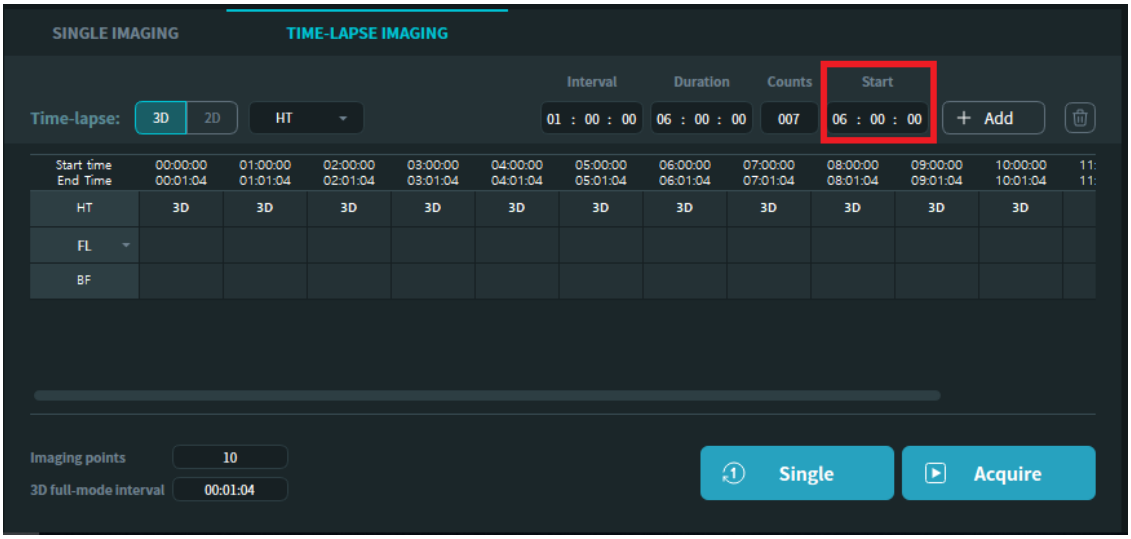
Two different intervals for HT imaging with different start time points

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 HT 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 HT time-lapse sequence with a different interval and duration in the Sequence time input field.
5. Enter the desired Start time of the second HT 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.

5.7 Time-lapse progress window

After starting a time-lapse acquisition, TomoStudio X displays a 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.

5.7.1 User interface description

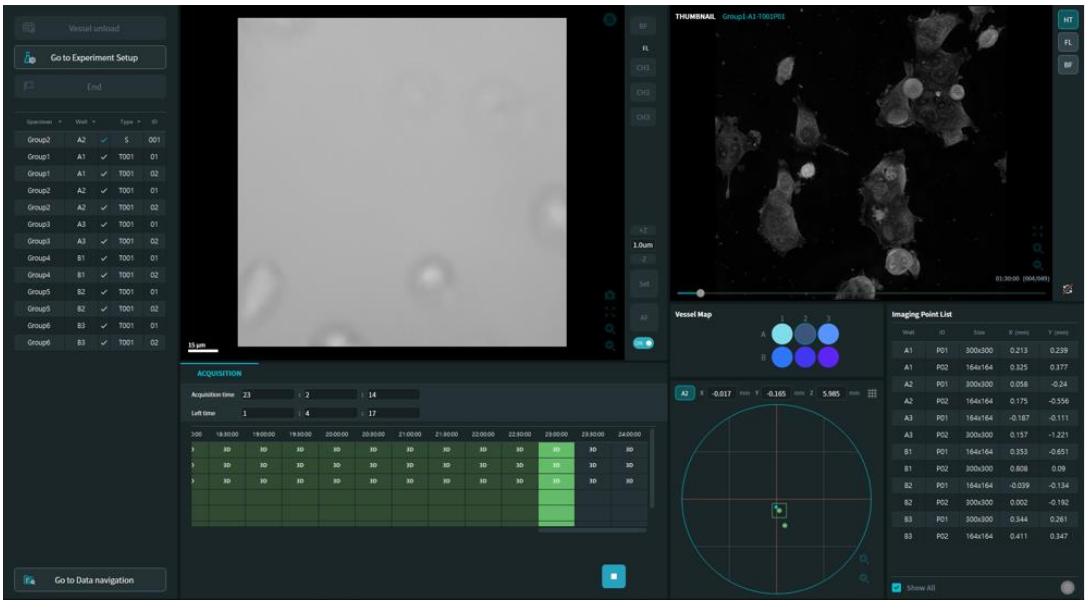
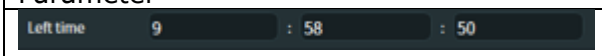
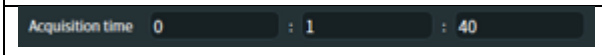
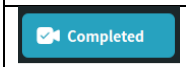


Figure 5.12 Time-Lapse Progress window

During time-lapse imaging, the Time-Lapse Schedule Table displays the status of the time-lapse schedule with various colors. The current imaging time frame 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 hidden.

Parameter		Description
	Time remaining	Shows the remaining time to finish the time-lapse sequence.
	Time elapsed	Shows the elapsed time after starting the acquisition.
	Stop	Immediately stops and exits the time-lapse sequence.
	Completed	Exits the Time-Lapse Progress window after finishing the time-lapse sequence.

Thumbnail viewer

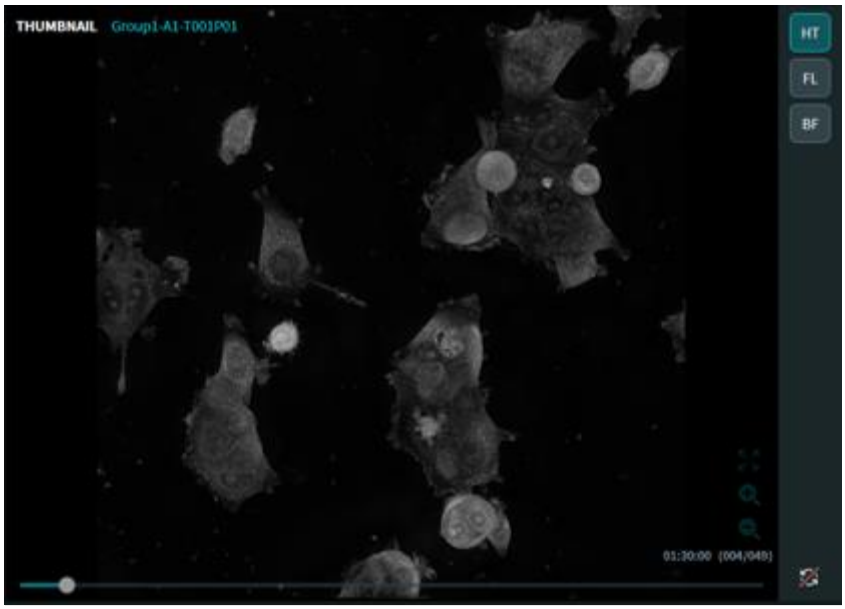


Figure 5.13 Thumbnail viewer

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 Page 69 for Image processing.

Parameter		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

TomoStudio™ X utilizes a proprietary file format, TCF (Tomocube Common File), for storing the image data. A TCF file stores not only images but also annotation datasets that include multiple types of images such as refractive index (RI) tomograms, maximum intensity projection (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 3D tomograms and other FL or BF images. Image Processing is performed by the HTX processing server, which runs as a separate module.

6.1.1 User interface description

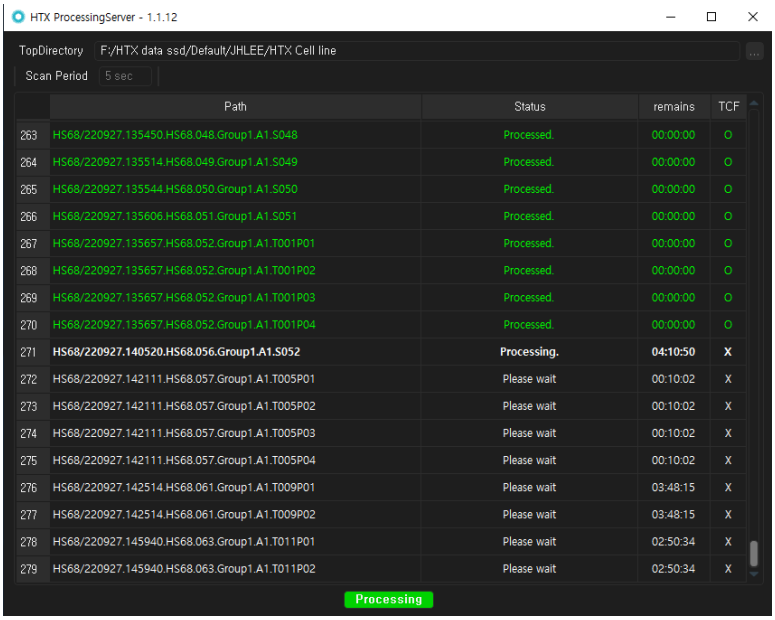


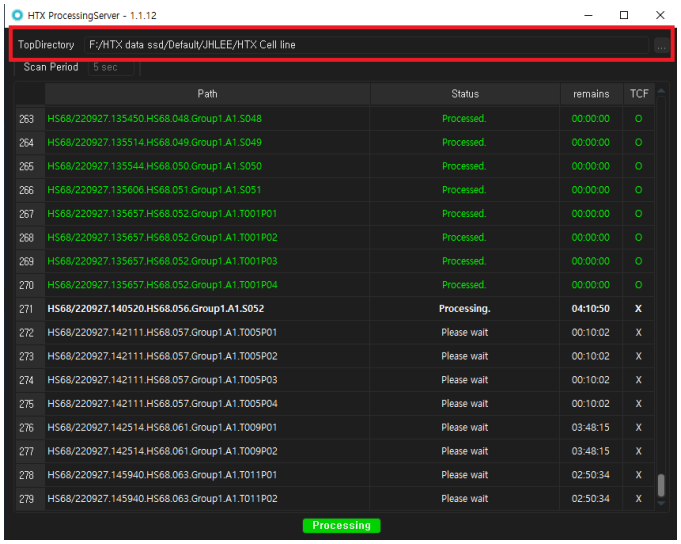
Figure 6.1 Processing server

Parameter		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.

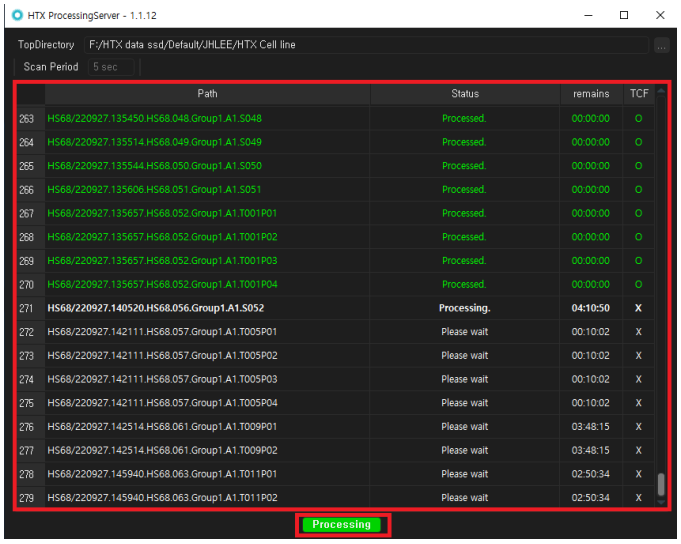
6.1.2 Running image processing

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.



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 panel in the Experiment Manager 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.

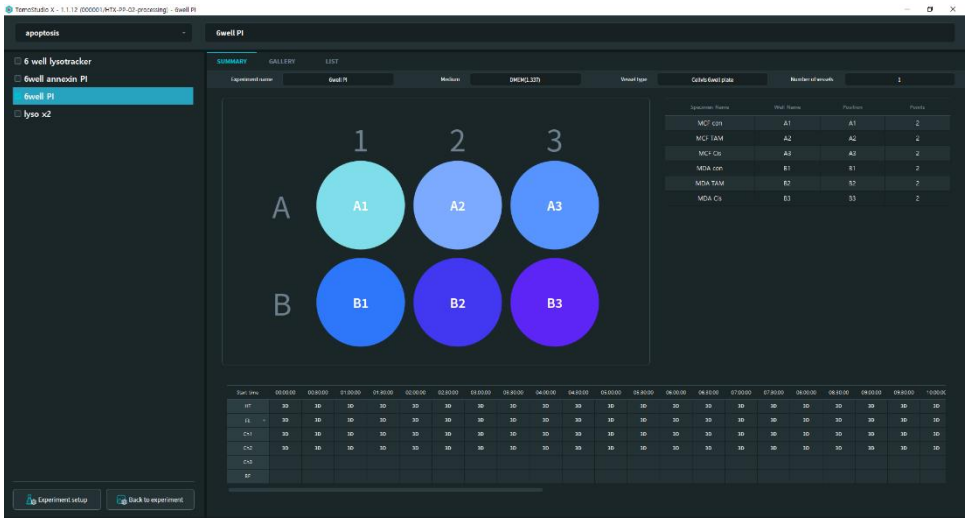


Figure 6.2 Data navigation (Summary mode)

Gallery mode

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

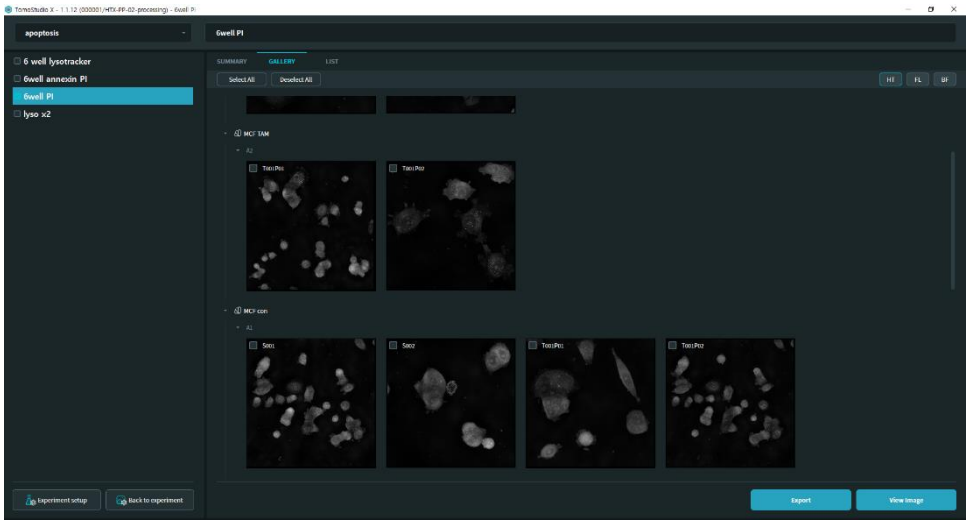


Figure 6.3 Data navigation (Gallery mode, HT view)

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

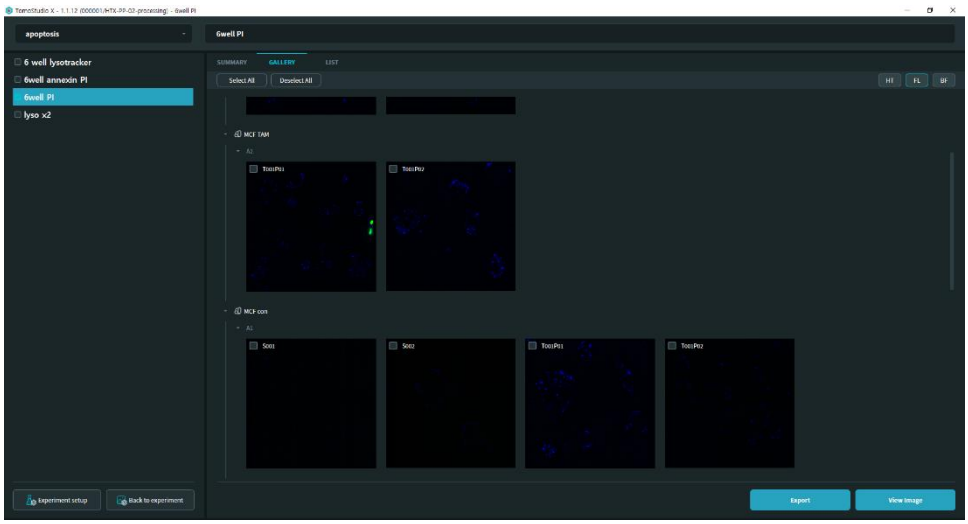


Figure 6.4 Data navigation (Gallery mode, FL view)

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.

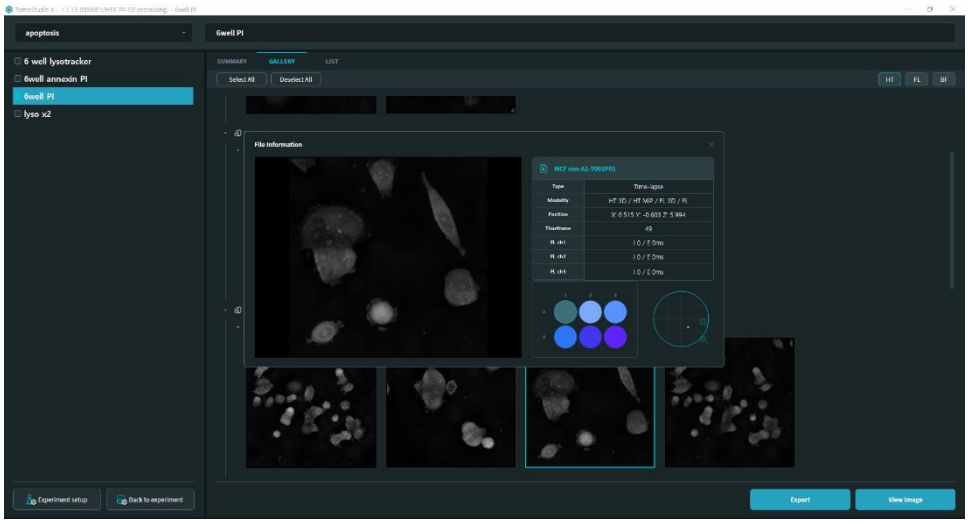


Figure 6.5 Data navigation (Gallery mode, file information window)

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 a 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.

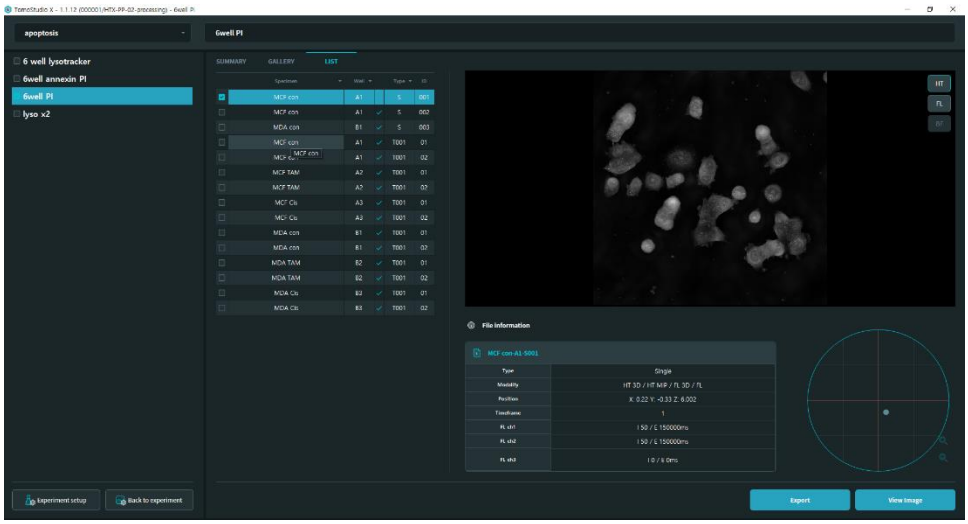
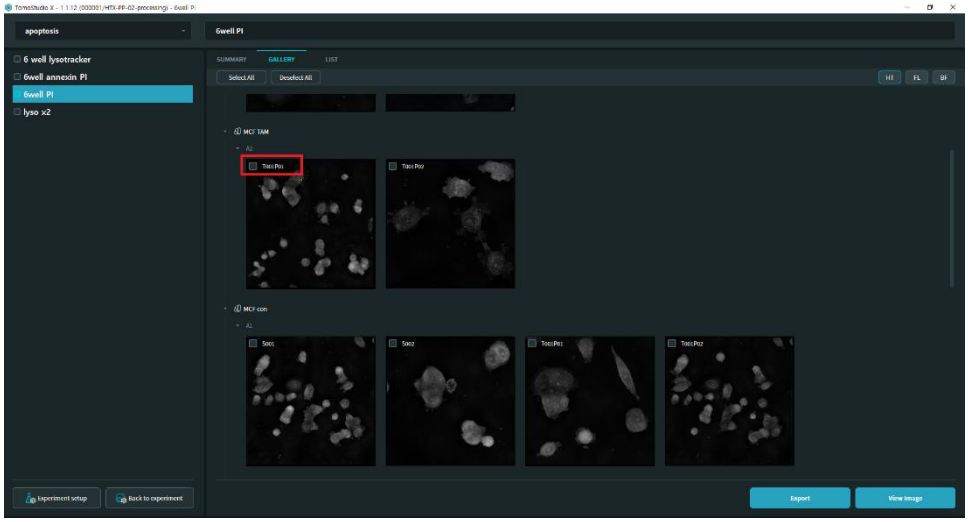


Figure 6.6 Data navigation (List mode)

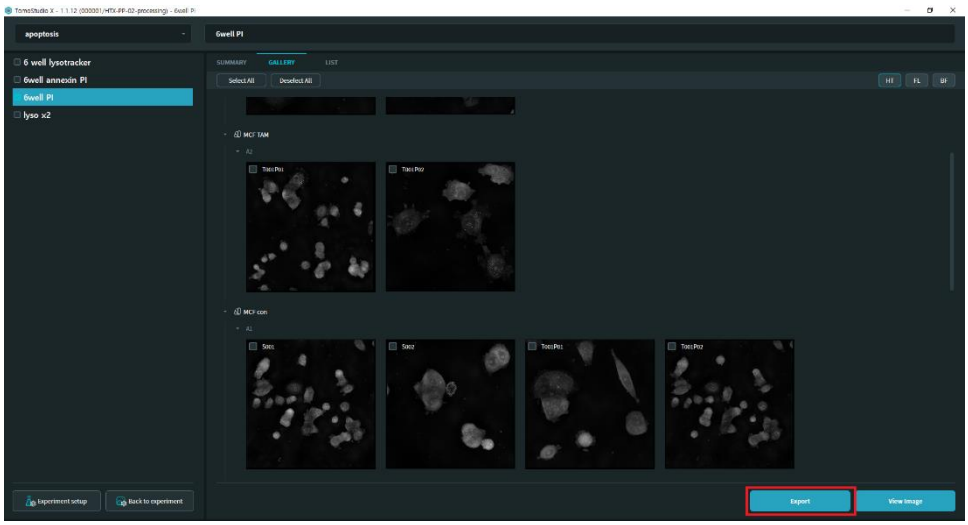
6.2.2 Data export as RAW file

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 TomoStudio X.

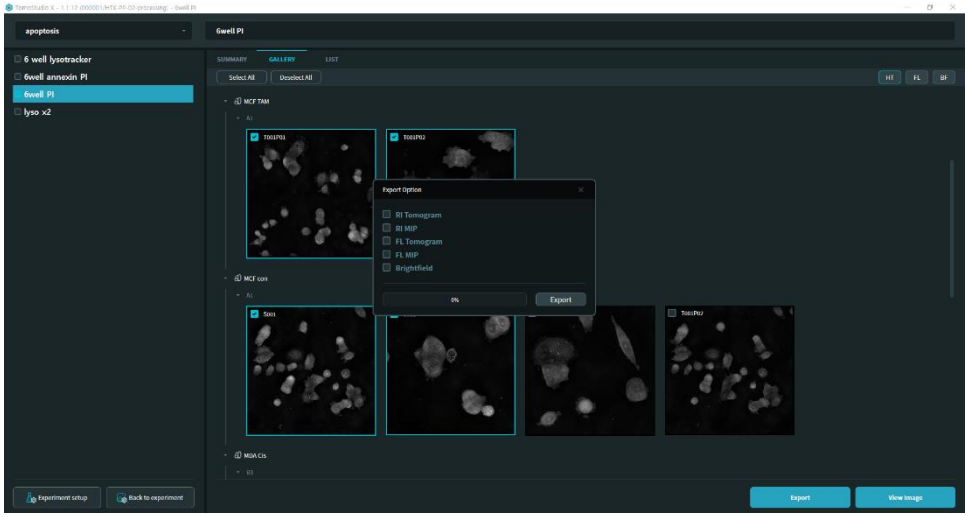
1. In Gallery mode or List mode, click the data file to be exported.



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.



6.3 Image viewer

To examine the TCF data, the Image Viewer in TomoStudio X can be used. The Image Viewer shows a 2D slice of the selected TCF data along the Z-axis. Users can adjust the image brightness and contrast as well as export the data as an image or a video.

6.3.1 User interface description

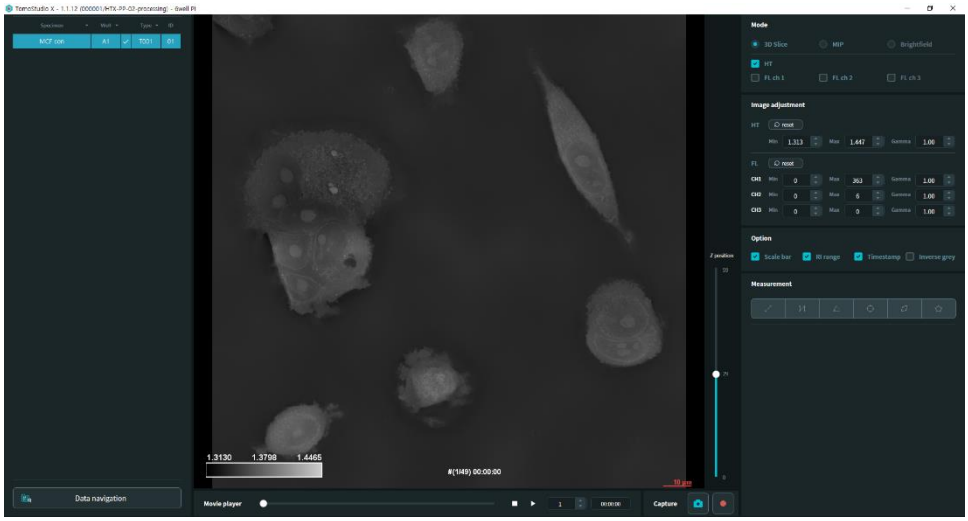
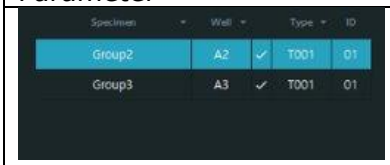
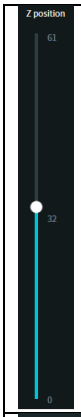
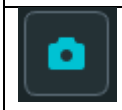
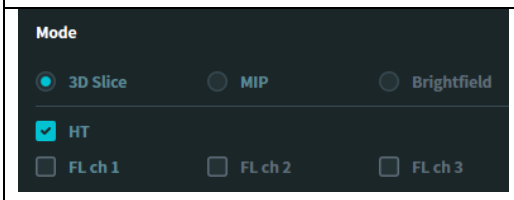
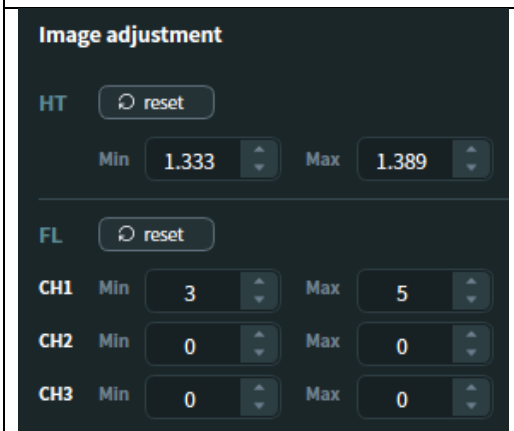
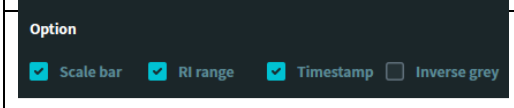


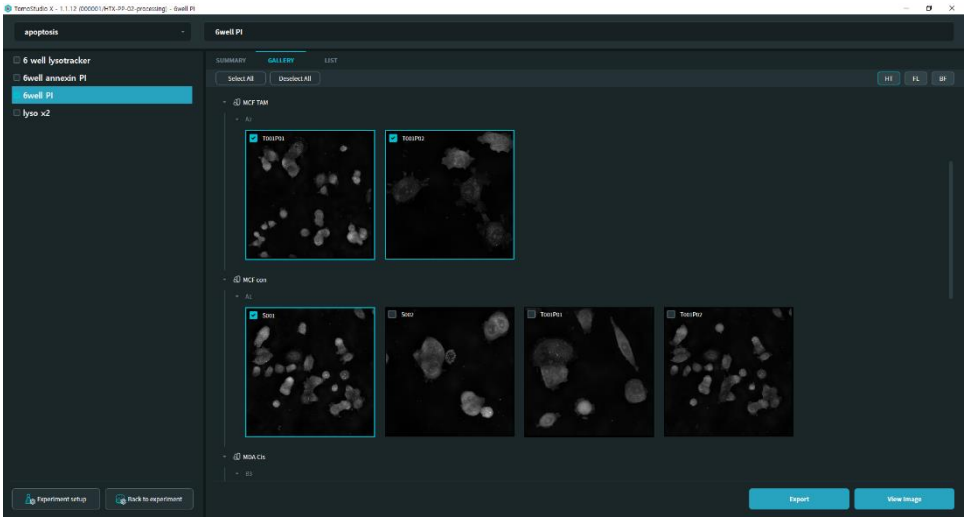
Figure 6.7 Image Viewer

Parameter		Description
	Image list	Located on the left side of the Image Viewer, the image list shows the data files currently loaded in the Image viewer.
	XY image panel	Located in the center of the Image Viewer, the XY image panel displays 2D slices of the data along the Z-axis.

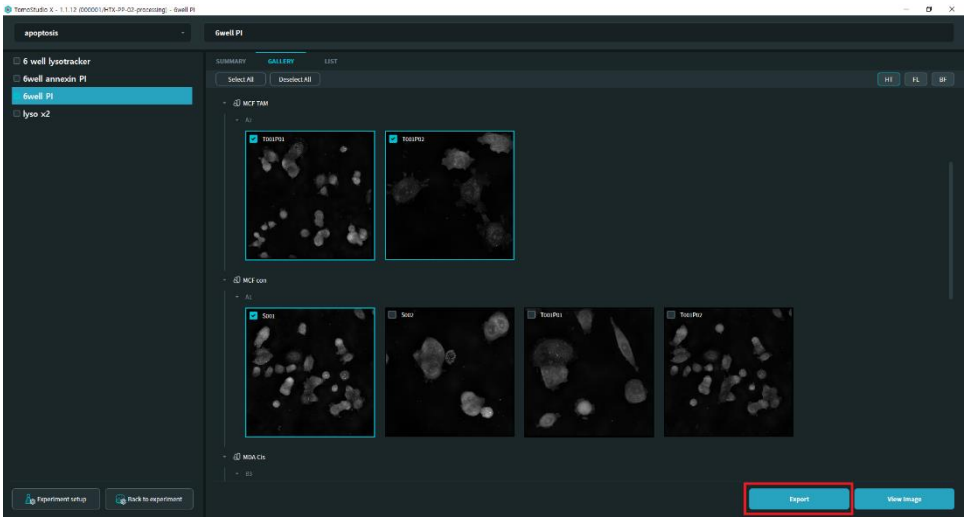
	Z-axis slider	Located on the right side of the XY image panel, the Z position slider is used to change the Z position of the displayed 2D slice.
	Movie player slider	Located under the XY image panel, the movie player slider is used to select the time frame to display from the selected time-lapse data file.
	Capture	Saves the current image in the XY image panel in PNG format.
	Record	Saves the current time-lapse data in MP4 video file format.
	Mode selection	Selects the image mode to display the data file with three options: 3D Z slice, 2D MIP, and BF. For 3D Z slice and 2D MIP, the HT and FL channels to display can be selected.
	Image adjustment	Sets the value for the minimum and maximum intensity for each image channel.
	View option	Checkboxes show/hide the scale bar, the range of RI, and the timestamp, and to select inverted contrast.

6.3.2 Loading multiple data files into the Image Viewer

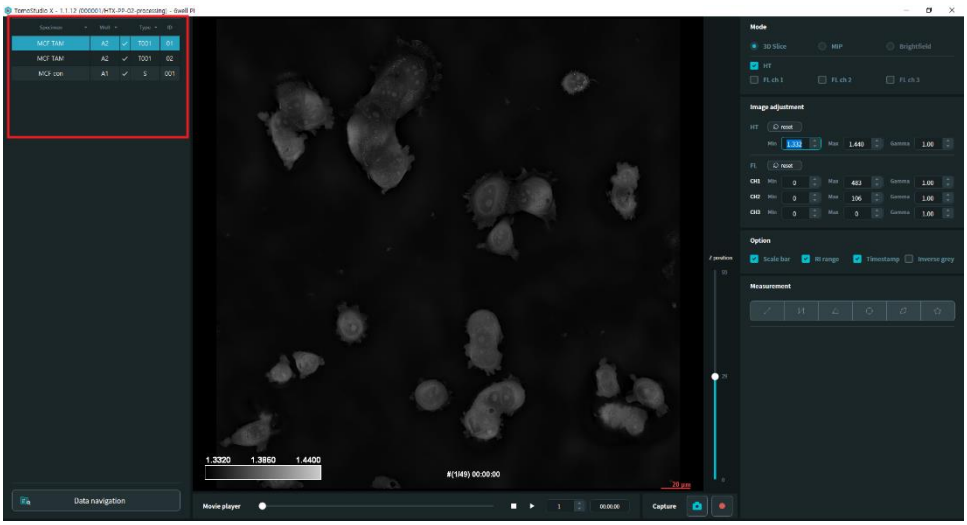
1. Select multiple images to load into the Image Viewer from the Gallery mode or List mode by clicking the desired images while holding the Ctrl key.



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



3. In the Image Viewer window, switch the view to other images by double-clicking the data in the image list.



CHAPTER 7. Maintenance

7.1 Daily care

The HT-X1 imaging system does not require routine maintenance or calibration.

To clean the surface of the instrument, use only a soft, dry tissue or cloth. Do not use liquids (e.g., alcohol, water, bleach).

> ⚠ **CAUTION** The use of harsh cleaning products or liquids to clean the instrument may scratch, damage, or stain its surface.

7.2 Decontamination

Contamination of the sample loading area can be removed by using 70% ethanol swabs.

For more information, contact technical support at support@tomocube.com.

Appendix A. Technical Specifications

A.1 Physical specifications

Dimensions (mm)	Instrument: 563 x 732 x 921 mm (W x D x H) Package: 800 x 900 x 1050 mm (W x D x H)
Weight (kg)	90 kg
Power supply*	100-240 VAC, 50/60 Hz, 5 A (max.)
Objective lens	Olympus UPLXAPO40X - 40x, NA 0.95, air
Optical resolution	Holotomography: lateral 156 nm, axial 1.07 µm Fluorescence*: lateral 270 nm, axial 1.13 µm at 488 nm wavelength
Image sensor	2.8 MP CMOS QE: 76%
Field-of-view (µm)	218 µm x 165 µm
Acquisition speed	Single 3D HT acquisition: 6 sec - 24 well requires 8 sec
Stage	Travel range - XY: 150 mm x 160 mm (resolution: 0.3 µm) - Z: 8 mm (resolution: 0.04 µm) Imaging area - 100 mm x 60 mm
Autofocus sensor	Laser wavelength: 780 nm Range: +100 to –300 µm Speed: < 1 sec with 100 µm displacement
Light source	Holotomography: 450 nm, LED Fluorescence: 4 channels, LED
Fluorescence filter	Size: ø25 mm / t = 3 to 5 mm (excitation, emission) Users can apply up to 5 filters Exchange time: 100 ms

A.2. Environmental specifications

Temperature	20 °C to 28 °C (68 °F to 82 °F)
Relative humidity	RH 35–85 %, non-condensing
EMC characteristics	Conforms with IEC 61326-1 (Class A, Group 1)
Safety	Conforms with IEC 61000-3-2

Appendix B. Stage-Top Incubator

The HT-X1 system supports a stage-top incubator to maintain an optimal temperature and gas concentration for live cell imaging. The incubator contains a built-in gas mixer unit and a chamber unit for mounting specimens onto the stage.

For more information about the product including safety instructions, refer to the STX-E series instruction manual (document number: MA-STX-E-EN-01-08) included in the package.

Important! Confirm that the stage-top incubator is included in the purchasing list.

Important! Only the stage-top incubator unit provided by Tomocube is compatible with the HT-X1 imaging system.

B.1 Physical specifications

Model	STXG-WSKMXA22B (Tokai HIT)
Dimensions (mm)	151 x 263 x 196 mm (W x D x H)
Weight (kg)	3.8 kg
Temperature setting range	Sample temperature: 37 °C Top heater: 10 °C – 65 °C Bath heater: 10 °C – 50 °C Stage heater: 10 °C – 50 °C Lens heater: 10 °C – 45 °C
Rise time	10 min to reach 50 °C
Temperature accuracy	Within 0.3 °C
Humidity control	Heated humidification by the heating bath unit - Recommended water volume: 32 mL
CO_2 concentration range	5% – 20% - Control method: PID control - Accuracy: ±0.1%
Gas type	100% CO_2
Gas pressure	Input: 0.1 MPa – 0.15 MPa Output: 160 mL per mine
Power source	100 V – 240 V AC ±10%, 50/60 Hz
Maximum power consumption (W)	110 W

B.2 Packing list

Index	Item	Quantity	Box
1	Warranty Policy	1	Main box
2	Instruction Manual	1	Main box
3	STXG Controller	1	Control Unit Box
4	220/110V Power cord	1	Control Unit Box
5	Gas tube	1	Control Unit Box
6	Dish Attachment for Well Plate	1	Accessory Box
7	Dish Attachment for 35/60 mm Dish	1	Accessory Box
8	Precision Screwdriver	1	Accessory Box
9	Disposable Syringe	1	Accessory Box
10	Dish Fixing Lid for 60 mm dish	1	Accessory Box
11	Dish Fixing Lid for 35 mm dish	1	Accessory Box
12	(Lid for Well Plate) *Spare glasses are included	1	Accessory Box
13	Temperature Sensor and Extension Wire	1	Accessory Box
14	Spare Cover Glass	1	Accessory Box
15	Chamber	1	Chamber Unit Box
16	Communication cable	1	Chamber Unit Box

B.3 Installation

Important! The stage-top incubator must be installed by a professional technician authorized by Tomocube.

1. Prepare a 100% CO_2 gas cylinder with a regulator.
2. Connect a gas tube between the regulator on the CO_2 gas cylinder and the 'CO_2 IN' port on the rear panel of the incubator controller.
3. Connect the opposite end of the gas tube connected to the incubator controller to the gas inlet of the chamber.
4. Connect the power cable between the incubator controller and power source.

B.4 How to start

1. Turn the pressure adjustment knob of the gas cylinder in the clockwise direction to adjust the secondary pressure indicated in the output meter of the regulator to the range from 0.1 MPa to 0.15 MPa.

Important! Start the gas supply 30 min before the experiment.

2. Turn the main switch on.
3. Pour distilled water into the water bath of the incubator chamber. The water level should be up to about 70% of the bath.
4. Place the white plastic cover of the incubation chamber over the water reservoir in the incubator chamber.

B.5 Maintenance

1. Turn the main switch off.

2. Carefully remove the top cover of the incubator chamber and wipe away any condensed water (dew) from the inner surface.

3. Remove the remaining water in the water bath with a syringe.

4. Clean the chamber using ethanol swabs.

Note: To prevent contamination, make sure to completely remove all water drops inside the water bath.

Appendix C. Image Analysis Using TomoAnalysis

C.1 Overview

TomoAnalysis is an independent analysis software for the image data acquired from Tomocube's Holotomography imaging systems. The software is optimized for comparative studies of samples via quantification of multiple images among different experimental groups, in addition to the three-dimensional (3D) visualization of cells and subcellular organelles. The software provides an intuitive user interface that presents numerous forms of information about the image data. Based on the 3D refractive index distribution captured by the holotomography system, the software displays 3D slices, namely *xy*, *yz*, and *xz* slices, along with maximum intensity projection (MIP) images. The refractive index distribution is also applied to calculate the concentration of a segmented volume, which is a distinctive feature of holotomographic imaging.

C.2 Applications

AI-enhanced automatic single-cell analysis

The cell segmentation by artificial intelligence (AI) application allows users to analyze quantitative measurements of single cells and their subcellular organelles, including the nucleus, nucleoli, and lipid droplets. Several AI models are available that can automatically segment cells and subcellular organelles from the data acquired by the HT-X1 system. Through AI cell segmentation, users can acquire separate cell instance masks and four subcellular organelle masks even if there are many cells in one FOV. The AI cell segmentation models work for both suspension and adhesion cells. However, the AI models were trained with cancer cell lines, and thus the prediction accuracy may vary for unusually shaped cells such as primary neuron cells, primary fibroblasts, or iPSC.

RI-threshold manual analysis

The application for manual segmentation by thresholding the RI allows users to segment one object by RI thresholding from the FOV. Through RI manual segmentation, users can create a mask based on the refractive index by determining the minimum and maximum values of the refractive index for thresholding. For segmented objects consisting of more than one shape, TomoAnalysis provides a function to label each area with a unique ID. RI manual segmentation is advantageous for analysis by simply segmenting cells only or segmenting lipids with a high refractive index. RI manual segmentation is useful to measure non-mammalian cell images like those of bacteria and biomaterials.

Fluorescence signal matching analysis

The FL mask generator application allows users to segment specific regions based on the fluorescently positive areas. Using the FL mask generator, users can segment the region with the desired fluorescence intensity by adjusting the minimum and maximum values of fluorescence. Up to three fluorescence channels are supported. TomoAnalysis can measure quantitative values from the HT data with segmented masks using the fluorescence signals. Because this application enables the quantitative analysis of certain regions that can be specified by fluorescence staining, the FL mask generator can be used when the segmentation of organelles such as lysosomes and mitochondria is not supported by the AI cell segmentation model or by RI-threshold manual segmentation.

Report generation

Reports of the analyzed results are provided for the masks generated from all applications.

Each report includes the following quantitative information about the mask: volume, surface area, projected area, dry mass, concentration, and mean RI. The reports also provide all relevant information on the time-lapse images.

For more information, refer to the TomoAnalysis User Guide or contact technical support at support@tomocube.com.

Appendix D. Support

D.1 Supporting documents

For more information, refer to the following documents.

- HT-X1 Installation Manual (Doc. No. TPM-HTX-02)

D.2 Customer support

Please contact our technical support team and provide the following information.

1. Description of inquiry and problem
    a. The inquiry is regarding the instrument or software usage
    b. Details of the malfunction or defect
    c. Specific requests
2. Serial number: Alphanumeric code of 7 characters on the certificate or label
3. Contact information

Contact information:

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

D.3. Product warranty

Tomocube warrants that its products are free from defects in material and workmanship. In the event of defects in materials and workmanship, Tomocube reserves the right to inspect the Product and to investigate any claim to determine whether the Product is defective. If Tomocube determines that the Product is defective and is covered by this Limited Warranty, Tomocube reserves the right, in its sole discretion, to repair or replace the defective Product.

Tomocube's liability for defective products and Buyer's exclusive remedy shall be limited to such repair or replacement. No Product will be returned to Tomocube without Tomocube's prior written consent. The validity of the warranties contained herein, with respect to certain other defects, are subject to (a) Buyer's demonstration that the Product has been stored, maintained and operated in accordance with instructions provided by Tomocube to Buyer and standard industry; undamaged as a result of negligence, improper handling or accident by anyone other than Tomocube, (b) payment of all invoices by Buyer for any Product or other charges to which Tomocube is entitled, and (c) authorized by Tomocube for repair of the Product; or (d) Buyer's proof that the Product has not been modified or altered without Tomocube's prior written consent. Subject to the foregoing, the warranty contained herein is valid for twelve (12) months from the date of delivery of

the product by Tomocube, unless another warranty period is specified on the surface of the Certificate of Quality.

Tomocube makes no warranties with respect to parts with limited technical life, such as data storage, any parts need periodic replacement and consumables. Components or products produced by other manufacturers are warranted by Tomocube only to the extent that the manufacturer supplying such components to Tomocube warrants those components and to the extent that Tomocube may assign to the purchaser. If Tomocube's software is included in the Product, Tomocube warrants that, when properly installed, software designed for use with a particular hardware product will not be able to execute its programming instructions due to defects in materials and workmanship. If Tomocube is notified of a defect during the applicable warranty period, Tomocube will repair or replace the software media that does not execute programming instructions due to such defect. Tomocube does not warrant that the software will be uninterrupted or error-free.

For local contact information, visit:
www.tomocube.com/contacts/distributors

Tomocube, Inc.
4th floor, 155, Sinseong-ro, Yuseong-gu
Daejeon 34109, Republic of Korea
www.tomocube.com

Doc. No. TPM-HTX-01, Version 1, 1/2023